

## Wybrane prace naukowe dt. ryzyka związanego z metodą *in vitro*

### Badania z recenzowanych światowych pism naukowych dotyczą ryzyka, jakie niesie procedura *in vitro* zarówno dla dziecka, jak i dla matki

„Techniki używane w czasie *in vitro* oraz innych metod sztucznego zapłodnienia powinny być traktowane jako potencjalnie teratogenne (powodujące wady płodu), a informacje na ten temat powinny być przekazywane lekarzom oraz opinii publicznej” – apelują naukowcy w czasopiśmie „*Fertility & Sterility*” (R. Klemetti, M. Gissler, T. Sevón, S. Koivurova, A. Ritvanen, E. Hemminki, *Children born after assisted fertilization have an increased rate of major congenital anomalies*, w: „*Fertility & Sterility*” Vol. 84, No. 5, November 2005, s. 1300-1307).

„Pacjenci *in vitro* powinni być uświadamiani na temat zwiększonego ryzyka powikłań okołoporodowych” – czytamy w „*Obstetrics&Gynecology*” (R. Jackson, K. Gibson, Y. Wu, M. Croughan, *Perinatal Outcomes in Singletons Following In Fertilization: A Meta-Analysis*, w: “*Obstetrics&Gynecology*”, Vol. 103, no 3, March 2004).

### Przykładowe wyniki z załączonych badań:

- Australijskie badania 4000 dzieci urodzonych między 1993 a 1997 r. wykazały, że wśród dzieci poczętych w sposób naturalny 4,2% ma wady wrodzone, natomiast wśród dzieci poczętych metodą *in vitro* – 9%. Odsetek dzieci obciążonych więcej niż jedną wadą wrodzoną w grupie kontrolnej wynosi 0,5%, natomiast wśród dzieci poczętych *in vitro* 1,6% (M. Hansen, J. Kurinczuk, C. Bower, S. Webb, *The risk of major birth defects after intracytoplasmic sperm injection and in-vitro fertilization*, w: „*The New England Journal of Medicine*”, 2002 March 7; 346: 725–30)
- Metaanaliza 22 badań naukowych, w których dokonano porównania stanu zdrowia dzieci urodzonych w wyniku zastosowania metody *in vitro* i dzieci poczętych w sposób naturalny wykazała, że u tych pierwszych pochodzących z pojedynczej ciąży w porównaniu do grupy kontrolnej ryzyko względne urodzenia się przed 32. tygodniem ciąży wynosi - 3,27; urodzenia się przed 37. tygodniem - 2,04; bardzo niskiej masy urodzeniowej - 3,00; niskiej masy urodzeniowej - 1,7; urodzenia wskutek cesarskiego cięcia -1,54; przyjęcia na intensywny oddział opieki noworodkowej - 1,27; umieralności okołoporodowej 1,68. (F.M. Helmerhorst, D. Perquin, D. Donker, M. Keirse, *Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies*, w: „*BMJ*”, 2004; 328; 261)
- Badania przeprowadzone na 20 000 pojedynczych ciąż kobiet, u których zapłodnienie nastąpiło metodą *in vitro* albo poprzez wprowadzenie plemnika do cytoplazmy komórki jajowej wykazały ponad czterokrotny wzrost ryzyka martwych urodzeń w porównaniu z kobietami, które zaszły

w ciąży w sposób naturalny po innej terapii bezpłodności (K. Wisborg, H.J. Ingerslev, T.B. Henriksen, *IVF and stillbirth: a prospective follow-up study*, w: „Human Reproduction”, 2010, vol. 25, No. 5, s. 1312-1316).

- Badania przeprowadzone na 845 384 przypadkach ciąż zarejestrowanych w latach 1988-2002 w medycznym rejestrze urodzeń w Norwegii wykazały 6,3 razy większe ryzyko wystąpienia łożyska przodującego w pojedynczych ciążach powstałych w wyniku *in vitro* w stosunku do ciąż uzyskanych naturalnie. (L.B. Romundstad, P.R. Romundstad, A. Sunde, V. von Düring, R. Skjaerven, L.J. Vatten, *Increased risk of placenta previa in pregnancies following IVF/ICSI; a comparison of ART. And non-ART. Pregnancies in the same mother*, w: „Human Reproduction” Vol. 21, No. 9, 2006, s.2353-2358)
- Z badań nad 1500 5-letnich dzieci z 5 krajów europejskich wynika, że w porównaniu z dziećmi poczętymi naturalnie u dzieci poczętych *in vitro* 1,8 razy częściej występowały poważne wady rozwojowe. Częściej zapadały one na choroby dziecięce (odpowiednio 77% i 57%), częściej przechodziły operacje chirurgiczne (odpowiednio 22% i 14%) i częściej były hospitalizowane (odpowiednio 28% i 20%) (M. Bonduelle, U.-B. Wennerholm, A. Loft, B.C. Tarlatzis, C. Peters, S. Henriët, C. Mau, A. Victorin-Cederquist, A. Van Steirteghem, A. Balaska, J.R. Emberson, A.G. Sutcliffe, *A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception*, w: „Human Reproduction”, Vol. 20, No. 2, 2005, s.413-419)
- „Działanie globalnego przemysłu [IVF] opiera się na założeniu, że daje on gwarancję bezpieczniejszych ciąż, ale wysoki poziom wskaźnika umieralności matek sugeruje, iż jest odwrotnie” – napisano w artykule wstępnym „BMJ” (S. Bewley, L. Foo, P. Braude, Editorial. *Adverse outcomes from IVF*, w: „British Medical Journal”, 2011; 342: d436)
- Szwedzkie badania oparte na zebranych w ciągu 25 lat zapisach ze wszystkich krajowych klinik wykazały, że ryzyko zapadalności na nowotwory u dzieci poczętych *in vitro* wynosi 1,42 w stosunku do występującego u dzieci poczętych naturalnie. (O. Finnström, B. Källén, A. Lindam, E. Nilsson, K.-G. Nygren, P. Otterblad Olausson, *Maternal and child outcome after in vitro fertilization – a review of 25 years of population-based data from Sweden*, w: „Acta Obstetricia et Gynecologica Scandinavica”, 90(2011), s. 494-500)
- Holenderskie badanie przeprowadzone na ponad 19 000 kobiet, które były poddane procedurze *in vitro* w latach 1983-95 oraz 6 000 kobiet z grupy porównawczej (kobiety ze zmniejszoną płodnością, nie poddane procedurze *in vitro*) wykazały, że w ciągu kolejnych 15 lat od poddania się *in vitro* może zwiększyć blisko czterokrotnie ryzyko zachorowania na raka jajnika. W grupie po *in vitro* standaryzowany wskaźnik zapadalności (SIR) wynosił 1,59 wobec 1,02 w grupie porównawczej. Zaś w okresie po 15 latach od wykonania *in vitro* SIR miał wartość 3,54, zaś w grupie porównawczej (kobiet, które nie przeszły *in vitro*) 1,09. (F.E. Leeuwen, H. Klip, T.M. Mooij et al., *Risk of borderline and invasive ovarian tumours after ovarian stimulation for in vitro fertilization in a large Dutch cohort*, w: „Human Reproduction” 2011, 26(12): 3456-3465)

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# Cancer Risk in Children and Young Adults Conceived by In Vitro Fertilization



**WHAT'S KNOWN ON THIS SUBJECT:** No clear-cut increase in cancer risk after IVF has been found, but most studies were too small to answer the question. There are characteristics of children who are conceived by IVF that could increase cancer risk.



**WHAT THIS STUDY ADDS:** This study is large enough to demonstrate that a slight but statistically significant cancer risk exists for children who are conceived by IVF. An interesting finding is the seeming increase in the risk for histiocytosis.

## abstract

FREE

**OBJECTIVES:** Studies conducted so far have found no statistically significant increased risk for cancer among children who are born after in vitro fertilization (IVF).

**METHODS:** We followed 26 692 children who were born after IVF during the years 1982–2005 by using the Swedish Cancer Register and compared the number of children who had cancer and were born after IVF with children who were not conceived by IVF. Adjustment was made for year of birth.

**RESULTS:** Maternal age, parity, smoking, subfertility, previous miscarriages, BMI, and multiple births did not significantly affect cancer risk in offspring. High birth weight, premature delivery, and the presence of respiratory diagnoses and low Apgar score were risk factors for cancer. We identified 53 cases of cancer in children who were born after IVF against 38 expected cases: 18 of them with hematologic cancer (15 of them acute lymphoblastic leukemia), 17 with eye or central nervous system tumors, and 12 with other solid cancers. There were 6 cases of Langerhans histiocytosis against 1.0 expected. The total cancer risk estimate was 1.42 (95% confidence interval: 1.09–1.87).

**CONCLUSIONS:** We found a moderately increased risk for cancer in children who were conceived by IVF. Putative intermediary factors could be preterm birth and neonatal asphyxia. *Pediatrics* 2010;126:e270–e276

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### KEY WORDS

childhood cancer, histiocytosis, in vitro fertilization, leukemia, brain tumor

### ABBREVIATIONS

IVF—in vitro fertilization

CI—confidence interval

ALL—acute lymphoblastic leukemia

OR—odds ratio

LGA—large for gestational age

ICSI—intracytoplasmic sperm injection

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Children who are born after in vitro fertilization (IVF) have an increased risk for perinatal complications and congenital malformations.<sup>1</sup> Some studies have also demonstrated a continued increased morbidity during at least the first years of life. The question of cancer risk in children who are conceived by IVF has been discussed in some articles. Bruinsma et al<sup>2</sup> studied 5249 births by using population-based registers and found 6 cancer cases against 4.33 expected. Klip et al<sup>3</sup> investigated cancer risk among 9484 children who were born after IVF or other related fertility techniques (85% were IVF) and found 16 cancers against 15.5 expected. Information was based on questionnaires with only 67% response rate. Three previous studies from Sweden<sup>4–6</sup> did not find any statistically significantly increased cancer risk. In the latest Swedish report, which was based on a follow-up of 16 280 infants by using national health registers, a risk estimate of 1.42 (95% confidence interval [CI]: 0.98–2.03) was found (based on 29 reported tumor cases against 21.4 expected). After adjustment for maternal age, parity, and smoking, the risk estimate decreased only slightly (1.35 [95% CI: 0.93–1.97]). One study found an association with maternal subfertility and an increased risk for acute lymphoblastic leukemia (ALL).<sup>7</sup> This study expands the follow-up time of the previously studied children who were conceived by IVF<sup>6</sup> and adds a number of children who were born after the end of the latest previous study (2001).

## METHODS

Children who were born after IVF in Sweden were identified from reports from all IVF clinics in Sweden.<sup>1,8</sup> Perinatal information was obtained by linkage with the Swedish Medical Birth Register.<sup>9</sup> Information on cancer development was obtained from the Swedish Cancer Register<sup>10</sup> up to and includ-

ing 2006. Thus, the definition of a patient having cancer is that the individual has been registered at least once in the Cancer Register.

Linkage was made by using the personal identification number that is assigned to each person who lives in Sweden. The first infant who was born after IVF in Sweden was born in 1982. The study was restricted to infants who were born in the period 1982–2005, because no cancer case was yet (in 2006) identified in the cancer register among infants who were born later. The proportion of infants who were born after IVF among all infants who were born in Sweden increased from <1 per 1000 before 1988 up to 2% to 3% from 1997 onward.

Comparison was made with all infants who were recorded in the Medical Birth Register, survived the perinatal period, and had an identification number in the register. Maternal and infant characteristics were studied by using the Mantel-Haenszel method with odds ratio (OR) estimates and 95% CIs estimated with Miettinen's technique. For comparisons of 2 ORs, 2-tailed *z* tests were made on the basis of Mantel-Haenszel  $\chi^2$  tests. Trend tests for *i* ORs were made as weighted linear regression analyses of the log(OR<sub>*i*</sub>). The following putative maternal risk factors were analyzed: maternal age (5-year classes), parity (1, 2, 3,  $\geq 4$ ), maternal smoking (unknown, 0, <10 cigarettes per day,  $\geq 10$  cigarettes per day), number of previous miscarriages (0, 1, 2,  $\geq 3$ ), years of unwanted childlessness (unknown, 0, 1, 2, 3, 4,  $\geq 5$ ), BMI (unknown, <19.8, 19.8–25.9, 26.0–29.9, 30.0–34.9, 35.0–39.9,  $\geq 40$ ), and number of infants in birth (1, 2,  $\geq 3$ ). The following putative neonatal factors were analyzed in singletons: preterm birth (<32 and <37 completed weeks), low birth weight (<1500 and <2500 g), small for gestational age and large for gestational age (LGA; <2

and >2 SDs according to growth graphs on the basis of data in the Medical Birth Register<sup>11</sup>), presence of neonatal diagnoses of respiratory problems or use of continuous positive airway pressure, and low 5-minute Apgar score.

This statistical method used is sensitive to changes in population size with age as a result of deaths or emigration. The death rate after the perinatal period is low (0.2% among all children in the population). Emigration could not be controlled for. It is probably low for children whose mother was born in Sweden and those who had 2 parents of Swedish nationality but may be higher for children whose parents were not born in Sweden and/or did not have a Swedish nationality. A reanalysis was therefore made with exclusion of all children whose mother was not born in Sweden or had at least 1 parent who was not of Swedish nationality (18% of all). This study was performed within the responsibilities of the National Board of Health and Welfare; therefore, no ethical approval from outside ethical committees was needed.

## RESULTS

Putative maternal risk factors for cancer in their children are shown in Table 1. In all analyses, adjustment was for year of birth, and additional adjustments were made as indicated in the tables.

Compared with other women, women who had IVF were older, were more often of first parity, smoked less, had fewer previous miscarriages, had a period of unwanted childlessness, more often had high BMI, and had an increased rate of multiple pregnancies.<sup>1</sup> None of these characteristics showed a statistically significant association with the risk for cancer in the offspring. The highest OR (1.18) was seen for a period of unwanted child-

**TABLE 1** Maternal Risk Factors for Cancer in the Offspring

Variable	No. of Children		OR	95% CI	Test for Trend	
	With Cancer (n = 6458)	In the Population (n = 2 417 878)			z	P
Maternal age, y <sup>a</sup>					1.47	.14
<20	186	60 807	0.98	0.85–1.14		
20–24	1347	464 926	0.96	0.90–1.02		
25–29	2334	861 074	0.99	0.94–1.04		
30–34	1783	695 093	1.05	0.99–1.11		
35–39	699	284 173	1.03	0.95–1.11		
40–44	104	49 930	0.89	0.73–1.08		
≥45	5	1875	1.18	0.49–2.86		
Parity <sup>a</sup>					0.25	.39
1	2642	1 011 804	1.00	0.95–1.06		
2	2317	863 357	1.00	0.95–1.06		
3	1034	375 460	0.98	0.91–1.05		
≥4	465	167 256	1.03	0.93–1.14		
Smoking <sup>a</sup>					1.48	.13
Unknown	793	202 276	—	—		
No	4273	1 770 934	1.00	Reference		
<10 cigarettes per d	834	282 091	0.99	0.92–1.07		
≥10 cigarettes per d	558	162 577	1.08	0.99–1.19		
Previous miscarriage <sup>b</sup>					0.94	.26
0	5507	2 007 790	1.00	Reference		
1	737	320 300	0.96	0.88–1.03		
2	157	66 215	1.02	0.87–1.19		
≥3	57	23 573	1.06	0.82–1.38		
Years of unwanted childlessness <sup>b</sup>					1.18	.26
0	6051	2 267 021	1.00	Reference		
1	128	44 594	1.04	0.87–1.24		
2	95	40 135	0.94	0.77–1.15		
3	50	22 048	0.91	0.69–1.20		
4	38	13 466	1.13	0.80–1.55		
≥5	97	30 614	1.18	0.96–1.44		
BMI <sup>c</sup>					0.89	.27
Unknown	375	227 366	—	—		
<19.8	171	109 542	0.97	0.82–1.14		
19.8–25.9	1172	752 677	1.00	Reference		
26.0–29.9	272	180 748	1.02	0.89–1.16		
30.0–39.9	139	96 544	1.01	0.85–1.21		
≥40.0	8	6356	0.97	0.49–1.95		
No. of infants in birth <sup>d</sup>						
1	6295	2 358 046	1.00	Reference		
2	150	60 513	1.07	0.90–1.27		
3	4	1578	1.13	0.87–1.49		

Women with perinatally dead infants and with infants without complete identification numbers were excluded.

<sup>a</sup> Adjusted for year of birth and for the other variables. For maternal age and parity, each group is compared with all other groups. One infant in population lacked parity information.

<sup>b</sup> Adjusted for year of birth and for the other variables.

<sup>c</sup> Adjusted for year of birth, number of previous miscarriages, and years of unwanted childlessness. Restricted to children who were born 1992–2005.

<sup>d</sup> Adjusted only for year of birth.

lessness of >5 years, but it did not reach statistical significance, and there is no clear-cut trend with duration of childlessness.

Table 2 shows the impact of some neonatal characteristics on cancer risk. The only adjustment made was for

year of birth. There was an increased risk for cancer associated with preterm birth before week 37, for birth weight of ≥4500 g, for LGA, and for low Apgar score. Among these characteristics, infants who were born after IVF are characterized by an increased risk

for preterm birth, low birth weight, being small for gestational age, respiratory problems, and low Apgar score.

Among children who were conceived by IVF and survived the perinatal period, there were 53 cases of cancer (Table 3). Eighteen infants had hematologic neoplasms (12.3 expected), 15 of them ALL. Fifteen infants had central nervous system neoplasms (8.1 expected), 7 of them astrocytomas. Two infants had malignant retinal tumors (retinoblastoma); the expected number was 1.25. Six infants had a diagnosis of histiocytosis (expected number: 1.0).

After adjustment for year of birth, the OR for childhood cancer among infants who were born after IVF was 1.42 (95% CI: 1.09–1.87; *P* = .01). After exclusion of infants with histiocytosis from the analysis, the OR decreased to 1.34 (95% CI: 1.02–1.76). Despite that maternal age, parity, smoking, and years of unwanted childlessness did not seem to affect cancer risk in our material, we tried an adjustment for these variables besides year of birth; the resulting OR hardly changed, from 1.42 to 1.45 (95% CI: 1.10–1.91), supporting the lack of confounding. All additional analyses were therefore made after adjustment only for year of birth. When children whose mother was born outside Sweden or whose father or mother was of non-Swedish nationality were excluded, 49 cancer cases remained (5505 in the population), and the OR increased slightly to 1.52 (95% CI: 1.15–2.02).

Fifteen of the children who were conceived by IVF and had cancer had been conceived by intracytoplasmic sperm injection (ICSI). The expected number of children who were conceived by ICSI, calculated from the ICSI rate among all infants who were born after IVF and adjusted for year of birth, is 15.5.

Among the 53 children who were conceived by IVF and developed cancer, 28

**TABLE 2** Neonatal Risk Factors for Cancer

Variable	No. of Children		OR	95% CI
	With Cancer ( <i>n</i> = 6459)	In the Population ( <i>n</i> = 2 419 274)		
Gestational duration, wk <sup>a</sup>	6435	2 413 568	—	—
<32	55	18 702	1.21	0.93–1.58
<37	444	146 120	1.16	1.05–1.28
Birth weight, g <sup>a</sup>	6420	2 409 373	—	—
<1500	40	15 369	1.06	0.78–1.45
<2500	288	101 899	1.07	0.95–1.21
≥4500	256	85 690	1.21	1.07–1.38
Growth deviation <sup>a</sup>	6246	2 342 927	—	—
SGA, less than −2 SDs	141	54 780	0.91	0.77–1.08
LGA, >2 SDs	440	132 277	1.34	1.21–1.47
Respiratory diagnosis <sup>b</sup>	194	75 908	1.07	0.92–1.23
Apgar score <sup>c</sup>	6385	2 402 279	—	—
<7 at 5 min	93	27 313	1.33	1.08–1.63

Perinatally dead infants and infants without complete identification numbers were excluded. OR adjusted only for year of birth. Numbers for each variable give number of infants with relevant information. SGA indicates small for gestational age.

<sup>a</sup> Only singletons with known gestational duration or birth weight; for growth deviation, known gestational duration, birth weight, and infant gender.

<sup>b</sup> Includes infants who were treated with continuous positive airway pressure or mechanical ventilation. All infants.

<sup>c</sup> All infants with known 5-minute Apgar score.

were younger than 3 years at cancer diagnosis, 14 were aged 3 to 5 years, 7 were 6 to 10 years, and 4 were older than 10 years (1 was 19 years). The expected numbers calculated from the age distribution of all cancers and adjusted for year of birth was 26.3, 15.4, 8.1, and 3.2, respectively. The distribution of age at onset in children who were conceived by IVF thus agrees well with that of other children. The OR among children who were conceived after IVF and received a cancer diagnosis before the age of 3 was 1.87 (95% CI: 1.27–2.77) and from age ≥3 years was 1.32 (95% CI: 0.89–1.96). These 2 estimates do not differ significantly, however ( $z = 1.23$ ,  $P = .19$ ), so it is not certain that the risk actually decreases after the age of 3.

Among children who were born after IVF and developed cancer, 7 had malformation diagnoses. One child had a cleft lip/palate, 1 had a coarctation of the aorta, 1 had an unspecified musculoskeletal malformation, 1 had an arm reduction, 1 had a kidney malformation, and 2 had Down syndrome. One of the 2 infants with Down syndrome had

ALL, and the other had acute myeloid leukemia.

Twenty-five infants who developed cancer were born in multiple births (24 twins, 1 triplet); the expected number from the yearly distribution of cases and the yearly multiple birth rates among all infants who were born after IVF was 19.1. The excess is thus not statistically significant ( $\chi^2 = 3.3$ ,  $P = .09$ ).

## DISCUSSION

This is the first study to demonstrate a statistically significant increase in cancer risk among children who are born after IVF. It is also the largest of the published studies. In our previous study,<sup>6</sup> which was based on 16 280 children and included 29 of the 53 cancer cases, nearly the same risk estimate was obtained as in this study (1.41 vs 1.42), but statistical significance was not reached (95% CI: 0.98–2.03).

Mothers of children who were born after IVF differ in many characteristics besides subfertility from other women who give birth: higher maternal age, high percentage of first parity, less smoking, increased number with high

BMI, and fewer who were born outside Sweden. Whether such differences can confound the analysis depends on their possible effect on the risk for cancer in the offspring. In our analysis, none of the included characteristics affected the total cancer risk, and adjustment for them therefore did not change the risk estimate. For the main analysis, we therefore did not adjust for any 1 of them. Some previous studies that used different methods analyzed the possible associations between maternal characteristics and the general risk for cancer in the offspring. Low maternal age (<20) was associated with an increased risk for acute leukemia,<sup>7</sup> whereas high maternal age had no effect in another study<sup>12</sup> but was associated with acute myeloid leukemia in 1 study.<sup>13</sup> We saw no certain maternal age effect on the risk for cancer in the offspring. The putative effect on acute myeloid leukemia seems not to be of importance in our study, because only 1 such case occurred among the cancers in children who were born after IVF. An increased risk for childhood leukemia with parity ≥4 has been found.<sup>14</sup> An association between previous miscarriages and brain tumors has been described.<sup>15</sup>

Some articles discussed the putative effect of maternal smoking on cancer risk in the offspring, but the results varied and no clear association was seen.<sup>16</sup> A protective effect on ALL was described in 1 study<sup>17</sup> but was not found in another.<sup>7</sup> A specific association has been described between maternal smoking and neuroblastoma<sup>18</sup> or retinoblastoma<sup>19</sup> in the child.

Maternal education level was associated in 1 study with a decreased risk for childhood cancer.<sup>20</sup> The effect of maternal education on IVF is complex, but, if anything, high maternal education is associated with a higher use of IVF.<sup>8</sup>

Many neonatal characteristics have been associated with a generally in-

**TABLE 3** Nature of the 53 Cancer Cases Observed Among Infants Who Were Born After IVF

Cancer Diagnosis and Location	No. of Cases	IVF Method Used (n)
Hematologic neoplasms	18	
ALL	15	Standard IVF (7), fresh testicular ICSI (6), fresh epididymal ICSI (1), unspecified (1)
Prolymphocytic leukemia	1	Standard IVF (1)
Acute myeloid leukemia	1	Standard IVF (1)
Chronic myeloid leukemia	1	Standard IVF (1)
Histiocytosis	6	
Langerhans histiocytosis	6	Standard IVF (4), fresh testicular ICSI (1), cryopreserved ejaculated ICSI (1)
Central nervous system or eye neoplasms	17	
Pilocytic astrocytoma in temporal lobe	1	Standard IVF (1)
Pilocytic astrocytoma in cerebellum	3	Fresh ejaculated ICSI (1), fresh testicular ICSI (1), cryopreserved nonejaculated ICSI (1)
Pilocytic astrocytoma, unspecified	1	Standard IVF (1)
Astrocytoma, cerebellum	1	Standard IVF (1)
Astrocytoma, brain stem	1	Standard IVF (1)
Medulloblastoma, cerebellum	1	Standard IVF (1)
Medulloblastoma, unspecified	1	Fresh testicular ICSI (1)
Glioblastoma, cerebellum	1	Standard IVF (1)
Glioblastoma, unspecified	1	Fresh ejaculated ICSI (1)
Unspecified brain stem neoplasm	1	Standard IVF (1)
Plexus papilloma	1	Cryopreserved standard IVF (1)
Ependymoma, spinal cord	1	Standard IVF (1)
Unspecified malignant optic nerve neoplasm	1	Standard IVF (1)
Unspecified malignant retinal neoplasm	2	Standard IVF (2)
Soft tissue neoplasms	3	
Fibrosarcoma	1	Standard IVF (1)
Pleomorphic sarcoma upper limb	1	Standard IVF (1)
Unspecified soft tissue neoplasm	1	Standard IVF (1)
Adenocarcinomas	3	
Adenopapilloma in appendix	1	Cryopreserved nonfresh ICSI (1)
Squamous cell cancer in larynx	1	Standard IVF (1)
Cancer in situ, vulva	1	Standard IVF (1)
Other neoplasms	6	
Craniopharyngeoma	1	Standard IVF (1)
Malignant melanoma lower limb	1	Standard IVF (1)
Hepatoblastoma	1	Standard IVF (1)
Mature testicular teratoma	2	Standard IVF (2)
Medullary cancer in thyroid gland	1	Standard IVF (1)

creased risk for cancer or for specific cancer forms in the offspring. Two of the children who were born after IVF and developed cancer had Down syndrome. A strong association exists between Down syndrome and childhood leukemia. The risk to have a child with a chromosome anomaly seems not to be increased after IVF,<sup>21</sup> and in that material, the risk for birth of an infant with Down syndrome after IVF was estimated to be 1.09 (95% CI: 0.73–1.62), on the basis of 27 cases (unpublished data) and adjusted for year of birth, maternal age, parity, smoking, and BMI. The other malformations that

were observed among the children who were conceived by IVF and developed cancer are not known to be associated with an increased cancer risk.

Neonatal factors could act as intermediaries between the IVF procedure and cancer development. Two neonatal factors that have shown a relatively constant association with an increased childhood cancer risk are high birth weight and neonatal asphyxia. High birth weight as a risk factor has been described repeatedly<sup>7,15,22,23</sup> and was also seen in our material (Table 2) as a birth weight  $\geq 4500$  g, or LGA. This has

been looked on as an effect of fetal growth factors.<sup>24</sup> If anything, infants who are born after IVF show an increased risk for intrauterine growth restriction.<sup>25</sup> One study found an increased risk for childhood leukemia after preterm birth,<sup>23</sup> which was also indicated in our material.

An increased risk for childhood cancer after neonatal asphyxia or oxygen treatment has also been described,<sup>12,20,26–28</sup> and this was also indicated in our material as seen for low Apgar score. This could be an intermediary in the effect of IVF on cancer risk, because an increased risk for low Apgar score and respiratory problems is seen in infants who are born after IVF.<sup>25</sup>

Multiple births are common complications in IVF pregnancies. There was no certain effect of multiple births on cancer risk in our material. We found a slight excess of children who were born in multiple births after IVF and developed cancer, but this was not statistically significant. One publication even suggested that twins have a lower cancer risk than singletons.<sup>29</sup>

Two tumors that possibly are associated with IVF have been specifically discussed. One is retinoblastoma, on the basis of a Netherlands study.<sup>30</sup> A more recent study of the same authors found no significant increase in retinoblastoma risk after IVF in the years after the initial observation.<sup>31</sup> In our material, there were only 2 malignant eye neoplasms, a number that is close to the expected number. The second condition is Langerhans histiocytosis. This has been observed only in the Swedish material, but it is not clear whether such cases would have been included in previous studies. We found a total of 6 such cases against the expected number of 1. Five of them were observed in a previous study.<sup>6</sup> In addition, 2 children had Letterer-Siwe disease, a closely related condition. They were

not registered in the cancer register but were identified from the hospital discharge register.<sup>5</sup>

The biological nature of Langerhans histiocytosis is debatable; is it a malignant neoplastic disease or a reactive process?<sup>32–34</sup> Little is known about the epidemiology of Langerhans histiocytosis. One study identified maternal urinary tract infections, feeding problems during infancy, and blood transfusions during infancy<sup>35</sup> as risk factors. Another study found increased risks associated with neonatal infections, solvent exposure, and family thyroid disease.<sup>36</sup> If histiocytosis is not regarded as a true malignancy, then there could be reason to exclude them from the analysis of total cancer risk;

the OR then went down to 1.34 but remained statistically significant.

We can see no biological explanation for why children who are conceived after IVF would have an increased risk for histiocytosis. It is noteworthy that only 1 additional case appeared in the new data set compared with that previously published, and thus the follow-up study did not support the previous observation of an increased risk. Because the observation has not been verified in independent investigations, it is possible that it is a random event. Additional information on this putative association is needed.

## CONCLUSIONS

We found a moderately increased risk for cancer in children who were con-

ceived by IVF. This is probably not attributable to the IVF procedure itself but could be an effect of confounding from unidentified characteristics of women who undergo IVF or could act via the widely known increased risks for neonatal complication. It should be stressed that the individual risk for a child who is born after IVF to develop childhood cancer is low. Additional studies on large populations are needed to permit analysis of such a rare outcome as cancer and notably of specific types.

## ACKNOWLEDGMENT

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# Neonatal outcome and congenital malformations in children born after in-vitro fertilization

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**BACKGROUND:** To evaluate the neonatal outcome and the prevalence of congenital malformations in children born after IVF in northern Finland we carried out a population-based study with matched controls. **METHODS:** Firstly, 304 IVF children born in 1990–1995 were compared with 569 controls, representing the general population in proportion of multiple births, randomly chosen from the Finnish Medical Birth Register (FMBR) and matched for sex, year of birth, area of residence, parity, maternal age and social class. Secondly, plurality matched controls ( $n = 103$ ) for IVF twins ( $n = 103$ ) were randomly chosen from the FMBR and analysed separately. Additionally, IVF singletons ( $n = 153$ ) were compared with singleton controls ( $n = 287$ ). Mortality rates were compared with national figures from FMBR. **RESULTS:** Most mortality rates were twice as high as national figures in the general population. When compared with the control group representing the general population, the incidences of preterm birth [odds ratio (OR) 5.6, 95% confidence interval (CI) 3.7–8.6], very low birth weight (OR 6.2, 95% CI 2.0–19.0), low birth weight (OR 9.8, 95% CI 5.6–17.3), neonatal morbidity (OR 2.4, 95% CI 1.7–3.4) and hospitalization (OR 3.2, 95% CI 2.2–4.6) were significantly higher in the IVF group. The prevalence of heart malformations was four-fold in the IVF population than in the controls representing the general population (OR 4.0, 95% CI 1.4–11.7). **CONCLUSIONS:** Neonatal outcome after IVF is worse than in the general population with similar maternal age, parity and social standing, mainly due to the large proportion of multifetal births after IVF. The higher prevalence of heart malformations does not solely arise from multiplicity but from other causes. In order to improve neonatal outcome after IVF, the number of embryos transferred should be limited to a minimum.

*Key words:* congenital malformations/hospitalization/in-vitro fertilization/neonatal outcome

## Introduction

Since IVF has become an efficient and widely used treatment for infertile couples over the last 20 years there has been concern about the health of children born after this treatment. Because of the relative novelty of IVF, the follow-up of children born after IVF is still quite limited. IVF is responsible for the increasing number of multifetal pregnancies, multiplicity being an important risk factor for adverse neonatal outcomes due to preterm birth, low birth weight and small for gestational age (SGA) (Friedler *et al.*, 1992; Balen *et al.*, 1993; Gissler *et al.*, 1995; Bergh *et al.*, 1999; Buitendijk, 1999). A number of studies have shown that children born after IVF have more neonatal problems (Yeh *et al.*, 1990; D'Souza *et al.*, 1997) and need longer hospitalization and intensive care than spontaneously conceived ones (Gissler *et al.*, 1995; Addor *et al.*, 1998; Koudstaal *et al.*, 2000). In

spite of the strong effect of multiplicity on neonatal outcomes, children from singleton IVF pregnancies also seem to be more predisposed to adverse neonatal outcomes such as preterm birth, low birth weight and longer hospitalization than other children (Gissler *et al.*, 1995).

Infertile women are frequently of advanced age at the time of conception and they may have a variety of underlying causes for infertility. Furthermore, during the IVF process the embryo is exposed to mechanical, thermal and chemical alterations. Theoretically, these factors can increase the risk of congenital malformations. Lancaster's study from the late 1980s was the first to report a higher prevalence of neural tube defects and transposition of the great vessels among IVF children (Lancaster, 1987). The majority of later studies have been negative as far as congenital malformations are concerned. However, a few very recent studies have reported an increase

in the prevalence of neural tube defects, oesophageal atresias (Bergh *et al.*, 1999; Ericson and Källén, 2001), omphalocele and hypospadias (after ICSI) among IVF children compared with spontaneously conceived controls (Ericson and Källén, 2001).

The objective of this population-based cohort study is to compare neonatal outcomes, the need for hospitalization after birth and the prevalence of congenital malformations between IVF children and spontaneously conceived controls matched for maternal age, parity, social class, sex, year of birth, area of residence and fetal plurality. The aim of having a unique study design with two separate control groups, firstly representing general population and secondly controls matched for plurality, was to evaluate the effect of IVF and multiplicity separately from each other on the neonatal outcome. Our *a priori* hypothesis was that there are more neonatal complications leading to longer hospitalization as well as a higher incidence of congenital malformations in children born after IVF, which was based on a few observations in previous literature and on a pilot study we conducted in 1997.

## Materials and methods

A cohort study with a group of IVF exposed children born in 1990–1995 and two groups of unexposed naturally conceived controls was set up. The exposed children were recruited from the register at the IVF outpatient clinic in the University Hospital of Oulu and the Infertility Clinic of the Family Federation of Finland in Oulu. These two clinics, the latter being a private clinic, cover all IVF treatments in northern Finland, i.e. the provinces of Oulu and Lapland.

Pre-study sample size calculations—primary for the subsequent long-term follow-up—were based on the approximation of a frequency of 15% for developmental disorders including neurological signs (gross and fine motor, speech and other handicaps) among the unexposed population. For 80% power, 0.05 alpha-error, ratio of 2:1 (unexposed:exposed) and a risk ratio of 1.6 for the outcome between the groups, a sample size of at least 238 exposed and 476 unexposed children was required.

There were 306 liveborn (154 singletons and 152 children from multiple pregnancies; 123 twins, 25 triplets and four quadruplets) and three stillborn (one twin and two triplets) IVF exposed children born in 1990–1995 in the study group. One singleton was conceived by ICSI, the rest of the children were born after conventional IVF and only fresh embryos were used. To examine neonatal outcome two separate unexposed liveborn control groups from the Finnish Medical Birth Register (FMBR; includes all births after completion of the 22nd gestational week or with birth weight of 500 g or more) were to be chosen: (i) 618 live controls (i.e. 2:1, 2×309, at this point we were unaware of the three stillborn cases among the IVF population) were to be chosen at random for all exposed children and matched in the following order: sex, year of birth, area of residence (i.e. the provinces of Oulu and Lapland), parity, maternal age and social class defined by occupation of the mother [upper white collar, lower white collar, blue collar, entrepreneurs (small business) and farmers, students, housewives and unknown]. This control group (control I) represents the general population in the proportion of multiple births with similar maternal age, parity and social class. (ii) A second control group for multiples was randomly chosen (1:1, 152:152), and matched for plurality in addition to the matching

criteria listed above. Consequently, we were able to do the stratified analyses by plurality, i.e. exposed singletons had their own singleton controls derived from control I group (hereafter control SII), and multiple births their own controls (control TII, T refers to twin). However, in the final study population the numbers differ because data on some unexposed children (missing controls) were not found (i.e. an exposed child may have only one control instead of the planned two) (Figure 1).

Data were collected from hospital records by a resident physician (S.K.) regarding neonatal parameters, length of neonatal hospitalization at neonatal wards and intensive care units, and congenital malformations. Neonatal diagnoses in hospital records were set by resident or specialized paediatricians and were based on the International Classification of Diseases (ICD-10) codes. For the subsequent long-term follow-up study we used the hospital records for the previous 3 years, enabling us to get information of congenital malformations detected at any stage between birth and 3 years of age.

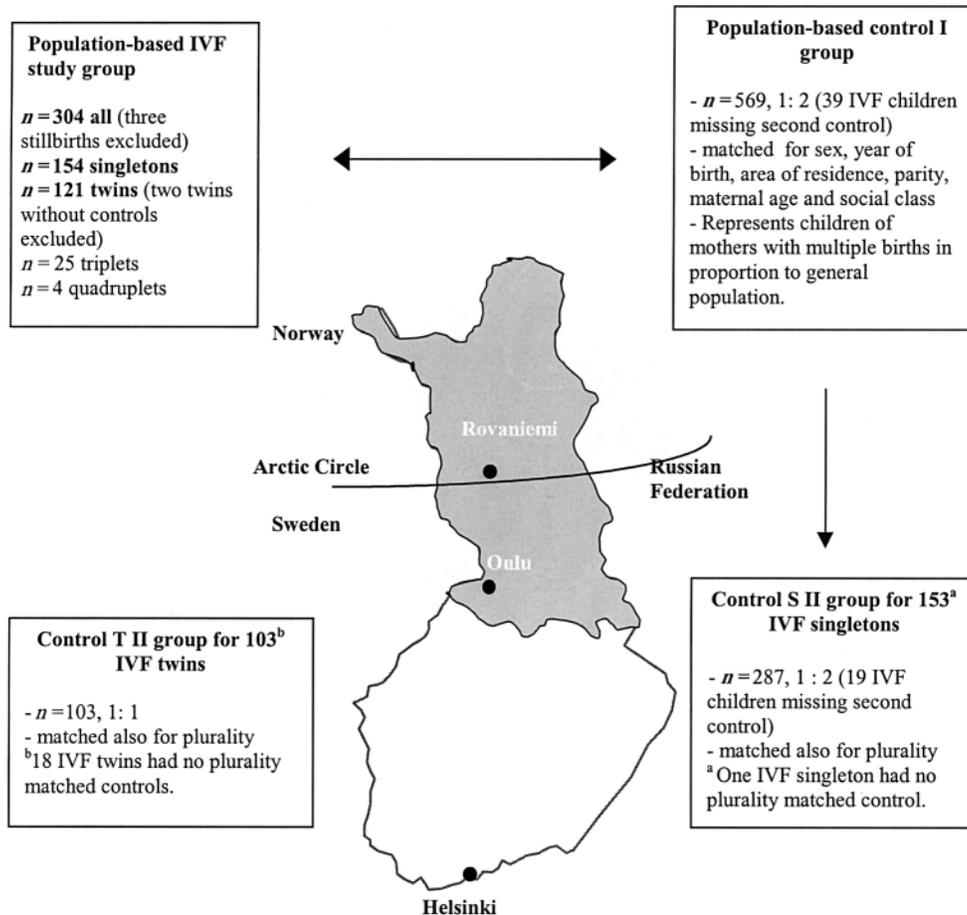
Neonatal morbidity was defined as having one or more of the following diagnoses based on ICD-10 codes: neonatal infections, hypoglycaemia, hyperbilirubinaemia needing blue light therapy, respiratory distress syndrome, bronchopulmonary dysplasia, patent ductus arteriosus, apnoea or intracranial haemorrhage. Ponderal index ( $\text{kg/m}^3$ ) is a neonatal measure of thinness reflecting intrauterine growth. Congenital malformations were defined according to the definition of the Finnish Register of Congenital Malformations and Birth Defects, which follows ICD-9.

The perinatal mortality rate (PNMR) includes stillbirths from the completed 22nd gestational week onwards or with birth weight of at least 500 g with early neonatal deaths. The early neonatal mortality rate includes neonatal deaths <7 days from birth, and the late neonatal mortality rate includes neonatal deaths during 7–27 days. Mortality rates were compared with national figures from FMBR regarding northern Finland, but stillbirths were excluded from further statistical analyses related to comparisons between the exposed children and their controls in terms of neonatal outcomes or congenital malformations.

Conditional logistic regression for matched sets was used to calculate odds ratios with 95% confidence intervals (CI) for categorical and categorized variables. The percentages were also calculated using matched sets, the denominator varies slightly due to some missing values, variable by variable. The aim of having a control cohort drawn from the general population was to compare the whole group of IVF children with the average population and to be able to estimate the consequences of the increased number of multiple pregnancies together with IVF treatment on the outcome of the child. The second set of controls matched for plurality was chosen to control the confounding caused by multiple birth on the outcome of the child. Success in matching was explored and it turned out that we were unable to follow only the matching criteria of the area of residence, because there were not enough eligible naturally conceived children from multiple pregnancies to be included in the control group. For the same reason, for triplets and quadruplets the matching criteria were not fulfilled at all and hence they were excluded from analyses stratified for plurality, but included in population-based analyses.

## Results

The final study population for population-based matched neonatal analyses and matched analyses stratified for plurality is presented in Figure 1. The male:female ratio was about 1:1 in both IVF and control populations.



**Figure 1.** Study design, final study population and the geographical catchment area of the study.

### Mortality

Mortality rates in the IVF group and in the general population in Northern Finland in 1990–1995 are presented in Table I. There were three stillbirths in the IVF group: one stillbirth occurred in a twin pregnancy and two stillbirths in a triplet pregnancy. Two infants in the IVF group and three infants in the control group died during the neonatal period. The causes of neonatal death in the IVF group were preterm birth in one of the cases and multiple anomalies (amniotic band sequence) in one case. In the control group the cause of death was preterm birth in all three cases.

### Neonatal outcome

The risk of preterm birth (<37th gestational week) was nearly six-fold higher in the IVF group when compared with controls representing the general population. The incidence of birth before the completion of the 32nd gestational week was over seven-fold and the incidence of birth between the 32nd and the 36th gestational weeks was over four-fold higher in the IVF group compared with control I group. No statistically significant differences in the proportion of children born preterm were found in analyses stratified for plurality (Table II).

The mean birth weight in the whole IVF group was 2917 g (SD 746.5) and in the control I group 3453 g (SD 585.7). In

the comparisons stratified for plurality the mean birth weights were 3364 g (SD 595.5) among the IVF singletons and 3483 g (SD 569.5) among the control singletons. For twins the figures were 2594 g (SD 528.1) versus 2547 g (SD 602.0) respectively. The risk of very low birth weight (<1500 g) was six-fold higher and the risk of low birth weight (1500–2499 g) was almost ten-fold higher in the whole IVF group when compared with control I group. Furthermore, a low Ponderal index (<25 kg/m<sup>3</sup>, which was the lower quartile in our study population) was significantly, almost three times, more common in the IVF population than in control I group. The analyses stratified for plurality showed no statistically significant differences for either very low/low birth weight or low Ponderal index (Table II).

The incidence of neonatal morbidity was significantly, over two-fold, higher in the IVF group when compared with the control I group. When stratified for plurality, no significant differences occurred. As a consequence of increased morbidity IVF children were significantly more often hospitalized and intubated during the neonatal period at neonatal intensive care units and/or neonatal wards than their controls representing group I. In analyses stratified for plurality no significant differences were found. No significant differences were found in the incidence of low Apgar score ( $\leq 7$ ) at 5 min in any of the comparisons (Table II).

**Table I.** The mortality rates in the IVF group and in the general population from the Finnish Medical Birth Register in northern Finland in 1990–1995

	IVF population/1000 births ( <i>n</i> = 309)	General population/ 1000 births ( <i>n</i> = 55 195)
Stillbirth rate (stillbirths from the completed 22nd gestational week onwards or with birth weight of at least 500 g)	9.7 (three stillbirths)	4.3
Perinatal mortality rate 1 (stillbirths from the completed 22nd gestational week onwards or with birth weight of at least 500 g with early neonatal deaths <7 days from birth)	16.2 (three stillbirths and two early neonatal deaths)	7.5
Perinatal mortality rate 2 (stillbirths from the completed 28th gestational week onwards or with birth weight of at least 500 g with early neonatal deaths <7 days from birth)	6.5 (one stillbirth and one early neonatal death)	5.1
Early neonatal mortality rate (neonatal deaths <7 days from birth)	6.5 (two early neonatal deaths)	2.8
Late neonatal mortality rate (neonatal deaths during 7–27 days from birth)	0 (no late neonatal deaths)	1.2
Total neonatal mortality rate (neonatal deaths during 0–27 days from birth)	6.5 (two neonatal deaths)	4.0

### Congenital malformations

There were in total 20 children with minor or major congenital malformations/syndromes in the IVF group, giving a malformation rate of 6.6%. No malformations occurred in stillborn children. Six cases of malformation were found in the IVF singletons, eight in twins and six in triplets. Fourteen cases of malformation were found in children born preterm (<37 gestational weeks) and six in children born at term. There were no major differences in the sex distribution of malformations in the IVF group (males, 11 versus females, 9). One case of trisomy 21 occurred in the IVF group. In the group of controls representing general population (control I) 25 children with minor or major congenital malformations/syndromes were found; 24 malformations in singletons and one in twins, giving a malformation rate of 4.4%. The majority of the malformations (*n* = 23) were found in infants born at term. The prevalence of congenital malformations in the control group was strongly associated with the male sex (males, 21 versus females, four), even if urogenital malformations were excluded. Specific congenital malformations are listed in Table III. In addition to the listed congenital malformations, a number of inguinal (IVF versus controls, nine versus eight) and umbilical hernias (10 versus three), unstable hips (two versus seven), undescended testes (two versus five) and hydroceles (one versus two) occurred in both groups, but were not considered to be malformations.

When all heart anomalies listed in Table III were analysed together, there was a four-fold increase in their prevalence in the IVF group compared with control I group [odds ratio (OR) 4.0, 95% confidence interval (CI) 1.4–11.7]. The difference was

not so prominent among singletons (OR 3.0, 95% CI 0.5–18.0) and was not found at all among twins (OR 0.8, 95% CI 0.2–3.4). For other malformations (including CNS, limb and visceral malformations) no significant differences were found among the groups (Table II).

### Discussion

The present study is a population-based cohort study consisting of all children born after IVF in northern Finland between 1990 and 1995, with carefully matched controls representing firstly the average population in the proportion of multiple births and secondly infants from multiple births in proportion to that in the IVF exposed group, to examine the effect of IVF on neonatal outcome independently of multiplicity. The unique study design with different control groups allows us to estimate the effect of IVF and multiplicity separately on the outcome of the infant born after infertility treatments. Furthermore, due to matching, the control population in this study represents children of old primiparous women minimizing the confounding effect of maternal age and parity. Another strength of this study is that we could trace nearly all children originally chosen for the study.

In our study, most mortality rates during the neonatal period were twice as high in the IVF group as in the general population from the same area and in the same time period. The power of the study in this respect was too small to make statistical inferences. Previous publications report stillbirth rates in IVF populations ranging between 4.3–19.7/1000 (MRC Working Party, 1990; Rizk *et al.*, 1991; Friedler *et al.*, 1992; FIVNAT,

**Table II.** Neonatal parameters and congenital malformations in matched IVF and control groups (The odds ratios and percentages were calculated using matched sets. The denominator varies slightly variable by variable due to some missing values.)

	Population-based analysis <sup>a</sup>		OR	Singletons		OR	Twins		OR
	IVF <i>n</i> = 304 <i>n</i> (%)	Control I <i>n</i> = 569 <sup>c</sup> <i>n</i> (%)	CI 95%	IVF <i>n</i> = 153 <i>n</i> (%)	Control SII <i>n</i> = 287 <sup>d</sup> <i>n</i> (%)	CI 95%	IVF <i>n</i> = 103 <i>n</i> (%)	Control TII <i>n</i> = 103 <i>n</i> (%)	CI 95%
Preterm birth <37 weeks	95 (31.5)	44 (7.8)	5.6 3.7–8.6	13 (8.6)	16 (5.6)	1.5 0.7–3.2	45 (43.7)	45 (43.7)	1.0 0.6–1.8
Birth before completed 32nd gestational week	16 (5.3)	6 (1.1)	7.5 2.6–21.5	3 (2.0)	3 (1.1)	2.0 0.4–9.8	2 (1.9)	11 (10.8)	<sup>b</sup>
Birth at 32–36 gestational week	81 (26.9)	38 (6.8)	4.4 2.8–7.0	11 (7.3)	14 (5.0)	1.2 0.5–2.9	44 (42.7)	34 (33.3)	1.3 0.7–2.3
Birth weight <1500 g	11 (3.6)	5 (0.9)	6.2 2.0–19.0	3 (2.0)	2 (0.7)	3.0 0.5–18.0	1 (1.0)	5 (4.9)	0.2 0.02–1.8
Birth weight 1500–2499 g	79 (26.1)	21 (3.7)	9.8 5.6–17.3	6 (4.0)	7 (2.5)	1.5 0.5–4.7	46 (44.7)	42 (40.8)	1.1 0.6–1.9
Ponderal index <25 kg/m <sup>3</sup>	82 (27.4)	70 (12.3)	2.6 1.8–3.8	24 (15.9)	37 (12.9)	1.2 0.7–2.1	29 (28.7)	36 (36.0)	0.7 0.4–1.3
Neonatal morbidity	85 (28.6)	82 (14.6)	2.4 1.7–3.4	27 (18.2)	43 (15.1)	1.3 0.7–2.2	31 (30.1)	35 (34.7)	0.8 0.5–1.5
Neonatal hospitalization	89 (30.0)	67 (11.9)	3.2 2.2–4.6	20 (13.4)	30 (10.6)	1.3 0.7–2.4	39 (38.2)	46 (44.7)	0.8 0.4–1.4
Intubation	42 (14.1)	26 (4.6)	3.3 2.0–5.5	7 (4.7)	15 (5.3)	0.9 0.4–2.2	17 (16.7)	19 (18.8)	0.9 0.4–1.8
Apgar ≤7 at 5 min	20 (6.7)	29 (5.2)	1.4 0.8–2.5	5 (3.4)	21 (7.5)	0.4 0.2–1.2	8 (7.9)	9 (9.0)	0.9 0.3–2.3
Heart malformations	10 (3.3)	5 (0.9)	4.0 1.4–11.7	3 (2.0)	2 (0.7)	3.0 0.5–18.0	4 (3.9)	5 (5.0)	0.8 0.2–3.4
Other malformations	11 (3.7)	20 (3.5)	1.1 0.5–2.4	3 (2.0)	8 (2.8)	0.8 0.2–3.1	4 (3.9)	4 (4.0)	1.0 0.3–4.0

<sup>a</sup>The whole IVF group was compared with matched controls, born to mothers of same age, representing average population in proportion of multiple births.

<sup>b</sup>*n* too small to calculate odds ratios (OR) and confidence intervals (CI).

<sup>c</sup>39 IVF children had only one control child.

<sup>d</sup>19 IVF children had only one control child.

1995; Tanbo *et al.*, 1995; Westergaard *et al.*, 1999), which is in accordance with our stillbirth rate. It should be noted that the definitions of birth differ by country and this may cause some variation in the stillbirth rates. The perinatal mortality rate for IVF children in this study (16.2/1000) was on average lower than most previously reported figures (17.0–39.7/1000) (MRC Working Party, 1990; Rizk *et al.*, 1991; Friedler *et al.*, 1992; Balen *et al.*, 1993; Olivennes *et al.*, 1993; FIVNAT, 1995; Gissler *et al.*, 1995; Westergaard *et al.*, 1999; Koudstaal *et al.*, 2000). Generally, the perinatal mortality rates in IVF populations are reported to be substantially higher than in the general population (MRC Working Party, 1990; Rizk *et al.*, 1991; Friedler *et al.*, 1992; FIVNAT, 1995). In previous reports, the total neonatal mortality rates range between 15.5–19.2/1000 (MRC Working Party, 1990; Rizk *et al.*, 1991; FIVNAT, 1995), being higher than our corresponding figure, 6.5/1000. The elevated mortality rates among IVF children are mainly due to the increased proportion of multifetal pregnancies following infertility treatments. Also, the characteristics of infertile women, especially advanced age, which may affect the course of IVF pregnancy, may, in turn, have an effect on the increased mortality rates.

There were significantly more preterm births and cases of

very low or low birth weight in the IVF group than in the control I group in our study, which is in accordance with numerous previous reports (MRC Working Party, 1990; Friedler *et al.*, 1992; Tan *et al.*, 1992; Gissler *et al.*, 1995; Tallo *et al.*, 1995; D'Souza *et al.*, 1997; Bergh *et al.*, 1999). No differences were found in the analysis stratified for plurality, thus not confirming the previous information of an elevated risk of preterm birth in IVF singletons (Gissler *et al.*, 1995; Tanbo *et al.*, 1995; Verlaenen *et al.*, 1995; Westergaard *et al.*, 1999; Koudstaal *et al.*, 2000). According to these results, multiplicity seems to be the most important factor behind the increased incidence of preterm birth and very low/low birth weight in IVF children.

As a whole, the IVF group survived the neonatal period worse than the general population-based control I group. Neonatal morbidity, including all the most common neonatal complications, was over twice as high in the IVF group as in the control I group. Consequently, the IVF children required admission to the neonatal intensive care unit or neonatal ward three times more often than controls. Similar results have been reported previously (FIVNAT, 1995; Gissler *et al.*, 1995; Tallo *et al.*, 1995; Tanbo *et al.*, 1995; D'Souza *et al.*, 1997; Koudstaal *et al.*, 2000). Since only small and statistically non-significant

**Table III.** Population-based comparison of the prevalence of children with congenital malformations/syndromes in the liveborn IVF ( $n = 304$ ) and control I groups ( $n = 569^a$ , represents general population in proportion of plurality)

	IVF children		Control I children	
	<i>n</i>	(%)	<i>n</i>	(%)
Heart malformations				
ASD	2	(0.7)	1	(0.2)
VSD	4	(1.3)	2	(0.4)
ASD and VSD	2	(0.7)	0	(0.0)
Aortic coarctation	0	(0.0)	2	(0.3)
Urological malformations				
Hypospadias	0	(0.0)	1	(0.2)
Pelvicourethral stenosis	0	(0.0)	1	(0.2)
Gastroenterological malformations				
Perianal fistula	1	(0.3)	0	(0.0)
Oesophagus atresia	0	(0.0)	1	(0.2)
Duodenal stenosis	0	(0.0)	1	(0.2)
Other				
Limb anomalies	4	(1.3)	7	(1.2)
Cleft palate	1	(0.3)	2	(0.4)
CNS malformations	0	(0.0)	3	(0.5)
Auricular atresia	1	(0.3)	0	(0.0)
Hemangioma	1	(0.3)	0	(0.0)
Pectoral muscle aplasia	0	(0.0)	1	(0.2)
Pulmonary hypoplasia	1	(0.3)	0	(0.0)
Congenital hypothyroidism	0	(0.0)	2	(0.4)
Syndromes/multiple malformations				
Amniotic band sequence <sup>b</sup>	1	(0.3)	0	(0.0)
Syndroma Goldenhar	1	(0.3)	0	(0.0)
Trisomy 21 <sup>c</sup>	1	(0.3)	0	(0.0)
Hemifacial microsomia	0	(0.0)	1	(0.2)
Total	20	(6.6)	25	(4.4)

ASD = atrial septal defect; VSD = ventricular septal defect; CNS = central nervous system.

<sup>a</sup>39 IVF children had only one control child.

<sup>b</sup>Includes a VSD and other malformations, which are not separately seen in this table, but are included in the OR calculations.

<sup>c</sup>Includes an ASD and a VSD, which are not separately seen in this table, but are included in the OR calculations.

differences occurred among singletons in our study, it can be concluded that multiplicity, along with preterm birth and low birth weight, are the most important determinants of neonatal outcome.

All children in the area are screened at birth for congenital malformations by paediatricians in specialized obstetric units or, occasionally, by general practitioners at local delivery units. Also later IVF and spontaneously conceived children go through the same medical examination protocol conducted by general practitioners and public health nurses in the public child welfare clinics. Therefore, we believe that no ascertainment bias was present regarding congenital malformations. The malformation rate in the population-based control group (4.4%) is similar to that of general Finnish population (The Finnish Register of Congenital Malformations and Birth Defects, unpublished data). The malformation rate in the IVF group (6.6%) in this study was higher than most previously reported rates after IVF or ICSI, which range between 2.2–6.1% (MRC Working Party, 1990; Rizk *et al.*, 1991; Friedler *et al.*, 1992; FIVNAT, 1995; Bonduelle *et al.*, 1996; Palermo *et al.*, 1996; D'Souza *et al.*, 1997; Addor *et al.*, 1998; Westergaard *et al.*, 1999) and are similar to the rates of congenital malformations in the general populations. Some of

these studies have taken therapeutic abortions due to prenatal diagnosis of congenital malformations into account with no notable increase in the malformation rates. The diagnoses of congenital malformations in these studies were mostly set at the neonatal period, unlike in our study, where we had information up to 3 years of age (for the subsequent follow-up study) enabling us to include also cases with malformations detected at any stage during the first 3 years of age. This might explain the higher malformation rate in our study. Still, it is difficult to make comparisons of international malformation rates, because the classifications of malformations are highly variable from one country to another. A recent Swedish study has reported an increased malformation rate of 7.6% (therapeutic abortions excluded) with an excess of hypospadias among ICSI children. They concluded this was mainly a result of a high rate of multiple births following ICSI and the excess of hypospadias was connected to paternal subfertility (Wennerholm *et al.*, 2000). Recent studies, also Swedish, with large study populations (no data on therapeutic abortions) reported an increase in the prevalence of neural tube defects, oesophageal atresias (Bergh *et al.*, 1999; Ericson and Källén, 2001), omphalocele and hypospadias (after ICSI) (Ericson and Källén, 2001) among IVF children compared with population-

based control groups. Our numbers on these malformations are too small to confirm this. Furthermore, we have no complete knowledge about possible therapeutic abortions or miscarriages in IVF pregnancies that did not lead to birth during the whole study period (no therapeutic abortions during 1992–1995; no knowledge during 1990–1991), which is a deficiency in this study. The starting point of our study was to choose IVF pregnancies ending in a birth, and thus all abortions were excluded. This may cause bias if the probability for a malformed fetus to be aborted varies between IVF and spontaneous pregnancies. Furthermore, our control groups were taken among live births, which was the reason why the group comparisons were made between liveborn children. However, no malformations were found among stillbirths in our study, and we believe that no major bias was caused, and if that was the case, it dilutes the differences between the groups.

Interestingly, there was a four-fold increase in the incidence of heart malformations, specifically septal defects, in the IVF group when compared with control I group, though this was not so prominent in the analysis stratified for plurality. To our knowledge, no previous reports of this kind have been published. An Australian re-analysis of a Belgian study on birth defects in ICSI children also found a four-fold excess of major cardiovascular defects after ICSI (Kurinczuk and Bower, 1997), but this re-analysis was quite controversial and was criticised by the original authors. Congenital heart malformations are due to complex multifactorial genetic and environmental causes, and fewer than 10% of all cardiac malformations arise from recognized chromosomal aberrations and mutations of single genes (Friedman and Child, 1998). Some investigators have noted that the mothers of children with VSD had a worse reproductive history than mothers without a child with VSD. They concluded that reproductive ability with differing levels of maternal hormones may be inversely related to the risk of VSD (Sands *et al.*, 1999), which might be supported by our results.

Although there were no statistically significant differences in the incidence of heart malformations in the analyses stratified for plurality, the excess of ASD and VSD in the IVF group cannot be solely explained by multiplicity. Heart defects do not arise directly from preterm birth, but from other background factors, and there was an increase, though non-significant, among singletons also. In most previous studies the sample sizes are too small to detect any differences in the prevalence of congenital malformations among IVF children compared with the general population. Larger population-based studies including data on therapeutic abortions are needed to investigate this further.

In conclusion, our results showed a poorer neonatal outcome for the IVF children when compared with controls representing general population in terms of preterm birth, lower birth weight and related problems arising mainly from multiplicity, since no statistically significant differences occurred in the analyses stratified for plurality. An excess of cardiac septal defects, but not of other congenital malformations, was found in the IVF children. This phenomenon probably results from a complex mechanism. Our control population represents children of old and primiparous women, so the differences would probably

be even greater compared with children from the population at common reproductive age. Due to a high rate of multiple pregnancies and births, IVF technology leads to increasing financial expenses to societies in the form of increased use of health services during pregnancy and the neonatal period. To date, the most important procedure, which could improve neonatal outcome and reduce financial costs, is to limit the number of embryos transferred, hence reducing the number of multiple pregnancies.

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# Increased risk of placenta previa in pregnancies following IVF/ICSI; a comparison of ART and non-ART pregnancies in the same mother

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**BACKGROUND:** The risk of placenta previa may be increased in pregnancies conceived by assisted reproduction technology (ART). Whether the increased risk is due to factors related to the reproductive technology, or associated with maternal factors, is not known. **METHODS:** In a nationwide population-based study, we included 845 384 pregnancies reported to the Medical Birth Registry of Norway between 1988 and 2002 and compared the risk of placenta previa in 7568 pregnancies conceived after assisted fertilization, with the risk in naturally conceived pregnancies. To study the influence of ART more directly, we compared the risk of placenta previa between consecutive pregnancies among 1349 women who had conceived both naturally and after assisted fertilization. Odds ratios (OR), adjusted for maternal age, parity, previous Caesarean section and time interval between pregnancies were estimated using logistic regression. **RESULTS:** There was a six-fold higher risk of placenta previa in singleton pregnancies conceived by assisted fertilization compared with naturally conceived pregnancies [adjusted OR 5.6, 95% confidence interval (CI) 4.4–7.0]. Among mothers who had conceived both naturally and after assisted fertilization, the risk of placenta previa was nearly three-fold higher in the pregnancy following assisted fertilization (adjusted OR 2.9, 95% CI 1.4–6.1), compared with that in the naturally conceived pregnancy. **CONCLUSIONS:** The use of ART is associated with an increased risk of placenta previa. Our findings suggest that the increased risk may be caused by factors related to the reproductive technology.

*Key words:* assisted reproduction technology/placenta previa/population study/sibling comparisons

## Introduction

Placenta previa, placentation in the lower segment of the uterine cavity, is associated with serious maternal and adverse fetal outcomes, including haemorrhage, prematurity and increased perinatal morbidity and mortality (McShane *et al.*, 1985; Ananth *et al.*, 1989). Its aetiology remains unclear, but several studies have reported higher frequencies of placenta previa in pregnancies of women with advanced maternal age, multiparity and previous Caesarean delivery and abortion (Faiz and Ananth, 2003). Lifestyle factors such as smoking and cocaine abuse during pregnancy have also been related to the increased risk of placenta previa (Handler *et al.*, 1994; Ananth *et al.*, 1996). In a meta-analysis of complications related to assisted reproduction, the investigators reported three-fold higher risk of placenta previa associated with the treatment (Jackson *et al.*, 2004). The result was, however, based on six small studies, with only 39 cases of placenta previa in 1610 pregnancies conceived by assisted fertilization.

The comparison group in the studies included in the meta-analysis has been women with naturally conceived pregnancies from the general population. However, these studies have not been able to separate effects of unfavourable maternal factors from factors that may be related to the reproduction technology. Worldwide, an increasing proportion of pregnancies are conceived by assisted fertilization, and therefore, possible iatrogenic side effects of the treatment should be clarified.

In this study, we first contrasted the prevalence of placenta previa in pregnancies following assisted fertilization with the prevalence in naturally conceived pregnancies. Second, we attempted to separate the influence of maternal factors from that of the assisted reproduction technology (ART) by studying women who had conceived both naturally and after assisted fertilization. Among these women, we compared the risk of placenta previa between consecutive pregnancies, where one sibling was conceived spontaneously and the other after

assisted fertilization. We hypothesized that if a pregnancy following assisted fertilization was more likely to result in placenta previa, the increased risk could be attributed to the ART and not only to maternal factors.

## Materials and methods

Data were derived from the Medical Birth Registry of Norway. This nationwide registry was established in 1967 and comprises records from more than  $2 \times 10^6$  deliveries (Irgens, 2000). Information on each pregnancy is based on standardized forms completed by midwives within 1 week of delivery. The reporting is mandatory and covers virtually all births in Norway. The form gives information related to the mother's health before and during pregnancy, complications during pregnancy and at delivery and perinatal data of the child.

From all fertility clinics in Norway, the Medical Birth Registry also receives separate notification of pregnancies conceived after assisted fertilization. This reporting is also mandatory and includes information related to the use of ART, notably IVF, ICSI and cryopreservation of embryos. In all these methods, fertilization occurs *in vitro*, and the resulting embryos are transferred to the uterus. Information on the date of embryo transfer, the number of embryos transferred and the number of fetuses with ongoing heart activity confirmed by ultrasound at gestational weeks 7–8 is also recorded. In this study, we did not include the induction of ovulation or insemination as methods of ART. Norwegian ART clinics have since mid-1990s almost exclusively replaced a maximum of two embryos. Fetal reduction is virtually never performed in Norway.

We used data for the period 1988–2002, comprising 882 040 pregnancies. According to recommendations from the WHO, we restricted the analyses to pregnancies where the length of gestation was 22 weeks or more and offspring birthweight was at least 500 g, resulting in 3607 excluded pregnancies. There were no pregnancies after assisted fertilization among mothers younger than 20 years of age or with parity of five or higher. Therefore, we excluded all pregnancies among mothers below 20 years of age ( $n = 29\ 998$ ) and parity of five or more ( $n = 1802$ ). We also excluded 335 triplet pregnancies (137 after assisted fertilization), 19 quadruplet pregnancies (six after assisted fertilization) and 895 pregnancies with missing data on the length of gestation and birthweight. This left 845 384 deliveries among 502 840 women; 832 490 were singletons, and 5581 (0.7%) of the singleton pregnancies were conceived after assisted fertilization. Among 12 894 twin pregnancies, 1987 (15.4%) were conceived after treatment with ART.

### Consecutive pregnancies in the same mother

In the study population of 502 840 women, 1349 women were registered with two consecutive singleton pregnancies, where one sibling was delivered after spontaneous conception and the other was delivered after ART. If the mothers had given birth to more than two singletons, we used the two first consecutive births eligible for the sibling comparison. Thus, among 2698 deliveries, 1349 children were conceived spontaneously and 1349 after ART. Among the pregnancies, 762 (56%) of those conceived by ART preceded the naturally conceived pregnancy.

### Placenta previa

Ultrasound screening is routinely offered to all pregnant women in Norway around week 18 of gestation. Approximately 98% of all pregnant women attend this ultrasound examination (Backe, 1997), and depending on the findings, some of these women are followed up throughout pregnancy with repeated ultrasound examinations. When

placenta previa is detected at the routine screening, the condition has to be verified at the follow-up examination around week 32 and subsequently confirmed at birth to be reported to the Medical Birth Registry. In births before the follow-up scan at week 32, the diagnosis is based on verification at birth. The standardized form sent to the Medical Birth Registry does not differentiate between placenta previa *marginalis* and placenta previa *totalis*.

### Statistical analysis

First, we used information from the general population of pregnant women and compared the risk of placenta previa between naturally conceived pregnancies and pregnancies conceived after assisted fertilization. Second, we restricted the analysis to consecutive pregnancies of singletons among mothers who had delivered both after spontaneous conception and after the use of assisted reproductive technology.

To account for the dependencies of pregnancies delivered by the same mother, we adjusted the SE for intra-group dependencies (Williams, 2000). We also used generalized estimating equation (GEE) and conditional logistic regression (Carlin *et al.*, 2005). The three techniques produced similar results, and we only present the results from the logistic regression analyses.

In the analyses, we evaluated possible confounding by other factors and adjusted for maternal age (20–29, 30–34, 35 years and older), parity (0, 1, 2 or higher), time interval between births (<3 years,  $\geq 3$  years), period of birth (5-year categories), previous Caesarean sections, sex of offspring and marital status. Stratified analyses were performed for combinations of period, maternal age and parity. In supplementary subanalyses, we adjusted for maternal smoking (before and during pregnancy) and the level of education. For the analyses, we used SPSS for Windows (Version 13, Chicago, IL, USA) and Stata (Version 9, College Station, TX, USA).

## Results

### Placenta previa in the general population of pregnant women

Women who gave birth after the use of ART were older and had fewer previous births than women who delivered after spontaneous conception (Table I). They also smoked less, but the level of education did not differ between the groups. Among 845 384 pregnancies, 1949 (0.23%) were diagnosed with placenta previa; 1910 in singleton pregnancies and 39 in twin pregnancies. The overall prevalence of placenta previa was fairly stable from 1988 to 2002, but in pregnancies following assisted fertilization, the prevalence was consistently higher throughout the period compared with spontaneously conceived pregnancies. Before any adjustments were made, the crude prevalence of placenta previa in naturally conceived singleton pregnancies was 0.22% as compared with 1.59% in singleton pregnancies conceived after assisted fertilization (Table I). In twin pregnancies, the corresponding proportions were 0.21 and 0.81%.

Adjustment for potentially confounding factors did not substantially alter the association between the use of ART and the occurrence of placenta previa (Table II). Thus, the odds ratio (OR) for singleton pregnancies was 5.6 [95% confidence interval (CI) 4.4–7.0] after adjustment for maternal age, parity, interval between deliveries, the year of delivery, the history of Caesarean section, offspring sex and marital status. In a separate subgroup analysis, we could also adjust for maternal smoking and the level of education, but the results were not

**Table I.** Maternal characteristics of pregnancies conceived by assisted fertilization and spontaneously conceived pregnancies in Norway, 1988–2002<sup>a</sup>

	Singleton pregnancies, <i>n</i> (%)		Twin pregnancies, <i>n</i> (%)	
	Assisted fertilization	Spontaneous conception	Assisted fertilization	Spontaneous conception
Maternal age (years)				
20–29	1036 (18.6)	493 403 (59.7)	428 (21.5)	5501 (50.4)
30–34	2601 (46.6)	235 609 (28.5)	999 (50.3)	3692 (33.8)
35–39	1883 (33.7)	97 897 (11.8)	560 (28.2)	1714 (15.7)
40+	61 (1.1)	7715 (0.9)	7 (0.4)	90 (0.8)
Parity				
0	3962 (71.0)	341 917 (41.3)	1388 (69.8)	4297 (39.4)
1	1457 (26.1)	301 642 (36.5)	543 (27.3)	4025 (36.9)
≥2	162 (2.9)	183 350 (22.2)	56 (2.8)	2656 (23.6)
Previous Caesarean section				
No	5319 (95.3)	765 050 (92.5)	1889 (95.1)	10 083 (92.4)
Yes	262 (4.7)	61 859 (7.5)	98 (4.9)	824 (7.6)
Smoking during pregnancy <sup>b</sup>				
Yes	261 (10.3)	30 834 (14.2)	83 (8.8)	447 (14.0)
No	1782 (70.0)	137 986 (63.6)	647 (68.9)	2008 (62.7)
Unknown	502 (19.7)	48 052 (22.2)	209 (22.3)	746 (23.3)
Method				
IVF <sup>c</sup>	4033 (72.3)	–	1533 (77.2)	–
ICSI <sup>c</sup>	981 (17.6)	–	305 (15.3)	–
Unknown	567 (10.2)	–	149 (7.5)	–
Placenta previa	89 (1.59)	1821 (0.22)	16 (0.81)	23 (0.21)

<sup>a</sup>Restricted to pregnancies among mothers 20 years or older with five or less previous births.

<sup>b</sup>Smoking data restricted to pregnancies after November 1998.

<sup>c</sup>Including thawed embryo replacements.

**Table II.** Odds ratio (OR) of placenta previa in pregnancies after assisted fertilization versus spontaneous conception adjusted for maternal age at birth, parity, duration between pregnancies, calendar period of birth and previous Caesarean section by plurality<sup>a</sup>

	Singletons					Twins				
	<i>N</i>	Cases	Crude OR	Adjusted OR	95% confidence interval (CI)	<i>n</i>	Cases	Crude OR	Adjusted OR	95% CI
Spontaneous conception	826 909	1821	1.0	1.0	Reference	10 907	23	1.0	1.0	Reference
Assisted fertilization	5581	89	7.3	5.6	4.4–7.0	1987	16	3.8	2.9	1.5–5.8
Maternal age										
20–29	494 439	711	1.0	1.0	Reference	5929	6	1.0	1.0	Reference
30–34	238 210	692	2.0	1.8	1.6–2.0	4691	21	4.4	3.4	1.3–8.9
35+	99 841	507	3.5	2.9	2.6–3.3	2274	12	5.2	3.7	1.3–10.8
Parity										
Para 0	345 879	591	1.0	1.0	Reference	5685	16	1.0	1.0	Reference
Para 1	303 099	750	1.4	1.2	1.0–1.3	4568	16	1.2	0.9	0.3–2.7
Para 2+	183 512	569	1.8	1.1	1.0–1.3	2641	7	0.9	0.7	0.2–2.0
Time between births										
Para 0 <sup>b</sup>	345 879	591	–	–	–	5685	16	–	–	–
<3 years	213 974	485	1.0	1.0	Reference	2827	6	1.0	1.0	Reference
>3 years	272 637	834	1.4	1.1	1.0–1.3	4382	17	1.8	1.5	0.6–3.9
Year of birth										
1988–1992	278 813	588	1.0	1.0	Reference	3548	8	1.0	1.0	Reference
1993–1997	282 386	617	1.0	1.0	0.9–1.1	4347	15	1.5	1.3	0.5–3.0
1998–2002	271 291	705	1.1	1.0	0.9–1.2	2641	16	4	1.0	0.4–2.2
Previous Caesarean section										
No	770 369	1670	1.0	1.0	Reference	11 927	6	1.0	1.0	Reference
Yes	62 121	240	1.8	1.4	1.2–1.6	922	33	2.4	2.2	0.8–5.9

<sup>a</sup>Analysis restricted to pregnancies among mothers giving birth at age 20 or older and with five or less previous births. SE corrected for intra-group dependencies.

<sup>b</sup>Dropped because of collinearity with parity.

substantially different from the main results (data not shown). In twin pregnancies, the adjusted OR was 2.9 (95% CI 1.5–5.8).

We analysed different methods of assisted reproduction separately and found that the increased risk of placenta previa was fairly similar for IVF and ICSI. Compared with the spontaneously conceived pregnancies, the prevalence was six-fold

higher in IVF pregnancies (adjusted OR 6.3, 95% CI 4.9–8.1) and four-fold higher in ICSI pregnancies (adjusted OR 4.4, 95% CI 2.5–7.8). In pregnancies after the replacement of thawed embryos, the numbers were too small to study any effects (three cases of placenta previa among 227 singleton pregnancies).

**Table III.** Characteristics of pregnancies complicated by placenta previa after assisted fertilization and spontaneous conception in Norway from 1988 to 2002 by plurality<sup>a</sup>

	Singletons		Twins	
	Assisted fertilization (N = 89)	Spontaneous conception (N = 1821)	Assisted fertilization (N = 16)	Spontaneous conception (N = 23)
Mean gestation in days (SD)	259.0 (20.2)	258.4 (23.3)	245.0 (16.0)	238 (23.0)
Mean birthweight in kg (SD)	2838 (638)	2904 (777)	2370 (716)	2507 (661)
Caesarean section (%)	96.6	85.1	100	87.0

<sup>a</sup>Restricted to pregnancies among mothers giving birth at age 20 or older and with five or less previous births.

In cases of placenta previa, mean gestational age and mean birthweight were fairly similar in pregnancies after naturally conceived and after assisted fertilization, whereas Caesarean delivery was more frequent if the pregnancy was conceived after assisted fertilization (Table III). Thus, the proportion of Caesarean section was 96.6% in singleton pregnancies with placenta previa as compared with 85.1% in spontaneously conceived pregnancies. To avoid the possibility that placenta previa was reported more often in pregnancies after assisted fertilization (potential surveillance bias), we restricted the diagnosis of placenta previa to pregnancies with Caesarean delivery. However, the results were similar to those of the main analysis, showing six-fold higher prevalence (OR 6.3, 95% CI 4.9–7.9) of placenta previa associated with assisted fertilization.

We also stratified the analysis according to the calendar period of birth (1988–1992, 1993–1997 and 1998–2002), parity and maternal age, but the results did not substantially differ across strata of these variables.

#### Comparison of consecutive sibling pregnancies

In the study population, 1349 women had delivered singletons both after natural conception and after assisted fertilization (Table IV). In pregnancies following assisted reproduction, women were slightly older but had fewer previous births and previous Caesarean sections than when the same women delivered after spontaneous conception. The crude prevalence of placenta previa was 2.0% in pregnancies conceived by ART as compared with 0.7% in pregnancies following natural conception, suggesting approximately three-fold higher prevalence. After adjustment for maternal age, parity and previous Caesarean section, placenta previa was nearly three times more likely to occur in pregnancies following assisted fertilization (OR = 2.9, 95% CI 1.4–6.1) compared with spontaneously conceived sibling pregnancies (Table V).

In additional analyses, we studied the association with placenta previa in pregnancies where the first child was conceived spontaneously and in pregnancies where the first child was conceived by assisted fertilization. After adjustment for maternal age and previous Caesarean section, the results showed a positive association with placenta previa regardless of whether the first (OR 2.5, 95% CI 0.5–12.5) or the second pregnancy (OR 2.6, 95% CI 0.4–16.8) was conceived by the use of ART.

**Table IV.** Maternal characteristics of consecutive singleton pregnancies among women who have given birth both after assisted fertilization and after spontaneous conception

	Assisted fertilization (N = 1349), n (%)	Spontaneous conception (N = 1349), n (%)
Maternal age at birth (years)		
20–29	254 (18.8)	461 (34.2)
30–34	676 (50.1)	489 (36.3)
35+	419 (31.1)	399 (29.6)
Parity		
0	675 (50.0)	537 (39.8)
1	617 (45.7)	704 (52.2)
≥2	57 (4.2)	108 (8.1)
Previous Caesarean section		
No	1225 (90.8)	1138 (84.4)
Yes	124 (9.2)	211 (15.6)
Method		
IVF	961 (71.2)	–
ICSI	194 (14.4)	–
Unknown	194 (14.4)	–
Smoking during pregnancy <sup>a</sup>		
Yes	57 (9.9)	51 (11.5)
No	402 (69.4)	302 (68.0)
Unknown	120 (20.7)	91 (20.5)
Placenta previa	27 (2.0)	10 (0.7)

<sup>a</sup>Smoking data restricted to pregnancies after November 1998.

#### Discussion

By comparing consecutive pregnancies, where the mother conceived spontaneously in one pregnancy and after assisted fertilization in the other, it seems reasonable to attribute differences in pregnancy complications to the reproduction technology rather than to maternal factors. Consequently, the nearly three-fold higher risk of placenta previa that we observed in the pregnancy following assisted fertilization may largely be attributed to factors related to the reproduction technology.

Within the large, unselected population, we found that placenta previa occurred six times more often in singleton pregnancies after assisted reproduction compared with naturally conceived pregnancies. In this setting, the higher prevalence of placenta previa is most likely due to a combination of maternal factors and factors related to the ART.

Previously, a few small studies have examined the association between assisted fertilization and the risk of placenta previa (Howe *et al.*, 1990; Tan *et al.*, 1992; Tanbo *et al.*, 1995; Verlaenen *et al.*, 1995; Reubinoff *et al.*, 1997; Koudstaal *et al.*, 2000; Shevell *et al.*, 2005). Most studies found that placenta previa is more common after assisted reproduction. Six of

**Table V.** Odds ratio (OR) of placenta previa in consecutive singleton pregnancies among women who have given birth both after assisted fertilization and after spontaneous conception

	Women	Placenta previa	Crude OR	AdjustedOR <sup>a</sup>	95% confidence interval
Spontaneous conception	1349	10	1.0	1.0	Reference
Assisted fertilization	1349	27	2.7	2.9	1.4–6.1
Order of mode of conception					
Spontaneous first					
Spontaneous conception	587	3	1.0	1.0	Reference
Assisted fertilization	587	17	5.8	2.6	0.4–16.8
Assisted fertilization first					
Spontaneous conception	762	7	1.0	1.0	Reference
Assisted fertilization	762	10	1.4	2.5	0.5–12.5

<sup>a</sup>Adjusted for maternal age at birth, parity and previous Caesarean section.

these studies were included in a meta-analysis of complications after assisted fertilization (Jackson *et al.*, 2004). The joint results indicated three-fold higher risk of placenta previa in pregnancies after assisted fertilization compared with naturally conceived pregnancies. However, the analysis was only based on 39 cases of placenta previa in 1610 pregnancies following assisted fertilization. However, no study could distinguish between the impact of maternal factors and factors related to the reproduction technology.

Our study includes an unselected nationwide population with compulsory reporting of all births to the Medical Birth Registry of Norway. The unique identification number of every citizen in the country enables pregnancies conceived after assisted fertilization to be identified and linked to pregnancy outcome. Information on potentially confounding factors, such as parity, maternal age and previous Caesarean section, allows us to adjust for these factors in the statistical analysis, and in a subset of the population, the information on the level of education and smoking could also be taken into account.

Although complete previa (*'totalis'*) tends to be associated with more severe bleeding and an absolute indication for Caesarean section, a less severe degree of placenta previa (*'marginalis'*) may also cause life-threatening haemorrhage and may therefore be regarded as clinically important even though the site of placentation allows vaginal delivery (Ghourab, 2001). Thus, we included all cases of placenta previa regardless of the mode of delivery in the primary analysis. In a secondary analysis, we restricted the diagnosis of placenta previa to Caesarean section deliveries. This restriction provided a slightly stronger association between ART and the risk of placenta previa (OR = 6.3), and the adjusted OR of 5.6 obtained in the primary analysis may be considered as a more conservative estimate.

Except for one extra ultrasound examination in weeks 7–8 of pregnancy, women who conceive after assisted fertilization attend the standard programme for prenatal care in Norway. Specially trained midwives perform the routine ultrasound examination at 17–18 weeks of gestation, and virtually, all pregnant women in the country attend this examination (Backe, 1997). If prenatal surveillance was more rigorous for women who received assisted fertilization, one consequence could be that placenta previa would be diagnosed more often in the assisted fertilization group than among women who conceived spontaneously. To reduce a possible diagnostic bias, we restricted the diagnosis of placenta previa to cases that were

registered after Caesarean deliveries. However, this did not attenuate the strong positive association with assisted fertilization. Twins constitute another group that receives close surveillance, regardless of whether the pregnancy is conceived spontaneously or after assisted fertilization. However, the higher frequency of placenta previa in twin pregnancies conceived by reproduction technology strengthens the validity of our findings and suggests that placenta previa is not diagnosed systematically different between the groups.

Nonetheless, women who seek infertility treatment represent a selected group of women. By using naturally conceived pregnancies from the general population as comparison, one cannot readily distinguish the impact of maternal factors from factors related to ART. By comparing consecutive pregnancies among women who have delivered after both assisted fertilization and spontaneous conception, one may, at least partly, solve this problem because confounding by maternal and environmental factors is less likely. The results for consecutive siblings, showing three-fold higher risk of placenta previa associated with assisted fertilization, suggest that a substantial proportion of the increased risk may be attributed to ART.

The underlying mechanism for this effect is not clear. In assisted fertilization, drugs are utilized to induce multiple follicular development. Fertilization and embryo development take place outside the body, and embryos enter the uterine cavity through the cervix by mechanical means.

The stimulation protocol used in assisted reproduction frequently results in very high levels of gonadal steroids that induce morphological and structural changes and disturbed expression of relevant genes in the endometrium (Horcajadas *et al.*, 2005). These effects are thought to be global, and given the current knowledge, this effect on the endometrium is not likely to contribute to a higher risk of placenta previa.

It is well documented that fertilization and embryo culture *in vitro* can change key metabolic pathways in the embryo (Leese *et al.*, 1998). These effects may interfere with implantation and early embryo development, but it is difficult to explain how the changes could result in more frequent implantation in the lower segment of the uterus.

In ART, embryos are placed in the uterine cavity by the transcervical route using a catheter. This procedure may induce uterine contraction, possibly due to the release of prostaglandins after mechanical stimulation of the internal cervical os (Fraser, 1992; Fanchin *et al.*, 1998; Mansour, 2005). It has

been demonstrated that as much as 15% of replaced embryos may be totally expelled from the uterus (Poindexter *et al.*, 1986). It is conceivable that these mechanically induced uterine contractions could lead to higher frequencies of implantation in the lower uterine segment and thereby increase the risk of placenta previa. Another study reported that 80% of embryos were implanted in the area in which they were transferred (Baba *et al.*, 2000), suggesting that the site of replacement could be particularly important. Also, lower deposition in the uterine cavity may improve the rate of successful implantation (Waterstone *et al.*, 1991; Coroleu *et al.*, 2002), and preference now tends to be lower replacement of the embryo. To evaluate whether the risk of placenta previa may be attributed to the depth of embryo replacement, however, the transfer distance from both the internal cervical os and the uterine fundus should be monitored and systematically recorded.

In summary, the risk of placenta previa in pregnancies following assisted reproductive treatment is considerably higher than in pregnancies following natural conception. Our results suggest that factors directly related to the reproduction technology contribute to the increased risk.

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# Perinatal Outcomes in Singletons Following In Vitro Fertilization: A Meta-Analysis

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**OBJECTIVE:** To estimate whether singleton pregnancies following in vitro fertilization (IVF) are at higher risk of perinatal mortality, preterm delivery, small for gestational age, and low or very low birth weight compared with spontaneous conceptions in studies that adjusted for age and parity.

**DATA SOURCES:** We searched MEDLINE, BIOSIS, Doctoral Dissertations On-Line, bibliographies, and conference proceedings for studies from 1978–2002 using the terms “in vitro fertilization,” “female infertility therapy,” and “reproductive techniques” combined with “fetal death,” “mortality,” “fetal growth restriction,” “small for gestational age,” “birth weight,” “premature labor,” “preterm delivery,” “infant,” “obstetric,” “perinatal,” and “neonatal.”

**METHODS OF STUDY SELECTION:** Inclusion criteria were singleton pregnancies following IVF compared with spontaneous conceptions, control for maternal age and parity; 1 of the above outcomes; and risk ratios or data to determine them. Study selection and data abstraction were performed in duplicate after removing identifying information.

**TABULATION, INTEGRATION, AND RESULTS:** Fifteen studies comprising 12,283 IVF and 1.9 million spontaneously conceived singletons were identified. Random-effects meta-analysis was performed. Compared with spontaneous conceptions, IVF singleton pregnancies were associated with significantly higher odds of each of the perinatal outcomes examined: perinatal mortality (odds ratio [OR] 2.2; 95% confidence interval [CI] 1.6, 3.0), preterm delivery (OR 2.0; 95% CI 1.7, 2.2), low birth weight (OR 1.8; 95% CI 1.4, 2.2), very low birth weight (OR 2.7; 95% CI 2.3, 3.1),

and small for gestational age (OR 1.6; 95% CI 1.3, 2.0). Statistical heterogeneity was noted only for preterm delivery and low birth weight. Sensitivity analyses revealed no significant changes in results. Early preterm delivery, spontaneous preterm delivery, placenta previa, gestational diabetes, preeclampsia, and neonatal intensive care admission were also significantly more prevalent in the IVF group.

**CONCLUSION:** In vitro fertilization patients should be advised of the increased risk for adverse perinatal outcomes. Obstetricians should not only manage these pregnancies as high risk but also avoid iatrogenic harm caused by elective preterm labor induction or cesarean. (Obstet Gynecol 2004;103:551–63. © 2004 by The American College of Obstetricians and Gynecologists.)

Since the birth of Louise Brown in 1978, in vitro fertilization (IVF) has become the standard treatment for many types of infertility, despite an initial absence of research examining possible adverse effects on the mother or child. Delayed child bearing and increased access to infertility treatment have resulted in a dramatic increase in demand for IVF. In several European countries, assisted reproductive technology pregnancies represent 2–3% of all births,<sup>1</sup> whereas 0.7% of all U.S. births are the result of assisted reproductive technology.<sup>2</sup> Until recently, research has focused primarily on the efficacy of various assisted reproductive technology methods and the rates of early pregnancy loss or multiple gestations. Now some researchers have begun to question the safety of assisted reproductive technology in terms of its effects on the patient’s health, her pregnancy, and her infant.

Recent studies have specifically addressed perinatal outcomes following IVF compared with spontaneous conception after controlling for maternal age, parity, multiple gestations, and other factors. Most studies found increases in preterm birth, low birth weight (LBW), or small for gestational age (SGA),<sup>3–16</sup> although a few did not.<sup>17–21</sup> Most individual studies had insufficient statistical power to detect significant differences in perinatal mortality.<sup>6,9,13,16,20</sup> Because of different study

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designs, populations, and reported outcomes, it is difficult to determine whether IVF pregnancies are at higher risk of adverse outcomes. Reviews on this topic have been neither systematic nor quantitative and have come to different conclusions.<sup>22-26</sup>

We conducted a meta-analysis to evaluate whether singleton pregnancies conceived with IVF are at higher risk of perinatal mortality, preterm delivery, LBW, or SGA when compared with naturally conceived singleton pregnancies. Given the influence of maternal age and parity on obstetric outcome,<sup>27,28</sup> only studies that controlled for maternal age and parity in either the design or analysis were included.

## SOURCES

We searched MEDLINE and BIOSIS using a search strategy combining the keywords and subject terms “in vitro fertilization,” “female infertility therapy,” and “reproductive techniques” with “mortality,” “fetal death,” “fetal growth restriction,” “small for gestational age,” “preterm delivery,” “premature labor,” “birth weight,” “infant,” “obstetric,” “perinatal,” and “neonatal” for articles published between 1978 (the date of the first IVF birth) and October 2002. Animal studies and case reports were excluded. Conference proceedings available online and Doctoral Dissertations On-Line were also searched as were abstracts from the 1999 to 2001 meetings of the American Society of Reproductive Medicine, Society for Maternal Fetal Medicine, European Society for Human Reproduction and Embryology, and International Federation of Gynecology and Obstetrics. Bibliographies were reviewed to identify other relevant studies. Studies published in languages other than English were considered if an English abstract was provided.

## STUDY SELECTION

Inclusion criteria were applied in 2 stages. First, we identified studies for blinded review that included a comparison of IVF singleton pregnancies to spontaneous conceptions and reported rates of perinatal mortality, preterm birth, LBW, very low birth weight (VLBW), or SGA in both groups. When the type of infertility treatment was unclear or the study group received a variety of treatments in addition to IVF, the study was submitted for further review. Two authors (R.J., K.G.) independently reviewed manuscripts meeting the above criteria after removing authors' names, journal titles, and funding sources. Final inclusion criteria were then applied: 1) comparison of IVF to spontaneous conception; 2) more than 50% in the IVF group had received standard IVF; 3) more than 50% in the spontaneous conception group

were fertile; 4) control for at least maternal age and parity; 5) singleton gestations reported and analyzed separately from multiple gestations; 6) outcomes explicitly defined and including 1 of the above; and 7) risk ratios with 95% confidence intervals (CIs) provided or sufficient data to enable calculation. Standard IVF was defined as ovulation induction, egg retrieval, IVF and intrauterine embryo transfer of a fresh embryo. A third author (M.C.), blinded to study author and journal, settled discrepancies regarding inclusion criteria and data abstraction.

We excluded studies that compared a series of IVF births with population rates unadjusted for age or parity, studies where more than 50% of subjects received variations of standard IVF such as gamete intrafallopian transfer, IVF with intracytoplasmic sperm injection or cryopreserved or donor embryos, and studies of spontaneous conception in infertile women. When multiple publications reported data for the same study subjects, the most recent publication was selected. Study authors were asked to provide further information when it was unclear whether a study met our inclusion criteria.

Two authors (K.G., R.J.) abstracted data about study design, population, data collection methods, and raw data onto standardized forms. Study designs were classified as 1) traditional cohort: IVF births (exposed) comprised a subpopulation of a larger birth cohort (unexposed), and outcomes were compared between the 2 groups in their entirety; 2) matched cohort: IVF births comprised a subpopulation of a larger birth cohort, but only a matched group of the unexposed cohort was used for comparison; and 3) external comparison cohort (double cohort): IVF births were compared with a group of spontaneous conceptions from a different population.

Primary outcomes were defined as follows: SGA = birth weight less than tenth percentile; preterm delivery = delivery less than 37 completed weeks of gestation; LBW and VLBW = weight less than 2,500 g and less than 1,500 g respectively; and perinatal mortality = stillbirths plus early neonatal deaths (7 days or less). The definition of stillbirth ranged from more than 20 weeks to more than 28 weeks depending on the definition used in the author's locale; we used the author's definition of stillbirth in our analyses. Meta-analysis of secondary obstetric and neonatal outcomes was performed when 3 or more studies provided data for a given outcome. These included early preterm birth (less than 32-33 weeks), type of labor, delivery method, malpresentation, gestational diabetes, pregnancy-induced hypertension, vaginal bleeding, placenta previa, and neonatal intensive care unit admission. Definitions of secondary outcomes were taken directly from the included reports and in some cases differed between studies. Malformation rates



were abstracted but are not analyzed because of extreme variability between studies in the definition and ascertainment of malformations.

Studies were assigned a quality score for use in stratified analysis. The score of 0 to 10 was based on 5 study design characteristics awarded 0 to 2 points as follows: traditional cohort = 2 points, matched cohort = 1, external comparison cohort = 0; enrollment begun on or after 1990 = 2, before 1990 = 0; adjustment for age and parity only = 1, for additional factors = 2; delivery of both groups at the same hospital(s) = 2, delivery of both groups region-wide = 1; delivery of IVF group at 1–3 hospitals and spontaneous group region-wide = 0; and more than 500 subjects in the IVF group = 2, more than 300 = 1, less than 300 = 0.

We used adjusted odds ratios (ORs) from reports that provided them. For reports that provided multiple ORs for a given outcome, we chose the one that adjusted for age, parity, and delivery date. For studies that provided primary data and matched subjects for at least age and parity, we calculated ORs and 95% CIs using the method of Woolf.<sup>29</sup> When a zero cell was encountered, a value of 0.5 was added to each cell in the table. When a published OR was inconsistent with the raw data provided by the study, the effect measure was recalculated using the available data. When data were insufficient to calculate an OR, we attempted to contact the original authors.

Summary ORs were calculated by taking a weighted average of individual study results using a general variance-based, random-effects model, weighing individual study results by the inverse of their variance.<sup>30</sup> The weight for each study was the inverse of the sum of 2 terms: the study variance and a term accounting for the between-study variability.<sup>30</sup> We chose the random-effects model because it is considered more conservative than a fixed effects model. In sensitivity analysis, we compared results of random and fixed effects models. We did not use the quality score in the calculation of weights of the individual studies. An OR less than 1.0 indicates a better outcome in the IVF group, whereas an OR greater than 1.0 favors the spontaneous conception group.

Heterogeneity was tested by using the general variance-based method<sup>30</sup> in which a conservative value of  $P < .10$  was used to classify study results as heterogeneous. We attempted to identify sources of heterogeneity and bias by performing stratified analyses. We compared ORs in subgroups with differing study designs, delivery sites, study dates, sample sizes, and quality. Differences in the stratified summary estimates were evaluated using a z score.<sup>29</sup>

A sensitivity analysis was performed to evaluate the stability of the overall risk estimate. Sensitivity analyses

were performed separately for each of the 5 primary outcomes. Potential publication bias was investigated visually using funnel plots and mathematically using Egger's regression asymmetry test<sup>31</sup> and Kendall rank correlation test.<sup>32</sup> The Egger test evaluated whether the intercept deviated significantly from zero in a regression of standardized effect estimates against their precision. The Kendall test examined the significance of the Kendall rank correlation between the standardized effect sizes and their variances. All calculations were performed using STATA (StataCorp, College Station, TX).

## RESULTS

Our initial search produced 1,452 citations. Of these, 1,415 did not meet initial screening criteria. These included case reports or series; unadjusted population-based IVF registry reports; reports of pregnancy rates, specific IVF methods, selective fetal reduction, and multiple gestation; and studies that did not report perinatal outcomes or that involved groups other than IVF and spontaneous conception. The remaining 37 articles were submitted for blinded evaluation. Of these, 13 were excluded because they did not match or adjust for maternal age and parity,<sup>21,33–44</sup> 4 had fewer than 50% IVF in the infertile group,<sup>10,11,45,46</sup> 2 did not analyze singletons separately from twins,<sup>47,48</sup> 1 used a composite neonatal morbidity outcome,<sup>49</sup> 1 included the same subjects in a more recent study,<sup>19</sup> and 1 did not match the standard IVF group to the spontaneous conception group.<sup>50</sup> The remaining 15 studies are summarized in Tables 1 and 2. The author of a study published in Norwegian<sup>7</sup> provided English translation.

The 15 studies include 12,283 IVF singleton pregnancies and 1.9 million spontaneously conceived singletons. Individual sample sizes ranged from 54<sup>20</sup> to 3,305<sup>3</sup> in the IVF group. All studies were retrospective and used variations of a cohort design: 3 were traditional cohort studies,<sup>3,5,7</sup> 8 were matched cohort studies,<sup>4,6,9,14–18</sup> and 4 were cohorts with an external comparison group.<sup>8,12,13,20</sup> None of the studies were blinded in that obstetricians caring for the patients could have known that patients had received IVF. The majority of studies were performed in Europe, with 1 performed in the United States.<sup>20</sup>

Study subjects were identified using hospital delivery logs, IVF clinic records, IVF registries, and regional or national birth registries. Two studies specifically excluded infertile women or women treated for infertility from the spontaneous conception group.<sup>4,16</sup> Many studies included small proportions with non-IVF treatments in the IVF group.<sup>3,4,8,9,13,14,18</sup> In addition to age, parity, and delivery date, about half of the studies controlled for other factors such as ethnicity, insurance, smoking, body



**Table 1.** Characteristics of Included Studies Comparing Perinatal Outcomes in IVF Versus Spontaneous Conceptions

Author	Study period	Country	Quality score*	Sample size (n)			IVF group	
				IVF	Spontaneous	Mean age (y)	Nulliparous (%)	Intracytoplasmic sperm injection (%)
Traditional cohort (single population, entire cohort examined)								
Bergh <sup>3</sup>	1982–1995	Sweden	5	3,305	1,490,667 <sup>†</sup>	...	...	7
Gissler <sup>5</sup>	1991–1993	Finland	9	746	188,381	...	77	0
Von Düring <sup>7</sup>	1988–1991	Norway	5	545	233,905	...	...	0
Matched cohort (single population, matched subgroup of cohort examined)								
Dhont <sup>4</sup>	1992–1997	Belgium	6	3,057	3,057	31.6	75	10
Koivurova <sup>15</sup>	1990–1995	Finland	5	153	287	...	...	0.6
Koudstaal <sup>16</sup>	?–1992 <sup>‡</sup>	Netherlands	5	307	307	32.8	72	0
Maman <sup>17</sup>	1989–1994	Israel	5	169	469	34.1	60	0
Reubinoff <sup>18</sup>	1983–1993	Israel	5	260	260	32.7	...	0
Verlaenen <sup>6</sup>	1979–1986	Belgium	5	140	140	31.7	84	0
Wang <sup>14</sup>	1986–1998	Australia	6	1,019	1,019	32.5	68	20
Westergaard <sup>9</sup>	1994–1999	Denmark	6	1,298	1,298	33.1	...	8
External comparison cohort (2 independent populations)								
Howe <sup>20</sup>	Not stated	United States	2	54	54	33	57	0
Tan <sup>12</sup>	1982–1989	England	3	494	978	34.2	74	0
Tanbo <sup>13</sup>	1978–1987	Norway	3	355	643	33	79	0
Wang <sup>8</sup>	1982–1989	Australia	3	465	21,547	...	73	0

\* 0 to 2 points each for study date, design, delivery site, sample size, and adjustment for confounders

<sup>†</sup> Exact number not given; estimate made by subtracting multiple gestations from total births

<sup>‡</sup> Start date not given.

mass index, or obstetric history.<sup>5,6,14–18,20</sup> Only 4 controlled for delivery site.<sup>6,16–18</sup> In the others, both groups delivered at hospitals throughout the region<sup>3–5,7,9,14,15</sup> or the IVF group delivered throughout the region and the spontaneous group delivered at 1 to 3 local hospitals.<sup>8,12,13,20</sup>

Individual study data and meta-analytic summary ORs for each primary outcome are shown in Figures 1–5. The number of eligible studies and subjects for each outcome ranged from 7 studies with 1,889 IVF pregnancies for the outcome of SGA (Figure 5), to 14 studies with 12,114 IVF pregnancies for the outcome of preterm delivery (Figure 2). The overall incidence of each outcome in the IVF group was perinatal mortality 19.6/1,000, preterm delivery 11.5%, LBW 9.5%, VLBW 2.5%, and SGA 14.6%. Meta-analysis of each outcome revealed that IVF singletons had significantly elevated odds of each adverse outcome as compared with spontaneously conceived singletons: perinatal mortality (OR 2.19; 95% CI 1.61, 2.98), preterm delivery (OR 1.95; 95% CI 1.73, 2.20), LBW (OR 1.77; 95% CI 1.40, 2.22), VLBW (OR 2.70; 95% CI 2.31, 3.14), and SGA (OR 1.60; 95% CI 1.25, 2.04).

Statistical heterogeneity ( $P < .10$ ) was not detected for perinatal mortality, VLBW, or SGA but was found for preterm delivery and LBW. Sources of heterogeneity for these outcomes were investigated by stratifying for study design, sample size, years of study, delivery site, and study quality (Figure 6). The majority of subgroups

had ORs that were similar to the overall summary OR and remained statistically significant. Heterogeneity that was present in the overall meta-analysis of preterm delivery and LBW was partially explained with stratification by study design features. For example, for both preterm delivery and LBW, smaller studies, those performed after 1990, with region-wide delivery of IVF births but local delivery of spontaneous conceptions, or with external comparison groups were not statistically heterogeneous ( $P > .10$ ). Furthermore, higher-quality studies showed significantly higher odds of preterm birth and LBW than did lower-quality studies.

Sensitivity analyses were performed for each of the 5 primary outcomes. Sequential removal of each study from the meta-analyses resulted in no significant changes in the overall summary ORs. Fixed effects models produced similar results as random-effects models. A number of studies that were originally excluded were added in the sensitivity analyses.<sup>10,11,21,41,46,50</sup> Addition of these did not change any of the summary ORs. Namely, when we included Schieve,<sup>10</sup> the largest study excluded, the OR for LBW changed from 1.77 to 1.76 and for VLBW from 2.70 to 2.51. For SGA, we added studies with slightly different definitions of SGA<sup>10,12,13</sup> with no change in the summary OR. Two studies that met our inclusion criteria were excluded in sensitivity analysis. Von Düring<sup>7</sup> reported adjusted relative risks instead of ORs. Tanbo<sup>13</sup> controlled for parity by including only nulliparous women in the spontaneous group. Exclusion



**Table 2.** Design of Included Studies Comparing Perinatal Outcomes in IVF Versus Spontaneous Conceptions

Author	Study population		Additional variables controlled for*	Method of control	Delivery site	Source of outcome data
	IVF	Spontaneous				
<b>Traditional cohort</b>						
Bergh <sup>3</sup>	All assisted reproductive technology pregnancies in Sweden obtained via query of the 14 IVF clinics and linked to the national birth registry; 99% successfully linked; 7% intracytoplasmic sperm injection, 8% cryopreserved embryos	All births during the same period from the national birth registry	...	Stratified analysis	Throughout Sweden	National birth and death registries
Gissler <sup>5</sup>	All births in the Finnish birth registry coded as IVF; approximately 20% of IVF births misclassified as spontaneous conceptions	All births during the same period from the national birth registry	County, smoking, marital status	Multiple logistic regression	Throughout Finland	National birth registry
Von Doring <sup>7</sup>	All births in the Norwegian birth registry coded as IVF; 11% excluded due to insufficient data in the birth registry	All births during the same period from the national birth registry	...	Stratified analysis	Throughout Norway	National birth registry
<b>Matched Cohort</b>						
Dhont <sup>4</sup>	All assisted reproductive technology births from a single Dutch region; method of classifying births as assisted reproductive technology not explicitly stated; approximately 10% intracytoplasmic sperm injection	Matched sample of births in the same region from the regional birth registry; births from ovulation induction excluded	Fetal sex	1:1 matching	Throughout Dutch-speaking Belgium	IVF: not stated; spontaneous: regional birth registry
Koivurova <sup>15</sup>	All IVF births in northern Finland identified from IVF clinic records	Matched sample of births in the same region from the national birth registry	Fetal sex, social class, region	1:2 matching	Throughout northern Finland	National birth registry
Koudstaal <sup>16</sup>	All IVF singletons that received IVF, prenatal care and delivery at the same hospital; excluded cryopreserved embryos and embryo reduction; excluded 19% because no appropriate match could be found	Matched singletons with prenatal care and delivery at the same hospital as the IVF subject; excluded infertility treatment and unknown gestational length	Delivery site, height, weight, smoking, race, medical and obstetric history	1:1 matching	4 university hospitals	Hospital charts
Maman <sup>17</sup>	All IVF singleton births to Jewish women at a single hospital; IVF received at multiple sites	Matched births to Jewish women at the same hospital	Gestational age, delivery site	1:3 matching	Single university hospital	Computerized hospital charts
Reubinoff <sup>18</sup>	All IVF pregnancies from 1 center, 14% cryopreserved embryos, 6% ovum donation	Matched sample delivering at the same hospital	Ethnicity	1:1 matching	"Most" IVF and all spontaneous at 1 university hospital	Hospital records
Verlaenen <sup>6</sup>	All IVF pregnancies from 1 center who also received prenatal care and delivered at the affiliated hospital; excluded embryo reduction	Matched sample of publicly insured patients with prenatal care and delivery at the same hospital	Height, weight, delivery site	1:1 matching	Single university hospital	Hospital records
Wang <sup>14</sup>	All assisted reproductive technology singletons from 1 center that delivered in South Australia and had birth information available in regional birth database; 11% GIFT, 20% intracytoplasmic sperm injection	Matched sample from the regional birth registry	Obstetric history, fetal sex, delivery type, malformations	1:1 matching	Throughout South Australia	Regional birth registry

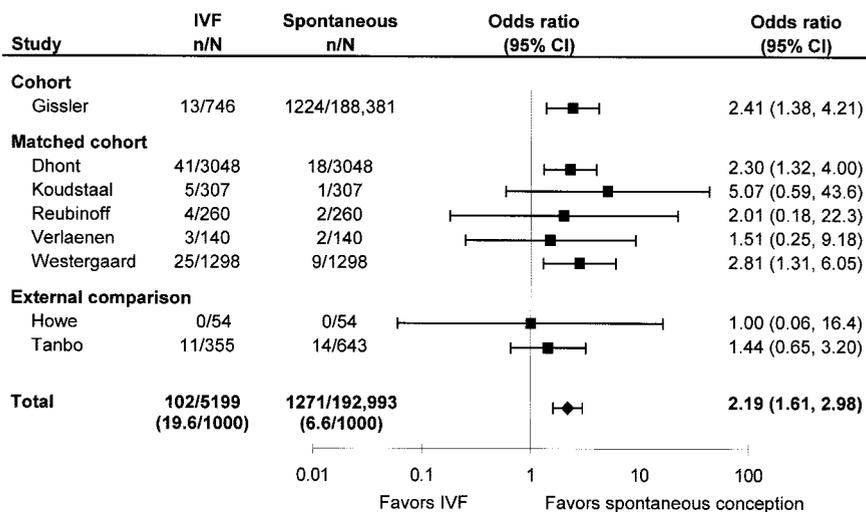
*(continued)*

**Table 2.** Design of Included Studies Comparing Perinatal Outcomes in IVF Versus Spontaneous Conceptions (*continued*)

Author	Study population		Additional variables controlled for*	Method of control	Delivery site	Source of outcome data
	IVF	Spontaneous				
Westergaard <sup>9</sup>	All assisted reproductive technology births in Denmark identified via mandatory assisted reproductive technology registry linked to national birth registry; 8.5% intracytoplasmic sperm injection, 5% cryopreserved and 2% ovum donation	Matched sample from national birth registry		1:1 matching	Throughout Denmark	National birth and death registries
External comparison cohort						
Howe <sup>20</sup>	First 100 IVF pregnancies > 20 weeks gestation from 1 center with available records	Matched sample from an affiliated university hospital	Race, medical problems, diethylstilbestrol, insurance	1:1 matching	IVF: 32 hospitals; spontaneous: 1 university hospital.	IVF: query of obstetrician; spontaneous: hospital records
Tan <sup>12</sup>	All IVF pregnancies from 1 center, identified via the British IVF registry; British residents only; 16% excluded because obstetric outcomes not available	All primiparous patients from 1988–89 from the database of 1 university and 1 community hospital	Not matched for parity but controls all primiparous	Stratum matching	IVF: throughout England; spontaneous: 2 hospitals	IVF: query of obstetrician; spontaneous: hospital records
Tanbo <sup>3</sup>	All assisted reproductive technology pregnancies >20 weeks gestations from 1 center; Scandinavian descent; vanishing twins excluded; 11% intrauterine insemination, 6.5% GIFT	Matched sample of healthy Scandinavian patients delivering at 1 hospital; 13% infertile but conceived spontaneously		1:2 matching	IVF: throughout Norway; spontaneous: 1 community hospital	Hospital records
Wang <sup>8</sup>	All IVF and GIFT singletons from 1 center	All singletons from obstetric database of a large university hospital	Gestational age	Logistic regression	IVF: throughout Australia; spontaneous: 1 university hospital	IVF: database at IVF center; spontaneous: labor and delivery database

IVF = in vitro fertilization; GIFT = gamete intrafallopian transfer.

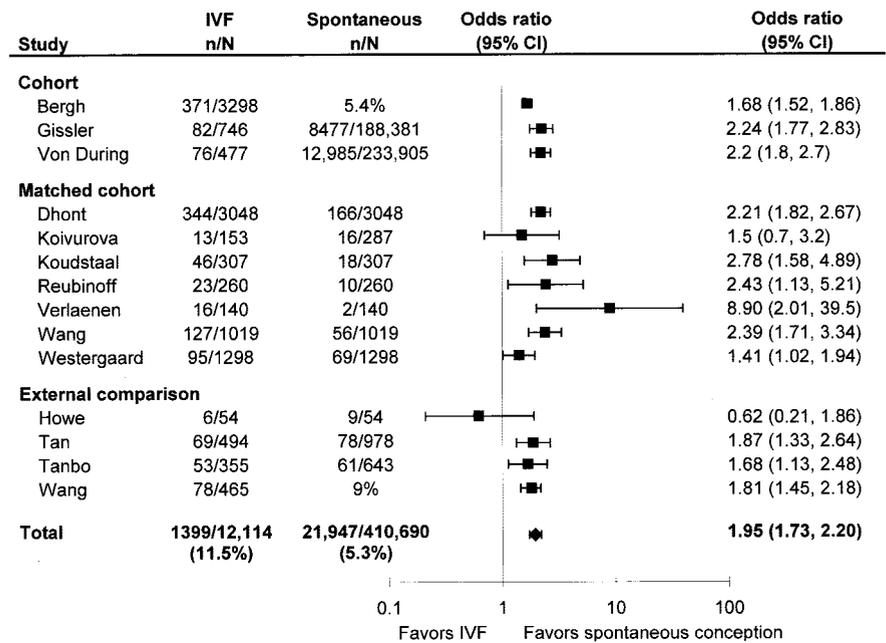
\* All studies controlled for at least maternal age, parity and delivery date.



**Figure 1.** Individual study data and meta-analytic summary odds ratios for perinatal mortality outcomes. CI = confidence interval; IVF = in vitro fertilization.

*Jackson. IVF Meta-Analysis. Obstet Gynecol 2004.*





**Figure 2.** Individual study data and meta-analytic summary odds ratios for preterm delivery outcomes. CI = confidence interval; IVF = in vitro fertilization.

*Jackson. IVF Meta-Analysis. Obstet Gynecol 2004.*

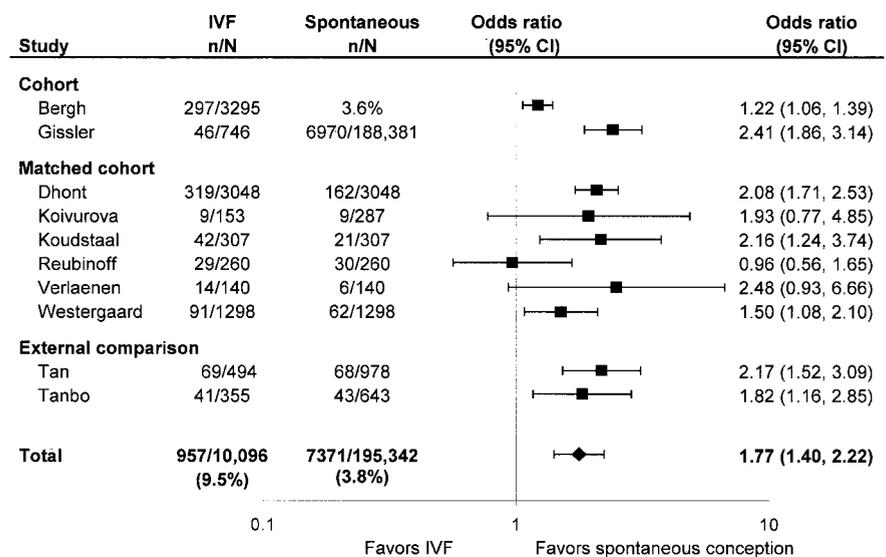
of these studies did not change summary ORs for the associated outcomes. Details of these analyses are available on request. No evidence of publication bias was observed using funnel plots, Egger's regression asymmetry test,<sup>31</sup> or the Kendall rank correlation test<sup>32</sup> (data available on request).

Meta-analyses of secondary outcomes revealed that the IVF group had significantly higher ORs for stillbirth, early preterm delivery, spontaneous preterm birth, gestational diabetes, preeclampsia, placenta previa, vaginal bleeding, labor induction, elective and emergent cesarean, neonatal death, and neonatal intensive care unit

admissions (Figure 7). Data were insufficient to examine premature rupture of membranes, antepartum hospitalization, fetal distress, or Apgar scores.

## DISCUSSION

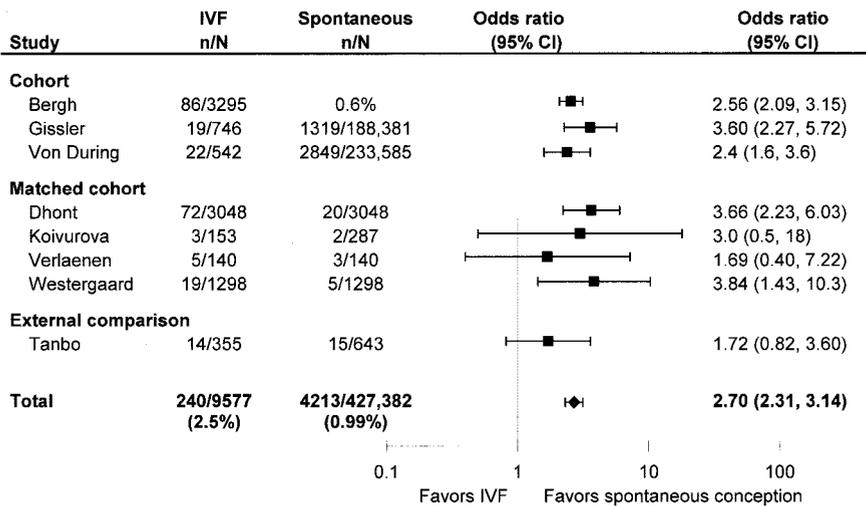
Our meta-analysis suggests that singleton IVF pregnancies are associated with numerous adverse perinatal outcomes, including perinatal mortality, preterm delivery, LBW, and SGA, even after controlling for maternal age and parity. The findings were remarkably consistent: significantly increased ORs ranging from 1.6 to 2.7 were



**Figure 3.** Individual study data and meta-analytic summary odds ratios for low birth weight outcomes. CI = confidence interval; IVF = in vitro fertilization.

*Jackson. IVF Meta-Analysis. Obstet Gynecol 2004.*





**Figure 4.** Individual study data and meta-analytic summary odds ratios for very low birth weight outcomes. CI = confidence interval; IVF = in vitro fertilization.

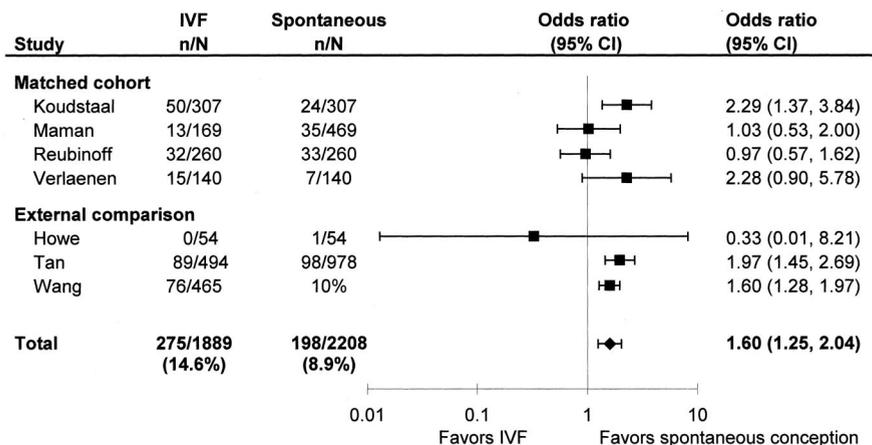
*Jackson. IVF Meta-Analysis. Obstet Gynecol 2004.*

observed for all of the major perinatal outcomes and nearly all of the secondary outcomes. This consistency was observed despite varying study designs, patient populations, and IVF and obstetric protocols. Many have ascribed the higher rates of adverse outcomes to the effects of multiple gestations and the increased age and nulliparity of women who obtain IVF.<sup>3,51-54</sup> Our results refute this assertion in that we observed increased adverse outcomes even in studies of singleton gestations that controlled for 2 major confounders: maternal age and parity.

Absolute risks and risk differences are generally more useful in counseling patients than relative risks. However, summary absolute risk differences could not be estimated using these studies given that several studies either did not provide raw incidence data in the spontaneous group<sup>3,14</sup> or provided incidence rates unadjusted for age and parity.<sup>5,7</sup> The absolute risks in the IVF group as a whole were clearly elevated as evidenced by the increased incidence of each outcome, ranging from 2.00%

for perinatal mortality to 14.6% for SGA. These numbers can be used to approximate the absolute risk in IVF singletons as a group but are simple arithmetic averages and cannot be applied to individual patients. Furthermore, individualized risks based on age and parity would be clinically useful but were also not estimable from the data given.

A limitation of any meta-analysis, especially one based upon observational studies, is that biases in individual studies will be reflected in the summary statistics. The most likely source of bias in our meta-analysis is related to altered management of IVF pregnancies. Because IVF pregnancies are highly valued by patients and their doctors, these patients may be more likely to be hospitalized or to undergo labor induction or cesarean for minor complications, thus leading to iatrogenic increases in preterm delivery and LBW. Indeed, most studies reported higher rates of induced labor<sup>6,13,16</sup> and elective cesarean<sup>6,9,13,16,18</sup> in the IVF group. Could the adverse outcomes we observed be due to treatment bias? Out-

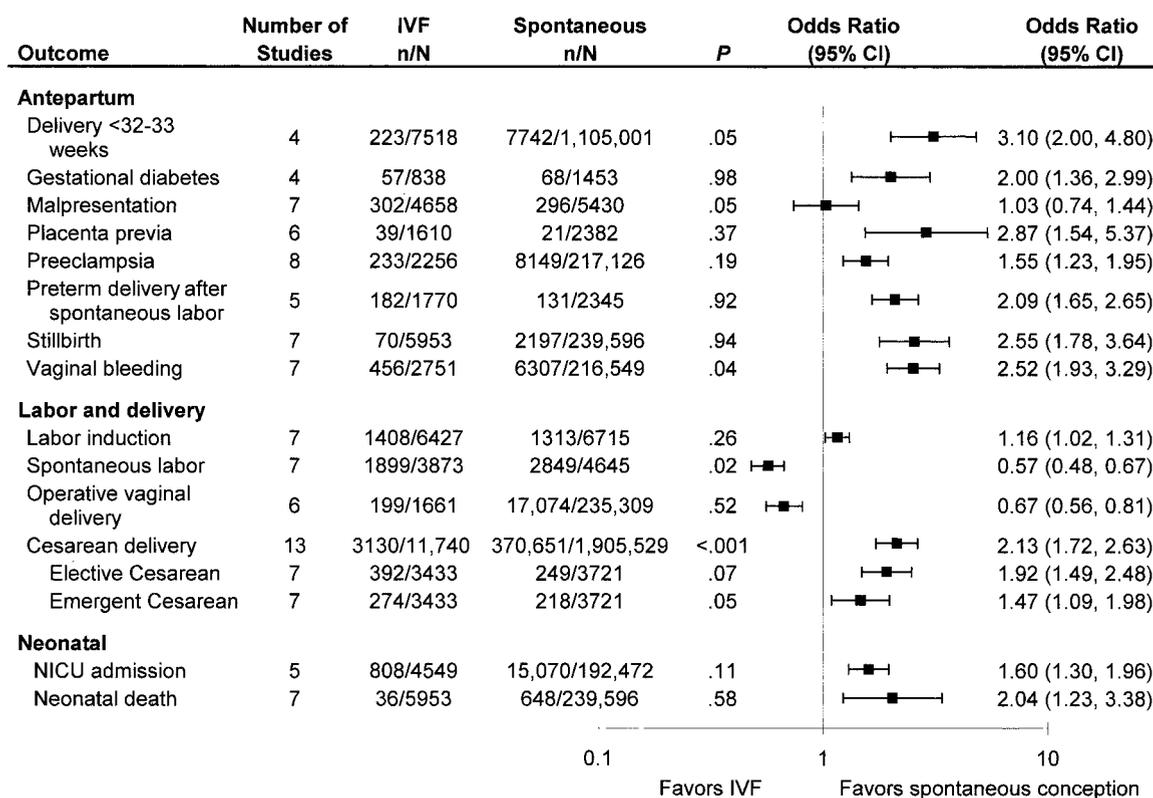


**Figure 5.** Individual study data and meta-analytic summary odds ratios for small for gestational age outcomes. CI = confidence interval; IVF = in vitro fertilization.

*Jackson. IVF Meta-Analysis. Obstet Gynecol 2004.*







**Figure 7.** Results of meta-analyses of secondary outcomes. Data were insufficient to examine premature rupture of membranes, antepartum hospitalization, fetal distress, or Apgar scores. CI = confidence interval; IVF = in vitro fertilization.

Jackson. *IVF Meta-Analysis. Obstet Gynecol* 2004.

for studies with these outcomes. Our search was limited to studies reporting on preterm delivery, LBW, VLBW, SGA or perinatal mortality. Second, for some of the outcomes, definitions varied from study to study. For example, definitions of vaginal bleeding ranged from any self-reported bleeding to bleeding severe enough to warrant hospital admission.

The causes of the increased risks we observed are unknown but could be due to the IVF procedure itself, to components of the IVF procedure, or to infertility per se. Recent studies by McElrath<sup>45</sup> and Draper<sup>41</sup> observed increased odds for VLBW and perinatal mortality in women with untreated infertility. To definitively address whether adverse outcomes are associated with infertility treatment as opposed to infertility would be ethically impossible, because it would require a randomized clinical trial comparing assisted conception with natural conception in fertile women. Clues can be obtained about the risks of assisted reproductive technology by comparing different subgroups of treated women. Wang<sup>14</sup> compared “low technology” treatments (intra-uterine insemination, donor insemination), assisted reproductive technology, and spontaneous conceptions

and found 50% increased odds for preterm birth in the low technology group and a 2-fold increase in the assisted reproductive technology group, indicating that both infertility itself and high technology treatments may be associated with increased preterm birth. Bergh<sup>3</sup> compared intracytoplasmic sperm injection, primarily performed for male factor infertility, with standard IVF, controlling for age and parity and found no statistically significant difference in preterm delivery or LBW, implying that fertile and infertile women undergoing IVF have similar outcomes. However, sample sizes were not given, so we were unable to determine whether there was adequate power. Several smaller studies have also compared intracytoplasmic sperm injection with IVF and generally found no difference in perinatal outcomes,<sup>55-59</sup> but it is unclear how many women in the intracytoplasmic sperm injection group were known to be fertile, and most studies did not report singleton gestations separate from multiples or control for maternal age and parity. Comparing stimulated with unstimulated cycles would isolate the effect of ovulation induction agents. Bergh<sup>3</sup> found no difference in outcomes for standard IVF compared with unstimulated IVF con-



trolled for age and parity. Wennerholm<sup>50</sup> found a lower preterm delivery rate in unstimulated IVF with cryopreserved embryos compared with standard IVF. Olive-ness<sup>36</sup> found no difference in preterm birth in those receiving only ovulation induction compared with IVF.

Future research should be conducted to further delineate the causes of the adverse outcomes observed in IVF singletons and attempt to better control for treatment biases. Given our findings, we recommend that informed consent for women undergoing IVF should include a discussion of possible perinatal risks. Furthermore, although obstetricians caring for these patients should consider IVF a risk factor for adverse perinatal outcomes, they should also be aware of the increased rates of labor induction and elective cesarean and attempt to avoid iatrogenic harm caused by preterm labor induction and cesarean.

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# Assisted Reproductive Technology and Pregnancy Outcome

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**OBJECTIVE:** To determine whether the use of assisted reproductive technology (ART) is associated with an increase in chromosomal abnormalities, fetal malformations, or adverse pregnancy outcomes.

**METHODS:** A prospective database from a large multi-center investigation of singleton pregnancies, the First And Second Trimester Evaluation of Risk trial, was examined. Subjects were divided into 3 groups: no ART use, use of ovulation induction (with or without intrauterine insemination), and use of in vitro fertilization (IVF). Multivariate logistic regression analysis was used to assess association between ART and adverse pregnancy outcomes (significance of differences was accepted at  $P < .05$ ).

**RESULTS:** A total of 36,062 pregnancies were analyzed: 34,286 (95.1%) were spontaneously conceived, 1,222 (3.4%) used ovulation induction, and 554 (1.5%) used IVF. There was no association between ART and fetal growth restriction, aneuploidy, or fetal anomalies after adjustment for age, race, marital status, years of education, prior preterm delivery, prior fetal anomaly, body mass index, smoking history, and bleeding in the current

pregnancy. Ovulation induction was associated with a statistically significant increase in placental abruption, fetal loss after 24 weeks, and gestational diabetes after adjustment. Use of IVF was associated with a statistically significant increase in preeclampsia, gestational hypertension, placental abruption, placenta previa, and risk of cesarean delivery.

**CONCLUSION:** Patients who undergo IVF are at increased risk for several adverse pregnancy outcomes. Although many of these risks are not seen in patients undergoing ovulation induction, several adverse pregnancy outcomes are still increased in this group. There was no increased incidence of fetal chromosomal or structural abnormalities in the women who used any type of ART compared with the women who conceived spontaneously.

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**LEVEL OF EVIDENCE: II-2**

The use of assisted reproductive technology (ART) is a highly successful and widely employed modality for the treatment of infertility. In 2001, more than 40,000 infants were born as a result of ART therapy, which represents 1% of the births in the United States.<sup>1</sup> Despite the success of ART, there is concern regarding both the safety of ART and its effect on maternal and fetal well-being. It is well-recognized that ART procedures significantly increase the risk of multiple gestations, both monozygotic and dizygotic, with the associated risks attributed to these pregnancies.<sup>2</sup> Additionally, some studies have suggested an increased risk of chromosome abnormalities, low birthweight, and preterm delivery in singletons.<sup>3–6</sup> Small studies have also suggested an association between the use of IVF and birth defects, adverse neurodevelopmental outcomes, preeclampsia, perinatal mortality, placenta previa, and an increased rate of cesarean delivery.<sup>7–13</sup>

Data derived from ART registries only provide results for overall pregnancy outcomes such as birth

\* For a list of members of the FASTER Research Consortium, see the Appendix.

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weight and number of multiple gestations. Limited data are available to describe patterns of anomalies and other adverse obstetric outcomes.<sup>10</sup> The few studies available are retrospective registry reviews, which exclude data regarding outcome with ovulation induction. Also, many prior studies fail to control for past obstetric history or other relevant variables. Our objective was to prospectively assess the effect of ART on the outcome of singleton pregnancies and to differentiate the effect of both IVF and ovulation induction.

## PATIENTS AND METHODS

The First And Second Trimester Evaluation of Risk (FASTER) trial, a National Institute of Child Health and Human Development–sponsored study, is a prospective multicenter investigation of singleton pregnancies enrolled from an unselected obstetric population. The study was undertaken from 1999 to 2002 and was approved by the institutional review boards at each of the participating centers. The study provided noninvasive assessment of Down syndrome risk using evaluation of first trimester nuchal translucency sonography, together with first and second trimester serum markers. A database was created containing detailed antenatal, birth, and pediatric outcomes on all enrolled patients.

Patients were enrolled into the FASTER trial at 10 3/7 to 13 6/7 weeks of gestation, at which time baseline demographic data and medical histories were recorded. Postdelivery follow-up was performed by telephone interview, personal interview, or medical record review by a trained research coordinator at each site. A purpose-designed computerized tracking system with up to 10 contacts per subject was used to ensure optimal outcome collection for all enrolled patients. In addition, a single perinatologist and a pediatric geneticist reviewed detailed maternal and pediatric medical records for the following patient subsets: all patients with abnormal first or second trimester screening, all pregnancies with adverse pediatric outcome, and 10% of normal subjects randomly selected at each site from the trial database.

For this analysis, women who elected to terminate their pregnancy before term for any reason were excluded. Patients were then categorized into 3 mutually exclusive groups: those who underwent an invasive ART procedure in this pregnancy, including the use of in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), gamete intrafallopian transfer or zygote intrafallopian transfer ( $n = 554$ ), women who used a noninvasive ART procedure only, such as ovulation induction or intrauterine insemination ( $n =$

1,222); and control patients who did not undergo any ART procedure ( $n = 34,286$ ).

The following adverse pregnancy outcomes were then evaluated: spontaneous fetal loss before 24 weeks, fetal loss or demise after 24 weeks, fetal growth restriction (estimated fetal weight by ultrasound below the 10th centile or birthweight below the 10th centile for gestational age), low birth weight (less than 2,500 g), gestational hypertension (blood pressure  $> 140/90$  on at least 2 occasions more than 6 hours apart without evidence of chronic hypertension), pre-eclampsia (criteria for gestational hypertension and significant proteinuria), preterm labor (before 37 weeks of gestation), preterm premature rupture of membranes (membrane rupture before 37 weeks of gestation), placental abruption (premature separation of a normally implanted placenta), placenta previa (placenta completely or partially covering the internal os), gestational diabetes (a minimum of 2 abnormal values on a 3-hour glucose tolerance test after a 100-g oral glucose load), cesarean delivery, fetal aneuploidy, and congenital anomalies (major or minor, confirmed at birth).

The effects of both invasive and noninvasive ART use were investigated simultaneously for each of the pregnancy outcomes. Crude and adjusted effects were estimated using multivariate logistic regression, and odds ratios (ORs), together with 95% confidence intervals (CIs), were calculated to quantify the effect.

Confounding variables for the adjusted models were selected in a 2-stage process. First, a series of statistical tests were performed to assess relationships of selected patient characteristics with ART use and each adverse pregnancy outcome. Tests included analysis of variance for continuous confounders and  $\chi^2$  tests for categorical confounders. Confounders that were significantly ( $P < .05$ ) associated with either ART use or the given adverse outcomes were considered in the next stage. The list of potential confounders was further reduced using multivariate logistic regression modeling and a backward elimination stepwise regression approach, keeping only those variables that were significant at  $P < .05$ .

In the final adjusted models, the following variables were considered as confounders: maternal age, maternal race, marital status, years of education, prior preterm delivery, prior pregnancy with anomaly, body mass index, smoking history, and bleeding in the current pregnancy.

Adjusted odds ratios with 95% confidence intervals were calculated to approximate relative risks of adverse outcomes. A  $P$  value of less than .05 was considered statistically significant (ie, the 95% confi-



dence intervals were calculated, and were considered significant if they did not include 1.0). However, due to the large sample size, statistical analysis was powerful enough to detect differences in risk between the ART groups that were statistically significant but where the actual size of the difference was small. In some cases the differences might be so small that they are not clinically meaningful. Therefore, since OR describes the magnitude of the effect between groups, an odds ratio cutoff of greater than 2.0 was selected to represent clinically meaningful risk to emphasize those outcomes with a marked association with invasive or noninvasive ART. All analyses were conducted using SAS 8.2 (SAS Institute Inc., Cary, NC).

## RESULTS

Complete obstetric and pediatric outcome data were available for 36,062 pregnancies for this analysis. The control group consisted of 34,286 (95.1%) patients who did not use any form of ART. A total of 1,222 (3.4%) patients used ovulation induction, and 554 (1.5%) underwent IVF (including gamete intrafallopian transfer or gamete intrafallopian transfer and zygote intrafallopian transfer or zygote intrafallopian transfer). Because only 33 patients reported use of ICSI, this group was not examined separately. The demographic characteristics of the 3 groups are summarized in Table 1. Those patients undergoing ART were significantly older, were more likely to be married, and had more years of education.

The overall incidences of pregnancy complica-

tions in this population are shown in Table 2. Multivariate analysis was then performed to calculate adjusted odds ratios for these adverse outcomes. The obstetric outcomes for patients undergoing ovulation induction and those using IVF are compared in Table 3 to patients who did not use ART. Patients who underwent ovulation induction were 2.4 times more likely to have a placental abruption (95% CI 1.3–4.2) and 2.1 times more likely to have a fetal loss after 24 weeks (95% CI 1.3–3.6) compared with controls. A significant association between the use of IVF and several adverse pregnancy outcomes was also noted. Patients using IVF were 2.7 times more likely to develop preeclampsia (95% CI 1.7–4.4), 2.4 times more likely to have a placental abruption (95% CI 1.1–5.2), 6.0 times more likely to have a placenta previa (95% CI 3.4–10.7), and 2.3 times more likely to undergo a cesarean delivery (95% CI 1.8–2.9) compared with controls. We did not observe an increase in the incidence of aneuploidy or congenital anomalies in patients undergoing IVF.

Although a statistically significant increase in the incidence of gestational hypertension in patients undergoing IVF and in the incidence of gestational diabetes in patients undergoing ovulation induction was noted, these findings did not meet criteria for achieving clinical significance, because the odds ratios were less than 2.0.

## DISCUSSION

Despite the widespread and increasing use of assisted reproductive technologies, there are few prospective

**Table 1. Use of Assisted Reproductive Technology and Pregnancy Outcome: Demographics of Population**

Characteristic	No ART (n = 34,286)	Ovulation Induction (n = 1,222)	IVF (n = 554)	P
Age (y)	29.9 (±5.7)	32.6 (±5.1)	34.5 (±5.2)	< .001
Race				< .001
African American	5.3	1.6	2.7	
Hispanic	23.3	4.5	4.5	
White	66.6	88.6	86.3	
Other	4.9	5.2	6.5	
Education (y)	14.2 (±2.6)	15.6 (±1.6)	16.1 (±1.3)	< .001
Marital status				< .001
Single	21.0	7.1	3.4	
Married	77.8	92.0	96.4	
Divorced	1.2	0.7	0	
Other	0.1	0.2	0.2	
Previous pregnancy (multiparous)	55.9	37.2	26.6	< .001
Prior preterm delivery	6.8	5.4	5.1	.048
Prior pregnancy with anomaly	3.6	7.4	12.0	< .001
Body mass index	25.0 (±5.3)	25.2 (±5.4)	24.4 (±4.8)	.009
Bleeding in pregnancy	14.0	15.8	28.8	< .001
Smoking	4.9	1.6	1.4	< .001

ART, assisted reproductive technology; IVF, in vitro fertilization. Values are % or mean (± standard deviation).



**Table 2. Incidence of Pregnancy Complications and Pediatric Outcomes**

Outcome	No ART (n = 34,286)	Ovulation Induction (n = 1,222)	IVF (n = 554)	P
Spontaneous fetal loss (< 24 wk)	0.3	0.4	0.2	.73
Fetal loss or demise (> 24 wk)	0.9	1.6	1.1	.09
Fetal growth restriction	1.1	2.1	0.9	.005
Gestational hypertension	4.6	5.8	6.4	.03
Preeclampsia	2.4	3.3	4.7	.001
Preterm labor	5.2	6.5	6.9	.03
PPROM	1.6	1.9	2.2	.42
Placental abruption	0.7	1.4	2.2	< .001
Placenta previa	0.6	0.5	3.6	< .001
Gestational diabetes	3.4	5.9	2.7	< .001
Cesarean delivery	23.6	26.2	47.2	< .001
Aneuploidy	0.4	0.3	0.4	.98
Congenital anomalies	1.9	2.3	3.5	.02
Low birth weight	5.1	7.4	5.9	.002

ART, assisted reproductive technology; IVF, in vitro fertilization; PPRM, preterm premature rupture of membranes. Values are %.

**Table 3. Adverse Pregnancy Outcomes: Comparison With Control Patients**

Outcome	Ovulation Induction Compared With No ART [Adjusted OR (95%CI)]	P	IVF Compared With No ART [OR (95% CI)]	P
Spontaneous fetal loss (< 24 wk)	1.6 (0.6–4.4)	.37	0.8 (0.1–5.6)	.80
Fetal loss or demise (> 24 wk)	2.1 (1.3–3.6)	.005	0.9 (0.3–2.4)	.78
Fetal growth restriction	1.5 (0.8–2.8)	.23	0.57 (0.1–2.2)	.39
Low birthweight	1.3 (1.0–1.8)	.09	0.9 (0.5–1.5)	.64
Gestational hypertension	0.8 (0.5–1.2)	.31	1.6 (1.0–2.5)	.036
Preeclampsia	1.1 (0.6–1.8)	.85	2.7 (1.7–4.4)	< .001
Preterm labor	1.1 (0.8–1.5)	.56	1.5 (1.0–2.2)	.07
PPROM	1.0 (0.5–1.8)	.93	1.1 (0.5–2.2)	.84
Placental abruption	2.4 (1.3–4.2)	.003	2.4 (1.1–5.2)	.03
Placenta previa	0.9 (0.3–2.3)	.75	6.0 (3.4–10.7)	< .001
Gestational diabetes	1.5 (1.1–2.2)	.01	0.5 (0.2–1.0)	.06
Cesarean delivery	1.1 (0.9–1.3)	.26	2.3 (1.8–2.9)	< .001
Aneuploidy	0.7 (0.2–2.1)	.48	0.4 (0.1–2.7)	.32
Congenital anomalies	1.1 (0.6–1.8)	.78	0.9 (0.4–2.0)	.78

ART, assisted reproductive technology; OR, odds ratio; CI, confidence interval; IVF, in vitro fertilization; PPRM, preterm premature rupture of membranes.

Adjusted odds ratios calculated after multivariate logistic regression analysis represent risk of treated group to experience outcome of interest compared with patients not undergoing any therapy.

studies published addressing the obstetric and pediatric outcomes with these therapies. Additionally, studies suggesting an increase in adverse outcomes such as congenital malformations are limited by small numbers and limited information on confounding variables.<sup>9</sup> A recent meta-analysis of a large number of IVF pregnancies suggested that such pregnancies are at increased risk for adverse perinatal outcome, including preterm delivery, low birthweight, placenta previa, gestational diabetes, preeclampsia, and neonatal intensive care admission.<sup>9</sup> The main strength of our study is that it incorporated a large number of ART pregnancies and collected prospectively from the general population, with appropriate controls.

Our findings corroborate those of the recent meta-analysis, demonstrating a significant increase in hypertensive disorders, placental abnormalities such as placenta previa and placental abruption, and the incidence of cesarean delivery.

We estimated a 2.7-fold increased risk of preeclampsia in IVF pregnancies compared with controls. This association between IVF and preeclampsia has also been noted by other authors, including Jackson et al<sup>9</sup> in 2004, Wang et al<sup>14</sup> in 2002, Maman et al<sup>15</sup> in 1998, and Tan et al<sup>11</sup> in 1992. We have also shown an increased incidence of abnormal placentation with IVF use, including a 2.4-fold increased risk of placental abruption and a 6.0-fold increased risk of



placenta previa noted in IVF pregnancies compared with controls. This has also been substantiated by other authors, including Verlaenen et al<sup>16</sup> in 1995, Li et al<sup>17</sup> in 1996, Tan et al<sup>12</sup> in 1992, and Jackson et al<sup>9</sup> in 2004. Preeclampsia, placental abruption, and placenta previa are all related to abnormalities of location and function of the placenta. Therefore, when pregnancy and the formation of the chorion are initiated in vitro, an inherent difference in the nature of the placenta itself may predispose the patient to develop these conditions during gestation.

Previous authors have suggested an association between IVF use and increased cesarean delivery rate.<sup>13,18</sup> We found greater than a two-fold increase in the incidence of cesarean delivery in patients undergoing IVF. Although studies have linked advancing maternal age to the risk of cesarean delivery, this increase remained significant in our study after adjustment for maternal age and parity. Infertile women have been reported to be more anxious about the outcome of their pregnancies compared with women who conceive spontaneously.<sup>19</sup> It is possible, therefore, that the apparent increase in cesarean delivery rates may reflect patient and physician choice, rather than an inherent biologic abnormality in such pregnancies.

We did not observe an increased incidence of congenital malformations or fetal aneuploidy in the women who used ART to conceive. A recent study by Zadori et al<sup>6</sup> in 2003 reviewed outcomes of 301 neonates born as a result of IVF in a population of more than 12,900 deliveries and found no significant increase in the number of major birth defects. Additionally, in the United States, the Society for Assisted Reproductive Technology reported a prevalence of congenital malformations of 1.9% among patients undergoing IVF, which was similar to that seen in the general population. Retzlaff and Hornstein<sup>3</sup> in 2003 performed an analysis of 11 major studies from 1996 to 2002 and concluded that the vast majority showed neither an increase in malformations nor clustering of any single specific major malformation in ICSI pregnancies. This finding is complemented by the work done by Bonduelle et al<sup>20</sup> in 2002, who studied 2,840 ICSI children and 2,955 IVF children in Brussels. This study found no significant difference in the malformation rate between ICSI and IVF pregnancies. However, our study contradicts the work of Hansen et al<sup>7</sup> in 2002, which found an increased incidence of major birth defects in IVF pregnancies with an overall adjusted odds ratio of 2.0. This study found that infants conceived with ART were more likely to have multiple major defects and were also more likely to have chromosomal abnormalities.

However, increased diagnostic vigilance of the study population may have resulted in ascertainment bias given the low-risk nature of the control population.<sup>21</sup>

Additionally, prior work has suggested an association between use of ICSI and an increase in both autosomal and sex chromosome abnormalities.<sup>22,23</sup> This apparent association may be due to the known increase in prevalence of chromosomal abnormalities in both azoospermic and oligospermic men.<sup>3</sup> It is possible that any association between IVF use and fetal chromosomal abnormalities may be confined only to the subgroup of patients using ICSI. Although our study found no association between ART use overall and fetal chromosomal abnormalities, we had insufficient numbers of patients using ICSI to evaluate this subgroup individually. Also, not all of the FASTER infants had a chromosome analysis performed, so it is possible that there are FASTER infants who have a sex chromosome abnormality but do not know it. In fact, most cases of sex chromosome abnormalities go undiagnosed.

A report from the Centers for Disease Control and Prevention of IVF pregnancies from 1996 to 1997 suggested a 1.8-fold increased risk of low birth weight infants.<sup>4</sup> This study attempted to control for the confounding effects of multiple gestation on incidence of low birth weight, and the analysis was restricted to those singletons born of pregnancies that did not originate as multiple gestations. Similar findings for IVF singletons were also reported by Bergh et al<sup>24</sup> in 1999, who compared 5,856 ART children to 1,505,742 children born in the general population and found an odds ratio of 4.4 for delivery of a very low birth weight singleton. However, most recently, Schieve et al<sup>11</sup> in 2004 found that despite the report of their findings in 2002, from 1996–2000 in 62,551 infants born of IVF, the risk for term low birth weight was found to decline, with an overall standardized risk ratio of 1.62. Our study failed to demonstrate any association between ART and low birth weight. Our population of IVF pregnancies may not have been sufficiently large to detect a difference in birth weight. Alternatively, it is possible that earlier larger studies may have demonstrated statistical significance, but without clinical significance.

The current study objectively addresses the outcome of a subgroup of patients with “subfertility,” requiring only ovulation induction rather than IVF. Nuojua-Huttunen et al<sup>25</sup> in 1999 studied 111 patients who underwent ovulation induction and found no change in obstetric or perinatal risk compared with controls. However, in another report by Gaudoin et al<sup>26</sup> in 2003, ovulation induction patients were 4.85



times more likely to have a LBW infant compared with controls. Although we did not find an increased incidence of low birthweight in this population, patients undergoing ovulation induction were 2.4 times more likely to have a placental abruption, and 2.1 times more likely to suffer a fetal loss after 24 weeks. In a study undertaken by Maman et al<sup>15</sup> in 1998, patients undergoing ovulation induction were also found to have a greater incidence of gestational diabetes, which may reflect an increased prevalence of polycystic ovary syndrome (PCOS) requiring therapy. This underlying metabolic instability may be linked to risk of abnormal placentation (abruption) and of fetal loss. Due to limitations of data collection regarding specific underlying causes for infertility in patients requiring ovulation induction, we cannot distinguish whether some of the abnormal outcomes seen here represent a preexisting disease process, such as PCOS, or are secondary to the ovulation induction therapy itself.<sup>27</sup> Despite the fact that there is a slight increase in adverse outcome for patients undergoing ovulation induction, as evidenced by the increase in fetal loss after 24 weeks, there still is a striking difference between complications rates between this group and patients undergoing IVF. This might imply that perhaps the state of subfertility or infertility itself may not be the cause of these adverse outcomes, but that these risks may be related to the process of in vitro fertilization itself.

It is unlikely that a single pathophysiologic approach is responsible for the wide range of adverse obstetric outcomes noted in this study, because the causes of infertility, both identified and unidentified, are broad. Some of the risks apparently associated with ART may be confounded by the nature and presence of infertility itself or by other associated underlying conditions, such as PCOS. A limitation of our study is that no data were collected on the particular cause of infertility, and therefore we cannot comment on how these different causes may affect outcomes. However, we feel that knowledge of an association between overall ART use and adverse pregnancy outcome will be useful for practitioners. The odds remain strong that infertile couples seeking to conceive through the use of assisted reproductive technology will have relatively uncomplicated pregnancies and healthy children. Clearly, however, there is an increased risk of adverse events in a subgroup of these patients, and the information provided here should prove useful when counseling prospective patients before embarking on fertility therapy.

Additionally, an increase in antenatal surveillance may be warranted in this population, including

assessment for hypertensive complications and sonographic evaluation of the placenta. The possible associations between infertility, or its therapies, with a range of adverse obstetric outcomes should be discussed with prospective patients before embarking on fertility therapy. Clinicians caring for such patients should be aware of these possible associated adverse outcomes and may need to be vigilant for additional signs or symptoms of complications during antenatal care. However, we cannot conclude from our data whether any particular program of fetal surveillance is warranted or would cause any adverse outcome. It is important, however, for patients and clinicians to realize that although an extra level of surveillance may be warranted given the additional degree of risk, their chances of having a healthy child through ART are overall extremely high.

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## APPENDIX

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# Parental infertility and cerebral palsy in children

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**BACKGROUND:** Children born after *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) have been reported to have a higher risk of cerebral palsy (CP), perhaps due to the higher frequency of preterm birth, multiple births or vanishing embryo in the pregnancies. However, it has been suggested that the underlying infertility may be part of the pathway. In this study, we examined whether untreated subfecundity (measured by time to pregnancy) or infertility treatment was associated with an increased risk of CP in the offspring.

**METHODS:** Using the Danish National Birth Cohort (1997–2003), we compared children born after 0–2 months of waiting time to pregnancy ( $n = 35\,848$ ) with those born after a time to pregnancy of 3–5 months ( $n = 15\,361$ ), 6–12 months ( $n = 11\,528$ ) and >12 months ( $n = 7387$ ), as well as those born after IVF/ICSI ( $n = 3617$ ), ovulation induction with or without intrauterine insemination ( $n = 3000$ ), and unplanned pregnancies ( $n = 13\,462$ ). CP cases were identified through the Danish CP Register.

**RESULTS:** In total, 165 (0.18%) children were diagnosed with CP in the entire cohort. We found no significant association between time to pregnancy and the risk of CP in children conceived spontaneously. Children born after IVF/ICSI had an increased risk of CP, even after adjustment for preterm birth and multiplicity (hazard ratio 2.30, 95% confidence interval 1.12–4.73).

**CONCLUSIONS:** Subfecundity *per se* did not appear to be associated with the risk of CP in children, whereas being born after IVF/ICSI conferred an increased risk.

**Key words:** cerebral palsy / infertility / infertility treatment / time to pregnancy / Danish National Birth Cohort

## Introduction

Cerebral palsy (CP) is a rare but serious disorder, with a prevalence of about 2 per 1000 live births. It can confer lifelong disability with a substantial impact on family life and societal healthcare costs (Rosenbaum *et al.*, 2007; O'Shea, 2008). Children born after *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) have been reported to have a higher risk of CP in a number of studies (Ericson *et al.*, 2002; Stromberg *et al.*, 2002; Kallen *et al.*, 2005; Lidegaard *et al.*, 2005; Hvidtjørn *et al.*, 2006, 2010; Klemetti *et al.*, 2006), perhaps because of the higher frequency of preterm birth, multiple births or vanishing embryo in these pregnancies (Pinborg *et al.*, 2005; Hvidtjørn *et al.*, 2006, 2009b, 2010). One study, however, reported that the association between IVF and CP disappeared after adjustment for the years of unwanted childlessness, suggesting that low fecundity (or its determinants) may be part of the pathway leading to CP (Kallen *et al.*, 2005). A recent study also reported a higher risk of

CP among spontaneously conceived children of subfertile couples who had been registered for treatment at an IVF clinic (Reid *et al.*, 2010).

In this study, we examined whether untreated subfecundity (measured by time to pregnancy) or infertility treatment was associated with a higher risk of CP in the offspring.

## Materials and Methods

The study population, described in detail elsewhere (Hvidtjørn *et al.*, 2009a), comprises all women who participated in the first interview (administered around 16 weeks of gestation) of the Danish National Birth Cohort (Olsen *et al.*, 2001) and whose pregnancy resulted in a live birth between 1997 and 2003. In the interview, women were asked whether their pregnancy was planned, and if so, how long they had tried to become pregnant before succeeding. Response categories for time to pregnancy were: 'right away', 1–2, 3–5, 6–12 and >12 months.

Participants reporting a time to pregnancy of >6 months were further asked whether they or their male partner had received any infertility treatment, including ICSI, IVF, intrauterine insemination (IUI), ovulation induction (OI) and other treatments (e.g. surgery). We used information on IVF (including ICSI) and OI (with or without IUI), validated through the Danish IVF Register and the Danish Drug Prescription Register, respectively. We excluded pregnancies resulting from 'other' treatments ( $n = 99$ ) and treatments that were reported by the women but had no matching information in the IVF Register or the Drug Prescription Register ( $n = 675$ ). We grouped births into seven mutually exclusive categories, based on time to pregnancy, infertility treatment and planning status: time to pregnancy of 0–2 (reference group), 3–5, 6–12, >12 months, born after IVF or ICSI, born after OI with or without IUI and unplanned. Children born in the first four categories of time to pregnancy were not the result of infertility treatment, and those in the last category also included children whose parents partly planned their pregnancy or did not report a time to pregnancy.

Cases of CP were identified by data linkage to the Danish Cerebral Palsy Register (Uldall et al., 2001). The register includes all children with a diagnosis of CP validated by a neuro-pediatrician in Denmark since 1995, with approximately 170 incident cases each year. Criteria for inclusion in the register are: age of 4 years or older, pre- or perinatal etiology (before 28 days post-partum) and meeting the diagnostic criteria according to Surveillance of Cerebral Palsy in Europe [Surveillance of Cerebral Palsy in Europe (SCPE), 2002b]. All data linkages were based on the unique civil registration numbers assigned to all residents at birth. All data related to the birth (including gestational age) came from the Medical Birth Register.

We used Cox regression to assess the association between time to pregnancy and the risk of CP. Follow-up started at the time of birth and ended when the child died, emigrated, received a diagnosis of CP or when follow-up ended (1 July 2009), whichever came first. Information on covariates was obtained from the Medical Birth Register and Statistics Denmark: maternal age (20–34, 35+ years), parity (0, 1+), smoking during pregnancy (yes, quit, no) and education [basic school (9–10 years), high school (11–16 years) and university or higher (17+ years)], as well as sex of child (male and female). Multiplicity and preterm birth (<37 weeks of gestation) are potential intermediate factors, and we ran the models with and without them to check their influence on the studied associations. As there were no mothers younger than 20 years of age included in the IVF register, children born to mothers younger than 20 years were excluded ( $n = 519$ ).

## Results

Among 90 203 children, 165 were diagnosed with CP (0.18%): 145 (0.17%) in 86 223 singletons, 18 (0.47%) in 3834 twins and 2 (2.11%) in 95 triplets. Median follow-up time was 8.7 years.

There was no significant association between time to pregnancy and the risk of CP in children, regardless of adjustment (Table I). The risk of CP was higher among children born after IVF or ICSI, as these children had more than twice the risk of CP than children born after a waiting time to pregnancy of 0–2 months, even after adjustment for multiplicity and preterm birth (hazard ratio 2.30, 95% confidence interval 1.12–4.73) (Table I).

Restricting analysis to term-born singletons yielded similar estimates (Table II). Excluding triplets from the analysis did not change the estimates, either (data not shown).

There were no differences in type and severity (regarding motor function and mental retardation) of CP between CP cases born after fertility treatment and CP cases born without treatment (data not shown).

## Discussion

In this large cohort of prospectively followed children, we found no significant association between time to pregnancy and the risk of CP in children conceived spontaneously, whereas children born after IVF or ICSI had an increased risk of CP.

The absence of an association between time to pregnancy and the risk of CP is not in agreement with two previous studies (Kallen et al., 2005; Reid et al., 2010). A Swedish study reported that the association between IVF treatment and CP disappeared after adjustment for the years of unwanted childlessness (Kallen et al., 2005). We had, however, only one category to represent all waiting times of more than 1 year, and it is possible that couples undergoing IVF had a substantially longer waiting time. On the other hand, it is possible that time of unwanted childlessness does not accurately reflect the severity of infertility. All the children studied by the Swedish investigators were born after IVF or ICSI, which makes the two studies substantially

**Table I Hazard ratios for CP in children according to time to pregnancy and infertility treatment.**

	No. of children	No. (%) with cerebral palsy	Crude HR	Adjusted HR <sup>a</sup> (95% CI)	Adjusted HR <sup>b</sup> (95% CI)
Time to pregnancy of 0–2 months <sup>c</sup>	35 848	59 (0.16)	1.00	1.00	1.00
Time to pregnancy of 3–5 months <sup>c</sup>	15 361	22 (0.14)	0.87	0.79 (0.47–1.31)	0.77 (0.46–1.29)
Time to pregnancy of 6–12 months <sup>c</sup>	11 528	19 (0.16)	0.95	0.91 (0.53–1.55)	0.88 (0.52–1.50)
Time to pregnancy of >12 months <sup>c</sup>	7387	19 (0.26)	1.40	1.27 (0.73–2.22)	1.20 (0.69–2.09)
OI or IUI <sup>d</sup>	3617	7 (0.19)	1.18	1.19 (0.54–2.64)	1.00 (0.44–2.28)
IVF or ICSI <sup>e</sup>	3000	17 (0.57)	3.47	3.23 (1.77–5.88)	2.30 (1.12–4.73)
Unplanned pregnancies	13 462	22 (0.16)	1.00	0.73 (0.43–1.24)	0.72 (0.43–1.22)

Cox regression; HR, hazard ratio; CI, confidence interval; reference group: children born after time to pregnancy of 0–2 months.

<sup>a</sup>Adjusted for maternal age, parity, smoking, education and sex of child.

<sup>b</sup>Adjusted for maternal age, parity, smoking, education, sex of child, multiplicity and preterm birth.

<sup>c</sup>Without infertility treatment.

<sup>d</sup>OI, ovulation induction; IUI, intrauterine insemination.

<sup>e</sup>IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

**Table II Hazard ratios for CP in term-born singletons according to time to pregnancy and infertility treatment.**

	No. of children	No. (%) with cerebral palsy	Crude HR	Adjusted HR <sup>a</sup> (95% CI)
Time to pregnancy of 0–2 months <sup>b</sup>	33 409	43 (0.13)	1.00	1.00
Time to pregnancy of 3–5 months <sup>b</sup>	14 285	16 (0.11)	0.87	0.84 (0.46–1.51)
Time to pregnancy of 6–12 months <sup>b</sup>	10 746	10 (0.09)	0.72	0.72 (0.36–1.44)
Time to pregnancy of >12 months <sup>b</sup>	6771	13 (0.19)	1.26	1.17 (0.59–2.32)
OI or IUI <sup>c</sup>	2895	3 (0.10)	0.80	0.84 (0.26–2.75)
IVF or ICSI <sup>d</sup>	1496	5 (0.33)	2.60	2.55 (0.95–6.86)
Unplanned pregnancies	12 470	14 (0.11)	0.88	0.68 (0.36–1.29)

Cox regression; HR, hazard ratio; CI, confidence interval; reference group: children born after time to pregnancy of 0–2 months.

<sup>a</sup>Adjusted for maternal age, parity, smoking, education and sex of child.

<sup>b</sup>Without infertility treatment.

<sup>c</sup>OI, ovulation induction; IUI, intrauterine insemination.

<sup>d</sup>IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

different. An Australian study reported a higher risk of CP among children of subfertile couples who had been registered at an IVF clinic but received no treatment for the pregnancy (Reid *et al.*, 2010). As the authors noted, there was no information on the conceptions that resulted in the births of the children, and it was possible that IVF treatment might have taken place outside the study area.

We did find an increased risk of CP in children born after IVF or ICSI, a finding in line with most previous studies (Ericson *et al.*, 2002; Stromberg *et al.*, 2002; Kallen *et al.*, 2005; Lidegaard *et al.*, 2005; Hvidtjorn *et al.*, 2006, 2010; Klemetti *et al.*, 2006). However, we found that the association could not be entirely attributed to multiple births or preterm birth, which have been the given explanations in most studies (Kallen *et al.*, 2005; Klemetti *et al.*, 2006; Hvidtjorn *et al.*, 2006, 2010), although not all (Stromberg *et al.*, 2002; Lidegaard *et al.*, 2005). The number of CP cases in our study was small, but the estimated CP prevalence of 0.33% for term-born IVF/ICSI singletons was within the most frequently reported ranges among IVF/ICSI singletons (Stromberg *et al.*, 2002; Pinborg *et al.*, 2003, 2004; Lidegaard *et al.*, 2005; Hvidtjorn *et al.*, 2006). The vanishing embryo may play a part in the increased risk (Pinborg *et al.*, 2005; Anand *et al.*, 2007). Children born after OI have also been shown to have a higher risk of CP, though on a smaller magnitude than children born after IVF (Hvidtjorn *et al.*, 2010). Our data regarding OI were too limited to allow us to draw any meaningful conclusion.

About one-third of all Danish women who were pregnant during the study period participated in the cohort (~60% of those invited). Selection bias is, however, unlikely, since we recruited mothers before they gave birth, and we had no loss to follow up except for the few children who died or left the country.

The information on time to pregnancy and infertility treatment was collected during the first or second trimester of pregnancy. Although time to pregnancy reported by women is subject to some degree of recall bias (Cooney *et al.*, 2009), it is likely that short-term recall is valid, and women in our study were only required to report time to pregnancy approximately 4 months into their pregnancy. The self-reported information on IVF or ICSI was validated using the national IVF register, which is believed to be complete, and misclassification of IVF or ICSI is not likely to be a problem in this study. The

self-reported information on OI with or without IUI was validated using the Danish Drug Prescription Register. Hormones used in OI can be prescribed for the next three cycles, and misclassification may occur, but it is likely to be minor and of a non-differential nature. There was a high agreement between the women's report and the registers (Hvidtjorn *et al.*, 2009a). In case of disagreement, we classified pregnancies according to the registers.

We identified CP cases through the CP diagnoses recorded in the Danish Cerebral Palsy Register. A validation study reported a completeness of 85% for CP cases born between 1979 and 1982 (Topp *et al.*, 1997), and the National Patient Register has since been used as a supplementary source, resulting in more complete registration of cases. On the other hand, up to 50% of CP diagnoses recorded in the National Patient Register were not CP cases according to the criteria for inclusion in the CP register. A CP prevalence of 1.8 per 1000 children is marginally less than 2.0–2.5 per 1000 children in the populations [Surveillance of Cerebral Palsy in Europe (SCPE, 2002a)], probably because the children in the cohort are slightly healthier than the general population (e.g. they had a lower rate of preterm birth), as shown in a validity study (Nohr *et al.*, 2006). Only two studies used CP cases recorded in the CP register as outcome (Reid *et al.*, 2010; Hvidtjorn *et al.*, 2010), and in one of the studies, not all the children had reached 4 years of age (Reid *et al.*, 2010).

Our results do not suggest an association between the underlying subfertility and CP, at least for periods of up to 12 months, whereas IVF or ICSI treatment confers a risk of CP.

## Authors' roles

All authors contributed to the conception and design, interpretation of data and revision of the manuscript. D.H. did the data analysis and J.L.Z. drafted the manuscript.

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# Risk of borderline and invasive ovarian tumours after ovarian stimulation for *in vitro* fertilization in a large Dutch cohort

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**BACKGROUND:** Long-term effects of ovarian stimulation for IVF on the risk of ovarian malignancies are unknown.

**METHODS:** We identified a nationwide historic cohort of 19 146 women who received IVF treatment in the Netherlands between 1983 and 1995, and a comparison group of 6006 subfertile women not treated with IVF. In 1997–1999, data on reproductive risk factors were obtained from 65% of women and data on subfertility (treatment) were obtained from the medical records. The incidence of ovarian malignancies (including borderline ovarian tumours) through 2007 was assessed through linkage with disease registries. The risk of ovarian malignancies in the IVF group was compared with risks in the general population and the subfertile comparison group.

**RESULTS:** After a median follow-up of 14.7 years, the risk of borderline ovarian tumours was increased in the IVF group compared with the general population [standardized incidence ratio (SIR) = 1.76; 95% confidence interval (CI) = 1.16–2.56]. The overall SIR for invasive ovarian cancer was not significantly elevated, but increased with longer follow-up after first IVF ( $P = 0.02$ ); the SIR was 3.54 (95% CI = 1.62–6.72) after 15 years. The risks of borderline ovarian tumours and of all ovarian malignancies combined in the IVF group were significantly increased compared with risks in the subfertile comparison group (hazard ratios = 4.23; 95% CI = 1.25–14.33 and 2.14; 95% CI = 1.07–4.25, respectively, adjusted for age, parity and subfertility cause).

**CONCLUSIONS:** Ovarian stimulation for IVF may increase the risk of ovarian malignancies, especially borderline ovarian tumours. More large cohort studies are needed to confirm these findings and to examine the effect of IVF treatment characteristics.

**Key words:** ovarian stimulation / ovarian malignancies / fertility drugs / infertility / *in vitro* fertilization

## Introduction

Currently, 1.2–2.3% of children born in the Western world are conceived by assisted reproductive technologies (Kremer *et al.*, 2008; Wright *et al.*, 2008). In the Netherlands, it has been estimated that the number of treatment cycles increased by 40% from 1996 till 2005 (Kremer *et al.*, 2008). Fertility drugs (FDs) used in IVF treatment temporarily raise serum levels of exogenous gonadotrophins and gonadal hormones, and consequently increase the chances of multiple folliculogenesis and ovulations. The long-term effects of ovarian stimulation are unknown. In view of the assumed role of ‘incessant ovulation’ (Fathalla, 1972) and increased gonadotrophin levels in ovarian cancer pathogenesis (Cramer and Welch, 1983; Risch, 1998; Vlahos *et al.*, 2010) concerns have been raised that ovarian stimulation and multiple ovarian punctures as used in IVF may increase the risk of ovarian malignancies (Fishel and Jackson, 1989). Invasive ovarian cancer accounts for 6% of female cancer deaths in the USA (Jemal *et al.*, 2008).

Over the past decades, several studies reported a significant increase of ovarian cancer risk after FD use (Whittemore *et al.*, 1992; Rossing *et al.*, 1994; Brinton *et al.*, 2005; Sanner *et al.*, 2009; Källén *et al.*, 2011), but others did not observe such an elevated risk (Franceschi *et al.*, 1994; Bristow and Karlan, 1996; Mosgaard *et al.*, 1997; Modan *et al.*, 1998; Venn *et al.*, 1999; Parazzini *et al.*, 2001; Dor *et al.*, 2002; Doyle *et al.*, 2002; Ness *et al.*, 2002; Rossing *et al.*, 2004; Dos Santos Silva *et al.*, 2009; Jensen *et al.*, 2009), or reported non-significant risk increases for subgroups (Shushan *et al.*, 1996; Ness *et al.*, 2002; Brinton *et al.*, 2004). Some studies noted an elevated risk of borderline ovarian tumours following the use of FDs (Harris *et al.*, 1992; Rossing *et al.*, 1994; Shushan *et al.*, 1996; Parazzini *et al.*, 1998; Ness *et al.*, 2002; Sanner *et al.*, 2009). Borderline ovarian tumours are low-grade ovarian malignancies with far less aggressive behaviour than invasive ovarian cancer (Bell, 2005; Hart, 2005).

Short follow-up, low statistical power and lack of control for important confounders, such as cause of subfertility and parity, have limited the conclusions from previous studies. We report here on a large nationwide cohort study in the Netherlands (the OMEGA study) that was designed to examine long-term risk of ovarian malignancies (both invasive ovarian cancer and borderline ovarian tumours) after ovarian stimulation for IVF. A unique feature of our study is that data on reproductive factors were obtained from the participating women, whereas detailed information on subfertility cause and treatment was abstracted from the medical files.

## Patients and Methods

### Study population

In 1995–1996, we identified a nationwide historical cohort of 19 861 subfertile women who received at least one IVF cycle with ovarian stimulation between 1983 and 1995 in 1 of the 12 IVF hospitals with legal permission to provide IVF treatment in the Netherlands. Since the registration of IVF treatment was obligatory by law, all IVF clinics in the Netherlands could provide a minimal data set with names, birth dates and addresses of eligible women. The institutional ethics committees of all IVF clinics approved the study procedures, which have been described previously (Klip *et al.*, 2001; Klip, 2002; de Boer *et al.*, 2003).

To obtain a large enough comparison group of subfertile women not treated with IVF, we identified women who were diagnosed with fertility problems shortly before IVF became a routine procedure for subfertile patients. The non-IVF comparison group consisted of 6604 women whose subfertility was diagnosed in the four participating clinics that had a computerized registry of all subfertile women evaluated during 1980–1995. We attempted to frequency match the non-IVF comparison group according to the distribution of subfertility diagnoses in the IVF group. Most women in the non-IVF group registered for their first consultation in the 1980s (before IVF became a routine procedure) and underwent tubal surgery and/or hormonal treatments. The majority of those who registered after 1990 withdrew from the waiting list for IVF because they pursued other treatment options, reached the age of 40 years (the upper age limit for IVF at the time), became pregnant or decided to refrain from IVF for various reasons, such as divorce. When the non-IVF group was compared with the IVF group, it turned out that 911 women selected into the non-IVF comparison group subsequently received IVF. These women had subfertility treatments other than IVF in one centre and subsequently received IVF in a second centre. In the description of the cohort, these women are included in the IVF group (Table 1) (see also section ‘Statistical analysis’).

Based on names, birth dates and addresses at the time of subfertility treatment all cohort members were traced. Given that the subjects’ last visit to the fertility clinic could date back to 1980, extensive tracing techniques were required to obtain current addresses of all women (Klip *et al.*, 2001; Klip, 2002; de Boer *et al.*, 2003), using the municipal population offices that fully cover the Netherlands. From the initial 26 465 women, 4.2% was not approached (the OMEGA cohort study, Fig. 1).

### Risk factor questionnaire

Between 1997 and 1999, 25 353 women received a risk factor questionnaire, a study information letter, and a brochure. Each participant was asked written informed consent for medical record data abstraction and future linkage with disease registries. The study information letter was signed by the treating gynaecologist or, if he/she had left, the current head of the IVF department. In the study information letter as well as in the brochure, women were informed about the purpose, the design and the privacy aspects of the study. The purpose of the study was stated as follows: ‘to examine whether women who underwent an IVF treatment more frequently report gynaecological health problems compared with women who did not have an IVF treatment’. After 4–6 weeks, non-responders were sent a reminder. Non-responders to the second letter were approached by telephone. The 23 page questionnaire ascertained information on the women’s reproductive histories, subfertility treatment, use of exogenous hormones, lifestyle factors and family history of cancer.

A total of 16 343 women returned the questionnaire (response rate 65.2%). The response rate was substantially lower in the non-IVF group (48.7%) than in the IVF group (71.1%).

### Medical records

Trained abstractors collected information on cause of subfertility and all fertility treatments. Cause of subfertility was classified as tubal, male factor, endometriosis, ovarian disorders, cervical factor, uterine abnormalities or unexplained. Multiple causes of subfertility were registered if applicable.

For each IVF and insemination cycle, we recorded date, dosage and type of FDs used in each phase of the menstrual cycle (hMG, FSH, clomiphene, hCG, GnRH and progesterone), number of oocytes collected and outcome. For FDs used prior to inseminations/IVF, we also coded date, dosage and type of FDs used per cycle. We made special attempts to collect information on subfertility treatments provided outside the participating IVF

**Table 1** Population characteristics of the OMEGA cohort by exposure status.

	IVF group (n = 19 146)		Non-IVF group (n = 6006)		Total (n = 25 152)	
	n	%	n	%	n	%
Year of birth						
≤1953	2527	13.2	1711	28.5	4238	16.8
1954–1957	4991	26.1	1440	24.0	6431	25.6
1958–1960	5995	31.3	1506	25.1	7501	29.8
≥1961	5633	29.4	1349	22.5	6982	27.8
Age at first IVF treatment or visit (years)						
≤26	1425	7.4	1159	19.3	2584	10.3
27–29	3015	15.7	1233	20.5	4248	16.9
30–32	4929	25.7	1339	22.3	6268	24.9
33–35	4711	24.6	1152	19.2	5863	23.3
≥36	5066	26.5	1123	18.7	6189	24.6
Subfertility diagnosis <sup>a,b</sup>						
Tubal	6025	31.5	1938	32.3	7963	31.7
Endometriosis	1970	10.3	349	5.8	2319	9.2
Male factor	5492	28.7	809	13.5	6301	25.1
Hormonal factor <sup>c</sup>	1287	6.7	409	6.8	1696	6.7
Unexplained	3412	17.8	537	8.9	3949	15.7
Other factors	912	4.8	360	6.0	1272	5.1
Missing	3309	17.3	2388	39.8	5697	22.7
Number of IVF treatments <sup>b</sup>						
1–2 cycles	6304	32.9				
3–4 cycles	6271	32.8				
5 or more cycles	3352	17.5				
Missing	3219	16.8				
Time since first treatment or visit (years)						
≤5 years	493	2.6	31	0.5	524	2.1
5–9 years	689	3.6	147	2.4	836	3.3
10–14 years	10 343	54.0	1526	25.4	11 869	47.2
≥15 years	7621	39.8	4302	71.6	11 923	47.4
Median years of follow-up	14.3		16.4			

<sup>a</sup>Women could have more than one cause of subfertility, except for unexplained and missing, which were unique classifications.

<sup>b</sup>Information based on medical records; for women without medical record data, information was added from health questionnaire survey.

<sup>c</sup>Included ovulation disorders, polycystic ovary syndrome and premature menopause.

clinics, by screening intake forms and letters from other treating physicians. Due to limited funding, we could only complete medical record abstraction for 9 out of 12 centres, i.e. 13 807 women (76% of women in the IVF group) (Klip et al., 2001; Klip, 2002; de Boer et al., 2003).

## Incidence of ovarian malignancies

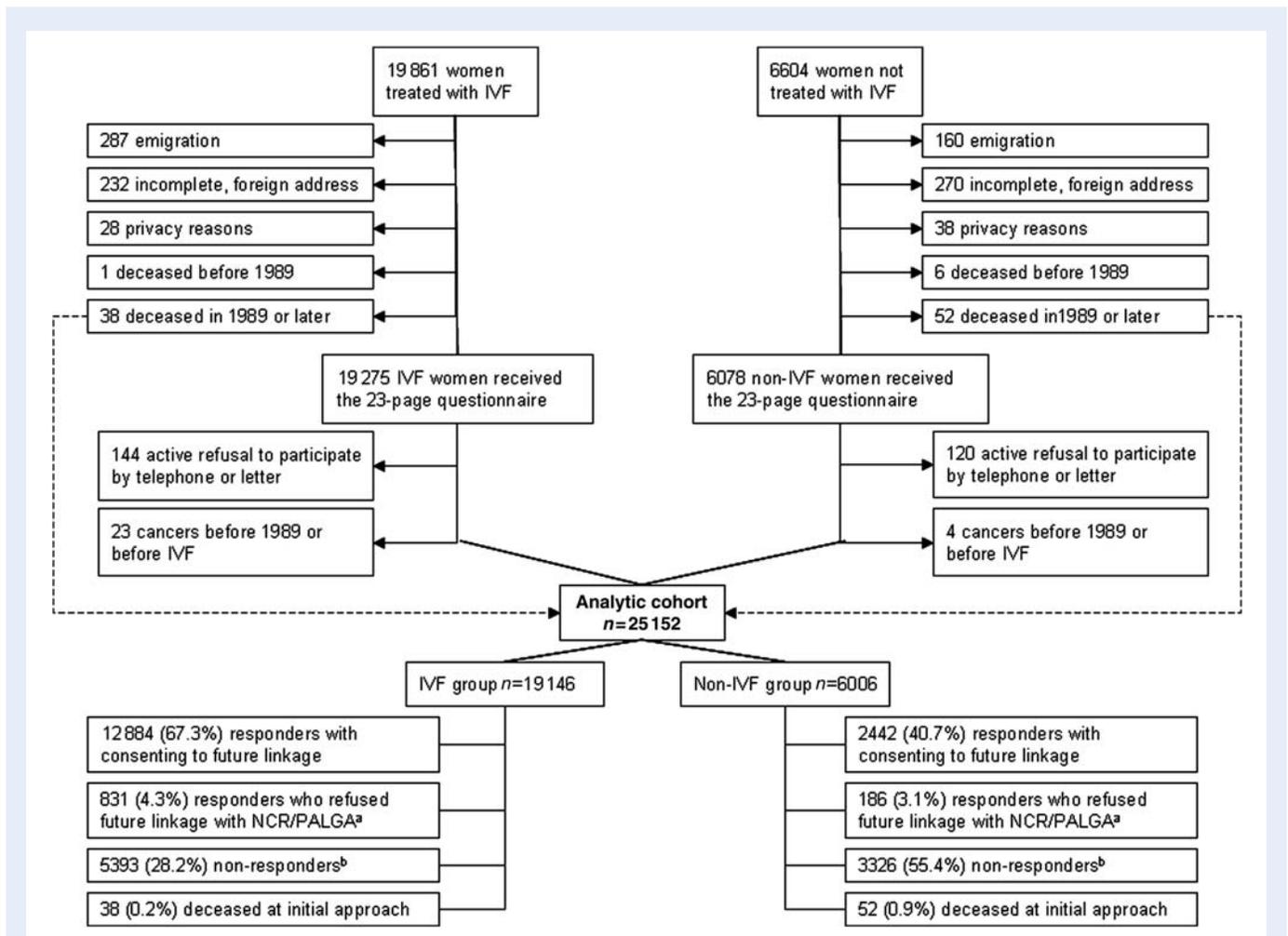
Cancer incidence in the period 1989–2003 was ascertained through linkage with the population-based Netherlands Cancer Registry (NCR)

(International Agency for Research on Cancer, 2003), and incidence of ovarian malignancies (including borderline ovarian tumours) through June 2007 was ascertained through linkage with the Dutch nationwide network and registry of histo- and cytopathology (PALGA). PALGA contains records of all histological diagnoses made in the Netherlands, with computerized data submission by all pathology laboratories, and nationwide coverage since 1989 (Casparie et al., 2007). We linked with PALGA since the NCR had incomplete data on borderline ovarian tumours; in addition PALGA case ascertainment is complete till 2 weeks prior to linkage, while the NCR lags a few years behind. We used a record linkage protocol developed previously (van den Brandt et al., 1990), which was based on the first four characters of the family name, gender and date of birth. All positive matches were checked for administrative twins by place of birth, postal code at cancer diagnosis and first initial. The NCR and PALGA granted us permission to not only link responders who gave permission, but also non-responders and deceased women, under additional privacy regulations. Only women who explicitly refused future linkage with disease registries (n = 1017; 4.0% of all women) were excluded from linkage. For each ovarian malignancy, we received information on date of diagnosis and morphology. Vital status as of June 2007 was obtained by linkage with the Central Bureau for Genealogy, which keeps computerized records of all deceased persons in the Netherlands since 1994.

## Statistical analysis

The analytic study cohort consisted of 25 152 women; 19 146 women in the IVF group and 6006 women in the non-IVF group (Fig. 1). Because the NCR and PALGA did not fully cover the Netherlands before 1989, the observation time for each participant started on 1 January 1989 or the date of first IVF treatment (IVF group), or clinic visit for subfertility evaluation (non-IVF group), whichever came last. Person-years of observation were calculated to the date PALGA follow-up ended (June 2007), date of ovarian cancer diagnosis or date of death, whichever came first. Women selected into the non-IVF comparison group who subsequently received IVF contributed person-time to the non-IVF group until the date of first IVF treatment, and switched to the IVF group after this date, according to standard cohort methodology regarding time-dependant allocation of person-years in case of changing exposure (Breslow and Day, 1987). Women diagnosed with ovarian cancer before entering the cohort (n = 14) or before 1989 (n = 13), were excluded from the analysis.

First, we compared ovarian cancer incidence in the IVF group and non-IVF group with incidence in the general population. We determined the standardized incidence ratio (SIR) as the ratio of the observed (O) and expected (E) number of cancers in the cohort. Expected numbers were based on age- and calendar period-specific reference rates for invasive ovarian cancer and borderline ovarian tumours from the NCR and PALGA, respectively (International Agency for Research on Cancer, 2003). Incidence rates for borderline ovarian tumours were calculated by the authors (T.M.M. and F.E.v L.), based on annual numbers of borderline ovarian tumour diagnoses obtained from PALGA. In all analyses, the subfertility cause(s) and treatments were preferably based on the medical records, and only derived from the woman's questionnaire if the records had not been abstracted. Information on reproductive factors was derived from the women's questionnaires, since these variables could change after IVF treatment. For non-responding women information from hospital databases was added when available. Previous FD use was defined as a combined variable relating to FD use during inseminations and FD use prior to inseminations/IVF, and was based on information from the medical records combined with the risk factor questionnaire.



**Figure 1** Identification of the OMEGA study cohort. <sup>a</sup>Women in this category contributed person time till date of questionnaire completion. <sup>b</sup>Including women who returned an empty questionnaire ( $n = 66$ ) and questionnaires that were returned to sender ( $n = 656$ ).

Cox proportional hazards models were used to compare cancer risk between the IVF group and the non-IVF group, adjusting for age and potential confounders such as parity and subfertility cause. Forward stepwise confounder selection, in which the effect of adding one confounder at a time was evaluated, was based on a  $> 10\%$  change in the risk estimate of the exposure variable of interest, irrespective of significance values.

In all analyses missing values were included as a separate category. Data were analysed with SPSS software (SPSS Inc., Chicago, IL, USA).

## Results

### Population characteristics

Characteristics of 19 146 IVF-treated women and 6006 women not treated with IVF are presented in Table 1. Women in the non-IVF group had a slightly longer median duration of follow-up than women in the IVF group (16.4 versus 14.3 years) and they were also older at the end of follow-up (mean age 49.4 versus 47.5 years). These differences reflect the initial inclusion criteria for the IVF and the non-IVF groups, with an over-representation of women in the non-IVF group seeking subfertility treatment in the years

before IVF treatment became a routine procedure. Cause of subfertility was related to tubal problems in 32% of women, 25% had male-factor subfertility, 9% endometriosis, 7% hormonal subfertility, 16% unexplained subfertility and 23% was missing (percentage add up to  $> 100\%$  due to multiple causes of subfertility). A total of 42% of the cohort was nulliparous at questionnaire completion. In the IVF group, 40% of women had one to two stimulated IVF cycles, 39% had three to four cycles and 21% received five or more cycles. IVF stimulation regimens used in the cohort have been described in detail previously (de Boer *et al.*, 2004). In brief, clomiphene/hMG or FSH/hMG stimulation protocols were used till 1988–1989, whereas stimulation with GnRH agonists became common after 1990 (from 20% in 1986 to about 90% after 1990). Furthermore, from 1984 to 1994, the number of ampoules of gonadotrophins strongly increased, as did the number of retrieved oocytes at the first IVF cycle (from 5.4 in 1986 to 10.7 in 1994) (de Boer *et al.*, 2004).

### Comparisons with external reference rates

After a median follow-up time of 14.7 years, 77 ovarian malignancies were observed in the full cohort [SIR = 1.43; 95% confidence interval

(CI) = 1.12–1.78]; 42 invasive ovarian cancers and 35 borderline ovarian tumours (Table II). Sixty-one ovarian malignancies were observed in the IVF group (SIR = 1.59; 95% CI = 1.21–2.04) and 16 in the non-IVF group (SIR = 1.02; 95% CI = 0.59–1.66). Compared with the general population rates, we observed a significantly increased risk for borderline ovarian tumours in the IVF group (SIR = 1.93; 95% CI = 1.31–2.73) and no increase in the non-IVF group (SIR = 0.67; 95% CI = 0.18–1.71). The SIRs for invasive ovarian cancer were not significantly raised in either IVF-treated women (1.35; 95% CI = 0.91–1.92) or non-IVF women (1.24; 95% CI = 0.64–2.17). The morphologies of the invasive ovarian cancers were serous (60%), mucinous (7%), clear-cell (7%), endometrioid (21%) and other (5%). Of the borderline ovarian tumours, 63% were serous and 37% were mucinous. Serous borderline ovarian tumours and invasive ovarian cancers occurred more frequently in the IVF group than in the non-IVF group ( $P = 0.04$ ).

The SIRs in both the IVF group and non-IVF group were strongly increased in the first year of follow-up (3- to 18-fold), possibly related to work-up for subfertility diagnosis and treatment. When we excluded the first year of follow-up, the SIR for all ovarian malignancies was 1.49 (95% CI = 1.12–1.94) in the IVF group and 0.85 (95% CI = 0.45–1.45)

in the non-IVF group. After 15 or more years, the SIR for invasive ovarian cancer in the IVF group was 3.54 (95% CI = 1.62–6.72,  $P$  for trend = 0.02), whereas the SIR in the non-IVF group was close to unity (Table II). No clear increase with longer follow-up was seen for borderline ovarian tumours ( $P$  for trend = 0.49).

Within the IVF group, SIRs of ovarian malignancy did not increase with a greater number of IVF cycles or ampoules of gonadotrophins (Table III). The mean number of oocytes harvested per stimulated cycle and the maximum number over all treatment cycles were used as a proxy for a woman's responsiveness to ovarian stimulation; the total number of oocytes collected over all cycles was used as a proxy for the amount of damage to the ovarian epithelium. The SIRs did not appear to be associated with any of these variables. FD use prior to IVF treatment was not associated with an increased SIR for all ovarian malignancies combined; for invasive ovarian cancer the SIR was non-significantly increased (SIR = 1.69; 95% CI = 0.95–2.79), while for borderline ovarian tumours the SIR was increased for women who did not use FDs prior to IVF treatment (SIR = 2.93; 95% CI = 1.71–4.69). These observations must be interpreted with caution since information on previous FD use was missing for 27% of women.

**Table II** Incidence of ovarian malignancies by years of follow up and exposure status.

Follow-up	IVF group				Non-IVF group				Total			
	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All ovarian malignancies												
< 1 years	6	1.52	3.94	1.44–8.57	3	0.31	9.55	1.97–27.91	9	1.84	4.90	2.24–9.30
1–4 years	9	7.52	1.20	0.55–2.27	1	1.74	0.57	0.01–3.20	10	9.27	1.08	0.52–1.98
5–9 years	16	12.41	1.29	0.74–2.09	3	3.58	0.84	0.17–2.45	19	15.99	1.19	0.72–1.86
10–14 years	18	13.22	1.36	0.81–2.15	4	4.63	0.86	0.23–2.21	22	17.85	1.23	0.77–1.87
≥ 15 years	12	3.73	3.22	1.66–5.62	5	5.36	0.93	0.30–2.18	17	9.08	1.87	1.09–3.00
All intervals	61	38.41	1.59	1.21–2.04	16	15.63	1.02	0.59–1.66	77	54.03	1.43	1.12–1.78
All intervals excl. first year	55	36.88	1.49	1.12–1.94	13	15.31	0.85	0.45–1.45	68	52.20	1.30	1.01–1.65
Invasive ovarian cancer												
< 1 years	2	0.78	2.57	0.31–9.26	3	0.16	18.35	3.79–53.60	5	0.94	5.30	1.72–12.37
1–4 years	5	3.94	1.27	0.41–2.96	1	0.93	1.07	0.03–5.97	6	4.88	1.23	0.45–2.68
5–9 years	4	6.90	0.58	0.16–1.48	2	2.03	0.99	0.12–3.56	6	8.93	0.67	0.25–1.46
10–14 years	10	8.13	1.23	0.59–2.26	2	2.85	0.70	0.09–2.54	12	10.98	1.09	0.56–1.91
≥ 15 years	9	2.54	3.54	1.62–6.72	4	3.68	1.09	0.30–2.79	13	6.22	2.09	1.11–3.57
All intervals	30	22.30	1.35	0.91–1.92	12	9.65	1.24	0.64–2.17	42	31.95	1.31	0.95–1.78
All intervals excl. first year	28	21.52	1.30	0.86–1.88	9	9.48	0.95	0.43–1.80	37	31.01	1.19	0.84–1.64
Borderline ovarian tumours												
< 1 years	4	0.74	5.38	1.46–13.77	0	0.15	0	0.00–24.59	4	0.89	4.47	1.21–11.45
1–4 years	4	3.58	1.12	0.03–2.86	0	0.81	0	0.00–4.55	4	4.39	0.91	0.25–2.33
5–9 years	12	5.51	2.18	1.13–3.81	1	1.55	0.64	0.02–3.59	13	7.06	1.84	0.98–3.15
10–14 years	8	5.09	1.57	0.68–3.10	2	1.79	1.12	0.14–4.04	10	6.87	1.45	0.70–2.68
≥ 15 years	3	1.18	2.53	0.52–7.40	1	1.68	0.60	0.02–3.32	4	2.86	1.40	0.38–3.58
All intervals	31	16.10	1.93	1.31–2.73	4	5.98	0.67	0.18–1.71	35	22.08	1.59	1.10–2.20
All intervals excl. first year	27	15.36	1.76	1.16–2.56	4	5.83	0.69	0.19–1.76	31	21.19	1.46	0.99–2.08

Obs, observed; Exp, expected; SIR, standardized incidence ratio; CI, confidence interval.

**Table III** Incidence of ovarian malignancies in IVF-treated women, according to IVF treatment characteristics, subfertility and parity.

IVF group	Person years	All ovarian malignancies				Invasive ovarian cancer				Borderline ovarian tumours			
		Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
Total number of IVF cycles <sup>a,b</sup>													
1–2 cycle(s)	82 599	21	13.99	1.50	0.93–2.29	11	8.12	1.35	0.68–2.42	10	5.87	1.70	0.97–3.74
3–4 cycles	84 025	22	14.46	1.52	0.95–2.30	10	8.43	1.19	0.57–2.18	12	6.04	1.99	1.22–4.14
≥5 cycles	47 661	12	8.43	1.42	0.74–2.49	7	4.97	1.41	0.57–2.90	5	3.45	1.45	0.47–3.38
Subfertility diagnosis <sup>b,c,d</sup>													
Tubal	84 822	35	14.96	2.34	1.63–3.25	15	8.90	1.69	0.94–2.78	20	6.06	3.30	2.02–5.10
Endometriosis	26 853	14	4.59	3.05	1.67–5.12	10	2.68	3.73	1.79–6.86	4	1.90	2.10	0.57–5.38
Male factor	70 793	16	11.53	1.39	0.79–2.25	11	6.58	1.67	0.83–2.99	5	4.95	1.01	0.33–2.36
Hormonal factor <sup>e</sup>	16 873	3	2.64	1.14	0.23–3.32	2	1.49	1.34	0.16–4.84	1	1.15	0.87	0.02–4.86
Unexplained	45 846	5	7.97	0.63	0.20–1.46	3	4.67	0.64	0.13–1.88	2	3.30	0.61	0.07–2.19
Other factors	12 005	4	2.02	1.98	0.54–5.07	2	1.17	1.71	0.21–6.19	2	0.85	2.35	0.28–8.48
Previous FD use <sup>c,f</sup>													
No	95 782	26	14.15	1.84	1.20–2.69	9	8.35	1.08	0.49–2.05	17	5.8	2.93	1.71–4.69
Yes	109 149	20	15.41	1.30	0.79–2.01	15	8.88	1.69	0.95–2.79	5	6.52	0.77	0.25–1.79
Missing	49 297	9	7.33	1.23	0.56–2.33	4	4.29	0.93	0.25–2.38	5	3.03	1.65	0.53–3.85
Parity <sup>a</sup>													
Nulliparous	86 058	24	12.82	1.87	1.20–2.79	9	7.58	1.19	0.54–2.25	15	5.24	2.86	1.60–4.72
Parous	123 242	21	17.38	1.21	0.75–1.85	14	10.03	1.40	0.76–2.34	7	7.35	0.95	0.38–1.96
Missing	44 928	10	6.68	1.50	0.72–2.75	5	3.91	1.28	0.41–2.98	5	2.77	1.81	0.59–4.22
Total no. of ampoules hMG/FSH <sup>g</sup>													
1–40 ampoules	48 033	10	6.85	1.46	0.70–2.69	5	3.99	1.25	0.41–2.93	5	2.86	1.75	0.57–4.08
41–80 ampoules	49 345	11	7.08	1.55	0.78–2.78	5	4.12	1.21	0.39–2.83	6	2.96	2.03	0.74–4.42
≥81 ampoules	57 749	14	8.60	1.63	0.89–2.73	8	5.06	1.58	0.68–3.11	6	3.54	1.69	0.62–3.69
Missing	99 101	20	14.35	1.39	0.85–2.15	10	8.35	1.20	0.57–2.20	10	6.00	1.67	0.80–3.07
Total no. of oocytes <sup>h</sup>													
0–19 oocytes	89 929	20	13.84	1.45	0.88–2.23	10	8.27	1.21	0.58–2.22	10	5.57	1.80	0.86–3.30
≥20 oocytes	79 186	16	10.63	1.50	0.86–2.44	7	6.01	1.16	0.47–2.40	9	4.62	1.95	0.89–3.70
Missing	85 113	19	12.42	1.53	0.92–2.39	11	7.24	1.52	0.76–2.72	8	5.18	1.55	0.67–3.05
Mean no. of oocytes <sup>h</sup>													
0–3 oocytes	21 468	6	3.81	1.57	0.58–3.43	3	2.40	1.25	0.26–3.65	3	1.41	2.12	0.44–6.20
4–6 oocytes	46 899	14	7.31	1.91	1.05–3.21	7	4.39	1.60	0.64–3.29	7	2.92	2.39	0.96–4.93
≥7 oocytes	100 747	15	13.35	1.12	0.63–1.85	6	7.50	0.80	0.29–1.74	9	5.85	1.54	0.70–2.92
Missing	85 113	20	12.41	1.61	0.98–2.49	12	7.24	1.66	0.86–2.90	8	5.17	1.55	0.67–3.05
Maximum no. of oocytes <sup>h</sup>													
0–5 oocytes	33 819	9	5.75	1.56	0.72–2.97	5	3.57	1.40	0.45–3.27	4	2.19	1.83	0.50–4.69
6–10 oocytes	58 581	13	8.75	1.49	0.79–2.54	5	5.16	0.97	0.31–2.26	8	3.59	2.23	0.96–4.39
≥11 oocytes	76 714	13	9.97	1.30	0.69–2.23	6	5.56	1.08	0.40–2.35	7	4.41	1.59	0.64–3.27
Missing	85 113	20	12.41	1.61	0.98–2.49	12	7.24	1.66	0.86–2.90	8	5.17	1.55	0.67–3.05

Obs, observed; Exp, expected; SIR, standardized incidence ratio; CI, confidence interval.

<sup>a</sup>Information based on health questionnaire survey; for non-responding women information was added from the medical records.

<sup>b</sup>Missing values of this variable were retrospectively completed for all cases; among non-cases with missing values, we distributed person time according to the distribution of person-years over categories of this variable.

<sup>c</sup>Information based on medical records; for women without medical record data, information was added from health questionnaire survey.

<sup>d</sup>Women may contribute person-years to more than one type of subfertility except for the categories unexplained and missing, which were unique classifications.

<sup>e</sup>Hormonal factors included ovulation disorders, polycystic ovary syndrome and premature menopause.

<sup>f</sup>Previous FD use was defined as a combined variable relating to FD use during inseminations and FD use prior to inseminations/IVF.

<sup>g</sup>Information based solely on medical records; no data abstraction could be done for 24% of the cohort that did give informed consent to do so.

**Table IV** Adjusted HRs for cancer risk in IVF group versus non-IVF group.

Cancer site	Overall		≥ 1 year follow-up		≥ 10 years follow-up	
	HR	95% CI	HR	95% CI	HR	95% CI
All ovarian malignancies <sup>a</sup>	2.05	1.10–3.82	2.14	1.07–4.25	2.08	0.86–5.00
Invasive ovarian cancer <sup>b</sup>	1.14	0.54–2.41	1.51	0.65–3.54	2.26	0.78–6.55
Borderline ovarian tumours <sup>c</sup>	6.38	2.05–19.84	4.23	1.25–14.33	2.26	0.46–11.05

HR, hazard ratio; CI, confidence interval.

<sup>a</sup>Adjusted for age at end of follow-up, endometriosis, tubal problems.

<sup>b</sup>Adjusted for age at end of follow-up, endometriosis.

<sup>c</sup>Adjusted for age at end of follow-up, tubal problems, parity.

Endometriosis was associated with significantly increased risk of invasive ovarian cancer, whereas tubal problems significantly increased the SIR for borderline ovarian tumours.

### Comparisons within the cohort

Direct comparison of the IVF group with the non-IVF group (Table IV) yielded an adjusted hazard ratio (HR) for all ovarian malignancies of 2.14 (95% CI = 1.07–4.25), excluding the first year of follow-up. The adjusted HRs for invasive ovarian cancer and borderline ovarian tumours were 1.51 (95% CI = 0.65–3.54) and 4.23 (95% CI = 1.25–14.33), respectively. No trends emerged with number of IVF cycles or other IVF treatment characteristics, but numbers in subcategories were small. Clomiphene use prior to IVF was not associated with increased risk of ovarian malignancies (HRs for all malignancies, invasive ovarian cancer and borderline ovarian tumours were 0.89 (95% CI = 0.45–1.77), 1.22 (95% CI = 0.50–2.99) and 0.62 (95% CI = 0.21–1.83), respectively). Finally, we compared the risk of all ovarian malignancies between the IVF group and women in the non-IVF group who never used FDs (HR = 1.83; 95% CI = 0.70–4.82, based on five cases in 2115 unexposed women).

## Discussion

This large nationwide cohort study with a median follow-up of 15 years shows that women treated with ovarian stimulation for IVF have a 2-fold increased risk of ovarian malignancies compared with subfertile women not treated with IVF. The excess risk was mostly due to borderline ovarian tumours, but 15 or more years after IVF treatment we also observed a SIR of 3.5 for invasive ovarian cancer.

Surprisingly, we observed that a high proportion (46%) of all ovarian malignancies in the IVF group concerned borderline ovarian tumours, whereas in the general population (below the age of 50 years) borderline ovarian tumours account only for 15–30% (Hart, 2005) of epithelial ovarian malignancies. So far only few studies examined FD use in relation to risk of borderline ovarian tumours, related to the fact that most population-based cancer registries do not record borderline ovarian tumours. Our cohort study is the first one examining

the risk of borderline ovarian tumours following IVF treatment. Strikingly, the few case–control studies that examined the risk of borderline ovarian tumours after FD use found 2- to 4-fold increased risks (Harris et al., 1992; Rossing et al., 1994; Shushan et al., 1996; Parazzini et al., 1998; Ness et al., 2002), though based on small numbers. In a case–cohort study (Rossing et al., 1994) reporting an 11-fold risk increase of ovarian malignancies after 12 or more cycles of clomiphene, 5 of the 11 ovarian tumours were borderline ovarian tumours. Although screening for ovarian tumours in IVF-treated women has never been recommended in the Netherlands, we considered whether the increased risk of borderline ovarian tumours in the IVF group might be due to increased medical surveillance. We sent a questionnaire about diagnostic procedures to the gynaecologists of all case subjects with a borderline ovarian tumour who had given permission to approach their physician ( $n = 18$ ). We received information for 14 subjects; in all cases, the diagnosis was made subsequent to complaints for which the woman visited her gynaecologist, rendering surveillance bias an unlikely explanation of our findings. Remarkably, we observed a high proportion of serous borderline ovarian tumours (63%), which was also seen in one case–control study (Ness et al., 2002). Mucinous borderline ovarian tumours are more frequent in the general population (Verbruggen et al., 2009).

Risk of borderline ovarian tumours was particularly strongly elevated in the first year after IVF, which is in line with several case reports of borderline ovarian tumours developing during or shortly after ovarian stimulation treatments (Atlas and Menczer, 1982; Goldberg et al., 1992; Nijman et al., 1992), providing support for speculations that ovarian stimulation may induce growth in existing highly differentiated tumours (Brinton et al., 2005). We excluded ovarian tumours occurring in the first year after IVF, because of concern that their diagnosis might be related to diagnostic and treatment procedures for infertility. The early increase in risk was followed by a SIR close to unity in the 1–4 year follow-up interval; subsequently, risk of borderline ovarian tumours remained elevated up to more than 15 years after first IVF treatment. Hence, our data suggest that IVF treatment may be causally related to a prolonged increase of the risk of highly differentiated tumours. The natural history of borderline ovarian tumours is unclear and it is unknown which part of borderline ovarian tumours, if undetected, would develop into invasive ovarian cancer (Singer et al., 2003; Sherman et al., 2004; Shih and Kurman, 2004).

A concerning finding of our study is the increased SIR of invasive ovarian cancer in the IVF group after more than 15 years of follow-up, which was not observed in the non-IVF group. We cannot compare this result with findings from others since our study is the first reporting on cancer risk more than 10 years after IVF treatment. However, Brinton et al. (2004) followed a large cohort of 12 193 women treated for infertility prior to the IVF era. After 15 or more years of follow-up they reported non-significantly elevated rate ratios of ovarian cancer, 1.48 (95% CI = 0.7–3.2) for clomiphene and 2.46 (95% CI = 0.7–8.3) for gonadotrophins (when compared with never use of these drugs). Sanner et al. (2009) reported on a Swedish cohort treated for infertility in the 1960s–1970s, with a median follow-up of 33 years. Gonadotrophins were associated with increased risk of invasive ovarian cancer (relative risk = 5.28, 95% CI = 1.70–16.47) but clomiphene was not (when compared with never use of these drugs) (Sanner et al., 2009). Ovulation stimulating drugs such as clomiphene were introduced in the late 1960s and IVF treatment with gonadotrophins,

resulting in much stronger ovarian stimulation, did not become widely available until the late 1980s. Consequently, women exposed to clomiphene have just recently reached the age range at which ovarian cancer frequently occurs (>70 years), while the oldest IVF-treated women have only recently reached their 50s. Since the induction period of ovarian cancer with respect to established risk factors amounts to 25 years or more (Risch, 1998), much longer follow-up is needed to fully evaluate the effects of gonadotrophins.

If ovarian stimulation were causally related to the risk of ovarian malignancy, we would expect increasing risks with greater number of IVF cycles or number of oocytes harvested. No such dose–response trends emerged. However, numbers in relevant dose categories were small, and data were missing for 17% of subjects, which reduced power for these analyses. In addition, the number of IVF cycles and number of harvested oocytes are only proxies for the number of ovarian punctures, which may have reduced the power to detect a dose–response relationship.

Case–control studies of the association between ovarian cancer risk and FD use have shown inconsistent results, with some studies reporting increased risks for subgroups (e.g. nulliparous women) (Ness *et al.*, 2002; Rossing *et al.*, 2004) and some suggesting a dose–response effect for clomiphene (Ness *et al.*, 2002; Rossing *et al.*, 2004). Treatment with hMG or FSH, as in IVF, may increase the number of ovulations to approximately six to nine times that of untreated women (Fishel and Jackson, 1989), which is a much stronger increase than the doubling of ovulations with clomiphene (Glasier, 1990; Derman and Adashi, 1994).

Nationwide cohort studies of IVF-treated women have only been reported from Australia (Venn *et al.*, 1999), Israel (Lerner-Geva *et al.*, 2003) and Sweden (Källén *et al.*, 2011). The first two cohort studies did not show increased risk of ovarian cancer in the IVF group compared with the general population (Venn *et al.*, 1999; Lerner-Geva *et al.*, 2003), while the recent Swedish study reported for parous women increased risk of ovarian cancer after IVF, compared with all other Swedish women who gave birth in the study period (HR = 2.09; 95% CI = 1.39–3.12) (Källén *et al.*, 2011). However, this study had no information on subfertility cause; therefore it is not clear whether the risk increase is attributable to IVF or subfertility. Of all cohort studies including IVF-treated women, our study includes the largest number of ovarian malignancies ( $n = 77$  versus 13, 3 and 26 cases in the cohort studies from Australia, Israel and Sweden) (Venn *et al.*, 1999; Lerner-Geva *et al.*, 2003; Källén *et al.*, 2011).

Our study design had several strengths and weaknesses. Advantages include the large size of our cohort and the long-term follow-up. Selection bias can be ruled out since we were able to link 96% of our cohort with the population-based cancer and pathology registries, enabling us to also evaluate the occurrence of borderline ovarian tumours. All ovarian malignancies were histologically confirmed. Furthermore, we collected reproductive variables after IVF directly from the participating women, whereas for the majority of women information on subfertility cause and treatment could be abstracted from the medical files. Our data also include information on FD use prior to IVF, although this was incomplete for 27% of women. A limitation of our study is, however, that the comparison group of women unexposed to IVF treatment was relatively small, and that a proportion of these women (40%), had used FDs (clomiphene) outside the IVF

setting (as did 54% of women in the IVF group), thus restricting the power for comparisons with a truly unexposed reference group. However, if multiple ovarian punctures rather than hormonal stimulation would induce ovarian malignancy, potential differences in FD use outside the IVF setting are not relevant.

Unfortunately, the response rate to the questionnaire was lower in the non-IVF group (49 versus 71% in the IVF group). Since we were allowed to link non-responders with the NCR and PALGA, differential non-response could not affect our overall risk estimates. However, the larger proportion of missing values for potential confounders (reproductive factors, cause of subfertility) among controls complicated our multivariable analyses. Adjustment for potential confounders did not materially affect our risk estimates, however.

We wondered whether the increased SIR of invasive ovarian cancer observed in the IVF group after 15 years might be due to less oral contraceptive (OC) use and/or lower parity in IVF-treated women. However, in the non-IVF group no increased SIR after long-term follow-up was seen. The proportion of long-term ( $\geq 7$  years) OC users was high in our cohort and very similar in the IVF group and the non-IVF group (39.2 and 38.1%, respectively). Dutch women start OC use early and have a late age at first birth (mean 1985–1995: 28 years (Statistics Netherlands; [www.cbs.nl](http://www.cbs.nl), 2011) and only 19.1% of the IVF group and 22.5% of the non-IVF group never used OC or used them <1 year. Consequently, OC use was not a confounder in our multivariable Cox analysis. IVF-treated women remained more often nulliparous than the non-IVF group (44 versus 35%), but adjustment for parity only affected our results for borderline ovarian tumours, not for invasive ovarian cancer.

Our study is the only IVF cohort including a comparison group of subfertile women not treated with IVF, in addition to a comparison with the general population. Such a comparison group is important since IVF-treated women differ from the general population with respect to several risk factors for ovarian malignancies, e.g. subfertility and nulliparity. We cannot exclude the possibility, however, that the severity of certain causes of subfertility in the IVF group was not the same as in the non-IVF group. Since adjustment for individual causes of subfertility only slightly affected our estimates of the risk associated with IVF (data not shown), residual confounding by severity of certain subfertility causes seems unlikely, however.

Another limitation of our study is that our results are based on IVF treatment protocols used until 1995, prior to the adoption of currently applied milder stimulation regimens.

In conclusion, our results suggest that ovarian stimulation for IVF may increase the risk of ovarian malignancies, especially borderline ovarian tumours. Knowledge about the magnitude of the risks associated with ovarian stimulation is important for women considering starting or continuing IVF treatment, as well as their treating physicians. Clearly, the outcome of weighing a wish to conceive against the potential risks associated with IVF may differ among couples considering fertility treatment. In the Netherlands the cumulative risk of ovarian malignancy (including borderline ovarian tumours) is small, i.e. 0.45% at the age of 55 years. If our results are true, we would estimate a 0.71% risk for women who underwent IVF. It should be explained to women opting for IVF treatment that a borderline ovarian tumour does not constitute a lethal disease, although it may require extensive surgery and cause substantial morbidity. Ovarian cancer, however, is a disease with a high case fatality rate, for which

effective screening methods are not available (Hermesen *et al.*, 2007). Although our findings give reason for some concern, they are still based on rather small numbers, no dose–response relationship was found and the risk increase for invasive ovarian cancer was not statistically significant in multivariable analyses. Even larger prospective cohort studies of IVF-treated women, with prolonged follow-up and a subfertile comparison group not treated with IVF, are needed to confirm or refute our findings and to conduct dose–response analyses with more power.

## Authors' roles

F.E.v L. and C.W.B. designed the OMEGA study and were principal investigators of the study. F.E.v L. also coordinated statistical analyses, contributed to interpretation of the data and drafted the paper. C.W.B. contributed to interpretation of the data and drafting of the manuscript. H.K. contributed to the design of the study, coordinated identification of the cohort and data collection, did statistical analyses and contributed to interpretation of data. T.M.M. coordinated data collection, did the statistical analyses, contributed to study design, interpretation of the data and drafting of the manuscript. A.M.G.vd S. contributed to data collection and statistical analysis. C.B.L., M.K., J.S.E.L., C.A.M.J., F.M.H., B.J.C., W.N.P., J.M.J.S., A.H.M.S., F.vd V., J.L.H.E., P.A.v D. and N.S.M. provided IVF patient data and contributed to interpretation of the data. All authors contributed to critical revisions of the draft manuscript. All authors saw and approved the final version of the report.

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## Conflict of interest

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# A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, *in vitro* fertilization and natural conception

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**BACKGROUND:** Over a million children have been born from assisted conception worldwide. Newer techniques being introduced appear less and less ‘natural’, such as intracytoplasmic sperm injection (ICSI), but there is little information on these children beyond the neonatal period. **METHODS:** 540 ICSI conceived 5-year-old children from five European countries were comprehensively assessed, along with 538 matched naturally conceived children and 437 children conceived with standard IVF. **RESULTS:** Of the 540 ICSI children examined, 63 (4.2%) had experienced a major congenital malformation. Compared with naturally conceived children, the odds of a major malformation were 2.77 (95% CI 1.41–5.46) for ICSI children and 1.80 (95% CI 0.85–3.81) for IVF children; these estimates were little affected by adjustment for socio-demographic factors. The higher rate observed in the ICSI group was due partially to an excess of malformations in the (boys’) urogenital system. In addition, ICSI and IVF children were more likely than naturally conceived children to have had a significant childhood illness, to have had a surgical operation, to require medical therapy and to be admitted to hospital. A detailed physical examination revealed no further substantial differences between the groups, however. **CONCLUSIONS:** Singleton ICSI and IVF 5-year-olds are more likely to need health care resources than naturally conceived children. Assessment of singleton ICSI and IVF children at 5 years of age was generally reassuring, however, we found that ICSI children presented with more major congenital malformations and both ICSI and IVF children were more likely to need health care resources than naturally conceived children. Ongoing monitoring of these children is therefore required.

*Key words:* birth defects/ICSI/IVF

## Introduction

Intracytoplasmic sperm injection (ICSI) was developed over 10 years ago and is now used worldwide for treating male factor infertility. However, concerns remain about the long-

term prospects for the children conceived with this technique and indeed of children conceived from ‘standard’ IVF (Hawkins *et al.*, 1999; Givens, 2000; Tournaye, 2003). Initial studies have investigated perinatal outcome, congenital anomalies and early neurodevelopmental ability (up to age 5 years) but there has been little to comprehensively assess these children’s physical well-being at later stages (Van Steirteghem *et al.*, 2002; Kurinczuk, 2003b).

We aimed to perform a detailed assessment of 5-year-old children and their families in a collaborative study, the International Collaborative Study of ICSI–Child and Family Outcomes (ICSI–CFO.) One of the primary objectives of the study was to assess whether ICSI is associated with

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significant health problems at age 5 years. Our initial report from ICSI–CFO (Barnes *et al.*, 2004) described our findings regarding family functioning and children's socio-emotional development. Here we report findings surrounding growth, morbidity and physical defects in the 540 5-year-old ICSI conceived children compared with similar numbers of naturally conceived children and an IVF conceived group.

## Materials and methods

### Study design

This was a European five-nation controlled cohort study. This involved a cross-sectional evaluation of three groups of children who were recruited at age 5 years according to their mode of conception. We recruited equivalent sized groups of ICSI, IVF and naturally conceived children (NC) according to centre size and number of ICSI cycles performed per year with targets of: 175 children per group in the UK/Belgium (UK/Be); 66 children per group in Denmark/Sweden (Dk/Sw); and 50 children per group in Greece (Gr). The study was conducted over a 24-month period, commencing November 2000.

### ICSI

In the UK and Belgium, ICSI conceived children were recruited mainly from established cohorts already assessed in their second year (Sutcliffe *et al.*, 2001; Bonduelle *et al.*, 2002b). Additional children (~10%) were recruited from major fertility clinics. In Sweden, ICSI children were recruited from complete cohorts of ICSI children, conceived after treatment at the two fertility clinics in Gothenburg. In Denmark, most ICSI children were recruited from one clinic but additional children were recruited from three other clinics. In Greece the children were recruited from several clinics.

### IVF comparison group

Due to differing national laws and quality of child health records, recruitment of comparison groups differed. Children born after IVF were recruited from participating fertility clinics in all countries via letters, which were sent to the families whose child fulfilled the matching criteria (listed below) for the study. In the UK, Belgium and Greece this involved five clinics, in Denmark one and in Sweden two clinics.

### Naturally conceived controls

Local school and nurseries were used to recruit a matched comparison group of naturally conceived children for the UK, Belgium and Greece. In Sweden, NC children were recruited from the medical birth registry selecting the children of the same age, matching for maternal age and sex and for the closest matching age. Five NC children were recruited for each ICSI child. Only children born in Western Sweden were chosen. In Denmark, a similar recruitment was performed from the participating hospital's birth registry.

### Criteria for inclusion and exclusion

We recruited ICSI and IVF conceived children between 4.5 and 5.5 years. Children were eligible if they were singleton, Caucasian, born at least 32 weeks gestation, first or second born and whose mother tongue was either English, Dutch, Danish, Swedish or Greek. Naturally conceived controls were selected according to the above criteria and were matched for age, sex, maternal education and parental socio-economic status.

### Participation rates

In the UK, 91% of the ICSI cohort assessed at age 18 months participated (Sutcliffe *et al.*, 2001), leading to the examination of 189/201 of the original ICSI cohort. Due to ethical restrictions it was not possible to ascertain the response rate in the IVF or NC groups. In Belgium, 45% of eligible children participated for ICSI and IVF groups and in the NC group 54% of those contacted responded. In Sweden, 96% of ICSI, 96% of IVF and 78% of NC children contacted participated. In Denmark, 68% of ICSI, 56% of IVF and 34% of NC contacted participated. In Greece, 25% of ICSI, 25% of IVF and 100% of NC children contacted participated. In all countries the ICSI conceived children were consecutive births.

### Study protocol

The children were assessed in a child friendly environment by trained paediatricians using an identical protocol. In the UK and Belgium two consecutive paediatricians (in each country) saw all children and in Denmark, Sweden and Greece children in each country were assessed by single paediatricians. The paediatrician was blinded for conception mode in Sweden, but not in the other countries, due to logistical settings. In all the centres, type of assisted reproductive technology (ICSI or IVF) was not revealed to the paediatrician.

A full history was taken from the parent(s) using a standard proforma. Socioeconomic status was classified according to the British system (Classification of Occupations. London: HM Stationery Office, 1970 and revised 1995) and equivalencies between the five participant countries were established for parental educational levels. The mother's health in pregnancy was recorded, including chronic maternal illnesses, maternal smoking/drinking and pregnancy complications.

Neonatal history included birthweight, gestational age, neonatal unit admission and treatments, and infant feeding details. Additional information was obtained from routine child health records for validation.

Significant childhood illnesses were recorded, including operations and periods in hospital. Illnesses described by the parent and considered significant by the paediatrician but not involving hospital admission/operation were also noted. Later these were coded by ICD10 (World Health Organization, 1992) and checked by the senior paediatrician (AGS, blind to mode of conception). Illnesses considered minor were not recorded. A general physical examination was conducted with special attention to the detection of malformations. Height and weight were measured using standard auxiological equipment. Pure tone audiometry (across standard screening frequencies down to 25dB), distance visual acuity (using Snellen charts) and stereotactic vision were assessed (Cooper *et al.*, 1979).

Congenital anomalies were classified according to the International Classification of Diseases 10th revision using Q codes (Q00–Q99). (World Health Organization, 1992). Malformations having a Q code were classified into major and minor by the geneticist (M.B., blind to mode of conception). Major malformations were defined as malformations that generally cause functional impairment or require surgical correction. (Smith, 1975). The remaining malformations were defined as minor. Minor malformations not having a Q code were also recorded according to a checklist containing 242 items (Bonduelle *et al.*, 2002a; Aase, 2004) used in previous studies.

### Statistical analysis

The study was designed to have 80% power (at a 5% significance level) to detect a 2-fold difference in congenital anomaly rates at

**Table I.** Parental socio-demographic characteristics and maternal health during pregnancy\*

	ICSI (n = 540)	IVF (n = 437)	NC (n = 538)	P-value
<b>Maternal characteristics</b>				
Age, years	33 ± 4	34 ± 4	31 ± 5	<0.001
Married, n (%)	491 (91)	370 (86)	357 (75)	<0.001
Current smoker, n (%)	97 (18)	96 (22)	85 (24)	0.079
Current drinker, n (%)	289 (55)	254 (60)	206 (53)	0.106
Manual social class n (%)	121 (23)	100 (23)	87 (18)	0.147
Education (university entry exam or higher), n (%)	278 (52)	219 (51)	271 (58)	0.055
<b>Paternal characteristics</b>				
Age, years	35 ± 5	35 ± 5	32 ± 5	<0.001
Current smoker, n (%)	132 (25)	96 (22)	107 (31)	0.018
Current drinker, n (%)	381 (71)	306 (71)	254 (74)	0.584
Manual social class, n (%)	157 (29)	127 (30)	123 (26)	0.417
Education (university entry exam or higher), n (%)	267 (49)	196 (45)	251 (47)	0.346
<b>Maternal health during pregnancy</b>				
Smoking while pregnant, n (%)	47 (9)	49 (11)	58 (13)	0.142
Drinking while pregnant, n (%)	38 (7)	42 (10)	54 (11)	0.129
<b>Maternal illnesses</b>				
Any chronic illness, n (%) <sup>a</sup>	42 (8)	52 (12)	36 (7)	0.011
Pregnancy complications, n (%) <sup>b</sup>	77 (14)	67 (15)	28 (5)	<0.001
<b>Labor onset</b>				
Induced, n (%)	139 (26)	108 (25)	110 (25)	0.055 <sup>c</sup>
Planned caesarean section, n (%)	75 (14)	49 (11)	38 (8)	
<b>Mode of delivery</b>				
Vaginal (including forceps/ventouse), n (%)	377 (71)	316 (73)	368 (79)	0.006 <sup>c</sup>
Caesarean section, n (%)	155 (29)	119 (27)	95 (21)	

\*± values are means ± SD. P-values are for the comparisons between the three subgroups;

<sup>a</sup>asthma, diabetes mellitus, endocrine, hypertension, renal disease, inflammatory bowel disease, epilepsy or other neurological disease;

<sup>b</sup>placental abruption, placenta previa, gestational diabetes, gestational hypertension/ preeclampsia;

<sup>c</sup>global test for difference across different types of labour onset (resp. mode of delivery).

NC, naturally conceived.

age 5 years. Differences in continuous measurements between the three study groups were assessed by analysis of variance; differences in categorical outcomes were assessed by Pearson's chi square test or, if cell counts were low (<5), by Fisher's exact test. If significant differences were observed between the three study groups ( $P < 0.05$ ), *post hoc* tests were employed to compare ICSI and IVF children separately to the NC group. Logistic regression (with adjustment for child's age and country) was used for dichotomous outcomes to compare ICSI and IVF children separately to the NC group, and to assess whether any observed differences could be explained by parental socio-demographic factors or oligozoospermia (<20 million/ml). All statistical tests were two-sided.

#### Role of the funding source

The protocol was approved by the ethics committee of each institution in accordance with national regulations in each country. The European Union 5th framework quality of life programme contract QL4-CT-2000-00545 paid for this project, entitled 'An International Collaborative Study of ICSI: Child and Family Outcomes (ICSI-CFO)'. The funding source had no responsibility for study design or interpretation of data.

## Results

### Recruitment and matching

In total, 1515 children were recruited into the study between November 2000 and November 2002. Recruitment targets were achieved with the exception of Greece (the smallest participant); matching was close and participating children's

parents were similar across a range of sociodemographic factors (Table I).

### Antenatal/perinatal characteristics

Table I shows the antenatal characteristics of the study population. Both maternal and paternal age was higher in the ICSI and IVF groups compared with the NC group ( $P < 0.001$ ). A history of chronic illness in mothers was more likely in those conceived with IVF (12%) than with ICSI (8%) or naturally conceived (7%). ICSI and IVF mothers were more likely to experience pregnancy complications. There was a higher rate of caesarean section delivery amongst ICSI and IVF children (29% ICSI, 27% IVF, 21% NC) mainly due to planned caesarean section.

The perinatal characteristics of the study population are shown in Table II. ICSI and IVF children were more likely to be born slightly less mature; mean gestational age was 39.2 weeks for ICSI children, 39.3 weeks for IVF and 39.7 weeks for NC children. ICSI and IVF conceived babies were more likely to be admitted to a neonatal unit and were more likely to have been admitted for longer than 7 days.

### Illness and morbidity up to age 5 years (Table III)

A number of differences between the three study groups were observed; 74% of ICSI children and 77% of IVF children experienced significant childhood illness compared with only 57% of NC children ( $P < 0.001$ ). Hospital admissions

**Table II.** Perinatal characteristics

Characteristic	ICSI ( <i>n</i> = 540)	IVF ( <i>n</i> = 437)	NC ( <i>n</i> = 538)	<i>P</i> -value
Male, <i>n</i> (%)	293 (54)	230 (53)	280 (52)	0.754
Gestational age, weeks	39.2 ± 1.7	39.3 ± 1.9	39.7 ± 1.6	<0.001
Birth weight, kgs	3.3 ± 0.6	3.3 ± 0.5	3.4 ± 0.5	0.081
Resuscitation required, <i>n</i> (%)	23 (4)	18 (4)	23 (4)	0.992
Neonatal admission required, <i>n</i> (%)	77 (14)	69 (16)	52 (10)	0.011
Neonatal admission (>7 days) required, <i>n</i> (%)	39 (7)	29 (7)	18 (3)	0.013
Ventilation required, <i>n</i> (%)	8 (2)	5 (1)	6 (1)	0.839
Exclusively breastfed, <i>n</i> (%)	178 (38)	117 (32)	115 (41)	0.040

\*± values are means ± SD. *P*-values are for the comparisons among the three subgroups.

**Table III.** Medical history at age 4.5 to 5.5 years\*

	ICSI ( <i>n</i> = 540)	IVF ( <i>n</i> = 437)	NC ( <i>n</i> = 538)	<i>P</i> -value
Age, years	5.0 ± 0.3	5.1 ± 0.3	5.1 ± 0.3	0.086
Height, cms	111 ± 6	111 ± 5	111 ± 5	0.857
Weight, kgs	19.5 ± 3.2	19.3 ± 2.8	19.7 ± 3.0	0.149
Head circumference, cms	51.6 ± 1.6	51.8 ± 1.4	51.5 ± 1.4	0.057
Medications, <i>n</i> (%)				
Asthmatic medication	53 (10)	44 (10)	34 (10)	0.959
Any medication	71 (13)	52 (12)	41 (11)	0.727
Admitted to hospital, <i>n</i> (%)	168 (31)	124 (28)	105 (20)	<0.001
Any therapy, <i>n</i> (%)	62 (11)	41 (9)	26 (5)	<0.001
Physiotherapy	11 (2)	6 (1)	2 (0)	0.032
Speech/language	37 (7)	30 (7)	19 (4)	0.028
Psychological	4 (1)	2 (0)	1 (0)	0.406
Any illness, <i>n</i> (%)	398 (74)	337 (77)	307 (57)	<0.001
Upper respiratory tract	167 (31)	122 (28)	122 (23)	0.009
Lower respiratory tract	108 (20)	92 (21)	71 (13)	0.002
Dermatological	69 (13)	72 (16)	59 (11)	0.038
Gastrointestinal	61 (11)	49 (11)	39 (7)	0.043
Other infections	174 (32)	146 (33)	154 (29)	0.234
Any surgery, <i>n</i> (%)	128 (24)	95 (22)	73 (14)	<0.001
Circumcision	15 (3)	13 (3)	8 (1)	0.236
Other GU surgery	27 (5)	15 (3)	8 (1)	0.005

\*± values are means ± SD. *P*-values are for the comparisons among the three subgroups.

NC, naturally conceived; GU, genitourinary.

were increased for ICSI and IVF children and these children were more likely to have had medical therapies (e.g. physiotherapy, speech therapy). Furthermore, a higher proportion of ICSI and IVF children required surgery (24% ICSI, 22% IVF, 14% NC; *P* < 0.001), particularly genitourinary surgery other than circumcision (5% ICSI, 3% IVF, 1% NC; *P* = 0.005).

### Physical examination at age 5 years

A thorough physical examination showed all children to have few problems. Audiological and vision measurements showed no differences between the three groups (data not shown). Height, weight and head circumference at 5 years of age were similar (Table III). After adjustment for maternal height, birth weight, gestational age and age of child at examination, the odds ratio for being above the 97th centile for height at 5 years (compared with NC children) was 0.98 (95% CI 0.44–2.21; *P* = 0.97) for ICSI children and 0.34 (95% CI 0.11–1.00; *P* = 0.05) for IVF children.

Table IV shows the prevalence of detected malformations. Compared with NC children, the age and country adjusted

odds of major malformation was 2.77 (95% CI 1.41–5.46) for ICSI children and 1.80 (95% CI 0.85–3.81) for IVF children. After adjustment for socio-demographic differences (maternal age, educational level, social class, maternal smoking and drinking, number of previous pregnancies), these odds ratios were attenuated only slightly (OR = 2.54, 95% CI 1.13–5.71 for ICSI children and OR = 1.66, 95% CI 0.70–3.95 for IVF children).

In the neonatal history, ICSI, IVF and NC children had comparable rates of previously observed major malformations. However, when re-examined in our study more major malformations became apparent either from the ICSI children's history of illness experience during their first 5 years (e.g. a history of corrective surgery or positive medical investigations) or as a result of our physical check. When considering organ specific major malformations it was noted that the increase in ICSI was partially due to increased defects in the urogenital system (3.7% ICSI, 2.1% IVF, 0.6% NC; *P* < 0.001). Furthermore, the increase in major malformations was mainly due to a higher malformation rate in ICSI boys (8.2%) compared to ICSI girls (3.6%), compared

**Table IV.** Prevalence of congenital malformations, *n* (%) counted per child for the items (a) to (g)

Type	ICSI ( <i>n</i> = 540)	IVF ( <i>n</i> = 437)	NC ( <i>n</i> = 538)	<i>P</i> -value
(a) Neonatal major malformations <sup>a</sup>	18 (3)	9 (2)	10 (2)	0.242
(b) Neonatal minor malformations	83 (15)	66 (15)	43 (8)	<0.001
(c) Major malformations detected after neonatal period (up to 5 years) <sup>a</sup>	16 (3)	10 (2)	2 (0)	0.002
(d) Minor malformations detected after neonatal period (up to 5 years)	86 (16)	80 (18)	72 (13)	0.108
(e) Total major malformation (a or c) <sup>a,b</sup>	33 (6)	18 (4)	12 (2)	0.006
(f) Total minor malformation (b or d)	154 (29)	137 (31)	109 (20)	<0.001
(g) Overall malformations (e or f) <sup>b</sup>	178 (33)	146 (33)	117 (22)	<0.001
Major malformations by organ type <sup>a,d</sup>				
Cardiac	4 (1)	1 (0)	2 (0)	0.637
Eyes, ears, face	2 (0)	2 (0)	1 (0)	0.861
Uro-genital <sup>c</sup>	20 (4)	9 (2)	3 (1)	<0.001
Gastrointestinal	4 (1)	2 (0)	2 (0)	0.751
Musculoskeletal	5 (1)	2 (0)	1 (0)	0.237
Skin	0 (0)	3 (1)	4 (1)	0.098

<sup>a</sup>All cases were allocated Q codes from ICD-10.

<sup>b</sup>These figures do not necessarily equal the sum of their components as some children will have had both types of malformation.

<sup>c</sup>Uro-genital anomalies were: congenital pelviureteric junction obstruction (two cases) bilateral congenital vesico-ureterorenal reflux (two cases), unilateral duplication of ureter (one), urethral stenosis (one), cervical cyst (one), hydrocele (one), hypospadias (three cases) labial cyst (one), other specified congenital malformations of male genital organs (one), undescended testicle, unilateral (one).

<sup>d</sup>Malformations are counted per organ type and not per child. NC, naturally conceived.

to their respective NC groups. Oligozoospermia (<20 million/ml) did not influence the presence of major or minor malformations.

Conversely, more minor malformations had already been detected in the neonatal period for ICSI and IVF children compared with the NC children (15% in both ICSI and IVF children compared with 8% in NC children;  $P < 0.001$ ). Similarly, by age 5 years minor malformations were still more frequent in ICSI and IVF children (29% ICSI, 31% IVF, 20% NC;  $P < 0.001$ ).

## Discussion

Our detailed study of 1515 children, which is the biggest cohort of ICSI and IVF children studied at the age of 5 years, showed that rates of major malformations were higher in ICSI compared to the NC group and were little affected by adjustment for socio-demographic differences. IVF children had a similar pattern of increased congenital anomalies, but this did not reach statistical significance. Bearing in mind that only children born at  $\geq 32$  weeks of gestation were included in the study, the ICSI/IVF children were nevertheless still a little less mature (Table II), a finding that is consistent with previous studies (Schieve *et al.*, 2002). Although these children were born at  $\geq 32$  weeks of gestation they still experienced greater neonatal morbidity. We did not include infants born less mature, to avoid possible confounding effects from extreme prematurity. We also excluded twins and higher order births for the same reason.

As most of the ICSI children were first born and rarely second born (as a consequence of the recent introduction of the ICSI technique) we limited the selection of the IVF and NC children to first and second born, trying to avoid a bias

in the interpretation of the results of delivery and birth parameters and in the study on psycho-emotional development and family functioning, which was done in parallel with this study on the same group of children.

Contrary to a previous report (Leslie *et al.*, 2003) we found that ICSI and IVF children appeared to experience higher illness morbidity compared with NC children in the first 5 years (Table III). It might be suggested that a higher recorded history of medical illnesses reflected excessive parental concern, but this would not explain why these children have had significantly more surgical interventions, especially genitourinary surgery, because of congenital malformations or higher rates of hospitalization overall (Table III). Parental concern might be expected to manifest as higher use of medication, which there was not.

On detailed physical examination, which included non-routine items such as vision and hearing assessments, children born after ICSI and IVF were reassuringly similar to the NC group (with the exception of congenital malformations; see Table IV). We did not detect any significant neurological findings, despite previous reports suggesting higher rates of cerebral palsy (Stromberg *et al.*, 2002) and fine motor defects (Sutcliffe *et al.*, 2001). We might, however, have missed cerebral palsy in children born  $> 32$  weeks since the power of this study to detect such rare events was limited. One strength of our study was that each child was examined; we did not rely on proxy measures or birth registry data. We did not detect an excess of tall children after ICSI conception. This is of interest in view of recent reports (Cox *et al.*, 2002; DeBaun *et al.*, 2003; Maher *et al.*, 2003) suggesting an increase in Beckwith Wiedemann syndrome (BWS) after IVF/ICSI. It is recognised that BWS is a spectrum and large size is one of those features. Our measurements are the first

documented data on the long-term growth of IVF/ICSI children.

Our study finding of increased risk of congenital malformations is in agreement with previous retrospective studies of Hansen *et al.* (2002b) and Wennerholm *et al.* (2000b). One prospective study on ICSI children ( $n = 3372$ ) (including a pre- and postnatal period up to 8 weeks) compared to a selected NC group out of the general population ( $n = 8016$ ) resulted in an initial relative risk (RR) of 1.44 (95% CI: 1.25–1.65) (Katalinic *et al.*, 2004). After adjustment for parental malformations, previous malformed child and maternal age the RR declined to 1.24 (95% CI: 1.02–1.50) suggesting that parental background played a role in the apparent increase of congenital malformations in ICSI. We examined our study children at the age of 5 years and did not have access to genetic data such as family history of malformations or number of miscarriages, which might influence the relative risk of malformations to ICSI children.

Major malformations were found to be present more often in the ICSI group, in particular in ICSI boys (data not shown), beyond the neonatal period and were due to an excess in uro-genital malformations. Some authors have already shown an excess risk of uro-genital malformations in ICSI compared to the general population (Wennerholm *et al.*, 2000a; Ericson and Kallen, 2001). Our data suggest a slight increase in major malformations in IVF children which is consistent with the literature, however, we had a smaller sample size than some previous reports (Westergaard *et al.*, 1999; Anthony *et al.*, 2002; Hansen *et al.*, 2002a). It is recognised that at least 30% of congenital anomalies are missed at birth and so the higher rates of anomalies at 5 years are unsurprising. The higher rates of genitourinary defects in the ICSI conceived boys (see Table IV) with a corresponding increased rate of genitourinary surgery are more likely to reflect paternal genetic factors than the ICSI procedure itself.

One limitation of our study is the potential for survivor bias to have been introduced by the recruitment of children who had already survived to the age of 5 years, as children with severe malformations and/or illnesses would have had a greater risk of infant death than children without such malformations. However, it is likely that any biases would have resulted in us underestimating, rather than overestimating, the true relative risks of severe congenital malformations associated with ICSI and IVF.

On the other hand, selection of the NC group from local schools and nurseries may have led to underestimation of the risk of severe malformations and/or illnesses in this group (since naturally conceived children with severe malformations may be less likely to attend normal school facilities). Our NC group could therefore have been healthier than average, leading to a false interpretation of the increase of congenital malformation associated with the ICSI/IVF groups. However, the observation that none of the children in the ICSI or IVF groups attended special schools or other institutions indicates that this is unlikely to have provided a major source of bias.

The main limitation of this study is the rather high rate of non-participation ('not reached and refusals') for ICSI

children (in two of the centres) and for IVF children (in three of the centres). Non-participation was especially high in Greece, where cultural influences led to a higher level of secrecy around the fertility treatment (and hence a lower participation rate among ICSI and IVF children) than was observed in other countries. Our non-participation rates were generally in accordance with other long-term follow-up studies, however (Bowen *et al.*, 1998). Despite the inter country differences in participation rate, results were nevertheless comparable between countries, with no country bias. For example, Sweden had virtually complete data and comparison of these data with other countries revealed no differences. Furthermore, our data showed internal consistency; IVF mothers, for example, experienced greater morbidity from chronic illnesses than ICSI mothers, which would be expected in view of the greater use of ICSI for male factor problems. In addition, after matching of cases, comparison and NC children, the three groups were similar for a broad range of sociodemographic factors (Table I). These observations therefore suggest that our findings are unlikely to have been greatly influenced by participation bias.

As discussed, cross-sectional recruitment of a proportion of a cohort has limitations when assessing the incidence of congenital anomalies, but is probably the most appropriate approach for assessing other outcomes, such as general health and development. As multiple outcomes were the focus of this study, it was felt that such compromises in study design were therefore necessary.

Overall, our study provides reassuring information about the physical health of children conceived after ICSI and standard IVF. On the other hand, it also shows the need for attentive health care of the growing-up ART children since more birth defects in ICSI and more hospital admissions, surgery and medical therapies were recorded in ICSI and IVF singletons compared to a NC comparative group. It also highlights the need for continuing monitoring of children conceived after assisted reproductive therapies, particularly regarding longer term issues such as a possible higher risk of imprinting disorders, cancers and reduced future fertility (Kurinczuk, 2003a). However the main risk (beyond the scope of this study) to couples having assisted reproductive therapies remains the morbidity and mortality associated with prematurity and multiple births.

#### **Conflict of interest statement**

None declared.

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# Assisted reproductive technology and major structural birth defects in the United States<sup>†</sup>

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**BACKGROUND:** With >1% of US births occurring following use of assisted reproductive technology (ART), it is critical to examine whether ART is associated with birth defects.

**METHODS:** We analyzed data from the National Birth Defects Prevention Study, a population-based, multicenter, case–control study of birth defects. We included mothers of fetuses or live-born infants with a major birth defect (case infants) and mothers who had live-born infants who did not have a major birth defect (control infants), delivered during the period October 1997–December 2003. We compared mothers who reported ART use (IVF or ICSI) with those who had unassisted conceptions. Multiple logistic regression was used to adjust for the following confounders: maternal race/ethnicity, maternal age, smoking and parity; we stratified by plurality.

**RESULTS:** ART was reported by 1.1% of all control mothers, and by 4.5% of control mothers 35 years or older. Among singleton births, ART was associated with septal heart defects (adjusted odds ratio [aOR] = 2.1, 95% confidence intervals [CI] 1.1–4.0), cleft lip with or without cleft palate (aOR = 2.4, 95% CI 1.2–5.1), esophageal atresia (aOR = 4.5, 95% CI 1.9–10.5) and anorectal atresia (aOR = 3.7, 95% CI 1.5–9.1). Among multiple births, ART was not significantly associated with any of the birth defects studied.

**CONCLUSIONS:** These findings suggest that some birth defects occur more often among infants conceived with ART. Although the mechanism is not clear, couples considering ART should be informed of all potential risks and benefits.

**Key words:** National Birth Defects Prevention Study / assisted reproductive technology / *in vitro* fertilization / birth defects / congenital anomalies

## Introduction

According to data from the 2002 National Survey of Family Growth, 11.9% of US women aged 15–44 years reported ever using any

infertility services (Chandra *et al.*, 2005). In the USA and worldwide, the use of assisted reproductive technology (ART) to treat infertility is increasing rapidly, with an estimated total of 200 000 babies born after use of ART worldwide in 2000 (Adamson *et al.*, 2006). ART is

<sup>†</sup> This work was completed by U.S. government employees as part of their official duties, and thus is a U.S. government work, and remains in the public domain.

defined as infertility treatments in which both oocytes and sperm are handled outside the body such as *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI). In the USA in 2005, more than 134 000 ART procedures were performed and more than 52 000 infants were live-born as a result of these procedures, representing > 1% of all US births (Wright *et al.*, 2008). This proportion is expected to continue to rise and research on short- and long-term health effects has not kept pace with rapid advances in treatment technology. Two meta-analyses were published in 2005, one that addressed the association between ART and birth defects (Hansen *et al.*, 2005), and the other more specifically addressed the association between ICSI and birth defects (Lie *et al.*, 2005). These two reviews included most of the existing literature on this topic and found an increased risk for birth defects overall after the use of IVF, but no additional risk from ICSI when compared with IVF (Hansen *et al.*, 2005; Lie *et al.*, 2005). However, the studies in these reviews were limited by a number of methodological problems, including small numbers of affected infants, heterogeneous case groups, lack of appropriate control groups and potential confounding (Schieve *et al.*, 2005).

In this study, we used data from an ongoing population-based, multicenter, case–control study of birth defects to examine possible associations between ART and major structural birth defects.

## Materials and Methods

### Study design and sample

The National Birth Defects Prevention Study (NBDPS) is an ongoing, multicenter, case–control study to investigate environmental and genetic risk factors for more than 30 selected major birth defects (Yoon *et al.*, 2001; Rasmussen *et al.*, 2002). For this study, case infants were identified through existing birth defects surveillance systems in 10 states (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Utah and Texas). These surveillance systems identify all children with birth defects from hospital records as part of their public health activities regardless of and with no prior information on conception method. Cases can be live-born, fetal deaths, or pregnancy terminations. For live-born children, birth defects are identified up to 1 year after birth at all surveillance sites, up to age 2 or 3 at some sites, and up to 6 years after birth at one site. Children who die after birth (either cases or controls) are eligible for inclusion in the study. Case information obtained from medical records was reviewed by a clinical geneticist at each study site to ensure that infants met the case definition. Case infants with a recognized or strongly suspected single-gene condition or chromosome abnormality were excluded. Infants with defects presumed to be secondary to another defect (e.g. cleft lip in a baby with holoprosencephaly) were included only in the primary defect category. Details of the clinical review methods of the study have been published elsewhere (Rasmussen *et al.*, 2003). Control infants were live-born infants without major birth defects; they were randomly selected from the same source populations as the case infants, either from birth certificates or birth hospital records. Only one case or control infant was eligible from each family; from multiple births where both babies had birth defects the first born infant was included. Case and control mothers completed a telephone interview in English or Spanish between 6 weeks and 2 years after the estimated date of delivery (Yoon *et al.*, 2001). This study was reviewed by institutional review boards at the Centers for Disease Control and Prevention and the collaborating institutions.

For this analysis, case and control infants were limited to those born on or after 1 October 1997, and with an estimated date of delivery on or before 31 December 2003. The response rate for the interview was

70.5% for case mothers and 67.2% for control mothers. For hypospadias, only infants with second- or third-degree hypospadias were included in the study, because of concern that first-degree hypospadias could be incompletely ascertained. In addition, for hypospadias, only male control infants were included in the analysis. Case and control infants whose mothers reported prepregnancy type 1 or type 2 diabetes were excluded from this analysis because of the strong association between diabetes and birth defects (Yang *et al.*, 2006).

### Measurement of exposure

The NBDPS telephone interview includes questions about a wide range of preconceptional and pregnancy exposures. Detailed questions about use of infertility treatments are included in the pregnancy history section, i.e. 'In the two months before you became pregnant... did you take any medications to help you become pregnant?' and 'Did you have any other procedures to help you become pregnant?' Women who respond affirmatively to either question are questioned further about specific treatments.

For this analysis, the main exposure of interest was use of ART, defined as the use of a treatment to conceive the index pregnancy, in which both sperm and egg or embryos were handled medically. These were IVF, ICSI, zygote intrafallopian transfer or gamete intrafallopian transfer. Mothers of case and control infants were considered unexposed if the mother answered 'No' to the screening question, 'Did you or the father take any medications or have any procedures to help you become pregnant?' Any mother who did not use ART but reported that she or the father used other infertility treatments (e.g. ovulation stimulating drugs or vasectomy reversal) were excluded from this analysis.

### Statistical analysis

All analyses were stratified into singleton and multiple (twins and higher order) births because multiple births are strongly associated with both ART and birth defects (Mastroiacovo *et al.*, 1999). Univariate analyses were used to calculate crude odds ratios (ORs) for exposure–outcome combinations that had at least three exposed case infants. If the expected number in any of the cells was less than five, the Fisher's exact test was used to estimate the confidence intervals (CIs). Adjusted ORs for those exposure–outcome combinations that had at least five exposed case infants were calculated using multiple logistic regression. Maternal age was considered an *a priori* confounder and was included in every model as a continuous variable. We defined potential confounders as factors associated with both ART and the birth defect, but by definition confounders do not have to be causally associated with birth defects. Confounders we considered were maternal race (non-Hispanic White or other), study center (Massachusetts or other), parity (no previous live births or one or more previous live births), history of miscarriages (none or one, or two or more), education (0–12 years or > 12 years), body mass index (<30 or ≥30), family income (<\$50 000 or ≥\$50 000), maternal smoking or alcohol use from 1 month before pregnancy through the end of the first trimester (any or none) and use of folic acid or multivitamin supplements during the month before pregnancy or first month of pregnancy (yes or no). Study center was analyzed as Massachusetts or other because more than half of the reports of use of ART were from Massachusetts. Preterm gestation (<37 weeks gestation) was included in the models for septal heart defects because preterm infants will more often have echocardiography performed than term infants.

Manual backward selection was used to create parsimonious models for singleton and multiple births separately by first modeling each of the defects; if the OR estimate changed more than 10% the factor was retained in the model. Based on this defect-specific modeling, two combined set of confounders were selected (one for singletons and one for twins) and used for each defect group.

Subgroup analyses were performed for infants with either an isolated defect (no other major unrelated birth defects) or multiple defects (more than one unrelated, major defect) (Rasmussen et al., 2003). Another subgroup analysis excluded case and control infants who had a family history of the specific defect in a first-degree relative. Sensitivity analyses were conducted to determine if results were different when infertility treatments with the use of donor eggs, sperm or embryos were excluded. All analyses were performed using SPSS 15.0.

## Results

Interviews were conducted with the mothers of 5008 control infants and 13 586 case infants. Of these, 39 participants (28 case and 11 control mothers) were excluded because data on infertility treatment were missing, and 876 (696 case and 180 control mothers) were excluded because they only used other non-ART infertility treatments, leaving 17 679 participants with complete exposure data. Of these women, 277 cases mothers and 25 control mothers with pre-existing diabetes were excluded. Limiting the data to the 25 defect categories that had at least three exposed singleton or multiple cases infants resulted in 9584 case and 4792 control mothers. A total of 683 case infants had more than one defect of interest and were included in more than one category. The mean time from date of birth to the interview was 8.8 months for control mothers and 11.5 months for case mothers.

ART use was reported by 51 (1.1%) control mothers and 230 (2.4%) case mothers. Twenty-one mothers reported ICSI (16 case and 5 control mothers), 36 mothers (27 case and 9 control mothers) reported use of a donor egg, sperm or embryo as part of ART and 45 case mothers and 10 control mothers reported using a frozen egg, sperm or embryo. All 230 case infants born after use of ART were live-born, whereas for case infants born after unassisted conception 1% ended in a fetal death and 1% ended in a pregnancy termination. Control infants conceived using ART differed from control infants conceived without use of ART for a number of factors, including multiple births, maternal age, race or ethnicity, parity, education, family income and maternal smoking (Table I). Also, more than half of all pregnancies conceived by ART were from Massachusetts. 23.4% of twins in the study occurred following ART, including 23.7% of twin case infants and 21.7% of twin control infants.

In an unadjusted analysis among singleton births, significant elevated ORs were observed for the association between ART and septal heart defects overall (and within that group, atrial septal defects [ASDs] secundum or not otherwise specified [NOS] and ventricular septal defects [VSDs] plus ASDs), esophageal atresia, anorectal atresia and hypospadias (Table II). Among multiple births, we observed no significantly increased ORs.

After adjusting for maternal age, study center, parity, family income and prematurity (septal heart defects only), we observed significant associations among singletons for the group of septal heart defects [OR 2.1, 95% CI 1.1–4.0 overall and within that group, ASD secundum/NOS (OR 3.0, 95% CI 1.5–6.1), and VSD plus ASD (OR 2.8, 95% CI 1.2–7.0)], cleft lip with/without cleft palate (CLCP) (OR 2.4, 95% CI 1.2–5.1), esophageal atresia (OR 4.5, 95% CI 1.9–10.5) and anorectal atresia (OR 3.7, 95% CI 1.5–9.1), and an elevated OR (2.1) for hypospadias (95% CI 0.9–5.2) (Table III). Again we did not observe any statistically significant associations among multiple births. For VSD plus ASD, the OR changed substantially after

**Table I** Characteristics of mothers who had a child without major birth defects, who reported either an unassisted conception or reported use of ART (National Birth Defects Prevention Study, 1997–2003)

	Unassisted conception (n = 4741) <sup>a</sup>	ART (n = 51) <sup>a</sup>	P-value
Multiple births			<0.00
Singletons	4635 (97.9%)	23 (45.1%)	
Twins	99 (2.1%)	23 (45.1%)	
Triplets or quadruplets	2 (0.0%)	5 (9.8%)	
Gestational age			<0.00
Very preterm (<32 weeks)	54 (1.1%)	4 (7.8%)	
Preterm (32–36 weeks)	359 (7.6%)	15 (29.4%)	
Term (37–45 weeks)	4325 (91.2%)	32 (62.7%)	
Birth weight			<0.00
Very low birth weight (<1500 g)	30 (0.6%)	3 (2.9%)	
Low birth weight (1500–2499 g)	226 (4.8%)	9 (17.6%)	
Normal birth weight (2500–3999 g)	3979 (83.9%)	37 (72.5%)	
Macrosomic (≥4000 g)	484 (10.2%)	2 (3.9%)	
Maternal age			<0.00
<25 years	1649 (34.8%)	0 (0%)	
25–29 years	1250 (26.4%)	4 (7.8%)	
30–34 years	1233 (26.0%)	19 (37.3%)	
35–39 years	520 (11.0%)	21 (41.2%)	
≥40 years	89 (1.9%)	7 (13.7%)	
Maternal race or ethnicity			<0.00
Non-Hispanic White	2797 (59.1%)	44 (86.3%)	
Non-Hispanic Black	568 (12.0%)	3 (5.9%)	
Hispanic	1096 (23.2%)	1 (2.0%)	
Other	268 (5.7%)	3 (5.9%)	
Study site			<0.00
Arkansas	560 (11.8%)	1 (2.0%)	
California	670 (14.1%)	2 (3.9%)	
Georgia	528 (11.1%)	4 (7.8%)	
Iowa	531 (11.2%)	5 (9.8%)	
Massachusetts	586 (12.4%)	27 (52.9%)	
New Jersey	551 (11.6%)	8 (15.7%)	
New York	447 (9.4%)	2 (3.9%)	
North Carolina	149 (3.1%)	1 (2.0%)	
Texas	589 (12.4%)	1 (2.0%)	
Utah	130 (2.7%)	0 (0%)	
Previous live births			0.003
None	1876 (39.6%)	29 (56.9%)	
One	1636 (34.5%)	19 (37.3%)	
Two or more	1227 (25.9%)	3 (5.9%)	
Previous miscarriages			0.415

Continued

**Table I** *Continued*

	Unassisted conception (n = 4741) <sup>a</sup>	ART (n = 51) <sup>a</sup>	P-value
None	3703 (78.1%)	36 (70.6%)	
One	788 (16.6%)	11 (21.6%)	
Two or more	248 (5.2%)	4 (7.8%)	
Maternal education			<0.00
< 12 years	824 (17.5%)	2 (4.0%)	
12 years	1196 (25.4%)	3 (5.9%)	
> 12 years	2692 (57.1%)	46 (90.2%)	
Body mass index			0.706
< 18.5 kg/m <sup>2</sup>	278 (6.1%)	2 (3.9%)	
18.5–24.9 kg/m <sup>2</sup>	2584 (56.9%)	29 (56.9%)	
25–29.9 kg/m <sup>2</sup>	1003 (22.1%)	14 (27.5%)	
≥30 kg/m <sup>2</sup>	679 (14.9%)	6 (11.8%)	
Family income			<0.00
<\$10 000	784 (18.6%)	0 (0%)	
\$10 000–\$49 999	2005 (47.5%)	7 (15.2%)	
\$50 000 or more	1436 (34.0%)	39 (84.8%)	
Alcohol use in the month before pregnancy or the first trimester	1781 (37.8%)	17 (33.3%)	0.509
Smoking during the month before pregnancy or the first trimester	932 (19.7%)	2 (3.9%)	0.005
Folic acid containing multivitamin use during the month before pregnancy or the first month of pregnancy	2319 (49.0%)	50 (98.0%)	<0.00
Child not alive at the time of the interview	9 (0.2%)	0 (0%)	NC

Participants with preexisting diabetes type 1 or 2 were excluded. <sup>a</sup>Due to missing values the contents of the cells do not always add up to the total number of subjects. NC, not calculated.

controlling for confounders. The difference is not completely explained by correcting for preterm; when not adjusted for preterm the OR was 3.4 (1.4–8.1), compared with 2.8 if preterm was included. When limiting the analysis to only term infants the OR decreased a little to 2.6, and was of borderline significance ( $P = 0.10$ ) due to the fact that only four exposed cases remained.

Subanalyses excluding those participants with a family history of the studied defects (data not shown) resulted in ORs very similar to those in Table III. Separate analyses of isolated and multiple defects were limited by small numbers for some defects. Defects for which there were sufficient numbers of isolated and multiple cases among the singleton births included septal heart defects, ASD secundum and esophageal atresia. For the heart defects, we observed higher ORs for the infants with multiple defects. For singleton infants with esophageal atresia, the ORs for infants with isolated and multiple defects were similar (5.1 and 4.3, respectively).

Clinical geneticists identified 1296 case infants as having multiple major defects. Of these, 37 (2.9%) were conceived using ART

compared with 191 of 8263 (2.3%) of infants with an isolated defect. When we looked at the patterns among infants with multiple defects, we found two phenotypes to be relatively common among infants conceived using ART, the VACTERL association (Vertebral defects, Anal atresia, Cardiac defects, Tracheo-Esophageal fistula, Renal malformations and Limb defects) and oculoauriculovertebral spectrum.

Excluding pregnancies conceived using donor eggs, sperm or embryos increased most of the estimates by 0.5, but the CIs remained similar. The biggest difference we noted was for hypospadias among singleton births; we observed a significant OR (increased from 2.1 to 3.3, 95% CI 1.1–9.8). For esophageal atresia among singleton births, the OR increased from 4.5 to 5.5 (95% CI 2.2–13.7).

## Discussion

In a population-based, multicenter, case–control study of birth defects, ART was significantly associated with ASD secundum/NOS, VSD plus ASD, CLCP, esophageal atresia and anorectal atresia among singleton births. Compared with singleton infants, infants from multiple births were more likely to have major defects. Because of the small numbers of multiple birth infants with birth defects and maternal reports of ART, it was not possible to reliably assess possible effect modification of the associations between multiple gestation and birth defects by ART.

The number of infants born after ART doubled in the USA from 1996 through 2004. By 2004, >1% of US births were estimated to have resulted from ART (Wright *et al.*, 2007). Data from several publications have shown increases in ART use worldwide (Adamson *et al.*, 2006; Wang *et al.*, 2006; Andersen *et al.*, 2007). A recent meta-analysis on ART and birth defects concluded that there was an ~40% increased risk of birth defects among infants conceived using ART compared with infants who were conceived without using any infertility treatments (Hansen *et al.*, 2005). However, it is difficult to assess the meaning or the biological plausibility of this finding because of the heterogeneity of the group of all birth defects combined. In contrast, the NBDPS afforded us an opportunity to assess associations between ART and more pathogenetically similar specific types of birth defects.

Our finding of an association between birth defects and ART among singletons was similar to the results of a large cohort study of IVF in Sweden (Kallen *et al.*, 2005). While we could not completely assess the reason for the lack of association in the multiple birth subsample, we demonstrated that infants of multiple births were more likely to have major birth defects, regardless of conception mode. Thus, the underlying mechanism by which ART increases the birth defect risk among singletons might have a smaller effect among multiple birth pregnancies. Another possibility is that the effect of ART on multiple births varies by zygosity, which we were unable to assess. Twins born to women not reporting ART use could be more likely to be monozygotic and these twins might be at higher risk for birth defects than dizygotic twins, as has been suggested by previous studies of twins (Ramos-Arroyo, 1991).

Our finding of septal heart defects, and more specifically ASD secundum/NOS, being associated with ART has been described to some extent in a recent study from Iowa (Olson *et al.*, 2005). Ericson and Kallen (2001) reported no associations between ART and cardiac defects, while Hansen *et al.* (2002) and Katalinic *et al.* (2004) reported

**Table II** Unadjusted associations between the use of ART and selected birth defects<sup>a</sup>, stratified by plurality (National Birth Defects Prevention Study, 1997–2003)

Birth defect categories	Singletons			Twins or higher		
	Unassisted conception	ART	Unadjusted OR (95% CI) <sup>b</sup>	Unassisted conception	ART	Unadjusted OR (95% CI) <sup>b</sup>
Controls	4635	23		101	28	
Anencephaly	222			14	3	0.8 (0.1–3.1)
Cataract	136	3	5.2 (0.9–19.0)	3		
Anotia, microtia	266	3	2.3 (0.4–7.6)	7	5	2.6 (0.6–10.2)
Conotruncal heart defects	996	8	1.6 (0.7–3.6)	46	6	0.5 (0.2–1.2)
d-Transposition of great arteries	320			8	3	1.4 (0.2–6.1)
Tetralogy of Fallot	441	5	2.3 (0.7–6.2)	21		
Septal heart defects	2001	27	2.7 (1.6–4.8)	122	38	1.1 (0.6–2.0)
Perimembraneous VSD	823	9	2.2 (0.9–5.0)	50	15	1.1 (0.5–2.2)
Multiple VSDs	32			6	4	2.4 (0.5–10.9)
ASD secundum or NOS	1080	18	3.4 (1.8–6.2)	74	22	1.1 (0.6–2.0)
VSD+ASD	301	8	5.4 (2.1–12.5)	19	5	0.9 (0.3–2.8)
Right outflow tract heart defects	723	4	1.1 (0.3–3.3)	52	11	0.8 (0.4–1.7)
Pulmonary valve stenosis	513	3	1.1 (0.2–3.7)	38	8	0.7 (0.3–1.7)
Left outflow tract heart defects	730	4	1.1 (0.3–3.2)	39	10	0.9 (0.4–2.1)
Coarctation of aorta	380			21	8	1.4 (0.6–3.4)
Cleft lip with or without palate	1173	12	2.0 (1.0–4.0)	45	13	1.0 (0.5–2.1)
Cleft palate	631	8	2.4 (1.0–5.8)	18	7	1.3 (0.5–3.5)
Esophageal atresia	266	9	6.8 (2.8–15.5)	18	11	2.2 (0.9–5.2)
Anorectal atresia	413	7	3.4 (1.2–8.3)	22	8	1.3 (0.5–3.3)
Hypospadias, second or third degree	785	14	4.6 (2.0–10.8)	40	25	2.2 (1.0–4.6)
Longitudinal limb deficiencies	187			8	3	1.4 (0.2–6.1)
Transverse limb deficiencies	291			14	3	0.8 (0.1–3.1)
Preaxial limb deficiencies	110			3	3	3.6 (0.5–24.7)
Craniosynostosis	464			17	7	1.5 (0.6–3.9)
Diaphragmatic hernia	334			14	4	1.0 (0.2–3.6)

<sup>a</sup>Only defects that had at least 3 exposed cases are included in this table; infants with multiple birth defects could be included in several categories; <sup>b</sup>If the expected number in a cell was less than 5, Fisher exact confidence limits were calculated. VSD, ventricular septal defect; ASD, atrial septal defect; NOS, not otherwise specified; ART, assisted reproductive technology; AOR, adjusted odds ratio; CI, confidence interval.

increased risks of cardiac defects in the aggregate in association with ART. None of these studies was sufficiently large to allow evaluation of specific cardiac phenotypes. However, because septal defects are the most prevalent of cardiac phenotypes (Hoffman and Kaplan, 2002), it is possible that the associations of ART with cardiac defects in the aggregate reported by Hansen *et al.* (2002) and Katalinic *et al.* (2004) reflect associations with septal defects as well.

We are not aware of any studies that looked at orofacial clefts and IVF specifically, but orofacial clefts (CLCP and cleft palate alone) have been included in studies looking at groups of birth defects. Only one study found an association, a crude OR of 5.11 (95% CI 1.26–20.80), for the association between cleft palate alone and ICSI (Kurinczuk and Bower, 1997). In our study, of the 16 mothers of children with cleft palate alone who reported use of ART, only one mentioned ICSI.

Consistent with our results, previous studies have suggested an association between ART and both esophageal atresia and anal atresia (Kallen *et al.*, 2005; Midrio *et al.*, 2006). Increased risks for

esophageal atresia (risk ratio [RR] 4.0, 95% CI 2.6–6.3) and anorectal atresia (RR 4.7, 95% CI 3.2–6.9) were observed among infants born in Sweden using IVF, compared with infants among the general population (Kallen *et al.*, 2005). Esophageal atresia and anorectal atresia are defects that often occur in association with other major defects (Robert *et al.*, 1993). However, when we evaluated esophageal atresia cases classified as having isolated and multiple defects separately, we found very similar results.

Hypospadias has been found to be associated with IVF and ICSI in several studies (Silver *et al.*, 1999; Wennerholm *et al.*, 2000; Ericson and Kallen, 2001; Hansen *et al.*, 2002). However, because hypospadias has been associated with multiple types of infertility treatments, as well as with advanced maternal age and primiparity, the true association might be with the underlying subfertility rather than with infertility treatments.

Although we did not observe associations between ART and birth defects among multiple births, this itself is known to be associated

**Table III Adjusted odds ratios for association between ART and birth defects stratified by plurality (National Birth Defects Prevention Study, 1997–2003)**

	Singleton <sup>a</sup> AOR (95% CI)	Twins or higher <sup>b</sup> AOR (95% CI)
Anotia/microtia		4.0 (0.7–21.8)
Conotruncal heart defects	1.4 (0.6–3.2)	0.8 (0.3–2.6)
Tetralogy of Fallot	1.6 (0.6–4.3)	
Septal heart defects <sup>c</sup>	2.1 (1.1–4.0)	1.3 (0.6–2.8)
Perimembraneous VSD <sup>c</sup>	1.4 (0.6–3.3)	1.1 (0.4–2.8)
ASD secundum/NOS <sup>c</sup>	3.0 (1.5–6.1)	1.7 (0.7–3.9)
VSD and ASD <sup>c</sup>	2.8 (1.2–7.0)	1.3 (0.3–5.4)
Right outflow tract heart defects		1.0 (0.4–2.9)
Pulmonary valve stenosis		1.0 (0.3–3.1)
Left outflow tract heart defects		1.0 (0.4–2.7)
Coarctation of aorta		1.1 (0.4–3.6)
Cleft lip with or without palate	2.4 (1.2–5.1)	1.3 (0.5–3.4)
Cleft palate	2.2 (1.0–5.1)	1.4 (0.4–4.8)
Esophageal atresia	4.5 (1.9–10.5)	2.2 (0.7–7.3)
Anorectal atresia	3.7 (1.5–9.1)	1.5 (0.4–5.2)
Hypospadias, second or third degree	2.1 (0.9–5.2)	2.1 (0.7–6.4)
Craniosynostosis		2.3 (0.6–9.3)

VSD, ventricular septal defect; ASD, atrial septal defect; NOS, not otherwise specified; ART, assisted reproductive technology; AOR, adjusted odds ratio; CI, confidence interval.

<sup>a</sup>Adjusted for maternal age, center (Massachusetts versus rest), family income, and parity; <sup>b</sup>Adjusted for: maternal age, family income, folic acid use, parity, and periconceptional alcohol use; <sup>c</sup>Also adjusted for preterm births.

with both ART (see Table I) and birth defects (Cragan *et al.*, 1993; Li *et al.*, 2003; Tang *et al.*, 2006). In this study, 23.4% of all twins occurred following ART. Thus, ART might contribute to the risk of major birth defects both directly by increasing the risk of defects among singletons, and indirectly by increasing the occurrence of twinning which is a strong risk factor for many types of major birth defects (Li *et al.*, 2003; Tang *et al.*, 2006). Even if the additional impact of any risk posed by ART is negligible among the already high-risk multiple births, the strong association between ART, as practiced in the USA, and multiple birth should nonetheless be considered as another pathway through which ART might indirectly contribute to birth defect risk.

The frequency of use of ART among control mothers in our study was comparable with that described among the general population (Wright *et al.*, 2004, 2008). Use of ART varied by state, with the highest prevalence of reported use among Massachusetts mothers. The higher rate of reported use in Massachusetts is likely due to the fact that since 1987 Massachusetts has mandated that fertility treatments be included in health insurance plans (Henne and Bundorf, 2008).

Our results were based on data from an ongoing, population-based case–control study of over 30 structural birth defects, for which

information on multiple maternal exposures were ascertained during a maternal telephone interview. Case infants were ascertained through existing population-based surveillance systems, which should limit ascertainment bias based on infertility treatment status. The etiologic heterogeneity of case groups was reduced by a careful review of clinical information on each case infant by a clinical geneticist and the use of standardized case definitions that excluded chromosomal and single-gene disorders. Our multicenter approach, combining data from 10 centers across the USA, has improved our capacity to evaluate the possible association between ART and a number of specific defects, which individually are quite rare outcomes, occurring in at most 1 in 700 births. Importantly, we were also able to adjust our analyses for several potential confounders.

There were two main limitations to this study. The first was the difficulty in distinguishing between the effects of the underlying subfertility and the infertility treatments used. Subfertile women might have a higher risk of having a child with a birth defect regardless of whether infertility treatments are used (Zhu *et al.*, 2006). In our study, women were not asked the time period required prior to conception, nor did we ask for the infertility diagnosis, so we were unable to adjust for these factors. The other issue was the potential for exposure misclassification; ART exposure was based solely on maternal report and not validated by medical records review.

There is also potential concern for ascertainment bias, since children born after ART may be monitored more intensely. While this is an important issue in cohort analyses, it is less of an issue in case–control studies that are based on population-based surveillance systems with active case finding. Moreover, many defects included in this study such as orofacial clefts, esophageal atresia and anorectal atresia have overt clinical manifestations that will be readily identified shortly after birth. We cannot completely discount the possibility of some ascertainment differences contributing to the association with septal heart defects as identification of these are linked to increased scrutiny. However, the association remained after adjusting for family income and other demographic factors. One last limitation especially relevant for the septal defects is the fact that we could not assess the quality of our gestational age variable, which was based on maternal report. However, a recent study found that for 86% of mothers of children between 8 and 18 years the difference between their report of gestational age and the vital records was one week or less (Adegbeye and Heitmann, 2008).

In this study, we examined the association between ART and major structural birth defects. The underlying biological mechanism by which this intervention might lead to phenotypes affecting diverse developmental pathways is unclear. Our findings could have been because of underlying infertility, small numbers or chance. Until further studies have corroborated our findings or clarified the basis for these findings, the practical application of our results is limited. Although the underlying mechanism of this effect could not be answered by this study, couples considering infertility treatments should be aware of all the possible benefits and risks posed for children conceived with these treatments.

## Authors' contribution

J.R. contributed to the design, acquisition, analysis and interpretation of the data, and drafting and editing the article. M.A.H. and C.A.H.

contributed to the design, acquisition and interpretation of the data, and drafting and editing the article. L.A.S. and A.C. contributed to the design, interpretation of the data, and drafting and editing the article. S.A.R. contributed to the conception, design, acquisition, analysis and interpretation of the data, and drafting and editing the article.

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## Articles

# Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection

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## Summary

**Background** Intracytoplasmic sperm injection (ICSI) was introduced as a new form of in-vitro fertilisation (IVF) in 1993 and is now accepted as the treatment of choice for severe male infertility in many centres around the world. However, there is little information about the long-term outcome of children conceived by ICSI. We aimed to find out the medical and developmental outcome of children conceived by ICSI at age 1 year.

**Methods** In this prospective study, we compared the medical and developmental outcome at 1 year of 89 children conceived by ICSI with 84 children conceived by routine IVF, and with 80 children conceived naturally. Formal developmental assessment was done with Bayley Scales of Infant Development (2nd edition) from which a mental development index (MDI) was derived.

**Findings** There was no significant difference in the incidence of major congenital malformations or major health problems in the first year of life. However, the mean Bayley MDI was significantly lower for the children conceived by ICSI than for the children conceived by routine IVF or naturally (95.9 [SD 10.7], 101.8 [8.5], and 102.5 [7.6], respectively,  $p < 0.0001$ ). 15 (17%) of 89 children conceived by ICSI experienced mildly or significantly delayed development (MDI < 85) at 1 year compared with two (2%) of the 84 children conceived by IVF and one (1%) of the 80 children conceived by natural conception ( $p < 0.0001$ ).

**Interpretation** Although most children conceived by ICSI are healthy and develop normally, there is an increased risk of mild delays in development at 1 year when compared with children conceived by routine IVF or conceived naturally. These findings support the need for ongoing developmental follow-up of children conceived by ICSI to see whether they are at increased risk of intellectual impairment or learning difficulties at school age.

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## Introduction

In 1992 and 1993 there were reports<sup>1,2</sup> of success with intracytoplasmic sperm injection (ICSI) for couples for whom fertilisation and pregnancy rates with conventional in-vitro fertilisation (IVF) had been poor. Because of these reports, ICSI has become the treatment of choice in many centres around the world for severe male infertility. Despite the rapid acceptance of this technique, there is little information about the long-term outcome of children conceived by ICSI.

The ICSI procedure involves fertilisation by injection of a single sperm directly into an oocyte, often with spermatozoa with impaired mobility and morphology. These defects may reflect an underlying abnormality in the sperm, therefore, use of these sperm may lead to an increased incidence of abnormalities in the child. Also, infertile men with oligozoospermia or azoospermia have an increased incidence of chromosomal anomalies, including Y-chromosome deletions and abnormal karyotypes that may be transmitted to their children.<sup>3-6</sup> By selecting a single sperm for injection the ICSI technique bypasses the usual process of natural selection which occurs both during natural conception and in conventional IVF, resulting in a greater chance of fertilisation with abnormal sperm. Finally, ICSI involves physical disruption of the cell membrane of the oocyte and introduction of extraneous material into the oocyte, together with the sperm. It is unclear whether this physical disruption has a detrimental effect on the oocyte or developing embryo. There have been few reports<sup>7,8</sup> on the medical outcome of children conceived by ICSI beyond the perinatal period and no reports of formal developmental assessments on these children.

The purpose of this study was to assess the medical and developmental outcome at 1 year of a cohort of children conceived by ICSI and to compare the physical and developmental outcome of these children with children conceived by conventional IVF and children conceived naturally.

## Patients and methods

Children enrolled into our study were conceived by ICSI in the North Shore assisted reproductive technology programme in Sydney, Australia. The first ICSI pregnancy through this programme occurred in May, 1993. All children conceived by ICSI over 25 months from May, 1993, through to the end of June 1995, were enrolled in a prospective study of outcome. Informed consent was obtained from the parents of all participants. The indication for ICSI was the presence of a triple-sperm defect with a sperm density of less than 10 million spermatozoa per mL, sperm motility less than 35%, and normal sperm morphology present in less than 20% of sperm. Many of these couples also had a history of failed fertilisation with conventional IVF, therefore, it was not possible to randomise

	ICSI (n=89)	IVF (n=84)	Natural conception (n=80)	p
<b>Babies</b>				
Boys	50 (56)	43 (51)	40 (50)	0.690
Twins	20 (22)	24 (29)	20 (25)	0.651
Gestational age (SD, weeks)	38.8 (2.1)	38.3 (2.1)	38.7 (2.3)	0.222
Gestation <37 weeks (SD; weeks)	12 (13)	11 (12)	13 (16)	0.820
Birthweight (SD; g)	3179 (581)	3107 (655)	3222 (628)	0.491
Birthweight <2500g (SD)	9 (10)	12 (14)	8 (10)	0.610
Birth length (SD; cm)	49.4 (2.6)	48.8 (3.3)	49.3 (3.5)	0.473
Birth head circumference (SD; cm)	34.3 (1.7)	34.3 (1.8)	34.5 (1.8)	0.810
Admitted to NICU	26 (29%)	18 (21%)	18 (23%)	0.582
<b>Mothers</b>				
Age (SD; years)	33.2 (4.0)	33.6 (3.2)	32.1 (2.6)	0.013
Education <10 years	16 (18%)	16 (19%)	5 (6%)*	0.039
Education >15 years	35 (39%)	32 (38%)	43 (54%)*	0.011
Unskilled occupation	7 (8%)	8 (10%)	6 (8%)*	0.738
NESB	27 (30%)	20 (24%)	10 (13%)*	0.02
<b>Fathers</b>				
Age (SD; years)	38.2 (6.4)	36.3 (5.3)	34.8 (4.6)*	0.0004
Education <10 years	17 (19%)	9 (11%)	8 (10%)	0.234
Education >15 years	46 (52%)	37 (44%)	43 (54%)*	0.420
Unskilled occupation	14 (16%)	3 (4%)*	4 (5%)*	0.008
NESB	28 (31%)	23 (27%)*	7 (9%)*	0.001

\*Significant difference compared with ICSI group using Bonferroni multiple comparison procedure. NICU=neonatal intensive care or special care baby unit. NESB=born in non-English speaking country.

Table 1: Neonatal and demographic characteristics

ICSI or routine IVF to patients. The use of epididymal sperm was introduced late in the study period and was only used successfully for two couples in the study. Details regarding sperm preparation and micromanipulative procedures used during this study have been described previously.<sup>9</sup>

During our study a total of 643 embryo-transfer procedures (383 fresh, 260 frozen, maximum of two embryos per procedure) were done from a total of 771 ICSI cycles. These embryo transfers resulted in 108 pregnancies (70 fresh, 38 frozen). A total of 26 early pregnancies were lost (12 pregnancy-test-positive pregnancies and 14 first trimester abortions), with 82 pregnancies continuing beyond 20 weeks. Of these 72 were singleton, and ten were twin pregnancies, resulting in 92 livebirths. These 92 babies formed the ICSI cohort for this study.

Children conceived by routine IVF and naturally, were recruited between September, 1992, and September, 1995, as part of a separate study assessing cognitive development and psycho-social adjustment of parents and their IVF concerned children during the first year of life.<sup>10-12</sup> Children conceived by IVF were enrolled by approaching women who had conceived by conventional IVF in the North Shore assisted reproductive technology programme at 28-30 weeks of pregnancy. 73 (80% acceptance) women agreed to participate. Of these pregnancies, 30% were the result of transfer of frozen embryos. Among the participants, 60 had singleton pregnancies and 13 had twin pregnancies.

Children conceived naturally were recruited by approaching women who were 28-30 weeks pregnant and were attending the Royal North Shore Hospital for obstetric care. To match the parental age, parity, and multiplicity of pregnancy of the babies conceived by natural conception with the babies conceived by ICSI and routine IVF, only older (>27 years) primiparous women were invited to participate in the study. After recruitment of 62 women (70% acceptance) with singleton pregnancies, ongoing recruitment of women with twin pregnancies continued until ten women with twin pregnancies conceived through natural conception had been recruited.

At birth, all children were assessed by a paediatrician or hospital doctor and information regarding the child's birth and neonatal status was recorded in a personal-health record book which is provided for all children born in New South Wales. The parents of ICSI, IVF, and naturally conceived children were contacted soon after birth, and information was collected on the babies' sex, gestational age, birthweight, length, head circumference, Apgar scores at 1 min and 5 min, need for

	ICSI (n=89)	IVF (n=84)	Natural conception (n=80)	p
<b>General health</b>				
Major malformations	4 (5%)	3 (4%)	4 (5%)	0.865
Hospital admissions	12 (13%)	16 (19%)	21 (26%)	0.112
Number of medical visits (SD)	6.9 (6.2)	7.6 (5.0)	8.0 (5.0)	0.410
<b>Growth</b>				
Weight (SD; kg)	10.3 (1.1)	10.3 (1.3)	10.9 (1.2)	0.067
Weight z score (SD)	0.3 (0.9)	0.2 (1.1)	0.7 (1.03)	0.124
Length (SD; cm)	76.9 (2.9)	76.6 (3.7)	77.3 (2.5)	0.265
Length z score (SD)	0.4 (0.9)	0.2 (1.4)	0.4 (0.9)	0.192
Head circumference (SD; cm)	47.0 (1.5)	47.4 (1.7)	47.6 (1.3)	0.609
Head circumference z score (SD)	0.8 (1.2)	1.2 (1.2)	1.4 (1.1)	0.277
<b>Bayley scores</b>				
MDI	95.9 (10.7)	101.8 (8.5)*	102.5 (7.6)*	<0.0001†
PDI	89.8 (16.6)	89.2 (15.1)	88.3 (15.7)	0.861

\*Indicates significant difference compared to ICSI group using Bonferroni multiple comparison procedure, after inclusion of demographic variables as covariates in analysis of variance. MDI=mental development index. PDI=psychomotor development index

Table 2: Outcome at 1 year

admission to a special-care or intensive-care baby unit, and presence of any major malformation noted by the paediatrician or hospital doctor on initial assessment.

Demographic information on the families, including parents' age, education level, occupation, country of origin, language spoken at home, maternal work status, childcare arrangements 1 year after the child's birth, and family history of medical and developmental problems was collected for all children.

The children were formally assessed at 1 year, with children who were born prematurely being assessed at 1 year corrected age. Information regarding major malformations or other major health problems diagnosed over the first year of life was collected by taking a history from the family, reviewing the child's personal-health record book, and when necessary, reviewing the child's hospital records. The children were given a physical assessment and their weight, length, and head circumference were measured. To standardise scores for children assessed at different ages, weight, length, and head circumference z scores (or SD scores) were calculated with the formula:<sup>13,14</sup>

$$\frac{\text{Individual's value} - \text{mean value for reference population}}{\text{SD for reference population}}$$

Major malformations were classified as malformations that generally cause functional impairment or require surgical correction, according to Bonduelle<sup>8</sup>

A formal developmental assessment was done on all children at 1 year with the Bayley Scales of Infant Development (2nd edition) (BSID-II).<sup>15</sup> The BSID-II was chosen because of its sound psychometric properties which enable the identification of performance differences among research groups, and its extensive restandardisation in 1991-92, which has resulted in an up-to-date, valid, and reliable test of infant development. The BSID-II assesses the current developmental functioning of infants and young children and consists of two major scales. The mental scale includes items that assess memory, problem solving, and language skills, and the motor scale assesses control of the gross and fine muscle groups. The child's performance on these scales is used to determine a mental development index (MDI) and psychomotor development index (PDI). The mean score for the MDI and PDI is 100 (SD 15). Performance on the scales is classified as follows: 115 or more as accelerated performance; 85-114 as within normal limits; 70-84 as mildly delayed performance; and 69 and less as significantly delayed performance.

The assessment at 1 year on each child conceived by ICSI was done by JB or FG. All of the assessments for the IVF and natural conception controls were done by FG. Inter-rater reliability for the two investigators using Kendall's  $\tau$  correlation for the Bayley's MDI was 0.953.

Statistical analysis was done with the statistical package SPSS for Windows 6.1. Between-group differences were analysed with

$\chi^2$  for categorical variables and analysis of variance for continuous variables. When analysis of variance revealed a difference within the three groups, the Bonferroni multiple comparisons procedure was done as a post-hoc comparison to determine which means were significantly different from each other.

ICSI, IVF, and natural conception groups were first compared for differences between perinatal and demographic variables. Where between-group differences were found, these variables were then included as covariates in the subsequent analyses comparing the groups for outcome at 1 year.

## Results

92 ICSI, 86 IVF, and 82 naturally conceived children were enrolled in the study. Of these 89 (97%) ICSI, 84 (98%) IVF, and 80 (98%) naturally conceived children were fully assessed at 1 year. The remaining seven infants had moved out of New South Wales. All parents were contacted by phone and none of the seven children were reported to have any major health problems or developmental delays. IVF and natural conception control mothers who agreed to participate in the study did not differ from those who declined to participate with regard to age or place of residence. However, additional socioeconomic data on those who did not participate was not available for comparison.

The proportion of children born following transfer of frozen embryos was similar for the ICSI and IVF groups (39% ICSI, 31% IVF). Demographic and neonatal data on the three groups are shown in table 1. We recruited additional naturally conceived twins so that the proportion of twins in each group was similar (22% ICSI, 29% IVF, and 25% naturally conceived). Eight (4%) of the 198 singletons and 28 (44%) of the 64 twins were born prematurely, thus twin pregnancies accounted for 28 (78%) of the premature births. The mean birthweight for the ICSI singletons was 3.38 (SD 0.46) kg and for twins was 2.47 (0.32) kg. There was no difference in the incidence of prematurity, mean birthweight, mean gestational age, or other neonatal parameters among the three groups.

25 (28%) children conceived by ICSI required admission to either a special-care or intensive-care baby unit in the neonatal period, which was not significantly different from the incidence of admission for children conceived by IVF or naturally (22% and 23%, respectively). About half of these admissions were because of prematurity.

On the whole, all three groups had well-educated, older parents, from middle and upper socioeconomic groups. Overall, 50% of fathers and 44% of mothers had 15 years of education or more (University degree or equivalent), 92% of fathers and 91% of mothers had skilled, professional, or managerial occupations; and 91% of fathers and 84% of mothers were aged at least 30 years. Parents of children conceived by ICSI had similar demographic characteristics to those of children conceived by IVF, although fathers of children conceived by ICSI were more likely than fathers of children conceived by IVF to have an unskilled occupation. When parents of children conceived by ICSI were compared with parents of naturally conceived children, we found that mothers of children conceived by ICSI were less well educated and more likely to be born in a non-English-speaking country than mothers of naturally conceived children. Fathers of ICSI-conceived children were older, more likely to have an unskilled

occupation, and more likely to be born in a non-English-speaking country than fathers of naturally conceived children. These differences reflect the demographic differences between the local population of patients attending Royal North Shore Hospital for obstetric care and the broader demographic range among patients attending the North Shore assisted reproductive technology programme.

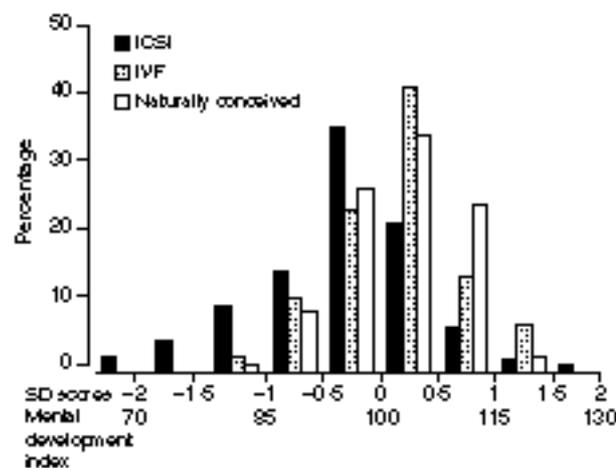
To control for these demographic differences in subsequent analyses, mothers' and fathers' age, occupation, education level, and country of origin were all included as covariates in subsequent analyses of outcome at 1 year.

### Medical outcome at 1 year

Children were seen for their 1-year assessment (table 2) at a mean age of 13.17 months. The mean age at follow-up was similar for each group (ICSI 13.1 [SD 0.9] months; IVF 13.2 [SD 0.7] months; and naturally conceived 13.2 [0.6] months).

There were no significant differences in the incidence of major malformations amongst the ICSI (4.5%), IVF (3.6%), or naturally conceived infants (5%). Two of the infants were from twin pregnancies (one IVF-conceived and one naturally conceived), the remainder were singletons. In the ICSI-conceived children the following malformations were noted: complex congenital heart disease, unilateral structural talipes requiring surgical correction, grade V vesico-ureteric reflux, and pelvi-ureteric junction obstruction of the left ureter. In the IVF-conceived children the following malformations were noted: complex congenital heart disease and craniostenosis, and two children with hypospadias. In the naturally conceived children the following malformations were noted: unilateral structural talipes, atrial septal defect, and two children with lambdoid craniostenosis requiring surgery.

There was no significant difference in the incidence of hospital admissions, major health problems, or visits to medical practitioners over the 12 months after birth. There was no significant difference between the groups in the mean weight, length, or head circumference of the children, or in the proportion of children whose weight, length, or head circumference was more than two SDs below the mean.



Distribution of Bayley MDI scores for ICSI, IVF, and naturally conceived children

*Developmental outcome at 1 year*

At 1 year, children conceived by ICSI had a significantly lower mean Bayley MDI than the IVF or naturally conceived children: ICSI 95.9 (SD 10.7, range 64–123), IVF 101.8 (8.5, 82–122) controls 102.5 (7.6, 82–118;  $p < 0.0001$ ). When data from subsets of boys and girls were analysed, between-group differences remained strong for boys (ICSI 91.4 [SD 10.3], IVF 101.0 [9.0], naturally conceived 101.7 [7.5];  $p = 0.002$ ), but were no longer significant for girls (ICSI 98.0 [11.0] IVF 102.7 [7.9], naturally conceived 103.3 [7.7];  $p = 0.057$ ), although there was a similar trend to lower scores among the ICSI girls. Children conceived by ICSI also had a significantly lower mean MDI than the groups of IVF-conceived or naturally conceived children when only singletons were considered (ICSI 97.0 [10.9] IVF 102.7 [8.2], naturally conceived 103.2 [7.0],  $p = 0.002$ ); and when only twins were considered (ICSI 92.0 [9.0], IVF 99.6 [8.9], naturally conceived 100.6 [9.1],  $p = 0.03$ ).

Figure 1 shows the distribution of MDI scores for the three groups. Although the developmental performance of most of the children conceived by ICSI was within the normal range, there was a shift to the left in the distribution of scores among the ICSI group, with 15% of the ICSI children in the mildly delayed range (1–2 SDs below the mean; MDI 70–84), and a further 2% with significantly delayed performance (>2 SDs below the mean, MDI <70). This developmental performance is in contrast to the IVF and naturally conceived groups where only 2% and 1%, respectively, were in the mildly delayed range and none were in the significantly delayed range ( $p < 0.0001$ ). Of the 15 children conceived by ICSI with an MDI of less than 85, nine were boys and six were girls.

Because children conceived by ICSI differed from both IVF and naturally conceived children in being more likely to have fathers with an unskilled occupation, we did a subset analysis on those children whose fathers had a managerial, professional, or skilled occupation and excluded all infants whose fathers had an unskilled occupation. Among this subset we continued to find the same between-group differences, with 12 (16%) of 75 ICSI infants having an MDI in the mildly or significantly delayed range compared with one (1%) of 81 IVF infants, and one (1%) of 73 naturally conceived infants (Fisher's exact test,  $p < 0.0001$ ).

There was no significant association at 1 year between the type of conception and mean Bayley PDI (ICSI 89.8 [SD 16.6], IVF 89.2 [15.1], and naturally conceived 88.3 [15.7],  $p = 0.861$ ), although it was noted that the mean PDI for all three groups was lower than for the Bayley standardisation sample.

## Discussion

Although most of the children conceived by ICSI in our study had Bayley MDI scores within the normal range, as a group they scored significantly lower than the IVF and naturally conceived children, with an average reduction of six points on the MDI and an increased proportion of ICSI children with MDI scores in the mildly delayed range at 1 year compared with the IVF and natural-conception groups.

Previous follow-up studies<sup>16–21</sup> on the outcome of children conceived by conventional IVF have shown no difference in the incidence of major malformations or

developmental outcome in children conceived by IVF compared with non-IVF controls. The introduction of ICSI as a new form of IVF, however, has raised new questions regarding the outcome of children conceived by this technique because of additional risks related to the invasive nature of the ICSI procedure and the use of poor-quality sperm.

Early reports of outcome after ICSI<sup>7,8,22–25</sup> have provided some reassurance regarding the medical outcome of these children, although a re-evaluation of some of these results has provided a less reassuring interpretation.<sup>26</sup> The incidence of major malformations detected at birth or in the perinatal period has been reported by several authors<sup>22,23,25,27</sup> to range from 0.95% to 3.6% of births after ICSI or other forms of microinsemination. It has been generally concluded by these authors that the incidence of major malformations following ICSI is not significantly different from that observed after standard IVF,<sup>8,25</sup> or to that expected in the general population.<sup>22,23</sup>

Our study supports these findings regarding the medical and perinatal outcome of children conceived by ICSI. Among the infants in our study there was no significant difference in the incidence of major malformations or major health problems in the first year of life in the children conceived by ICSI compared with the IVF or with naturally conceived children.

In contrast, there were significant differences between the infants conceived by ICSI and the IVF and naturally conceived infants found on developmental assessment at 1 year. Children conceived by ICSI were found to have an increased incidence of mild delays on the Bayley MDI, which assesses memory, problem solving ability, and language skills. There was no difference between the groups in the children's performance on the PDI, which assesses motor skills, although all groups had a lower mean performance than expected on test norms, possibly due to differences in racial mix among our Australian population compared with the population in the USA.

Although mild developmental delay at 1 year is not always strongly predictive of later intellectual impairment,<sup>28,29</sup> tests that assess cognitive or mental skills are generally felt to be more predictive of long-term outcome than tests that assess motor skills.<sup>30</sup> Our finding of an increased proportion of children in the mildly delayed range at 1 year on the Bayley MDI raises concerns regarding the developmental potential of children conceived by ICSI compared with IVF and naturally conceived children.

Possible reasons for poorer performance among the children conceived by ICSI include factors related to genetic abnormalities in the sperm, as well as factors relating to the ICSI procedure itself. Cytogenetic studies of subfertile men have shown an increased risk of chromosomal abnormalities (eg, sex chromosome abnormalities, balanced structural rearrangements, and meiotic chromosomal abnormalities).<sup>3–6</sup> Although some of these chromosomal abnormalities can be detected on standard cytogenetic testing it is possible that other genetic alterations may not be detectable on routine screening.<sup>31</sup> Karyotypes done on children conceived by ICSI have found an increased incidence of paternally transmitted structural aberrations and sex-chromosome abnormalities.<sup>22,32,33</sup> Liebaers and colleagues<sup>22</sup> reported results of prenatal karyotypes among a cohort of children

conceived before February, 1995. Of the 491 prenatal karyotypes, 12 (2.4%) were abnormal. Six were considered to be benign structural aberrations such as inversions or translocations inherited from the father. Of the other six, five (1.0%) were sex-chromosome aneuploidies, and one was a trisomy 21. The incidence of sex-chromosome aberrations was higher than expected as the population incidence of these aneuploidies is reported to be less than 0.3%.<sup>34</sup> We did not do cytogenetic tests on most of the children and it is therefore possible that the mild delays in development found in some of the children in this study may have been due to chromosomal abnormalities. Our finding that developmental differences were greater for boys than for girls in the ICSI group adds support to the possibility of transmission of chromosomal anomalies from father to son. Even when cytogenetic testing has been done and found to be normal, this does not exclude the possibility of an undetected chromosomal abnormality that may influence childhood development.

Incomplete or disturbed imprinting may be another source of concern in the use of abnormal sperm. Although the relative contribution of gamete methylation to genetic imprinting and regulation of gene expression is still not fully understood, it seems likely that a disruption in this process may contribute to later developmental abnormalities.<sup>35</sup> Whether immature or abnormal sperm may be incomplete or disturbed with regard to the process of imprinting needs to be clarified.

Because the ICSI procedure bypasses the usual process of natural selection of sperm selection of sperm with minor genetic abnormalities may occur that may have an influence on infant development resulting in an increased incidence of children with mild developmental delays. In natural conception or routine IVF, the sperm must penetrate the zona pellucida and cell membrane of the oocyte and it is theoretically possible that sperm with minor genetic abnormalities may be less efficient at accomplishing this task than sperm without these abnormalities.

Other factors in this study which may have influenced the outcome include a lack of blinding and demographic differences between the groups. Because the IVF and natural-conception controls were originally recruited as part of a separate study to compare IVF parents and children, the investigators were aware of the children's status. Although this may potentially have influenced the outcome, there was no evidence of consistent bias in the results, with only the MDI varying between groups. Because all participants had the BSID-II, with a standardised format, and with high inter-rater reliability between the examiners, we believe that the results found in this study are because of true differences between the groups, rather than investigator bias.

Because demographic differences may also influence developmental outcome, we collected information on a number of demographic variables for the three study groups. On the whole, all groups (including the ICSI group) had well educated, older parents from middle and upper socioeconomic groups. Although demographic differences were found between the groups they do not appear to account for the differences in developmental outcome seen at 1 year.

Our findings support for the need for developmental follow-up of children conceived by ICSI to determine whether they are at increased risk of intellectual impairment or learning difficulties at school age.

### Contributors

Jennifer Bowen was the principal investigator and was responsible for the study design, assessment of the ICSI-conceived children, statistical analysis, and preparation of the manuscript. Frances Gibson was responsible for the study on IVF and naturally conceived children, from which the controls for this study were recruited. She also did the assessments on the IVF, control, and some of the ICSI-conceived children, and supervised the assessments on the remainder of the ICSI children. Garth Leslie conceived the study, helped with study design, and collected perinatal data on the children. Douglas Saunders arranged for recruitment of IVF and ICSI-conceived patients, provided medical information on the ICSI and IVF pregnancies and supported the study design. All investigators contributed to the final manuscript.

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# A review of known imprinting syndromes and their association with assisted reproduction technologies

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**An association between assisted reproduction technologies (ART) and abnormal genomic imprinting in humans has been recognized for several years; however, the magnitude of this risk and the spectrum of imprinting syndromes to which the risk applies remains unknown. Nine human imprinting syndromes have been identified but current evidence links ART with only three: Beckwith–Wiedemann syndrome, Angelman syndrome and the newly described maternal hypomethylation syndrome. There is currently a lack of evidence linking ART with the remaining six imprinting syndromes: Prader–Willi syndrome, Russell–Silver syndrome, maternal and paternal uniparental disomy of chromosome 14, pseudohypoparathyroidism type 1b and transient neonatal diabetes. Evidence from clinical reports suggests that the association between imprinting syndromes and ART may be restricted to syndromes where the imprinting change takes the form of hypomethylation on the maternal allele. In contrast, studies of gametes and early embryos suggest that ART can be associated with hypermethylation as well as hypomethylation, with imprinting changes occurring on paternal as well as maternal alleles. The health effects of ART-associated imprinting changes may also extend beyond the nine recognized imprinting syndromes.**

**Keywords:** assisted reproduction; imprinting syndromes; Beckwith–Wiedemann syndrome; Angelman syndrome; maternal hypomethylation syndrome

## Introduction

It is now five years since a series of clinical studies raised concern about a link between assisted reproduction technologies (ART) and two imprinting syndromes, Beckwith–Wiedemann syndrome (BWS) (DeBaun *et al.*, 2003; Gicquel *et al.*, 2003; Maher *et al.*, 2003b; Halliday *et al.*, 2004) and Angelman syndrome (AS) (Cox *et al.*, 2002; Orstavik *et al.*, 2003). These studies relied on case records and questionnaire data, and a control group was used in only one study (Halliday *et al.*, 2004). For these reasons, subsequent interpretation and utilization of these data in the clinical setting has been difficult. Several subsequent clinic-based and population-based studies have attempted to strengthen the epidemiological links between imprinting syndromes and various types of ART including *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) (Lidegaard *et al.*, 2005; Sutcliffe *et al.*, 2006; Bowdin *et al.*, 2007; Doornbos *et al.*, 2007). Unfortunately, these studies have generally been underpowered or have suffered from other methodological difficulties, and there remains a lack of reliable data on the relative and overall risk of imprinting syndromes in children conceived using ART. It is also unclear whether a risk applies to all imprinting syndromes or just a subset thereof. Here we

provide a review of known imprinting syndromes, and examine the evidence for each being associated with ARTs.

## ***Diverse genetic and epigenetic changes underlie imprinting syndromes***

Genomic imprinting is the modification of the genome so that genes from only one (rather than two) parental alleles are expressed. The mechanism underlying imprinting is epigenetic, occurring via changes in DNA methylation and histone modifications rather than through alterations in DNA sequence. Among imprinted genes, those that are paternally expressed tend to promote growth whereas those that are maternally expressed tend to suppress growth, leading to the hypothesis that genomic imprinting may have evolved as a parental 'battle of the sexes' to regulate the maternal allocation of resources to the offspring (Moore and Haig, 1991). Approximately 1% of all human genes are thought to be imprinted, with ~50 imprinted genes identified to date, and an additional 150 genes predicted to be imprinted on the basis of DNA sequence characteristics (Luedi *et al.*, 2007).

Imprinting syndromes are a group of medical conditions that result from the altered expression of genes that are usually imprinted. The mechanisms that alter the expression of

imprinted genes are diverse and can be categorized into three 'genetic' mechanisms and one 'epigenetic' mechanism. The relative contribution of each mechanism varies for each imprinting syndrome. The three genetic mechanisms are: (i) large deletions or duplications of chromosomal regions that contain imprinted genes; (ii) DNA mutations in genes that are usually imprinted or in their imprinting control centers and (iii) uniparental disomy (UPD). In contrast, the epigenetic mechanism involves no alteration in DNA sequence, but changes in DNA methylation and modification of histones (epimutations) that can arise as a result of errors in imprint erasure, establishment or maintenance. Changes in DNA methylation can be further subdivided into four categories, comprising gain of methylation (hypermethylation) and loss of methylation (LOM) (hypomethylation) occurring on either the maternal or paternal allele.

To date, evidence from ART-conceived patients with imprinting syndromes suggests that the increased risk of imprinting syndromes associated with ART is confined to the category of epimutations. The pathway(s) that lead to epimutations in ART-conceived children remain unresolved, but possible contributing factors include the subfertility itself (Ludwig *et al.*, 2005; Doornbos *et al.*, 2007; Kobayashi *et al.*, 2007), the process of ovulation induction (Sato *et al.*, 2007; Fortier *et al.*, 2008), physical interference with embryos during IVF/ICSI/embryo transfer (Rivera *et al.*, 2008) and aspects of the *in vitro* culture of embryos (Doherty *et al.*, 2000; Fauque *et al.*, 2007). There is currently no evidence that genetic mechanisms (DNA mutations or cytogenetic deletions/duplications) are associated with ART, and there is no particular reason to expect that such mutations would favour imprinted genes. Maternal UPD (matUPD) is however associated with advanced maternal age (Kotzot, 2004) and therefore matUPD is expected to occur more commonly in ART pregnancies because women using ART are typically older.

This review will focus on nine recognized imprinting syndromes: AS, BWS, Prader–Willi syndrome (PWS), Russell–Silver syndrome (RSS), maternal and paternal UPD (patUPD) of chromosome 14 (matUPD14, patUPD14), pseudohypoparathyroidism type 1b (PHP-1b), transient neonatal diabetes (TND) and the newly described 'maternal hypomethylation syndrome'. These phenotypes typically result from altered expression of imprinted genes; that is, rather than being expressed from one allele, the imprinted genes are either expressed from two alleles, or not expressed at all. The proportion of cases in which the underlying mechanism is epimutation varies considerably between these syndromes (Table I).

### **Angelman syndrome**

AS (OMIM 105830) affects ~1 in 16 000 children and is characterized by severe intellectual disability, speech impairment, ataxia, a happy demeanor, seizures and microcephaly (Williams and Driscoll, 2007). The most common molecular mechanism underpinning AS is a deletion of 4–6 Mb at 15q11.2–15q13, found in ~70% of AS patients. PatUPD of chromosome 15 accounts for 7% of AS patients and an additional 11% have mutations in the gene *UBE3A*.

Approximately 3% of patients with AS have an 'imprinting defect', evidenced by a paternal-only pattern of methylation but biparental inheritance of 15q11.2–15q13. In a small proportion (10%) of these AS patients, the imprinting defect is actually due to a deletion of the imprinting centre (Buiting *et al.*, 2003), but the remainder are thought to represent epimutations.

ART has been implicated in AS by reports of five ART-conceived patients with epimutation-AS (Cox *et al.*, 2002; Orstavik *et al.*, 2003; Ludwig *et al.*, 2005). Of these, four were conceived using ICSI (Cox *et al.*, 2002; Orstavik *et al.*, 2003; Ludwig *et al.*, 2005) and one using ovarian hyperstimulation alone (Ludwig *et al.*, 2005). A tentative link has also been drawn between AS and subfertility by the report of two patients with epimutation-AS in whom the parents had taken >24 months to become pregnant (Ludwig *et al.*, 2005), and by a third patient with epimutation-AS who was conceived using donor insemination after unsuccessful IVF (Sutcliffe *et al.*, 2006). The link between AS and ART is based on the rarity of AS (1/16 000), the rarity of epimutations as a mechanism of AS (~3%) and the relatively infrequent use of ART as a method of conception (2–3%). These three events are expected to coincide by chance only once every ~20 million births.

Given the rarity of epimutations as a mechanism for AS, the relative risk of epimutation-AS associated with ART would need to be high to have any detectable impact on the incidence of AS in population-based studies; it is therefore not surprising that none has been detected. In a cohort of 63 AS patients ascertained through a Dutch AS support group, there were no AS patients conceived using IVF/ICSI, although three were born following ovulation induction, one by artificial insemination and four following a time-to-pregnancy >12 months (Doornbos *et al.*, 2007). Importantly, none of these patients were documented as having an epimutation. Similarly in a British study of 75 AS patients, none were conceived using IVF/ICSI, although two were conceived using artificial insemination (both had deletion-AS) and one was conceived naturally following previous use of ART (Sutcliffe *et al.*, 2006). Interestingly, the latter patient had an imprinting defect, suggesting the presence of an epimutation.

### **Beckwith–Wiedemann syndrome**

BWS (OMIM 130650) is an overgrowth syndrome estimated to affect 1 in 13 700 children (Shuman *et al.*, 2005). Clinical features are highly variable but include prenatal and post-natal overgrowth, neonatal hypoglycaemia, exomphalos, macroglossia, hemihyperplasia, an increased risk of embryonal tumours (particularly Wilms tumour), but normal intellect.

The majority of BWS patients have an epimutation affecting the maternal allele of one of two differentially methylated regions (DMRs) at chromosome 11p15, DMR1 (regulating the genes *H19* and *IGF2*) and DMR2 (regulating the genes *CDKN1C* and *KCNQ1*). In over half of all BWS patients, the epimutation is hypomethylation at DMR2, whereas 2–7% of patients have hypermethylation at DMR1 (Shuman *et al.*, 2005). The remaining BWS patients have patUPD of chromosome 11p, a cytogenetically visible chromosome abnormality, or a DNA mutation in the gene, *CDKN1C*.

**Table I.** Molecular mechanisms underlying known imprinting syndromes.

	Angelman syndrome	Prader–Willi syndrome	Beckwith–Wiedemann syndrome	Russell–Silver syndrome	MatUPD14 syndrome	PatUPD14 syndrome	Pseudo-hypoparathyroidism 1B	Transient neonatal diabetes	Maternal Hypomethylation syndrome
Prevalence	1 in 16 000	1 in 17 500	1 in 13 700	1 in 100 000	Unknown	Unknown	Unknown	1 in 500 000	Unknown
Molecular mechanism									
Cytogenetic deletion/rearrangement, %	70	70	1–2	<1	Rare	0	0	40	0
Uniparental Disomy, % (UPD)	7 (Pat UPD15)	25 (Mat UPD15)	20 (Pat UPD 11p)	5 (Mat UPD7)	>95 (Mat UPD14)	100 (Pat UPD14)	1 case (Pat UPD 20q)	40 (Pat UPD6)	0
DNA mutation in gene/imprinting centre, %	5–10	<1	10	Unknown	0	0	Most familial cases+some sporadic cases	0	0
Epimutations (total), %	2.5	<1	65	64	1 report	Unrecorded	Unknown	20	100
Maternal hypomethylation, %	<b>2.5, 5 ART patients</b>		<b>50–60, &gt;60 ART patients</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>Possibly some sporadic cases</b>	<b>20</b>	<b>100, 3 ART patients</b>
Maternal hypermethylation, %	0	0	2–7	0	0	0	0	0	0
Paternal hypomethylation, %	0	<1	0	<b>64 (11p), 1 ART patient with RSS-like phenotype</b>	1 report	0	0	0	0
Paternal hypermethylation, %	0	0	0	<b>1 report (7q) (ART patient)</b>	0	0	0	0	0
References	Williams and Driscoll (2007)	Cassidy and Schwartz (2006)	Shuman <i>et al.</i> (2005)	Kagami <i>et al.</i> (2007), Netchine <i>et al.</i> (2007), Saal (2007)	Martin <i>et al.</i> (1999), Temple <i>et al.</i> (2007)	Kotzot and Utermann (2005)	Bastepe <i>et al.</i> (2001), Bastepe <i>et al.</i> (2005), Liu <i>et al.</i> (2005)	Temple <i>et al.</i> (1996), Temple (2007)	Mackay <i>et al.</i> (2006), Rossignol <i>et al.</i> (2006)

ART-conceived patients are shown in bold type.

To date, more than 60 BWS patients who were conceived using IVF/ICSI have been reported (DeBaun *et al.*, 2003; Gicquel *et al.*, 2003; Maher *et al.*, 2003b; Halliday *et al.*, 2004; Chang *et al.*, 2005; Lidegaard *et al.*, 2005; Rossignol *et al.*, 2006; Sutcliffe *et al.*, 2006; Bowdin *et al.*, 2007; Doornbos *et al.*, 2007; Gomes *et al.*, 2007). In the great majority of these patients the underlying molecular mechanism has been hypomethylation on the maternal allele of DMR2. BWS patients have also been reported where conception has been assisted by ovulation induction (Chang *et al.*, 2005; Sutcliffe *et al.*, 2006; Doornbos *et al.*, 2007), and others have been conceived without ART, but following >12 months subfertility (Doornbos *et al.*, 2007); the significance of these observations remains uncertain.

BWS remains the only imprinting syndrome for which data exist to allow an estimation of the relative risk of imprinting syndromes in ART pregnancies. Halliday *et al.* (2004) calculated that BWS patients were approximately nine times more likely to have been conceived by ART than patients without BWS. If we assume that epigenetic mechanisms account for ~65% of BWS cases (Table I), and that the increased risk from ART applies only to epimutations and not to genetic mutations, then it can be estimated that BWS patients with an epimutation are approximately 14 times more likely to have been conceived by ART than patients without epimutation BWS.

#### **Prader–Willi syndrome**

PWS (OMIM 176270) affects approximately 1 in 17 500 children and is characterized by neonatal hypotonia, childhood onset obesity, cognitive impairment, distinctive behavioural characteristics, hypogonadism and a characteristic facial appearance (Cassidy and Schwartz, 2006). There is not a single gene responsible for PWS, but most aspects of the PWS phenotype result from absence of paternal expression of a cluster of non-coding RNAs known as ‘HBII-85’ (Sahoo *et al.*, 2008). In the great majority of PWS patients, the underlying molecular mechanism is either a 4–6 Mb chromosome deletion at 15q11.2–15q13 (70%) or matUPD of chromosome 15 (25%). Epimutations causing PWS are very rare, accounting for <1% of PWS patients, and take the form of hypomethylation on the paternally-inherited allele (Buiting *et al.*, 2003). To date, there have been no reports of patients conceived using ART who have PWS as a result of an epimutation.

Several studies have attempted to find an association between PWS and ART. One study surveyed 163 patients with sporadic PWS and found 9 of the 163 that were conceived using ART (2 using ICSI and 7 using fertility drugs), however, molecular data were available for only 2 patients, both of whom had a chromosome deletion (Sutcliffe *et al.*, 2006). Another study surveyed 86 patients with PWS and found 4 of the 86 were conceived using various forms of ART (2 using IVF/ICSI, 1 using artificial insemination and 1 using ovulation induction); an additional 5 of the 86 reported prior subfertility, however, a 15q deletion was found in all ART-conceived patients for whom molecular data were available (Doornbos *et al.*, 2007). Lidegaard *et al.* (2005) undertook a registry study of 6052 children conceived using ART and did not find any patients with PWS; however, this result must be interpreted

with caution because only 3 PWS patients were detected out of 442 349 controls, suggesting that the study methodology failed to ascertain most PWS diagnoses.

The example of PWS illustrates the epidemiological difficulties in detecting possible ART-associated risks for imprinting syndromes that are themselves very rare. If there were no increase in the risk of epimutation-PWS associated with ART, then even in countries where ART use is common, an ART-conceived baby with epimutation-PWS would be expected to be born only once every ~70 million births. An increase in the risk of epimutations of similar magnitude to that observed for BWS ( $\times 14$  increase) would lead to the birth of a baby with epimutations-PWS only once every 5 million births, a frequency that would be virtually impossible to detect.

#### **Russell–Silver syndrome**

RSS (OMIM 180860) is a disorder of decreased growth that is estimated to affect 1 in 100 000 children. RSS is characterized by intrauterine and post-natal growth retardation plus variable additional features including fifth finger clinodactyly, limb length asymmetry, a typical facial phenotype and variable learning disabilities (Saal, 2007).

RSS differs from other imprinting syndromes in that three distinct imprinted loci on two different chromosomes have so far been implicated. The most important mechanism underlying RSS is hypomethylation on the paternal allele of DMR1 at 11p15 (Gicquel *et al.*, 2005; Netchine *et al.*, 2007), the same locus for which hypomethylation on the maternal allele causes BWS. This epimutation is responsible for over half of all patients with RSS (Gicquel *et al.*, 2005; Eggermann *et al.*, 2006; Netchine *et al.*, 2007). Maternally inherited chromosome duplications involving the 11p15 DMR1 can also cause an RSS-like phenotype (Fisher *et al.*, 2002; Eggermann *et al.*, 2005), and a maternally inherited duplication at the 11p15 DMR2 has been reported in one RSS patient (Schonherr *et al.*, 2007). Approximately 5% of patients with RSS have matUPD of chromosome 7 (Netchine *et al.*, 2007). Two imprinted loci have been implicated in the matUPD7 phenotype, a DMR on 7p12.2 (including the gene GRB10) and a DMR at 7q32.2 (including the gene MEST), however, to date, no patients have been reported with RSS due to epimutations at these loci. Maternally inherited duplications that include the 7p DMR have been associated with an RSS-like phenotype (Joyce *et al.*, 1999; Monk *et al.*, 2000). In ~30% of patients with RSS the underlying mechanism is unknown.

There is currently little evidence linking RSS with ART. To date, there have been only five patients reported with RSS and who were conceived using IVF/ICSI (Svensson *et al.*, 2005; Bliiek *et al.*, 2006; Kagami *et al.*, 2007; Galli-Tsinopoulou *et al.*, 2008), and molecular data are available for only two. One ICSI-conceived girl with an RSS-like phenotype was found to have hypomethylation at the paternal allele of DMR1 on 11p15 (Bliiek *et al.*, 2006) and an IVF-conceived girl with RSS was found to have hypermethylation on the paternal allele of the 7q DMR (MEST) (Kagami *et al.*, 2007). The significance of the latter result is uncertain because MEST hypermethylation is not a recognized cause of RSS, and partial hypermethylation of MEST was also detected in the girl’s father.

**MatUPD14 syndrome**

Approximately 50 patients with maternal UPD14 (matUPD14) have now been reported (Kotzot and Utermann, 2005; Mitter *et al.*, 2006; Kagami *et al.*, 2008), however, matUPD14 may be significantly more common because the phenotype is relatively non-specific and molecular testing is not routinely undertaken. Some matUPD14 patients are detected because of the presence of a Robertsonian translocation involving chromosome 14 (Kotzot and Utermann, 2005).

MatUPD14 is characterized by pre- and post-natal growth retardation, hypotonia, facial dysmorphism, obesity, early onset puberty and variable intellectual outcome (Kotzot and Utermann, 2005). The phenotype is thought to result from altered gene expression at a 14q32 DMR that is usually paternally methylated (Geuns *et al.*, 2007). To date, only one patient has been reported with matUPD14 occurring as a result of an epimutation, paternal hypomethylation at the 14q32 DMR in the presence of biparental inheritance of chromosome 14 (Temple *et al.*, 2007). Three patients have also been described with matUPD14 syndrome resulting from a submicroscopic deletion at 14q32 on the paternal allele (Kagami *et al.*, 2008). There have been no reported ART-conceived patients with matUPD14 syndrome.

**PatUPD syndrome**

PatUPD14 (OMIM 608149) is very rare, with ~30 patients described in the literature to date (Kotzot and Utermann, 2005; Kagami *et al.*, 2008). PatUPD14 is characterized by polyhydramnios, premature labour, skeletal abnormalities including small chest and frequently early death (Kotzot and Utermann, 2005). Like matUPD14, the phenotype is thought to result from altered gene expression at the 14q32 DMR. Although originally defined by the presence of UPD14, more recently some patients with a patUPD14 syndrome-like phenotype have been found to have either sub-microscopic chromosome deletions at 14q32 or epimutations resulting in hypermethylation at 14q32, both involving the maternal allele (Kagami *et al.*, 2008). There has been no report of patUPD14 syndrome resulting from an epimutation, nor has there been report of any patient with patUPD14 syndrome having been conceived using ART.

**Pseudohypoparathyroidism 1b**

PHP-1b (OMIM 603233) manifests as hypocalcaemia and hyperphosphataemia due to resistance to parathyroid hormone. PHP-1b is caused by mutations or epimutations in regulatory regions of the gene *GNAS1*. *GNAS1* encodes the  $\alpha$ -subunit of the stimulatory G protein, and in the proximal renal tubule, transcripts of the  $\alpha$ -subunit are derived only from the maternal allele.

PHP-1b occurs in familial and sporadic forms. The great majority of familial cases result from a genetic mutation, a 3-kb microdeletion located 220 kb upstream of *GNAS1* that results in hypomethylation on the maternal allele (Bastepe *et al.*, 2003; Bastepe *et al.*, 2005). Patients with sporadic PHP-1b also show hypomethylation on the maternal allele of *GNAS1*, but in sporadic PHP-1b the hypomethylation is more extensive and there is usually no microdeletion or detectable alteration in DNA sequence (Bastepe *et al.*, 2005; Liu *et al.*,

2005). It is likely that some sporadic cases of PHP-1b result from an epimutation involving hypomethylation of the maternal allele of *GNAS1* (Liu *et al.*, 2005). To date, only one patient has been described with PHP-1b caused by patUPD of chromosome 20q (Bastepe *et al.*, 2001). There have been no patients with PHP-1b reported to have been conceived using ART.

**Transient neonatal diabetes mellitus**

TND (OMIM 601410) presents in the neonatal period with growth retardation and hypoglycaemia. At 6q24 there exists a DMR that is usually methylated on the maternal allele and unmethylated on the paternal allele and regulates two imprinted genes, *ZAC* and *HYMAI* (Gardner *et al.*, 2000; Arima *et al.*, 2001). TND results from a 'double dose' of the paternal epigenotype, with the underlying molecular mechanism being paternal chromosome duplication in 40% of patients, patUPD6 in 40% and in 20% hypomethylation at the maternal allele of 6q24 DMR (Temple, 2007).

To date, there have been no documented instances of patients with TND born following IVF/ICSI. Sutcliffe *et al.*, (2006) sought conception data from 23 registry-based TND patients and found one patient in whom the parents had previously used IVF; however, this patient was shown to have patUPD6. Mackay *et al.* (2006) also reported two patients with TND who were conceived naturally, but whose parents had previously experienced infertility. Both TND patients had maternal hypomethylation at 6q24, with one also having maternal hypomethylation at other loci (see Section maternal hypomethylation syndrome below).

**Maternal hypomethylation syndrome**

Three recent publications have indicated the existence of a novel imprinting syndrome resulting from maternal hypomethylation at multiple loci (Mackay *et al.*, 2006; Rossignol *et al.*, 2006; Boonen *et al.*, 2008). Rossignol *et al.* (2006) studied 40 BWS patients and found that 10 of them had LOM at loci other than the 11p15 DMR2. Eleven of the 40 BWS patients in the study had been conceived using ART, and three of these exhibited LOM at multiple loci. These results indicate that the maternal hypomethylation syndrome can be associated with, but is not limited to, ART conceptions. Mackay *et al.* (2006) studied 12 patients with TND resulting from maternal hypomethylation at the 6q24 DMR, and found that 6 had hypomethylation at other loci. None of these patients were conceived following ART, although one was conceived following a period of subfertility. A recent report described two siblings with maternal hypomethylation syndrome, exhibiting features of TND and BWS (Boonen *et al.*, 2008). The siblings and their mother were the product of consanguineous relationships, raising the possibility of a novel autosomal recessive defect of the imprinting mechanism being present either in the siblings or their mother.

**Other imprinted loci may be associated with unknown syndromes**

The nine imprinting syndromes outlined above have been defined because they present a recognizable phenotype that is usually present from early life. As noted previously, there are

an estimated 200 imprinted genes in the human genome (Luedi *et al.*, 2007), and the majority of these are yet to be associated with a clinical syndrome or phenotype. Epimutations at some of these loci might contribute to the increased incidence of pregnancy complications and birth defects that have been observed in ART pregnancies (Horsthemke and Ludwig, 2005), while other epimutations might result in more subtle effects, such as altered predisposition to common diseases (e.g. psychiatric disorders, cancers), which could significantly influence the long-term health of ART-conceived children (Maher *et al.*, 2003a).

Follow-up studies examining the health, growth and development of children conceived using ART have generally been reassuring (Knoester *et al.*, 2008; Leunens *et al.*, 2008), although one study found that IVF-conceived children were taller than their naturally conceived counterparts (Miles *et al.*, 2007). Future studies of young adults conceived using ART may be helpful for delineating subtle phenotypes affecting health, growth or behavior that might ultimately be shown to result from aberrant imprinting.

### Summary of clinical data

Table I shows a summary of the clinical data reviewed above, and allows two conclusions to be drawn.

First, evidence of imprinting syndromes resulting from epimutations in ART-assisted pregnancies is so far confined to three syndromes: BWS, AS and the maternal hypomethylation syndrome. It is notable that for all three syndromes the observed epigenetic defect is hypomethylation on the maternal allele of the relevant DMR. This is the same category of epimutation that was found to cause large offspring syndrome in sheep, which results from hypomethylation of the maternal IGF2 receptor gene (Young *et al.*, 2001), and suggests that the risk of imprinting syndromes associated with ART might be confined to the subgroup of imprinting syndromes caused by hypomethylation of the maternal allele; this subgroup also includes TND and PHP-1b. The situation with RSS remains unclear; there is one report of an ART-conceived patient with hypomethylation at the paternal 11p15 DMR1, however, this patient had a 'RSS-like' phenotype that did not meet the diagnostic criteria for RSS (Bliet *et al.*, 2006). A second ART-conceived RSS patient had an unusual epimutation at the 7q32 DMR that is not a recognized cause of RSS (Kagami *et al.*, 2007).

The second conclusion is that an effect of ART is only likely to be detectable for syndromes where epimutations comprise a significant proportion of cases. It is no surprise that the best evidence for an effect of ART on imprinting comes from studies of BWS, a disorder where 65% of patients have an epimutation. For syndromes in which epimutations are infrequent, such as PWS, an effect of ART may be almost impossible to detect, particularly if the increased risk of epimutations is similar in magnitude to that observed in BWS ( $\sim 14\times$ ).

### Lessons from the study of gametes and preimplantation embryos

Additional insight into the association between ART and imprinting syndromes can be gained from studying gametes and preimplantation embryos. Such studies in humans have

considerable limitations because the primary source of oocytes and embryos is infertile couples who by definition are undergoing IVF/ICSI. Although several studies have examined the patterns of gene expression or methylation in human gametes/preimplantation embryos (Huntriss *et al.*, 1998; Monk and Salpekar, 2001; Salpekar *et al.*, 2001; Geuns *et al.*, 2003, 2007), the fact that these samples have been obtained from infertile couples undergoing IVF/ICSI prevents comparison with a suitable control group comprising naturally conceived embryos or naturally ovulated oocytes.

One study was able to study the methylation patterns in superovulated oocytes from infertile women and compare them with the methylation patterns in immature oocytes collected from fertile women undergoing laparoscopic procedures (Sato *et al.*, 2007). Compared with oocytes from fertile women, superovulated oocytes from some infertile women were shown to have hypomethylation at the 7q32 DMR and hypermethylation at the 11p DMR1. It could not be determined whether these epimutations were the result of infertility itself, the ovulation induction process, or maternal age, however similar results were obtained from superovulated oocytes from fertile mice, implicating the ovulation induction procedure (Sato *et al.*, 2007). Consistent with these observations, hypermethylation at the maternal 11p DMR1 has also been detected in human ES cells derived from IVF blastocysts (Li *et al.*, 2005).

Human spermatozoa are more readily available from fertile males, providing a suitable control group, yet there is conflicting evidence regarding the effects of infertility on imprinting in spermatozoa. The 11p15 DMR1, which is normally methylated on the paternal allele, was found by one study to be normally methylated in the spermatozoa of infertile men (Hartmann *et al.*, 2006), however, another study found hypomethylation of the 11p15 DMR1 in infertile men and this also correlated with the severity of oligospermia (Marques *et al.*, 2004). In the same cohort there was no evidence of hypermethylation at the 7q32 DMR, which is normally unmethylated in males (Marques *et al.*, 2004). Kobayashi *et al.* (2007) analysed the methylation profile of seven genes (2 paternally methylated, 5 maternally methylated) in the sperm of infertile men, and found that the sperm of oligospermic men were more likely to carry epimutations than the sperm of normospermic males. Detected epimutations fell into two groups: hypomethylation at loci that are usually methylated in sperm (11p15 DMR1; 14q32 DMR) implies a failure of imprint acquisition at these loci, whereas hypermethylation at DMRs that are usually unmethylated in sperm (7q32, 6q24, 15q DMRs) presumably results from a failure of imprint erasure.

More extensive studies have been possible in mouse embryos because embryos are available from normally fertile mice, in the absence of ovulation induction and *in vitro* culture. In mice, IVF and embryo culture appear to result in a higher frequency of epimutations. Extensive studies of the H19 locus in mouse embryos have yielded inconsistent results: some studies have found paternal hypomethylation (Doherty *et al.*, 2000; Mann *et al.*, 2004), whereas others have found maternal hypermethylation (Khosla *et al.*, 2001; Li *et al.*, 2005). These contrasting results might reflect the use of different mouse strains and culture media. Another

possibility is that the use of pooled embryos in these studies masked individual differences between embryos; a recent study analysing individual embryos found that some exhibited maternal hypermethylation while other had paternal hypomethylation (Fauque *et al.*, 2007). Rivera *et al.* (2008) also studied individual embryos and found that IVF and embryo manipulation resulted in hypomethylation at the paternal H19 locus and hypomethylation at the maternal 11p15 DMR2 locus, the latter being the same epimutation that is associated with BWS in human ART conceptions.

The presence of epimutations affecting both maternal and paternal alleles suggests that defective maintenance of the imprint after fertilization is an important underlying mechanism. This hypothesis is supported by a recent study of methylation patterns in mouse placentae obtained following ovulation induction, which showed hypomethylation at paternal as well as maternal alleles (Fortier *et al.*, 2008). The same epimutations were not observed in the embryos themselves, indicating a failure of imprint maintenance in the placenta, where the mechanism for imprint maintenance appears to be less robust. These findings were not observed in placentae that had not been subjected to *in vitro* culture or manipulation, implicating the ovulation induction process as the cause of defective imprint maintenance.

Although these studies of mouse and human gametes and embryos have yielded inconsistent results, they collectively suggest that epimutations associated with ART: (i) appear to be associated with subfertility, ovulation induction and embryo culture; (ii) can affect both maternally and paternally methylated genes and (iii) can involve either hypomethylation or hypermethylation.

## Concluding comments

Despite emerging evidence of an association between ART and several imprinting syndromes, there is still limited knowledge about the cause of epimutations in ART pregnancies, the syndromes for which a risk applies and the level of risk. For syndromes in which epimutations make up only a small proportion of cases, such as PWS, it is unlikely that evidence of an increased risk associated with ART will ever be obtained. Future studies should focus on syndromes where a significant proportion of cases are caused by epimutations, such as RSS and TND. Given the rarity of all imprinting syndromes, another focus of future studies should be the long-term follow-up of the health of ART-conceived adults. Such studies may shed light on imprinting effects that extend beyond those associated with the recognized imprinting syndromes.

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# Assisted Reproductive Technology and Placenta-Mediated Adverse Pregnancy Outcomes

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**OBJECTIVE:** To estimate whether the use of specific types of assisted reproductive technology (ART) is associated with an increased risk of placenta-mediated pregnancy complications, which include preeclampsia, stillbirth, small for gestational age at birth, and placental abruption.

**METHODS:** A population-based retrospective cohort study was conducted on singleton pregnancies conceived by different types of ART based on the 2004–2007 Ontario Niday Perinatal Database. Patients with fetal anomalies and maternal health problems were excluded as important confounders. Three exposed groups were created by the subtype of ART, including in vitro fertilization with or without intracytoplasmic sperm injection, intrauterine insemination, and ovulation induction. The non-exposed groups were the singleton pregnancies conceived naturally. For each exposed woman, four women from the nonexposed group were randomly matched by maternal age and parity.

**RESULTS:** There were 2,118 exposed participants and 8,420 matched nonexposed participants in the study. The sample size provided 80% power for a relative risk of 2.0 of placenta-mediated adverse pregnancy outcomes with ART. After adjustment of potential confounders, includ-

ing smoking, delivery hospital level, initiating time of prenatal care, average neighborhood income, fetal sex, and previous cesarean delivery, there was no association observed between different types of ART groups and the composite of placenta-mediated pregnancy complications. Intrauterine insemination was associated with a significantly increased risk of preeclampsia (12 [2.67%] odds ratio 2.2, 95% confidence interval 1.04–5.04) compared with the corresponding control group (23 [1.29%]).

**CONCLUSION:** Assisted reproductive technology is not associated with an increased risk of the composite outcome of placenta-mediated pregnancy complications among singleton pregnancies.

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**LEVEL OF EVIDENCE: II**

The widespread application of assisted reproductive technology (ART) has remarkably increased the pregnancy rate for subfertile patients in the past 30 years. In the United States, nearly 1% of total births and 18% of multiple gestations were associated with ART.<sup>1</sup> Unlike earlier researches, in recent years the increased number of studies have begun to focus on the obstetric, perinatal, and long-term outcomes after ART rather than the pregnancy rate alone.<sup>2,3</sup> Based on these studies, there was consensus in a workshop in 2005 held by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development that patients should be informed of the unfavorable perinatal mortality and morbidity followed by ART compared with natural conception.<sup>4</sup> It has been widely accepted that the ART-related increased risk for adverse obstetric and perinatal outcomes is mainly due to the high frequency of multiple pregnancies.<sup>5</sup> However, a series of studies has also demonstrated that the singleton pregnancies after ART treatment are at high risk of poor perinatal outcomes when compared with spontaneously achieved pregnancies although

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the etiology for this is still unknown.<sup>6,7</sup> One possible explanation is that ART may adversely affect the maternal–fetal interface and early phase of placentation.<sup>8</sup> There is some evidence to suggest that the techniques and processes in ART, such as the transfer of the conceptus to uterine cavity through cervix, and the altered hormonal environment during maternal adaptation to pregnancy and its effect on the local endometrium, may interfere with the development of maternal–fetal interface at the early stage of implantation consequently resulting in placenta insufficiency and corresponding adverse perinatal outcomes.<sup>8,9</sup> In addition, Jauniaux et al<sup>10</sup> and Daniel et al<sup>9</sup> investigated the pathologic features in singleton pregnancies after ART and found that placentas after ART had significantly higher weight, appear thicker, and had a higher incidence rate in abnormal placental shapes and cord insertion than placentas from spontaneously conceived pregnancies. So it is plausible to consider that ART may be associated with placenta-mediated pregnancy complications.

Placenta-mediated pregnancy complications comprise a group of diseases, including preeclampsia, intrauterine fetal restriction, pregnancy loss and placenta abruption.<sup>11,12</sup> These conditions have similar pathophysiologic mechanisms attributable to diseased placental vessels, abnormal placental shapes, inadequate uteroplacental circulation, or abnormal placental placentation.<sup>13</sup>

This purpose of this study was designed to compare the risk of placenta-mediated pregnancy complications in singleton pregnancies conceived by different types of ART with matched spontaneously conceived pregnancies and to explore whether there is relationship between ART and placenta-mediated pregnancy complications.

## MATERIALS AND METHODS

This study was approved by the Ottawa Hospital Research Ethics Board. The data in this retrospective cohort study were from 2004 to 2007 Ontario Niday Perinatal Database, which is web-based data entry, and perinatal data from 82 participating sites including both hospitals and midwife groups were collected. Sites could either enter data directly into the database (64 sites) or upload data from their own database (18 sites). Data can be downloaded and analyzed by applicants with passwords. The database is managed by Ontario Perinatal Surveillance System steering committees and has included more than 95% of total births in Ontario, Canada. Extensive sets of data quality checks are included in every data entry module. A user guide provided the definitions for each variable to make sure the consistency for the different

participants. A final set of checks is done by a well-trained analyst to ensure data quality. Each organization received training to manage the system's data entry and reporting capabilities.

The prenatal database provided extensive prenatal information, including maternal demographic information, maternal behavior, maternal health problems, maternal complications, intrapartum complications and interventions, birth outcomes, and infant health. In the database, the province was divided into 16 residence areas according to geographic location, and delivery hospitals were divided into four different levels—small community hospital, large community hospital, teaching hospital, and midwife group.

All the patients with multiple gestations, maternal health problems, and fetal anomalies were excluded from this study because they might be important confounding variables in the study. Maternal health problems in this database included alcohol dependence syndrome, asthma, chronic hypertension (hypertension that predates the pregnancy or was diagnosed before the 20th week of gestation), insulin-dependent and non-insulin-dependent diabetes mellitus, heart disease (any preexisting cardiac disease including dysrhythmia, congenital anomalies, etc), hepatitis B, human immunodeficiency virus (HIV), lupus, psychiatric disorder, thyroid disease (hypothyroidism, hyperthyroidism). The exposed group was the singleton pregnancies conceived through different types of ART treatment. In this study, three exposed groups were set up based on the types of ART available in the database, which are in vitro fertilization (IVF-ET) with or without intracytoplasmic sperm injection (ICSI) (IVF-ET is the spontaneous fertilization of eggs by sperm in vitro and then embryos are transferred into uterus through the cervix; IVF/ICSI is a procedure in which a single sperm was injected directly into an egg with a micromanipulator in vitro), intrauterine insemination (IUI, a fertilization procedure in which sperm are washed, concentrated, and injected directly into a women's uterus), ovulation induction (involves the use of medication to stimulate one or more mature follicles). The nonexposed group consisted of singleton pregnancies conceived naturally. For each exposed patient, four patients from the nonexposed group were randomly matched by maternal age (within 2 years) and parity.

The primary outcome was a composite of placenta-mediated pregnancy complications defined as one or more of preeclampsia, small for gestational age (SGA, less than the 10th percentile for growth), placental abruption (premature separation of a normally implanted placenta after the 20th week of gestation and before the fetus is delivered) or stillbirth



(death of fetus at less than 20 weeks of gestation). Secondary outcomes were the individual outcomes of the composite of placenta-mediated pregnancy complications, ie, preeclampsia, placenta abruption, stillbirth, and SGA.

We first described the distribution of maternal and fetal information (maternal age, parity, smoking during pregnancy, initiating time for prenatal care, delivery hospital level, fetal sex, average neighborhood income) among three exposed groups and the nonexposed group. Then the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of placenta-mediated pregnancy complications related to the three types of ART were estimated through conditional logistic regression. Potential confounding variables in the multivariable regression model, such as smoking during the pregnancy, delivery hospital level, initiating time of prenatal care, average neighborhood income, fetal sex, and previous caesarean delivery, were further adjusted. To a study of large sample size with strong statistical power, a small difference that could be easily detected and considered as having statistical significance might not be clinically meaningful. To guarantee the marked association between different types of ART and adverse pregnancy outcomes to be detected, OR greater than 2.0 was chosen to represent a clinically meaningful association. Sample size was calculated with the assumption of  $\alpha=5\%$ , power  $(1-\beta)=80\%$ , relative risk of 2.0 in the exposed group. The rate for the composite of placenta-mediated adverse pregnancy outcomes in the control group was estimated to be 15%. This assumed an SGA prevalence of 10%, preeclampsia of 3.5%, fetal demise of 0.5%, and placental abruption of 0.9%.<sup>14</sup> Therefore, any sample with 2,118 exposed and 8,420 unexposed patients will have adequate power. All analyses were performed using SAS-PC statistical software 9.1 (SAS Inc., Cary, NC).

## RESULTS

From 2004 to 2007, there were 2,118 exposed patients and 8,420 matched nonexposed patients in the database. In total, almost 99% of exposed patients were matched with 4 nonexposed subjects. The exposed patients were further divided into three subgroups of 870 IVF, 471 IUI, and 777 ovulation induction. Three corresponding matched nonexposed groups were created. The maternal and fetal characteristics of study groups are shown in Table 1. The exposed patients had tendency to be older (aged more than 30 years), nulliparous, and nonsmoking, had initiating prenatal care in the first trimester, had higher economic in-

come, and delivered in the large community hospital and teaching hospital. Significant differences were found in smoking during the pregnancy, initiating prenatal care in the first trimester, delivery hospital level, and average neighborhood income between exposed groups and corresponding nonexposed groups.

Table 2 demonstrated the prevalence, ORs, and 95% CIs of the composite of placenta-mediated pregnancy complications, preeclampsia, SGA births, stillbirth, and placenta abruption for three study groups and the corresponding patients in the control group. After further adjusting for smoking during the pregnancy, initiating prenatal care in the first trimester, delivery hospital level, and average economic incomes, there was no association between different types of ART groups and the composite of placenta-mediated pregnancy complications. As for as secondary outcomes were concerned, the uses of IVF/ICSI and ovulation induction were not associated with the increased risk of preeclampsia, SGA birth, stillbirth, and placenta abruption. Although there was a statistically significant increase in SGA for the ovulation induction group, the strength of the association was weak with an OR of 1.40 (95% CI 1.06–1.86). The patients who underwent IUI were 2.2 times more likely to have preeclampsia (95% CI 1.04–5.04) compared with the corresponding nonexposed group. However, the use of IUI did not increase the risk of stillbirth, placenta abruption, and SGA.

## DISCUSSION

Our population-based study suggested that there was no significant association between different types of ART and the composite of placenta-mediated pregnancy complications. The sample size in the study was more than enough to capture a relative risk of 2.0 with 80% power for the composite of adverse pregnancy outcomes after ART. For the secondary outcomes, there was no increased risk in preeclampsia, SGA, placental abruption, or stillbirth with IVF/ICSI, but there was a slightly increased risk in SGA with ovulation induction and a significantly increased risk in preeclampsia with IUI.

The results of our study were not consistent with those of Jackson et al.<sup>15</sup> The meta-analysis, which included 12,283 IVF pregnancies and 1.9 million naturally conceived pregnancies for singletons, revealed that preeclampsia, SGA, and stillbirth were significantly associated with the IVF group with an OR of 1.55, 2.19, and 2.55 respectively. Nevertheless, biases brought from individual studies with different study designs, populations, definitions of outcomes, and obstetric management were the important limita-



**Table 1. Maternal and Fetal Characteristics of the Study Population**

	IVF (N=870)	Nonexposed Group 1 (N=3,433)	<i>P</i>	Ovulation (N=777)	Nonexposed Group 2 (N=3,103)	<i>P</i>	IUI (N=471)	Nonexposed Group 3 (N=1,884)	<i>P</i>
Maternal age (y)									
Less than 25 y	10 (1.15)	40 (1.17)	.989	46 (5.92)	181 (5.83)	1.0	11 (2.34)	44 (2.34)	1.0
25–29 y	93 (10.69)	372 (10.84)		223 (28.70)	892 (28.75)		74 (15.71)	296 (15.71)	
30–34 y	319 (36.67)	1,276 (37.17)		329 (42.34)	1,316 (42.41)		199 (42.25)	796 (42.25)	
35 y or more	448 (51.49)	1,745 (50.83)		179 (23.04)	714 (23.01)		187 (39.70)	748 (39.70)	
Smoking in pregnancy									
No	771 (88.62)	2,921 (85.09)	<.001	699 (89.96)	2,647 (86.17)	.017	413 (87.69)	1,621 (86.04)	.145
Yes	68 (7.82)	269 (7.84)		44 (5.66)	203 (6.54)		24 (5.10)	143 (7.59)	
Not reported	31 (3.56)	243 (7.08)		34 (4.38)	226 (7.28)		34 (7.22)	120 (6.37)	
Initiating prenatal care									
First trimester (yes)	591 (67.93)	1,915 (55.78)	<.001	680 (87.52)	1,784 (57.49)	<.001	338 (71.76)	1,094 (58.07)	<.001
First trimester (no)	60 (6.90)	377 (10.98)		54 (6.95)	346 (11.15)		41 (8.70)	202 (10.72)	
Not reported	219 (25.17)	1,141 (33.24)		43 (5.53)	973 (31.36)		92 (19.53)	588 (31.21)	
Delivery hospital level									
Small community	33 (3.79)	298 (8.68)	<.001	61 (7.85)	369 (11.89)	<.001	25 (5.31)	180 (9.55)	<.001
Large community	591 (67.93)	2,459 (71.63)		635 (81.72)	2,239 (72.16)		315 (66.88)	1,335 (70.86)	
Teaching hospital	242 (27.82)	653 (19.02)		76 (9.78)	478 (15.40)		130 (27.60)	351 (18.63)	
Midwife	4 (0.46)	23 (0.67)		5 (0.64)	17 (0.55)		1 (0.21)	18 (0.96)	
Fetal sex									
Male	432 (49.71)	1,776 (51.29)	.27	382 (49.29)	1,592 (51.34)	.31	237 (50.32)	990 (52.60)	.37
Female	437 (50.29)	1,653 (48.21)		393 (50.71)	1,509 (48.66)		234 (49.68)	892 (47.40)	
Parity									
Nulliparous	607 (69.77)	2,404 (70.03)	.88	496 (63.84)	1,984 (63.94)	.96	334 (70.91)	1,336 (70.91)	1.00
Multiparous	263 (30.23)	1,029 (29.97)		281 (36.16)	1,119 (36.06)		137 (29.09)	548 (29.09)	
Income									
Less than 20% quintile	112 (13.49)	721 (21.59)	<.001	108 (14.30)	650 (21.43)	<.001	54 (11.79)	402 (22.05)	<.001
20–40% quintile	152 (18.31)	682 (20.43)		157 (20.79)	600 (19.78)		88 (19.21)	369 (20.24)	
40–60% quintile	188 (22.65)	647 (19.38)		180 (23.84)	578 (19.06)		109 (23.80)	346 (18.98)	
60–80% quintile	168 (20.24)	665 (19.92)		173 (22.91)	585 (19.29)		104 (22.71)	352 (19.31)	
More than 80% quintile	210 (25.30)	624 (18.69)		137 (18.15)	620 (20.44)		103 (22.49)	354 (19.42)	
Previous cesarean delivery									
Yes	72 (8.29)	274 (8.03)	0.79	70 (9.10)	272 (8.85)	0.82	37 (7.86)	142 (7.57)	.83
No	796 (91.91)	3,138 (91.97)		699 (90.90)	2,802 (91.15)		434 (92.14)	1,735 (92.43)	

IVF, in vitro fertilization, IUI, intrauterine insemination.

Data are n (%).

For each exposed woman, four women from the nonexposed group were randomly matched by maternal age (within 2 years) and parity.

tions. In addition, although this meta-analysis study included the studies that controlled for maternal age and parity, other known important confounders, such as smoking, preexisting maternal disease, delivery hospital level, and socioeconomic status, were not controlled for.

Our findings showed there was no increased risk of preeclampsia with IVF/ICSI, which is not consistent with the study of Chen et al.<sup>16</sup> However, the singleton pregnancies were not separated from the twin pregnancies, and some important confounding factors, such as preexisting lupus, thyroid disease, and the information of socioeconomic status, were not controlled for in the study. In the present study, we

found that IUI had a significantly increased risk of preeclampsia. The increase in preeclampsia in the IUI group could be secondary to the use of donor semen. Smith et al<sup>18</sup> demonstrated that there is an increase risk of preeclampsia in women who received donor semen compared with their partners' semen. However, we did not have information on whether it was donor or partner semen in IUI in our dataset.

Although the women after ovulation induction demonstrated a statistically increased risk of SGA compared with corresponding nonexposed group, the association was weak with an OR of 1.4 (less than 2.0). In a prospective study with 34,286 spontaneous gestations, 1,222 pregnancies after ovulation induction



**Table 2. Placenta-Mediated Adverse Pregnancy Outcomes in Different Types of Assisted Reproductive Technology**

	IVF (N=870)	Nonexposed Group 1 (N=3,433)	Adjusted OR (95% CI)	Ovulation (N=777)
Primary outcome	95 (11.73)	399 (12.36)	0.92 (0.73–1.19)	86 (11.48)
Secondary outcomes				
Preeclampsia	11 (1.36)	45 (1.39)	1.12 (0.56–2.22)	8 (1.06)
SGA	74 (8.52)	354 (10.38)	0.83 (0.63–1.10)	84 (10.87)
Placenta abruption	12 (1.48)	23 (0.71)	1.26 (0.54–2.92)	6 (0.80)
Stillbirth	10 (1.15)	19 (0.55)	1.96 (0.84–4.57)	3 (0.39)

IVF, in vitro fertilization; OR, odds ratio; CI, confidence interval; IUI, intrauterine insemination; SGA, small for gestational age. Data are n (%).

Primary outcome is defined as a composite of placenta-mediated pregnancy complications.

Odds ratio and 95% CI adjusted for initiating time of prenatal care, smoking during the pregnancy, delivery hospital level, average neighborhood income, fetal sex, and previous cesarean delivery.

and 554 pregnancies after IVF demonstrated that ovulation induction was not associated with SGA and preeclampsia, which was consistent with our findings, but it was related to a statistically significant increased risk of placenta abruption with an OR of 2.4 and stillbirth with an OR of 2.1.<sup>19</sup> This study also suggested the association between IVF and an increased risk of preeclampsia and placenta abruption. However, this study did not exclude fetal anomalies as our study did, and important confounders, such as preexisting maternal diseases, socioeconomic status, and delivery hospital level, were not controlled for, which may account for the different results with our study.

There are several strengths for our study. The most important is that extensive maternal and newborn information was available in the database, which enables the important confounders to be effectively controlled for. In this study, we first excluded all the patients with multiple pregnancies, fetal anomalies, and many preexisting maternal diseases, such as alcohol dependence syndrome, asthma, chronic hypertension, insulin-dependent and non-insulin-dependent diabetes mellitus, heart disease, hepatitis B, HIV, lupus, psychiatric disorders, and thyroid disease, to explore the effect of ART alone on the placental-mediated pregnancy complication in singletons. Then maternal age and parity, as two major confounding variables, were matched in study groups. Other potential confounders, such as delivery hospital level, fetal sex, smoking, initiating prenatal care in the first trimester, and average neighborhood income were further adjusted in this study. In addition, all the exposure and nonexposure patients were randomly selected from the same source of the database. Therefore, it is less likely to have selection bias in our study. Lastly, unlike most of the previous studies that focus only on the relationship between perinatal outcomes

and in vitro fertilization with or without ICSI,<sup>20,21</sup> the Niday database also enabled us to investigate the link between non-IVF ART including ovulation induction, IUI, and adverse pregnancy outcomes.

Limitations in our study cannot be overlooked. Our study was based on an administrative database, and it was inevitable to encounter administrative errors, such as incomplete and missing data or coding errors. We also had a limited sample size for our secondary analysis. Furthermore, in our secondary analysis, we did not adjust our *P* values for multiple testing. However, this was meant to be an exploratory analysis and hypothesis generating. For example, the ovulation induction of SGA of 1.4 may be a type I error and should be interpreted with caution. Last, the information on maternal obesity, the causes of infertility, time to pregnancy, the medication, and laboratory technique used in the treatment, which may also relate to adverse pregnancy outcomes following ART,<sup>22</sup> were not available in our database for analysis.

There are two possible explanations for the negative finding in our study. First, ART may not have any effect on trophoblastic invasion. Although some studies consistently observed that the placenta from ART-related pregnancies had a significant higher incidence of marginal or velamentous cord insertion, abnormal placenta shape, higher mean placenta weight, and placental-fetal weight ratio, there was no significant difference found in the risk of placental histopathological features, including placenta infarcts, fibrin deposition, chorioamnionitis, fibrinoid necrosis, and lesions of villi between IVF/ICSI-conceived pregnancies and spontaneously conceived pregnancies in singletons.<sup>9</sup> Interestingly, in another study of Prefumo et al,<sup>23</sup> uterine artery Doppler velocimetry at 11–14 weeks of gestation was performed on 31 singleton IVF/ICSI pregnancies and 62 matched spon-



Nonexposed Group 2 (N=3,103)	Adjusted OR (95% CI)	IUI (N=471)	Nonexposed Group 3 (N=1,884)	Adjusted OR (95% CI)
315 (10.77)	1.19 (0.91–1.57)	62 (13.81)	216 (12.20)	1.30 (0.94–1.80)
44 (1.50)	0.72 (0.31–1.68)	12 (2.67)	23 (1.29)	2.28 (1.04–5.02)
273 (8.83)	1.40 (1.06–1.86)	47 (9.98)	193 (10.28)	1.07 (0.76–1.52)
23 (0.78)	1.63 (0.60–4.42)	3 (0.67)	13 (0.73)	1.57 (0.41–6.03)
7 (0.23)	1.66 (0.40–7.01)	8 (1.70)	14 (0.74)	2.48 (0.96–6.38)

taneously conceived pregnancies. There was no difference found in uterine artery Doppler indices between two study groups. The study suggested that ART conception per se is not associated with inadequate or defective trophoblastic invasion of maternal spiral arteries in the early pregnancy, which is postulated to be part of the development of early-onset preeclampsia and intrauterine growth restriction.<sup>17</sup> Furthermore, although our study found IUI was associated with a higher incidence of preeclampsia, there was no association observed between IUI and early-onset preeclampsia. Second, our negative study finding could be a result of having a robust data set and strong study design allowing for adequate adjustment of potential confounders. For example, our study excluded the pregnancies with fetal anomalies, which may also account for an increased risk of SGA, preeclampsia, placenta abruption, or stillbirth in other studies. Psychiatric disorders, average economic income, and delivery hospital level are highly related to the patient treated by ART.

In summary, after controlling for multiple confounding variables, we did not find an increase in the composite of placenta-mediated adverse pregnancy outcomes among singleton pregnancies regardless of the type of ART. The finding of our study may indirectly reflect that ART will not interfere with the development of maternal–fetal interface at the early stage of implantation. Also the findings can be somewhat reassuring to patients undergoing ART treatment who have a singleton pregnancy. Although ovulation induction is associated with a slightly increased risk of SGA in our study, the association was weak by our a priori definitions, and it may be premature for us to recommend an intensive prenatal surveillance (eg, serial ultrasonography to follow-up fetal growth assessment). Future research could focus on pharmacologic interventions, hormone levels,

methods of retrieval, embryo culture, and embryo transfer procedure on pregnancy outcomes.

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# Asthma in children born after infertility treatment: findings from the UK Millennium Cohort Study

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**STUDY QUESTION:** Is asthma more common in children born after subfertility and assisted reproduction technologies (ART)?

**SUMMARY ANSWER:** Yes. Asthma, wheezing in the last year and anti-asthmatic medication were all more common in children born after a prolonged time to conception (TTC). This was driven specifically by an increase in children born after ART.

**WHAT IS KNOWN ALREADY:** Few studies have investigated any association between ART and asthma in subsequent children, and findings to date have been mixed. A large registry-based study found an increase in asthma medication in ART children but suggests underlying infertility is the putative risk factor. Little is known about asthma in children after unplanned or mistimed conceptions.

**STUDY DESIGN, SIZE, DURATION:** The Millennium Cohort Study is a UK-wide, prospective study of 18 818 children recruited at 9 months of age. Follow-up is ongoing. This study analyses data from follow-up surveys at 5 and 7 years of age (response rates of 79 and 70%, respectively).

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** Singleton children whose natural mothers provided follow-up data were included. Mothers reported whether their pregnancy was planned; planners provided TTC and details of any ART. The population was divided into 'unplanned' (unplanned and unhappy), 'mistimed' (unplanned but happy), 'planned' (planned, TTC < 12 months), 'untreated subfertile' (planned, TTC > 12 months), 'ovulation induced' (received clomiphene citrate) and 'ART' (IVF or ICSI). The primary analysis used the planned children as the comparison group; secondary analysis compared the treatment groups to the children born to untreated subfertile parents. Outcomes were parent report of asthma and wheezing at 5 and 7 years, derived from validated questions in the International Study of Asthma and Allergies in Childhood, plus use of anti-asthmatic medications. A total of 13 041 (72%) children with full data on asthma and confounders were included at 5 years of age, and 11 585 (64%) at 7 years.

**MAIN RESULTS AND THE ROLE OF CHANCE:** Compared with planned children, those born to subfertile parents were significantly more likely to experience asthma, wheezing and to be taking anti-asthmatics at 5 years of age [adjusted odds ratio (OR): 1.39 (95% confidence interval (CI): 1.07, 1.80), OR: 1.27 (1.00, 1.63) and OR: 1.90 (1.32, 2.74), respectively]. This association was mainly related to an increase among children born after ART (adjusted OR: 2.65 (1.48, 4.76), OR: 1.97, (1.10, 3.53) and OR: 4.67 (2.20, 9.94) for asthma, wheezing and taking anti-asthmatics, respectively). The association was also present, though reduced, at the age of 7 years.

**LIMITATIONS, REASONS FOR CAUTION:** The number of singletons born after ART was relatively small ( $n = 104$ ), and as such the findings should be interpreted with caution. However, data on a wide range of possible confounding and mediating factors were available and analysed. The data were weighted for non-response to minimize selection bias.

**WIDER IMPLICATIONS OF THE FINDINGS:** The findings add to the growing body of evidence suggesting an association between subfertility, ART and asthma in children. Further work is needed to establish causality and elucidate the underlying mechanism. These findings are generalizable to singletons only, and further work on multiples is needed.

**STUDY FUNDING/COMPETING INTEREST(S):** This study was funded by a Medical Research Council project grant. No competing interests.

**Key words:** infertility / assisted reproduction techniques / asthma / unplanned pregnancy

## Introduction

Asthma is a chronic, complex, obstructive lung disease characterized by acute symptomatic episodes of bronchial restriction, breathlessness and wheezing. The prevalence of childhood asthma is high in the UK: approximately one in five children are diagnosed by a doctor, making it one of the most common chronic childhood conditions (Kaur et al., 1998; Patel et al., 2008). Asthma can limit a child's daily life, social activities and may result in missed school days which then impacts on parents working life (Sennhauser et al., 2005). Children with asthma require more contact with doctors than non-sufferers, and may require medication and hospitalization. In 2003, it was estimated that 1–5-year-old children with wheezing in the UK cost the health service a total of £53 million (Stevens et al., 2003).

There is still limited understanding of the aetiology of asthma but there are many identified risk factors for the condition, including pre-natal, environmental and genetic factors, and gene-by-environment interactions (Subbarao et al., 2009). Pregnancy-related risk factors for asthma include preterm birth, low birthweight and Caesarean delivery. A recent, large registry-based study of Swedish children concluded that those born after assisted reproduction techniques (ART), such as IVF, were more likely to be prescribed anti-asthmatic medication compared with naturally conceived children but the underlying duration of subfertility appeared to be the putative risk factor rather than an effect of treatment (Kallen et al., 2012). Others analysing Scandinavian registry data have also reported a significant increase in the use of asthma medications and hospitalization among children conceived after infertility treatment (Ericson et al., 2002; Koivurova et al., 2007; Finnstrom et al., 2011), though some researchers have found no effect (Pinborg et al., 2003; Klemetti et al., 2006). Smaller clinic-based studies in Turkish and American populations have found no increased risk of asthma after infertility treatment (Cetinkaya et al., 2009; Sicignano et al., 2010). The inconsistent results could be related to variations in participation rates, consideration of confounding factors and differing measures of asthma, such as prescription records or self-report.

In this study we assess the effects of pregnancy planning, time to conception (TTC) and ART on asthma and wheezing in children at 5 and 7 years of age. We present findings from the UK Millennium Cohort Study (MCS), one of the few large observational studies where data are available on conception status, asthma diagnosis and key confounding factors.

## Materials and Methods

### The MCS

The MCS is a nationally representative prospective cohort study of 18 818 children across the UK (Hansen, 2008). A random two-stage sample of all

infants born in 2000–2002, and resident in the UK at 9 months, was drawn from the Department of Social Security Child Benefit Registers. Baseline interviews captured socio-demographic and health data, including information on pregnancy and infertility treatment, and the children were subsequently followed up at 3, 5 and 7 years. Data for 5 and 7 years are presented here. Ethical approval for the Millennium Cohort Study was granted from the multi-centre research ethics committee.

### Conception history

Mothers were asked if they had planned to conceive, and how they felt when they discovered they were pregnant. 'Planners' were then asked how long they took to conceive and if they received fertility treatment. Women were grouped into the following categories:

- (i) unplanned (unplanned, unhappy about pregnancy);
- (ii) mistimed (unplanned, happy about pregnancy);
- (iii) planned (planned, TTC < 12 months);
- (iv) untreated subfertile (planned, TTC > 12 months);
- (v) ovulation induction (OI) (planned, used ovulation inducing drugs such as clomiphene citrate);
- (vi) ART (planned, used ART such as IVF or ICSI).

### Asthma and wheezing illness outcomes

At 5 and 7 years mothers were asked about asthma and wheezing illnesses including occurrence, frequency and severity indicators for each child. The questions were taken from the International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaire for asthma. This validated instrument has been widely used to measure childhood asthma and wheezing illnesses (Asher et al., 1995; Asher et al., 1998). In this study the main outcomes analysed are 'ever had asthma' and 'wheezing in the last 12 months' at the age of 5 and 7 years.

At each interview mothers were also asked whether their child was taking any medication (pills, syrups or other liquids, inhalers, patches, creams, suppositories or injections) prescribed by a doctor or hospital on a regular basis (every day for two weeks or more), and if so what was the name of the medication. We describe the prevalence of asthma medications, identified from the British National Formulary codes. Since these children were taking a daily dose it is likely that this reflects a maintenance dose of inhaled corticosteroids, and therefore reflects severity of the condition.

### Potential confounding factors

The factors which may potentially explain any observed association between conception history and asthma were identified from the literature, which indicated a potential association with both the outcome and the exposure. These included the following.

*Age and sex of cohort member*

*Known risk factors:* mother's history of asthma—reflecting a possible genetic component in the development of asthma in the child, and also a possible link between maternal asthma and fertility owing to shared metabolic roots (Real et al., 2007) or effects of anti-asthmatic

medication on fertility (Kallen and Olausson, 2007); mother's BMI (because of link between maternal pre-pregnancy BMI and childhood asthma (Reichman and Nepomnyaschy, 2008), and maternal BMI and subfertility (van der Steeg et al., 2008)); parental smoking (coded as mother smoked during pregnancy, parent smoked earlier in childhood, one parent currently smokes, both parents currently smoke); number of siblings in household, type of childcare at the age of 3 years (both to try to capture some exposure to infections); furry pets in household, damp/condensation in the home (potential irritants and allergens); polluted residential area (environmental effects).

**Sociodemographic factors:** household socioeconomic position (higher of mother/father using UK National Statistics socioeconomic class, four categories); family income; mother's qualifications (National Vocational Qualifications or equivalent groups); mother's age at birth of child; family type (lone parent, cohabiting or married); ethnicity (white, non-white).

### Potential mediating factors

Possible mediating factors considered to be on the causal pathway between conception group and childhood asthma included gestational age (in weeks); delivery type (vaginal, instrumental or Caesarean section); breastfeeding (coded as 'none', '<4 months', '>4 months', included as there is an evidence of a protective effect).

### Statistical analysis

First, we examined the effect of overall subfertility, by combining the untreated subfertile, OI and ART groups and comparing them to the children born after planned pregnancies. Next, we explored whether there were different effects dependent on degrees of infertility or treatment by separating out the untreated subfertile, OI and ART groups and comparing these to the planned pregnancies. Finally, we investigated whether the treatment groups were at a higher risk than the untreated subfertile group, by using children of untreated subfertile couples as the comparison group.

For each of these comparisons, logistic regression was used to estimate odds ratios (OR) for the three outcomes (asthma, wheezing and medications) adjusting for potential confounding factors. Potential confounders were included if they were statistically significantly associated with the outcome at the 5% level (indicated by a Wald,  $P < 0.05$ ) after controlling for other factors in the model. A final model, adjusted for gestational age, delivery type and breastfeeding, assessed whether there was any evidence that the effect of conception group on asthma was mediated via these factors.

All analyses took the clustered, stratified study design into account by using the 'survey commands' in Stata version 11SE (StataCorp, 2009). All reported estimates are weighted by sampling and non-response weights to account for missing data owing to non-response at later sweeps (Hansen, 2008; Plewis, 2007).

## Results

### Description of study population

Figure 1 shows the study population, with details of exclusions and non-response. 42% (5684/13 041) of children were born after an unplanned pregnancy; 15% of mothers reported that they felt unhappy or ambivalent about the pregnancy ('unplanned'  $n = 2039$ ), while 27% of mothers were happy ('mistimed'  $n = 3645$ ). 52% of mothers (6575/13 041) reported a planned pregnancy, conceived in <12

months ('planned group'), a further 4% (505) conceived after 12 months or longer (untreated 'subfertile group'), while 1.4% (173) had ovulation inducing drugs and 0.9% (104) were born following ART (Table I).

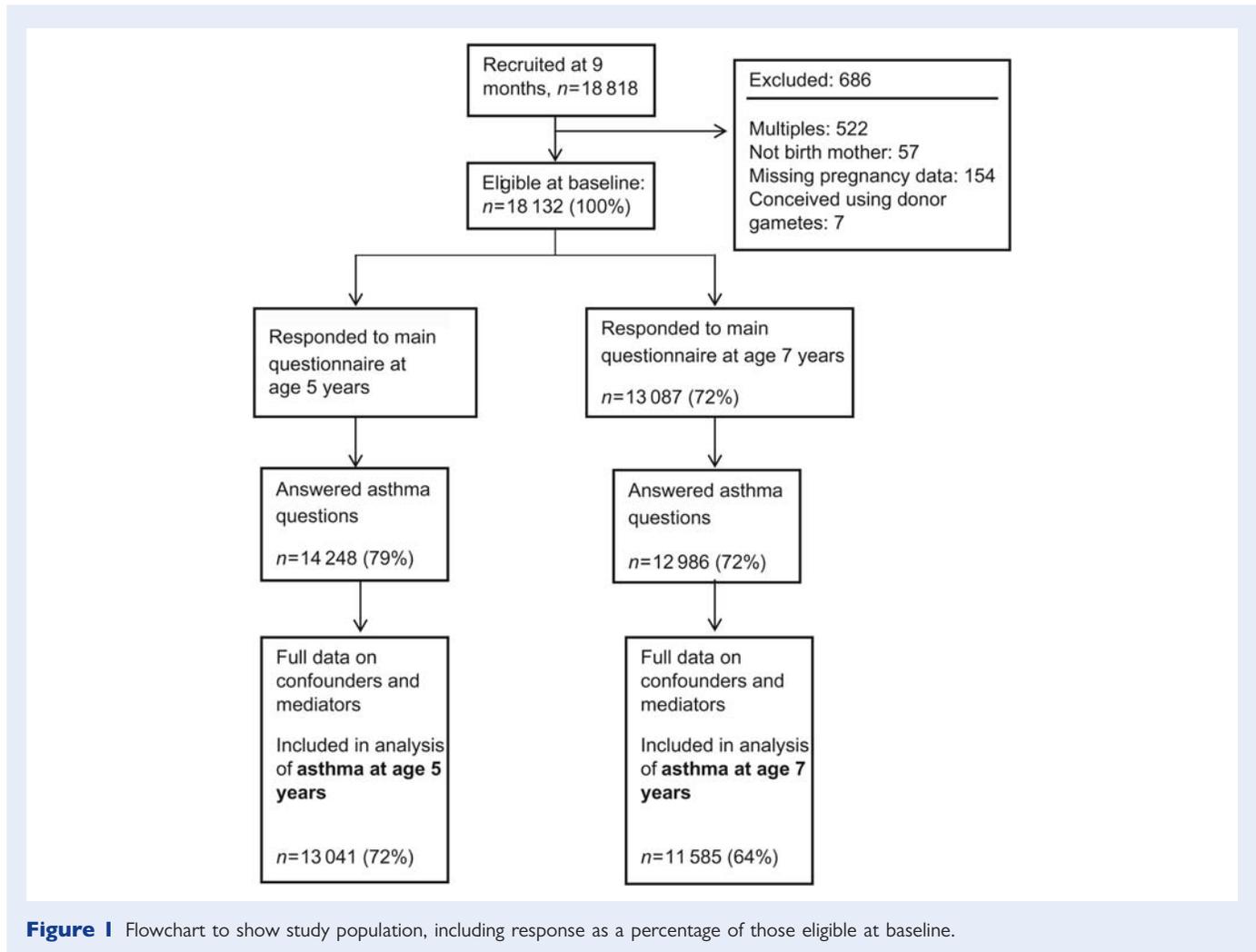
Table I shows clear and consistent differences across the conception groups in terms of demographic, socioeconomic and behavioural factors. Children born after ART were, on average, born at an earlier gestation and lower birthweight than the children from other groups, and were more likely to experience a Caesarean delivery (all  $P$ -values  $< 0.001$  for the ART group compared with the normal TTC group). Compared with the planned, fertile group, the unplanned children were generally born to younger mothers, who were less likely to be in a relationship, had lower educational attainment and a more disadvantaged socioeconomic position. Mothers in the unplanned groups were also more likely to smoke in pregnancy, less likely to breastfeed and more likely to report damp housing or polluted residential areas (all  $P$ -values  $< 0.001$  for the unplanned group compared with the normal TTC group).

Approximately 15% of the study population had asthma at ages of 5 and 7 years (the prevalence of asthma and related symptoms is shown in Table II). Wheezing in the last year was more prevalent in the younger age group (16 versus 12% at ages of 5 and 7 years). Table I shows many of the expected differences in key risk factors between children with and without a doctor diagnosis of asthma. Boys are more likely to be asthmatic than girls (17 versus 12%). Children with asthma are more likely to have a family history of asthma, to be born to less wealthy and less well-educated parents, to be born earlier and at a lower birthweight and were less likely to be breastfed than the children without asthma.

### Asthma in subfertile and infertility treatment groups

In comparison with the planned group, children born to parents who experienced a prolonged TTC (>12 months) regardless of subsequent fertility treatment were significantly more likely to report asthma [at 5 years, unadjusted OR: 1.28 (95% confidence interval (CI): 1.00, 1.42), fully adjusted OR: 1.39 (1.07, 1.80)].

Considered separately, children born after a prolonged TTC but conceived without fertility treatment (untreated subfertile group) showed a small increase in the odds of asthma or wheezing symptoms at both 5 and 7 years, though this did not reach statistical significance at the 5% level [e.g. for asthma OR: 1.34 (0.98, 1.83) and OR: 1.15 (0.84, 1.58) at 5 and 7 years, respectively, see Table III]; they were more likely to be taking anti-asthmatic medications than their planned peers. The children of mothers who underwent OI treatment showed no increase in asthma or wheezing symptoms, though the use of anti-asthmatic medications was again more common. Children born after ART, however, were significantly more likely to have asthma at both 5 and 7 years of age and adjustment for confounding factors strengthened the observed association. A doubling in risk was seen at 5 years [adj OR: 2.65 (1.48, 4.76) and 1.97 (1.10, 3.53) for asthma and wheezing, respectively]; the effect was reduced at 7 years [OR: 1.84 (1.03, 3.28) and OR: 1.50 (0.77, 2.92)]. This group of children born after ART was also considerably more likely to be taking anti-asthmatic medications than the planned, normal TTC



group [adj OR: 4.67 (2.20, 9.94) and 2.29 (1.00, 5.24) at 5 and 7 years, respectively].

When compared with the children born to untreated subfertile couples, the ART group was significantly more likely to report an asthma diagnosis and asthma medications at 5 years. The effect was smaller at 7 years and no longer statistically significant. There was no evidence of a difference between the untreated subfertile and the OI groups (see Table IV).

Among the potential mediators, gestational age and breastfeeding behaviour were seen to be independently associated with asthma but the effect of including these variables in the models of the association between conception group and asthma was minimal [adjusted OR for asthma in the ART group at 5 years, including gestational age, breastfeeding and Caesarean section in the model: OR: 2.38 (1.34, 4.24)].

### Asthma and pregnancy planning

Unadjusted analyses consistently indicate that unplanned children are more likely to have an asthma diagnosis than their planned peers [OR: 1.42 (1.20, 1.67) and OR: 1.30 (1.09, 1.54) at 5 and 7 years, respectively], but this is attenuated when adjusted for confounding factors [OR: 1.11 (0.94, 1.31) and OR: 0.96 (0.79, 1.16)]. A similar

pattern is seen for wheezing in the last year at the age of 5 years, but by the age of 7 years there is no evidence of an increase even in the unadjusted analysis. Reported daily use of anti-asthmatic medications in the unplanned group is higher at the age of 5 years than among the planned group, though this is not seen at the age of 7 years.

The pattern is identical for the mistimed group, though the observed effects in the unadjusted analyses are smaller.

## Discussion

In this study of 13 000 UK children we found some evidence that subfertility is associated with an increased risk of asthma in subsequent children, and that this effect is greatest among those conceived following ART. This association varies in strength but appears consistently across a number of indicators, including 'ever had asthma', 'wheezing in the last year' and prescribed asthma medications. While unadjusted results also suggest that unplanned and mistimed children are at greater risk of asthma and wheezing, it is apparent from the adjusted analyses that this is likely to be a result of confounding, particularly by social circumstances.

**Table 1** Description of the study population from the UK Millennium Cohort Study (MCS) at 5-year survey (n = 13 041).

	Reported asthma				Conception group			
	Yes	No	Unplanned	Mistimed	Planned	Subfertile	OI	ART
Unweighted n (wt%)	1956 (14.7)	11 085 (85.3)	2039 (15.3)	3645 (26.7)	6575 (51.8)	505 (4.0)	173 (1.4)	104 (0.9)
Socio-demographic factors								
Mother's age at birth of cohort member (years)	29.1	27.8	26.6	27.5	29.9	31.5	31.7	33.6
Socioeconomic position, Prof/mgt (wt%)	29.7	40.6	20.9	28.6	48.3	48.1	51.7	57.5
Maternal education, NVQ 4/5 (wt%)	30.7	38.1	22.0	28.5	44.8	41.9	51.2	48.4
Highest household income band $\geq$ £52 000 (wt%)	9.7	16.5	7.3	10.1	20.2	17.4	28.2	21.6
Single parent families (wt%)	24.2	16.8	36.1	24.4	10.2	9.8	7.1	8.9
Ethnicity (non-white) (wt%)	11.4	12.4	13.0	14.9	10.8	11.2	5.2	13.6
Possible risk factors								
Male child (wt%)	69.4	49.6	50.1	50.1	51.4	48.9	49.2	46.2
Mother's history of asthma (wt%)	28.7	14.9	18.5	18.6	16.0	13.9	15.0	6.8
Mother's pre-pregnancy BMI $>30$ kg/m <sup>2</sup> (obese)	10.8	8.7	8.8	9.2	8.5	13.6	20.0	3.0
One or both parents currently smoke (wt%)	21.8	20.7	22.7	24.0	18.3	26.4	17.1	21.5
No siblings in household (wt%)	18.4	15.7	20.7	20.5	11.5	20.2	20.9	48.0
Attended formal childcare (wt%) <sup>a</sup>	26.2	29.5	22.9	23.7	33.0	30.4	38.9	33.6
Furry pets in household (wt%)	46.2	42.9	45.2	43.4	42.6	48.5	41.8	37.3
House has damp problem (wt%)	9.2	6.6	9.6	8.8	5.6	5.5	2.5	1.7
Residential area considered polluted (wt%) <sup>b</sup>	6.8	5.5	8.8	6.6	4.3	5.2	6.9	7.6
Potential mediating factors								
Caesarean delivery (wt%)	22.6	20.9	16.7	20.2	21.5	32.0	34.7	40.3
Birthweight (kg, mean)	3.29	3.39	3.33	3.34	3.43	3.33	3.32	3.16
Gestational age (weeks, mean)	38.9	39.9	39.1	39.1	39.3	39.2	39.0	38.2
Preterm birth ( $<37$ weeks gestation)	10.3	6.4	8.0	7.7	5.9	7.9	12.3	18.5
Breastfeeding $\geq 4$ months (wt%)	25.3	35.0	21.2	29.2	39.2	32.1	39.0	44.7

NVQ 4/5, National Vocational Qualifications level 4/5 are equivalent to certificates of Higher education and above. Prof/mgt, professional/management; OI, ovulation induction; ART, assisted reproduction technologies. wt% indicates percentages weighted for effects of sampling and non-response.

<sup>a</sup>At 3 years.

<sup>b</sup>Parents reported that residential area has problems with pollution, grime, environmental problems—only available at 9 months.

## Asthma in children born to subfertile and infertile couples

The effects of subfertility on asthma in the subsequent children were assessed in two ways. The first compared the children of subfertile couples (who conceived with no treatment, with OI only or following ART) with the children of parents who planned their conception and conceived in less than 12 months. The second compared the different groups of subfertile couples to explore the influence of severity of infertility and the effects of infertility treatment. The overall picture presented by our results is one of an increased risk of asthma in children born after infertility treatment. The existing literature on asthma after ART conception is limited but our findings are consistent with the

findings of the larger Scandinavian registry-based studies which found an increase in both asthma medications (Finnstrom *et al.*, 2011; Kallen *et al.*, 2012) and hospitalizations among ART-conceived children (Ericson *et al.*, 2002; Koivurova *et al.*, 2007; Finnstrom *et al.*, 2011). However, this is not always identified (Pinborg *et al.*, 2003; Klemetti *et al.*, 2006). Other observational studies, which have been smaller and clinic based, have not reported any increase in asthma in ART children but these studies suffered from low participation (and thus potential selection bias) and were unable to control for key confounding factors (Cetinkaya *et al.*, 2009; Sicignano *et al.*, 2010).

In the present study we found that in comparison with the planned, normal TTC group the children born to subfertile parents were more likely to suffer from asthma and to be taking anti-asthmatic

**Table II** Prevalence of asthma and related symptoms in the MCS cohort (wt %, using appropriate weights for each sweep).

Asthma indicator	Conception status						
	Unplanned	Mistimed	Planned	Subfertile	OI	ART	Total
Unweighted <i>n</i> (asthma)/ <i>N</i>							
Age 5 years	372/2039	580/3645	885/6575	83/505	18/173	18/104	1956/13 041
Age 7 years	347/1827	559/3217	896/5837	83/463	23/154	18/87	1926/11 585
Asthma							
Age 5 years	17.7	15.8	13.2	16.0	11.8	23.7	15.0
Age 7 years	18.4	17.8	14.9	17.0	15.9	22.9	16.5
Wheezing in the last year							
Age 5 years	18.2	16.8	14.3	16.8	13.8	22.4	15.7
Age 7 years	12.3	12.6	11.3	13.4	7.6	17.0	11.9
Asthma medications <sup>a</sup>							
Age 5 years	5.0	3.8	3.2	4.7	5.2	11.8	3.8
Age 7 years	4.8	5.4	4.0	6.1	6.1	9.2	4.7

wt% indicates percentages weighted for effects of sampling and non-response.

<sup>a</sup>These are prescribed medications (any pills, syrups or other liquids, inhalers, patches, creams, suppositories or injections) taken every day for 2 weeks or more. Parent reported the name of the medication and those used to treat asthma were identified from British National Formulary codes.

medications. When the subfertile group was divided up into untreated subfertility, OI and ART it becomes clear that it is the ART group that is at highest risk of asthma. At 5 years ART children were >2.5 times as likely to have asthma as the planned group [adjusted OR: 2.65 (1.48, 4.76)], and twice as likely as the untreated subfertile group [adj OR: 1.98 (1.06, 3.72)]. The observed effects are reduced by 7 years of age, though this appears to be a result of increases in asthma prevalence in the comparison group between 5 and 7 years, rather than a reduction in the ART group, perhaps suggesting earlier diagnosis in the ART children.

A larger Swedish study has indicated that once duration of unwanted childlessness is accounted for, there is little effect of infertility treatment on patterns of asthma (measured by prescribed asthma medications) (Kallen et al., 2012). The higher risk we observed in the ART group could be related to an increased severity of infertility, or a consequence of the treatment: these data do not allow us to distinguish between the two possibilities. If the risk of asthma in children was associated simply with prolonged infertility you may also expect to see an increase in risk among the OI group, albeit smaller than that experienced by the ART group. This is not seen in our data; however, it should be noted that in this population the OI group appear to be quite different from the other subfertile groups: they have a higher prevalence of obesity [which is associated with anovulation and polycystic ovary syndrome (PCOS)] and on average they are also wealthier, more highly educated and their children are more likely to be in formal childcare by 3 years (perhaps indicating a return of parent(s) to the workforce). They also have a shorter average TTC than the untreated subfertile group (mean: 29.2 months versus 38.3 months), perhaps suggesting that they are not representative of a 'more infertile' group but instead represent a more demanding subgroup who actively sought intervention and earlier treatment.

If the observed increase in asthma among ART children is real, one must consider what may be driving this association. One possibility is over-reporting by excessively protective ART parents. Similarly, parents who sought medical help to conceive may be more likely to seek medical help for their child and therefore get a diagnosis of asthma or medication prescribed. However, there is no increase in other atopic conditions in this population [eczema and hayfever (data on request)], suggesting that over-reporting is not a major issue because you might expect to see it across all these relatively common conditions.

Another possibility is that the results are related to residual confounding. Our findings are adjusted for recognized risk factors, and we have tried to account for theories such as the 'hygiene hypothesis' (Strachan, 1989; Subbarao et al., 2009) by looking at firstborn status, siblings and childcare type to try to capture some measure of exposure to infections but there could still be an unknown or unmeasured confounder driving the observed association. There are other hypotheses about causal factors which we could not address in this analysis as the data were not available [e.g. supplement use might be higher in women who have had fertility problems, and it has been suggested that folate supplementation in pregnancy can result in poorer respiratory outcomes in young children (Whitrow et al., 2009)].

Asthmatic women are more likely to report prolonged childlessness (Kallen and Olausson, 2007), though there is little evidence that this affects the eventual number of pregnancies or live births (Forastiere et al., 2005; Tata et al., 2007). Oligomenorrhoea (common in PCOS) has been found to be associated with both asthma and lung function, and a shared aetiology (such as insulin resistance) has been hypothesized (Real et al., 2007). It has also been reported that a greater than expected proportion of women undergoing IVF report the use of anti-asthmatics (Kallen and Olausson, 2007), and that these medications are associated with anovulatory infertility (Svanes et al., 2005).

**Table III** Odds ratios for asthma, wheezing and medications at 5 and 7 years in each conception group, compared with 'planned, normal TTC' group.

Group	Age 5 years			Age 7 years		
	n	Adjusted for sex only	Fully adjusted model	n	Adjusted for sex only	Fully adjusted model
Asthma						
Unplanned	2039	1.42 (1.20, 1.67)	1.11 (0.94, 1.31)	1827	1.30 (1.09, 1.54)	0.96 (0.79, 1.16)
Mistimed	3645	1.24 (1.08, 1.42)	1.03 (0.89, 1.19)	3217	1.23 (1.07, 1.41)	0.98 (0.84, 1.15)
Planned	6575	Comparison group		5837	Comparison group	
All Subfertile	782	1.28 (1.00, 1.65)	1.39 (1.07, 1.80)	704	1.22 (0.95, 1.57)	1.23 (0.95, 1.59)
<i>Untreated</i>	505	1.27 (0.94, 1.72)	1.34 (0.98, 1.83)	463	1.18 (0.86, 1.60)	1.15 (0.84, 1.58)
<i>OI</i>	173	0.88 (0.53, 1.46)	0.96 (0.57, 1.61)	154	1.10 (0.68, 1.78)	1.17 (0.72, 1.90)
<i>ART</i>	104	2.10 (1.16, 3.81)	2.65 (1.48, 4.76)	87	1.73 (0.97, 3.11)	1.84 (1.03, 3.28)
Wheezing in the last year						
Unplanned	2039	1.33 (1.15, 1.55)	1.13 (0.98, 1.30)	1826	1.10 (0.91, 1.34)	0.95 (0.77, 1.17)
Mistimed	3645	1.21 (1.06, 1.38)	1.08 (0.94, 1.24)	3217	1.12 (0.95, 1.34)	1.01 (0.84, 1.22)
Planned	6575	Comparison group		5837	Comparison group	
All Subfertile	782	1.23 (0.97, 1.57)	1.27 (1.00, 1.63)	704	1.15 (0.87, 1.51)	1.08 (0.82, 1.43)
<i>Untreated</i>	505	1.22 (0.93, 1.60)	1.24 (0.94, 1.63)	463	1.22 (0.88, 1.68)	1.15 (0.83, 1.60)
<i>OI</i>	173	0.97 (0.58, 1.60)	1.00 (0.61, 1.66)	154	0.66 (0.34, 1.28)	0.64 (0.33, 1.22)
<i>ART</i>	104	1.76 (1.00, 3.12)	1.97 (1.10, 3.53)	87	1.65 (0.85, 3.20)	1.50 (0.77, 2.92)
Anti-asthmatic medications taken daily						
Unplanned	2039	1.59 (1.20, 2.10)	1.34 (1.02, 1.78)	1827	1.21 (0.92, 1.58)	1.03 (0.76, 1.39)
Mistimed	3645	1.18 (0.92, 1.51)	1.04 (0.81, 1.34)	3217	1.37 (1.07, 1.76)	1.21 (0.95, 1.55)
Planned	6575	Comparison group		5837	Comparison group	
All Subfertile	782	1.87 (1.30, 2.70)	1.90 (1.32, 2.74)	704	1.67 (1.16, 2.42)	1.63 (1.13, 2.34)
<i>Untreated</i>	505	1.51 (0.94, 2.41)	1.51 (0.94, 2.41)	463	1.56 (1.00, 2.43)	1.52 (0.97, 2.38)
<i>OI</i>	173	1.65 (0.77, 3.51)	1.65 (0.77, 3.55)	154	1.57 (0.72, 3.44)	1.57 (0.73, 3.41)
<i>ART</i>	104	4.11 (1.96, 8.64)	4.67 (2.20, 9.94)	87	2.48 (1.05, 5.86)	2.29 (1.00, 5.24)

Odds ratios (95% confidence interval) presented for all subfertile, and by subgroup. Fully adjusted models, controlling for effects of confounding factors: Age 5: maternal history of asthma, family smoking, social class, maternal age; Age 7: maternal history of asthma, family smoking, family income, family type, siblings and pets in the home. Italics indicate subgroups within the 'All subfertile' category. The comparison group remains the 'Planned, normal time to conception' children.

Asthma has a genetic component (Subbarao *et al.*, 2009), so it may be that higher asthma in subfertile women would lead to higher asthma prevalence in their children. We controlled for maternal asthma and the effects persist; among the children for whom we had data on paternal asthma, controlling for this made little difference (data on request).

Finally, we must consider that the observed association could be causal but we cannot as yet explain the mechanism. Further investigation is needed to disentangle the relative effects of prolonged subfertility and its treatment on asthma in the children.

### Asthma in children born after unplanned and mistimed pregnancies

Though the unadjusted analyses suggest that unplanned and mistimed children are more likely to be asthmatic, it is clear from the adjusted analyses that it is differences in social circumstances that explain most of the association. There is a well-recognized socioeconomic gradient in childhood asthma (Subbarao *et al.*, 2009), and this is reflected in our results.

At the age of 5 years, the parents of the unplanned pregnancy group report higher use of anti-asthmatic medication, an effect which persists after adjustment for confounding.

### Strengths and Limitations

To our knowledge, this is the only observational study of conception status and childhood asthma in a UK population. However, the sample included only 104 singletons born after ART, which precluded analysis by type of infertility treatment (IVF, ICSI). The relatively small ART sample also demands that findings should be interpreted with caution.

We were able to use a validated outcome measure (ISAAC questions), not just hospitalization or medication records. The use of anti-asthmatic medications may be considered a more stringent outcome definition, identifying only the most serious cases; in the MCS only children taking daily medications in the last 2 weeks are identified. It should also be noted that asthma medications may be prescribed more to the children with the most concerned and health-conscious parents and that this may therefore be higher in the ART group (Cardol *et al.*, 2005; Zuidgeest *et al.*, 2009).

**Table IV** Comparing the subfertile groups: odds ratios (95% confidence interval) for asthma, wheezing and medications at 5 and 7 years in each infertility treatment group, compared with the untreated subfertile group.

Group	Age 5 years			Age 7 years		
	n	Adjusted for sex only	Fully adjusted model	n	Adjusted for sex only	Fully adjusted model
Asthma						
Subfertile, no treatment	505		Comparison group	463		Comparison group
OI	173	0.70 (0.39, 1.25)	0.72 (0.39, 1.32)	153	0.93 (0.52, 1.67)	1.01 (0.57, 1.81)
ART	104	1.65 (0.87, 3.13)	1.98 (1.06, 3.72)	87	1.48 (0.80, 2.71)	1.59 (0.88, 2.89)
Wheezing in the last year						
Subfertile, no treatment	505		Comparison group	463		Comparison group
OI	173	0.79 (0.46, 1.37)	0.81 (0.47, 1.39)	153	0.54 (0.27, 1.08)	0.55 (0.28, 1.09)
ART	104	1.45 (0.81, 2.58)	1.59 (0.88, 2.87)	87	1.35 (0.64, 2.85)	1.30 (0.62, 2.75)
Anti-asthmatic medications taken daily						
Subfertile, no treatment	505		Comparison group	463		Comparison group
OI	170	1.09 (0.44, 2.74)	1.10 (0.43, 2.79)	153	1.01 (0.42, 2.39)	1.03 (0.44, 2.44)
ART	104	2.73 (1.26, 5.94)	3.10 (1.42, 6.79)	87	1.59 (0.61, 4.12)	1.50 (0.60, 3.79)

Fully adjusted models, controlling for effects of confounding factors: Age 5: maternal history of asthma, family smoking, social class, maternal age; Age 7: maternal history of asthma, family smoking, family income, family type, siblings and pets in the home.

The MCS is designed to provide a representative sample of the UK population; however, missing data owing to loss to follow-up can result in bias in cohort studies. Response was socially patterned and non-response weights, which take into account factors such as socio-economic position, were used in the analysis to minimize the effects. This data set included data on the most important potential explanatory factors, so unlike previous studies we were able to control for many key confounding factors; however, there remains a possibility of residual confounding by unmeasured or unknown risk factors. Although we attempted to examine the effects of possible mediating variables, the data did not allow the exploration of possible causal pathways. Detailed data on conception history also provided the opportunity to examine the effect of a full range of conception histories, rather than simply comparing ART conceptions to all other children.

The prevalence of asthma in our population (15%) is lower than estimates for prevalence in children in the UK in the 1990s from either ISAAC [23% in 1864 6–7 year olds in Sunderland (1998)] or the Health Survey for England [21% of 2–15 year old children in England (Gupta and Strachan, 2004)]. However, this is consistent with recognized temporal changes in the prevalence and diagnosing of asthma. Since the analysis was restricted to singletons, the findings are only generalizable to the singleton population

## Conclusion

Our findings suggest that asthma, wheezing and the use of anti-asthmatic medications are higher among children born to subfertile couples than those who conceived in less than 12 months. This is most apparent at the age of 5 years, and remains evident at 7 years, though the size of the effect is diminished. Children born after ART have a much higher risk, though we cannot determine if this is indicative of a treatment effect or related to a greater degree of subfertility in this group of parents. If the observed association is

causal, then the mechanism driving it remains unknown and further research in this area is warranted.

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## Authors' roles

M.Q. is the principal investigator for this project. All authors were involved in the design of the study and the interpretation of findings. C.C. completed the analysis and the first draft of the manuscript. All authors contributed to the writing of the final document. C.C. acts as a guarantor.

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## Conflict of interest

None declared.

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# Asthma in Swedish children conceived by in vitro fertilisation

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## ABSTRACT

**Objectives** To investigate a proposed association between in vitro fertilisation (IVF) and child asthma.

**Design** The risk for asthma after IVF was estimated as ORs using Mantel–Haenszel analysis.

**Setting** The Swedish Medical Birth Register.

**Patients** Of the 2 628 728 children born in 1982–2007 and surviving the perinatal period, 31 918 were conceived by IVF. Presence of asthma was defined as at least five prescriptions of antiasthmatic drugs during the period 1 July 2005–31 December 2009 according to the Swedish Prescribed Drug Register (115 767 children, 2323 of whom were born after IVF).

**Results** A significantly increased risk for asthma, albeit small, was found in children conceived by IVF (aOR 1.28, 95% CI 1.23 to 1.34), increasing the absolute risk from 4.4% to 5.6%. The risk increase for asthma was the same in boys and girls, in singletons and twins, and after caesarean section and vaginal delivery. The risk was higher for preterm than term singletons. For children with a low Apgar score, respiratory diagnoses, mechanical ventilation, continuous positive airway pressure or neonatal sepsis, the effect of IVF on asthma risk was low and statistically non-significant. Adjustment for length of involuntary childlessness eliminated the effect, and removal of infants whose mothers had used antiasthmatics in early pregnancy reduced the risk.

**Conclusions** This study verifies an association between IVF and asthma in children. This can be partly explained by neonatal morbidity and by maternal asthma acting as mediators, but the main risk factor is parental subfertility. The mechanism for this is unclear.

## INTRODUCTION

It has repeatedly been demonstrated that infants born after in vitro fertilisation (IVF) show an increased risk for preterm birth, low birth weight and neonatal morbidity.<sup>1–3</sup> There are more multiple births following IVF than in the general population even though the rate of twins and higher order multiples has declined during the last decade as policy on the number of transferred embryos has changed.<sup>4</sup> However, among singletons neonatal complications also occur at an increased rate after IVF and include preterm delivery, low Apgar score and respiratory problems.<sup>1 2 4</sup> These conditions may result in increased morbidity later in life. Some late sequelae are more common than expected among children born after IVF, such as cerebral palsy,<sup>5</sup> mainly due to the high rate of multiple births. The risk will therefore probably decline with the decreasing rate of multiple births after IVF. Other long-term morbidity has also been described as a consequence of IVF, for example, a slightly increased risk for cancer.<sup>6</sup>

## What is already known on this topic

- ▶ Only a few previous studies have investigated the possible risk of asthma in children conceived by in vitro fertilisation.
- ▶ There are two relatively large studies, one of which did and one of which did not find an increased risk for asthma.

## What this study adds

- ▶ This is the largest study yet carried out and demonstrates a statistically significant increase in asthma risk after in vitro fertilisation (IVF).
- ▶ The increased risk is explained by parental subfertility, but neonatal pathology among IVF infants may be a factor.

In recent years, some epidemiological studies have identified premature birth, neonatal morbidity and caesarean section as risk factors for childhood asthma.<sup>7–11</sup> As such neonatal conditions are more common among infants conceived by IVF, these children may have an increased risk of developing asthma. The literature on the subject is limited. Some studies investigating morbidity in children conceived by IVF mentioned asthma and described an approximately 30% risk increase.<sup>1 12</sup> However, one study found no such risk,<sup>13</sup> while other small studies gave inconsistent results.<sup>14–16</sup>

## MATERIAL AND METHODS

The Swedish Medical Birth Register contains information on most births in Sweden since 1973 (1–2% are missing).<sup>17</sup> Information on year of delivery, maternal age in completed years (5-year groups: <20, 20–24, etc), parity (number of previous infants born: +1, 2, 3, ≥4), smoking in early pregnancy (unknown, none, <10 cigarettes per day, ≥10 cigarettes per day) and prepregnancy weight, height and body mass index (BMI; <19.8, 19.8–24.9, 25–29.9, ≥30) was collected. Pregnancy duration (in completed weeks) was known in nearly all cases and was usually estimated from a second trimester ultrasound. Small for gestational age (SGA) and adequate for gestational age (AGA) infants were identified using sex and parity specific growth graphs based on data from the Medical Birth Register.<sup>18</sup> Caesarean section or vaginal delivery was recorded and information on



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neonatal diagnoses was coded according to the International Classification of Diseases. Register information on cohabitation in early pregnancy was obtained. Information on maternal use of antiasthmatic drugs was acquired from midwife interviews at the first antenatal visit. Records for infants dying in the perinatal period were excluded from analysis.

### Identification of births after conception using IVF

In Sweden IVF is performed in only a limited number of clinics, and the National Board of Health and Welfare has collected data on all IVF procedures which resulted in a delivery for the period 1982–2006.<sup>19</sup> As all residents of Sweden have a unique personal identification number, the maternal identification number was used for linkage with the Medical Birth Register.

### Identification of asthma in children and young adults

Since 1 July 2005 a register of all filled prescriptions with the identification numbers of the patients has been maintained in Sweden.<sup>20</sup> From this register, records referring to antiasthmatic drugs (ATC code R03) were extracted up to the prescription filling date of the 31 December 2009 for children born in 1982–2007. For each child, the number of prescriptions filled and the number of events when prescriptions were filled (as more than one drug could be prescribed at each event) were calculated but restricted to prescriptions filled after the age of 2 years as an asthma diagnosis before the age of 2 is regarded as imprecise.<sup>9</sup>

### Statistical analysis

Children without data in the Medical Birth Register including all immigrant children, were excluded from analysis. To adjust for putative confounders, Mantel–Haenszel analyses were performed to generate ORs, and the approximate 95% CIs were estimated with Miettinen's method. Adjustments were made for year of delivery, maternal age, parity, smoking and BMI. Various subanalyses were carried out after stratification, for example, by infant gender.

Two ORs were compared based on the variance estimates in the Mantel–Haenszel analyses. Weighted linear regression analyses of the log (ORs) were used to detect a linear trend in the ORs.

### Ethics

The study was performed within the responsibilities of the National Board of Health and Welfare and therefore no ethics approval from external ethics committees was required.

### RESULTS

During the observation period, 2 628 728 infants surviving the perinatal period were registered in the Medical Birth Register, 31 918 of whom were conceived through IVF. Among all children, 388 328 (15%) had at least one prescription for an antiasthmatic drug, while the corresponding number was 6597 (21%) among IVF children. The OR for having an antiasthmatic drug prescribed after adjustment for year of birth, maternal age, parity, smoking and BMI was 1.18 (95% CI 1.14 to 1.21).

Table 1 shows that the OR for asthma among children born after IVF increases with the number of prescription events for each child, while the number of cases simultaneously decreases. Further analysis will be restricted to children who had at least five prescriptions filled, constituting 7.3% of IVF children and 4.4% of all children in the population who had antiasthmatic drugs. The adjusted OR was 1.28 (95% CI 1.23 to 1.34).

**Table 1** Number of infants born following in vitro fertilisation (IVF) with asthma according to number of prescriptions for antiasthmatic drugs

Number of prescription occasions	Asthma, IVF	Asthma, population	OR (95% CI)
1	2003	138623	1.06 (1.02 to 1.11)
2–4	2271	133398	1.12 (1.07 to 1.17)
5–9	1289	34912	1.24 (1.17 to 1.31)
10–19	758	37499	1.29 (1.20 to 1.39)
≥20	276	13406	1.37 (1.21 to 1.54)
≥5	2323	115767	1.28 (1.23 to 1.34)

ORs with 95% CIs are shown adjusted for year of birth of child, maternal age, parity, smoking and body mass index. Total number of IVF children 31918, total number in population 2628728.

### Putative confounders among maternal characteristics

Table 2 shows that there is a higher chance of an infant being born after IVF as maternal age increases, but only a weak increase in the risk that the child will have asthma. There is a strong over-representation of first parity children after IVF and a weaker increase in asthma risk in first parity children. Maternal smoking is lower with IVF but results in a slight increase in asthma risk. With increasing BMI, the chance of IVF and risk of the child having asthma both increase. Subfertility is obviously always a factor for couples who have IVF and table 3 shows that there is a moderately increased risk for childhood asthma with subfertility (excluding cases with known IVF).

Women who immigrated to Sweden have a lower rate of IVF than Swedish-born women. Their children may have other risk factors for asthma compared to the children of Swedish-born women but exclusion of women born outside Sweden did not change the OR which was 1.27 (95% CI 1.21 to 1.33). Nearly all women who undergo IVF are cohabiting. Restricting the analysis to cohabiting women gave an OR of 1.29 (95% CI 1.23 to 1.35).

The reported maternal use of antiasthmatic medicines in early pregnancy was analysed in order to estimate the impact of maternal asthma on the association between IVF and asthma in their offspring. Such data were only available after 1 July 1995, and for this period, the OR for asthma in children conceived by IVF was calculated as 1.28 (95% CI 1.22 to 1.34). Of all infants, 4833 had asthma among the 36 397 whose mother had reported use of antiasthmatic drugs in early pregnancy (13.3%), while among 732 such infants conceived by IVF, 127 had asthma (17.3%). Among women using antiasthmatic drugs, the risk for asthma in children conceived by IVF was 1.15 (95% CI 0.94 to 1.42), representing only about 55% of the risk for all children and not statistically significant.

When adjustment was made for parental subfertility (measured as number of years of unwanted childlessness), the effect of IVF on the risk of childhood asthma disappeared: OR 1.02 (95% CI 0.98 to 1.09).

### Infant characteristics as mediators of the effect of IVF on asthma risk

As seen in table 4, there was no difference in the increase in risk for asthma in IVF children according to infant gender. As expected, the sex ratio of children with asthma was significantly increased, both (males/females) after IVF and in the population. The oldest children in the cohort had the highest risk for

**Table 2** Number of infants according to in vitro fertilisation (IVF) and asthma status

Variable	Asthma, IVF	Total IVF	Asthma population	Total population	OR (95% CI), IVF	OR (95% CI), asthma
Maternal age, years						
<20	0	2	2239	64473	–	0.78 (0.74 to 0.81)
20–24	35	377	19567	490721	0.16 (0.15 to 0.18)	0.92 (0.91 to 0.94)
25–29	392	4874	40642	921296	1.00 (reference)	1.00 (reference)
30–34	971	13705	35463	771437	3.36 (3.25 to 3.46)	0.99 (0.98 to 1.01)
35–39	780	11211	15064	321194	7.62 (7.37 to 7.85)	1.01 (0.99 to 1.03)
40–44	142	1741	2669	57411	6.56 (6.23 to 6.91)	1.01 (0.97 to 1.05)
≥45	3	8	123	2196	–	1.21 (1.01 to 1.46)
Parity						
1	1395	18680	52175	1105484	1.00 (reference)	1.00 (reference)
2	654	9085	41216	940109	0.38 (0.37 to 0.39)	0.91 (0.90 to 0.92)
3	203	3116	15513	403864	0.24 (0.23 to 0.25)	0.80 (0.78 to 0.82)
≥4	71	1037	6863	179271	0.15 (0.14 to 0.16)	0.76 (0.74 to 0.78)
Smoking						
Unknown	220	2776	9537	217316	–	–
None	1939	26996	88340	1962302	1.00 (reference)	1.00 (reference)
<10 cigarettes/day	116	1547	11597	292997	0.73 (0.59 to 0.67)	1.04 (1.01 to 1.06)
≥10 cigarettes/day	48	599	6293	166113	0.58 (0.53 to 0.63)	1.06 (1.03 to 1.09)
Body mass index						
Unknown	455	6167	43346	1297103	–	–
<19.8	101	1656	5859	125288	0.83 (0.78 to 0.87)	0.90 (0.88 to 0.93)
19.8–24.9	1149	17029	45219	868443	1.00 (reference)	1.00 (reference)
25–29.9	378	4631	12536	212653	1.15 (1.11 to 1.18)	1.13 (1.11 to 1.16)
≥30	240	2435	8807	125231	1.10 (1.05 to 1.15)	1.35 (1.32 to 1.38)
Total number	2323	31918	115767	2628728	–	–

ORs with 95% CIs are shown for IVF and for asthma according to some maternal characteristics. Each variable is adjusted for year of birth of child and all other tabulated variables.

asthma, but the trend between the three birth periods did not reach statistical significance ( $z=1.40$ ,  $p=0.15$ ).

Twins had a higher OR than singletons, but this difference did not reach statistical significance ( $z=1.94$ ,  $p=0.06$ ). Among singletons, the OR was higher in children born preterm than in children born term and this difference was statistically significant ( $z=2.17$ ,  $p=0.04$ ). There was no significant difference in the risk for asthma associated with IVF between children born after caesarean section and children born vaginally ( $z=0.53$ ,  $p=0.35$ ).

For five conditions (5 min Apgar score <7, neonatal respiratory diagnoses, use of mechanical ventilation, use of continuous positive airway pressure (CPAP) and neonatal sepsis), the ORs were low and not statistically significant.

When the analysis was restricted to term singleton AGA children, delivered vaginally, with an Apgar score at 5 min of ≥7,

and without a neonatal diagnosis of respiratory problems, mechanical ventilation or CPAP, sepsis or neonatal icterus, the OR was 1.16 (95% CI 1.08 to 1.24). This estimate is based on 14 512 children conceived by IVF of whom 909 had asthma, and 1 940 731 children in the population of whom 79 150 had asthma. Thus, this selected group had about half of the risk increase.

## DISCUSSION

The advantage of the present study is its size (seven times bigger than the previous largest study<sup>13</sup>) which was made possible by linking two national registers using national personal identification numbers. Both exposure (IVF treatment) and outcome (treatment for asthma) were thus based on information collected independently of this research. This also allowed for adjustment for various putative confounders and examination of mediators, based on prospectively collected register data. As always in epidemiological studies, there may be unidentified confounders which can explain part or even all of the association. When information on a specific variable is missing in a large proportion of cases (such as information on BMI), adjustment will be incomplete. However, exclusion of cases lacking information on smoking and/or BMI usually had little effect on the OR estimates.

Various methods can be used to identify asthma in children including detailed parental questionnaires (eg, the international ISAAC study<sup>21</sup>) or hospital discharge diagnoses.<sup>1–12</sup> We used prescriptions for antiasthmatic drugs as an indicator of asthma.<sup>9–11</sup> However, as such drugs can be used for indications other than asthma, resulting in dilution with non-asthma cases, we decided to use five or more prescription events as the

**Table 3** Association between subfertility (years of unwanted childlessness) and risk for asthma in the child

Years of unwanted childlessness	With asthma	Total number	OR (95% CI)
0	106685	2471548	1.00 (reference)
1	2496	50635	1.10 (1.05 to 1.14)
2	2502	45329	1.17 (1.12 to 1.22)
3	1436	25028	1.22 (1.15 to 1.30)
≥4	2650	48288	1.16 (1.11 to 1.22)

ORs with 95% CIs are shown adjusted for year of birth of child, maternal age, parity and body mass index. Children born after in vitro fertilisation are excluded from the analysis.

**Table 4** Number of infants according to in vitro fertilisation (IVF) and asthma status

Stratum	Asthma, IVF	Total IVF	Asthma, population	Total population	OR (95% CI)
All children	2323	31918	115767	2628728	1.28 (1.22 to 1.33)
Boys	1421	16452	65418	1350716	1.29 (1.21 to 1.36)
Girls	902	15458	50340	1277484	1.27 (1.19 to 1.37)
Born 1982–1989	38	762	25667	922279	1.65 (1.19 to 2.29)
Born 1990–1997	839	14089	44407	1015355	1.30 (1.21 to 1.40)
Born 1998–2007	1446	17240	45695	691094	1.25 (1.19 to 1.31)
Singletons	1660	22673	112456	2560483	1.22 (1.16 to 1.28)
Twins	633	8705	3201	66056	1.37 (1.23 to 1.51)
Preterm singletons (<37 weeks)	241	1860	8398	129372	1.39 (1.20 to 1.60)
Term singletons (≥37 weeks)	1416	20783	103870	2425468	1.17 (1.11 to 1.24)
SGA singletons	73	859	3032	56160	1.13 (0.88 to 1.46)
AGA singletons	1660	22673	112456	2560483	1.22 (1.16 to 1.28)
Caesarean section	928	11003	20274	353240	1.25 (1.17 to 1.35)
Vaginal delivery	1395	20915	95493	2275488	1.22 (1.16 to 1.29)
Apgar 5 min <7	58	693	1854	29167	1.00 (0.74 to 1.34)
Neonatal respiratory diagnoses	219	2009	4730	58504	1.11 (0.95 to 1.29)
Mechanical ventilation	21	144	331	3749	0.75 (0.43 to 1.31)
CPAP	65	601	1041	12281	1.06 (0.78 to 1.42)
Neonatal sepsis	48	542	1300	18862	1.02 (0.74 to 1.40)
Neonatal icterus	70	1003	2060	44372	1.36 (1.05 to 1.77)

ORs with 95% CIs are shown for IVF and for asthma according to some infant characteristics. Each OR is adjusted for year of birth of child, maternal age, parity, smoking and BMI. AGA, adequate for gestational age (between -2 and +2 SDs of expected weight); CPAP, continuous positive airway pressure; SGA, small for gestational age (<2 SDs of expected weight).

criterion for inclusion in the study. Nevertheless, some non-asthmatic cases may remain which will bias the ORs towards 1.0. Selection of the cut-off value is a balance between getting a high proportion of true asthma and sufficient numbers for acceptable statistical power.

A weakness in the study is that there was no information on post-neonatal death or emigration of children. Therefore, some such children will be included in the denominators and this will reduce risk estimates slightly. There is no major difference in risk of post-neonatal death between children born after IVF and other children, but immigrant parents may be more inclined to emigrate again and may also have used IVF less. Exclusion of non-Swedish born women did not markedly change the OR.

There was no difference in the OR for asthma after IVF between boys and girls. The same was true for caesarean section versus vaginal delivery: the ORs for the two groups were similar even though caesarean section is a known risk factor for childhood asthma.<sup>7</sup> There is a slight and not quite significant difference in ORs between children born as twins and as singletons. However, among singletons, there is a higher risk of asthma in preterm IVF infants than in term IVF infants. If the effect of IVF on asthma risk was due to an increased rate of preterm birth after IVF, the OR for preterm children should instead be lower than for term births. This phenomenon was seen for six neonatal conditions: SGA in singletons, low Apgar score, respiratory problems, use of mechanical ventilation, use of CPAP and neonatal sepsis. These factors thus behaved as mediators and when stratified for them, the effect of IVF declined and lost statistical significance. However, these factors explain only a small amount of the total effect because of the relatively small proportion of infants with these conditions.

When analysis was restricted to 'normal' outcomes (singleton AGA term infants delivered vaginally, with normal Apgar score and no neonatal diagnosis of respiratory problems, no mechanical ventilation or CPAP, and no sepsis or jaundice), a lower but

significantly increased risk remained, which was a little more than half of the initial risk. This indicates that the remaining risk may not be due to neonatal effects seen after IVF but may be more directly linked to IVF per se or to the subfertility underlying IVF.

An effect of parental subfertility (estimated as years of unwanted childlessness) on the risk for asthma was demonstrated. Adjustment for the length of unwanted childlessness when known showed that the effect of IVF on asthma nearly disappeared, leaving a non-significant OR of 1.02. This indicates that the main effect is related to the subfertility of the couple, partly via neonatal pathology. It does not seem to be directly related to the IVF methodology.

One can only speculate on possible links between subfertility and asthma in children. There is a genetic component in the risk of asthma and if parental asthma was associated with an increased risk of subfertility, such a link could at least partly explain the association. In a previous study we showed that women who had IVF had used a surfeit of antiasthmatics (OR 1.39, 95% CI 1.22 to 1.58).<sup>22</sup> Women using antiasthmatics have an excess of unwanted childlessness of 2 years or more.<sup>23</sup> A link has been suggested between the use of asthma medication and the risk of ovulatory infertility.<sup>24</sup> On the other hand, no effect on final fertility rates in women with asthma was found.<sup>25</sup> When we analysed women who had reported own use of antiasthmatic drugs in early pregnancy, the association between IVF and asthma in the offspring was reduced, but the number was low and the CI large. All mothers with a genetic load for asthma were certainly not identified and the contribution of paternal genetics was not considered. A genetic link between asthma, subfertility and therefore IVF, and childhood asthma may exist but can probably explain only part of the association between IVF and asthma.

In conclusion, a link between IVF and asthma in children was found which seems only partly due to increased neonatal

morbidity. The association is mainly caused by underlying fertility problems. The link between subfertility and asthma risk has no obvious explanation so further studies are needed.

**Contributors** BK carried out the data analysis and drafted the manuscript. KGN and OF took part in the planning of the study, discussed the results, and read and commented on the manuscript draft. POO supervised data collection, took part in the planning of the study, discussed the results, and read and commented on the manuscript draft.

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## Beckwith-Wiedemann syndrome and assisted reproduction technology (ART)

E R Maher, L A Brueton, S C Bowdin, et al.

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## LETTER TO JMG

## Beckwith-Wiedemann syndrome and assisted reproduction technology (ART)

E R Maher, L A Brueton, S C Bowdin, A Luharia, W Cooper, T R Cole, F Macdonald, J R Sampson, C L Barratt, W Reik, M M Hawkins

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Beckwith-Wiedemann syndrome (BWS) is a model imprinting disorder resulting from mutations or epimutations affecting imprinted genes on chromosome 11p15.5.<sup>1</sup> The classical clinical features of BWS are macroglossia, pre- and/or postnatal overgrowth, and anterior abdominal wall defects (umbilical hernia or exomphalos). Additional more variable features include hemihypertrophy, neonatal hypoglycaemia, facial naevus flammeus, ear pits and creases, renal anomalies, and an increased risk of embryonal tumours.<sup>2</sup> Most cases of BWS are sporadic and ~20% of these have uniparental disomy (paternal isodisomy) for a variable region of chromosome 11 which always includes the 11p15.5 imprinted gene cluster.<sup>3-5</sup> Up to 60% of sporadic BWS patients have epigenetic changes at differentially methylated regions within 11p15.5 that are associated with alterations in the imprinting or expression of paternally expressed genes, such as *IGF2* and *KCNQ1OT*, or maternally expressed genes, such as *H19* and *CDKN1C*.<sup>1</sup> Thus, 5-10% have epigenetic alterations at the *IGF2/H19* loci (the maternal *H19* and *IGF2* alleles display paternal allele methylation and expression patterns with biallelic *IGF2* expression and silencing of *H19* expression),<sup>6</sup> and 40-50% have loss of maternal allele methylation at a differentially methylated region (KvDMR1) within an intron of *KCNQ1*. KvDMR1 loss of methylation is associated with biallelic expression of *KCNQ1OT*.<sup>7-9</sup> The epigenetic alterations at *H19/IGF2* or KvDMR1 are thought to result from defects at two putative imprinting control centres (BWSIC1 and BWSIC2, respectively).<sup>1</sup> The precise nature of the putative BWSIC2 is unknown and therefore the origin of these putative BWSIC2 defects is unknown. Weksberg *et al*<sup>10</sup> showed a clear association between monozygotic twinning and BWS with KvDMR1 loss of methylation and suggested two possible explanations: (1) that discordance for BWS in monozygotic twins is caused by unequal splitting of the inner cell mass during twinning resulting in differential maintenance of imprinting at KvDMR1, or (2) that loss of imprinting associated with KvDMR1 demethylation predisposes to twinning as well as to discordance for BWS.

Recently, a possible association between another human imprinting disorder, Angelman syndrome, and intracytoplasmic sperm injection (ICSI) was reported.<sup>11</sup> Angelman syndrome occurs in ~1 in 15 000 newborns and most cases have a deletion of 15q.<sup>12</sup> Although only a minority of cases (<5%) of Angelman syndrome are caused by an imprinting defect,<sup>13 14</sup> both of the two cases associated with ICSI that were described by Cox *et al*<sup>11</sup> had an imprinting defect. This led to the suggestion that ICSI may be associated with an increased susceptibility to imprinting errors. To investigate this hypothesis further, we have determined whether there is evidence of an association between BWS and a history of assisted conception techniques.

## METHODS AND RESULTS

We reviewed the notes of 149 BWS patients who had been referred to the BWS Research Group at the Birmingham Uni-

versity Section of Medical Genetics and/or the West Midlands Molecular Genetics Service (for uniparental disomy analysis) and for whom detailed clinical information had been collected. A history of assisted conception techniques was recorded for six cases (4%) (table 1). To estimate whether this was likely to be a significantly increased proportion, we compared the frequency of in vitro fertilisation (IVF) and ICSI births in the BWS cohort with that in the general population. The first ART associated BWS case was born in 1989 and the most recent in 2002. Data for the number of children born after ART are available for 1995, 1996, 1997, 1998, 1999, and 2000 ([www.hfea.gov.uk](http://www.hfea.gov.uk)) and during these years there was a total of 43 074 births after IVF or ICSI to UK residents. The corresponding number of total births in the UK was 4 320 482, so that 0.997% of births in the general population were after IVF or ICSI. Based on these data, if the proportion of births after IVF and ICSI in BWS patients and in the general population were similar, we would have expected 1.7252 of the 149 BWS patients studied to have been born as a result of IVF or ICSI. To test the significance of the observed and expected frequencies we used a Poisson approximation to the binomial distribution and obtained a two tailed p value of 0.018. Thus, the observed frequency (n=6) of IVF and ICSI births in the BWS series is significantly greater than the expected (1.7252), with an associated 95% confidence interval on the excess risk

## Key points

- Beckwith-Wiedemann syndrome (BWS) is a model imprinting disorder resulting from mutations or epimutations affecting imprinted genes on chromosome 11p15.5.
- Recently a possible association of Angelman syndrome, another human imprinting disorder, and intracytoplasmic sperm injection (ICSI) has been described.
- To determine if there might be an association between ICSI and BWS births, we reviewed the incidence of assisted reproduction technology (ART) births in a cohort of 149 sporadic BWS births.
- Six of 149 (4%) BWS children were born after ART (three after ICSI and three after in vitro fertilisation (IVF)) compared to ~1.2% in the general population (p=0.009).
- These observations support an association between ART and human imprinting disorders.
- As both IVF and ICSI procedures were associated with BWS, loss of maternal allelic methylation at differentially methylated regions within imprinted gene clusters associated with in vitro embryo culture may be an important factor in the pathogenesis of ART associated imprinting disorders.

**Table 1** Clinical features of six patients diagnosed with Beckwith-Wiedemann syndrome after ICSI and IVF therapy

Patient ID	ART history	Clinical features	Genetic investigations
1	Twin 1 of dichorionic, diamniotic male twins conceived by ICSI (twin 2 was unaffected)	Macroglossia, umbilical hernia, ear pits, facial naevus flammeus, neonatal hypoglycaemia	Normal chromosome analysis, UPD excluded, hypomethylation KvDMR1
2	Male singleton pregnancy conceived by ICSI	Macroglossia, umbilical hernia, unilateral inguinal hernia, birth weight 3515 g, postnatal weight >97th centile, naevus flammeus, single posterior helical pit	Normal male karyotype, molecular genetic investigations not yet performed
3	Singleton male infant conceived by ICSI	Macroglossia, macrosomia (birth weight 4030 g, 137 cm cord), facial naevus flammeus	Normal male karyotype, UPD excluded, hypomethylation KvDMR1
4	IVF conception, non-identical twin pregnancy (twin unaffected)	Macroglossia, macrosomia, hepatomegaly, ear lobe creases, facial naevus flammeus	Normal male karyotype, UPD excluded
5	IVF conception, single pregnancy	Exomphalos, adrenal cytomegaly, nephromegaly. Died of prematurity related complications (born at 27 weeks' conception)	Normal chromosome analysis, UPD excluded
6	IVF conception, twin 2 of monozygotic female twins. Twin 1 unaffected	Macroglossia, diastasis recti, neonatal hypoglycaemia, helical ear pits, hemihypertrophy, facial naevus flammeus	Normal cytogenetic analysis, no molecular genetic results available

of 1.5, 8.8. It should be noted that (1) although these calculations do not take account of maternal age, there is no reported evidence that maternal age in BWS births differs from that in the general population and (2) data on the frequency of IVF and ICSI births are not available for years before 1995. Had such data been available, then this would have very probably reduced the expected number of BWS births after IVF or ICSI, as the birth rate in the general population has been declining since 1989 and the annual number of ART births before 1995 would have been less than during the five years included in the comparison. Therefore our comparison is likely to be conservative in relation to calendar year.

Of the six BWS cases, three occurred after ICSI treatment and three after IVF. Molecular genetic studies for uniparental disomy were performed in four cases and were negative in each case. Two cases were assessed for KvDMR1 methylation status and both cases showed loss of methylation on the maternal allele. One of the IVF associated cases (case 6) occurred in a discordant pair of monozygotic twins, which is of interest in the light of the report of Weksberg *et al.*,<sup>10</sup> who showed an association between monozygotic twinning in BWS and KvDMR1 loss of methylation. The KvDMR1 methylation status of case 6 is not known (DNA was not available), but it is interesting to note that Weksberg *et al.*<sup>10</sup> speculated that KvDMR1 is vulnerable to demethylation at a critical stage of preimplantation development and that this loss of imprinting predisposes to twinning and discordance for BWS.

## DISCUSSION

We have described an apparent increased frequency in children born with the aid of assisted reproductive technology (ART) in patients with the BWS imprinting disorder. Furthermore, as a detailed reproductive history had not been specifically requested, we may have overlooked additional cases. Our findings are compatible with those of Cox *et al.*,<sup>11</sup> who reported an apparently increased risk of Angelman syndrome after ICSI. We note that in both the ART associated BWS cases in which a molecular alteration was identified, we detected loss of maternal allele methylation at KvDMR1. This is consistent with the loss of maternal allele methylation at *SNRPN* in ART associated Angelman syndrome cases. However, whereas such imprinting defects are uncommon in Angelman syndrome, KvDMR1 loss of methylation is the most common molecular abnormality in sporadic BWS (~40-50% of cases).<sup>7-9</sup> We are not aware of any other surveys of the incidence of ART among a series of BWS cases, but we note that in two reports of children born after ART the frequency of BWS was 1 in 73 and 1 in 91 children, respectively.<sup>15, 16</sup> As the (minimal) incidence of BWS has been estimated recently at 0.13 per 10 000 liveborn

infants,<sup>17</sup> these findings would further support our hypothesis of a causal link between ART and disordered genomic imprinting.

Although Cox *et al.*<sup>11</sup> linked Angelman syndrome with ICSI, in our series the association of ART with BWS was not limited to ICSI. The occurrence of BWS after in vitro fertilisation without ICSI suggests that a common feature of the ICSI and IVF procedures might predispose to abnormal imprinting. We note that in animals in vitro culture of embryos and ES cells can affect DNA methylation and imprinting<sup>18-20</sup> and in vitro culture of sheep pre-embryos may be associated with fetal overgrowth and hypomethylation of a differentially methylated region of the maternal *Igf2r* allele.<sup>21</sup> This raises the possibility that in vitro embryonal cell culture per se might predispose to maternal allele demethylation and imprinting errors. Further studies are required to determine the precise relationship between human imprinting disorders and ART, but with the trends towards increasing use of ICSI and for extending in vitro culture times in ART,<sup>22</sup> it will be increasingly important to address these questions in large scale studies of children born after ART.

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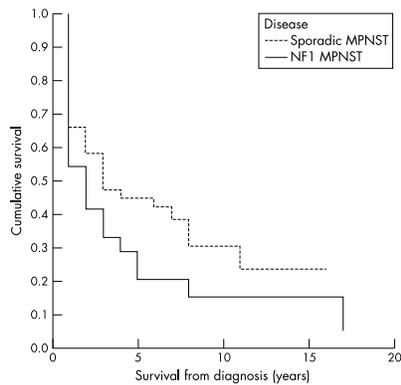
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# PostScript

## CORRECTIONS

In the May 2002 issue of the journal, in the paper by Evans *et al* on "Malignant peripheral nerve sheath tumours in neurofibromatosis 1", the Kaplan Meier curve published in the article was an analysis from birth to current age or death rather than from diagnosis. The p value attached to the curve related to the analysis from diagnosis. The correct figure of survival from diagnosis is shown below.



In the letter by Maher *et al* on "Beckwith-Wiedemann syndrome and assisted reproduction technology (ART)" in the January 2003 issue of the journal (vol 40, pp 62-64), there were four errors in the first paragraph of the Methods and result section and in the Key points. The correct paragraphs are reproduced below with the errors noted in bold.

## METHODS AND RESULTS

We reviewed the notes of 149 BWS patients who had been referred to the BWS Research Group at the Birmingham University Section of Medical Genetics and/or the West Midlands Molecular Genetics Service (for uniparental disomy analysis) and for whom detailed clinical information had been collected. A history of assisted conception techniques was recorded for six cases (4%) (table 1). To estimate whether this was likely to be a significantly increased proportion, we compared the frequency of in vitro fertilisation (IVF) and ICSI births in the BWS cohort with that in the general population. The first ART associated BWS case was born in 1989 and the most recent in 2002. Data for the number of children born after ART are available for 1995, 1996, 1997, 1998, 1999, and 2000 ("<http://www.hfea.gov.uk>") and during these years there was a total of 43 074 births after IVF or ICSI to UK residents. The corresponding number of total births in the UK was 4 320 482, so that 0.997% of births in the general population were after IVF or ICSI. Based on these data, if the proportion of births after IVF and ICSI in BWS patients and in the general population were similar, we would have expected **1.4855** of the 149 BWS patients studied to have been born as a result of IVF or ICSI. To test the significance of the observed and expected frequencies we used a Poisson approximation to the binomial distribution and obtained a two tailed p value of **0.009**. Thus, the observed frequency (n=6) of IVF and ICSI births in the BWS series is significantly greater than the expected (**1.4855**), with an associated 95% confidence interval on the excess risk of 1.5, 8.8. It should be noted that (1) although these calculations do not take account of maternal age, there is no reported evidence that maternal age in BWS births differs from that in the general population and (2) data on the frequency of IVF and ICSI births are not available for years before 1995. Had such data been available, then this would have very probably reduced the expected number of BWS births after IVF

or ICSI, as the birth rate in the general population has been declining since 1989 and the annual number of ART births before 1995 would have been less than during the five years included in the comparison. Therefore our comparison is likely to be conservative in relation to calendar year.

## Key points

- Beckwith-Wiedemann syndrome (BWS) is a model imprinting disorder resulting from mutations or epimutations affecting imprinted genes on chromosome 11p15.5.
- Recently a possible association of Angelman syndrome, another human imprinting disorder, and intracytoplasmic sperm injection (ICSI) has been described.
- To determine if there might be an association between ICSI and BWS births, we reviewed the incidence of assisted reproduction technology (ART) births in a cohort of 149 sporadic BWS births.
- Six of 149 (4%) BWS children were born after ART (three after ICSI and three after in vitro fertilisation (IVF)) compared to ~1.0% in the general population (p=0.009).
- These observations support an association between ART and human imprinting disorders.
- As both IVF and ICSI procedures were associated with BWS, loss of maternal allelic methylation at differentially methylated regions within imprinted gene clusters associated with in vitro embryo culture may be an important factor in the pathogenesis of ART associated imprinting disorders.

# Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis

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**Objective:** To conduct a meta-analysis of studies assessing the effect of IVF and intracytoplasmic sperm injection (ICSI) on birth defects.

**Design:** Meta-analysis.

**Setting:** Centers for reproductive care.

**Patient(s):** Patients treated by IVF and/or ICSI.

**Intervention(s):** We identified all studies published by September 2011 with data related to birth defects in children conceived by IVF and/or ICSI compared with spontaneously conceived children, or birth defects in the children conceived by IVF compared with those by ICSI. Risk ratios from individual studies were pooled with the fixed and random effect models.

**Main Outcome Measure(s):** Risk of birth defects in children conceived by IVF and/or ICSI.

**Result(s):** Of 925 studies reviewed for eligibility, 802 were excluded after screening titles and abstracts, 67 were excluded for duplicated data, data unavailable, or inappropriate control group, 56 were included in the final analysis. Among the 56 studies, 46 studies had data on birth defects in children conceived by IVF and/or ICSI (124,468) compared with spontaneously conceived children. These studies provided a pooled risk estimation of 1.37 (95% confidence interval [CI]: 1.26–1.48), which is also evident in subgroup analysis. In addition, 24 studies had data on birth defects in children conceived by IVF (46,890) compared with those by ICSI (27,754), which provided an overall no risk difference.

**Conclusion(s):** Children conceived by IVF and/or ICSI are at significantly increased risk for birth defects, and there is no risk difference between children conceived by IVF and/or ICSI. (Fertil Steril® 2012;97:1331–7. ©2012 by American Society for Reproductive Medicine.)

**Key Words:** Birth defects, IVF, ICSI, meta-analysis

Assisted reproductive technologies (ART), including IVF and intracytoplasmic sperm injection (ICSI), have been widely used in the treatment of human infertility. Since the first child conceived by IVF was born in the United Kingdom in 1978 (1), more than 1 million babies worldwide have been born from IVF and/or ICSI (2). There has always been a concern that the infants conceived

by ART are at an increased risk of birth defects. Most publications reported increased risks of birth defects in infants born after ART compared with spontaneously conceived (SC) children (2–24). However, controversial results were also abundant (25–47). Intracytoplasmic sperm injection involves the selection of a single sperm cell and the manual injection of the cell into the egg, thus, it is interesting to compare birth defects

risk between IVF and ICSI (2, 19–24, 41–57). Actually, case reports reported severe defects in children conceived by ICSI (58–60). To systematically evaluate published evidence on the association between birth defects and ART and compare the risk difference between IVF and ICSI, we conducted an extensive literature search and meta-analysis.

## MATERIALS AND METHODS

### Search Strategy and Selection Criteria

We retrieved Medline and Embase databases using a broad combination of search terms that included in vitro fertilization/IVF, intracytoplasmic sperm injection/ICSI, assisted reproductive technology/ART, infertility treatment and birth defect, congenital defects or

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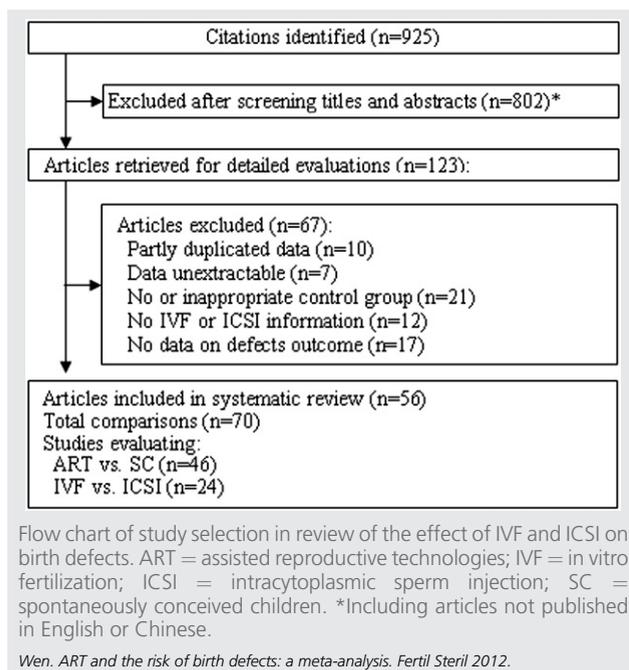
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FIGURE 1



congenital abnormality. All publications appearing before September 2011 in these databases were included. Furthermore, we reviewed reference lists in the retrieved articles. Institutional Review Board approval was obtained.

Studies were included if 1) the exposure of interest was IVF and/or ICSI; 2) the outcome of interest was birth defects; 3) comparison of IVF and/or ICSI to SC, or comparison of IVF to ICSI; and 4) risk ratios (RR) with 95% confidence intervals (CI) provided or could be calculated (Fig. 1). Because of language barrier, only studies published in English or Chinese were included for further analysis. We excluded studies that were not published as full reports; studies that were case reports; studies with inappropriate comparison group or without control subjects.

### Independent Assessment

Two investigators (J. Wen and J. Jiang) independently reviewed all the articles, and data were checked by other investigators. The two investigators were blinded to identify information from each study, and judged the inclusion and exclusion of the study. Where a study provided birth defect data for IVF and ICSI infants separately compared with a single SC comparison group (24, 47), the data were pooled to form one risk estimate of IVF plus ICSI versus SC. If the sources of study population recruitment overlapped in two or more articles, the one with the more detailed birth defect information was selected (4, 22, 61–64).

Authors, publication year, study location, types of treatment, number of ART infants and control subjects, study design, birth defect subgroup, adjustment for confounders, and other related information were extracted. The concordance rate between the two investigators was 98.4%. Discrepancies were resolved by consensus.

### Statistical Analysis

If adjusted RR was not given, crude RR was used. In all the included studies, only an American study was a case-control study (5). Because birth defects are rare, we assumed equivalence of the odds ratio and the relative risk. Therefore, we apply RR for the effect measure of this study. Statistical heterogeneity among studies was evaluated by using the  $\chi^2$  test,  $P$  values, and  $I^2$  statistics (65). Where the homogeneous test was not significant ( $P > .10$ ), a fixed-effects model was used to obtain summary RR, otherwise, a random-effects model was used. Publication bias was evaluated by using funnel plots and the Begg's test (66). A  $P$  value less than .10 was considered to indicate significant publication bias. All statistical tests were two-sided and calculated using the Stata software (version 9.1; Stata Corp).

### RESULTS

Of the 56 included studies, 14 included data on both IVF and/or ICSI children compared with SC children and IVF children compared with ICSI children. A summary of the 56 included studies is given in Supplemental Table 1 (available online). The earliest study was published in 1989 and the latest one in 2011, but more than half of the studies were published within the past 6 years. The sample size of the IVF and/or ICSI patients in each study ranged from 34–16,280. Thirty of 56 studies (54%) had <1,000 IVF and/or ICSI patients, and about half of the studies (54%) were population-based. A few of the studies matched the cases and controls, but the majority of the studies (64%) adjusted confounding factors such as maternal age, parity, sex, year of birth, social class, and/or smoking. Furthermore, 45% of the studies stratified birth defects to various organ systems.

Overall, 46 studies accessed birth defects of IVF and/or ICSI children compared with SC children, involving 124,468 IVF and/or ICSI infants. As shown in Table 1, for IVF and/or ICSI children compared with SC children, a significantly increased risk of birth defects was observed (RR = 1.37, 95% CI 1.26–1.48). The individual risk estimate for these studies ranged from 0.56–5.53 (Fig. 2).

In our analysis, a small Australia study had a very low RR of 0.56 (95% CI 0.37–0.84) (39), significantly different from other studies. In that study, birth defects were assessed at 2 years of age, and neonatal deaths were excluded. Thus, we removed that study in all subsequent analysis. After removal of this study, the adjusted/crude RR of ART versus SC was 1.39 (95% CI 1.29–1.50).

The pooled adjusted RR or crude RR was 1.36 (95% CI 1.25–1.47) and 1.45 (95% CI 1.33–1.59), respectively, indicating an adjustment of the potential confounding factors that may help clarify the true risk estimates, although the heterogeneity test was not significant between the two subgroups ( $P = .298$ ) (Table 1). We also stratified our analyses to IVF or ICSI compared with SC, population or clinic-based studies, study sample size, and defect-affected systems. In our results, the RR of ICSI compared with SC (1.58) was larger than that of IVF compared with SC (1.30), but statistical significance was not reached (heterogeneity test:  $P = .113$ ). The RR of clinic-based studies (1.67) was significantly larger than that of

TABLE 1

## Results of birth defects in children conceived by IVF and/or ICSI.

	No. of comparisons	Pooled RR <sup>a</sup> (95% CI)	Pooled RR <sup>b</sup> (95% CI)	P value <sup>c</sup>	I <sup>2</sup> (%) <sup>c</sup>
ART vs. SC					
All defects (adjusted/crude data <sup>d</sup> )	46		1.37 (1.26–1.48)	.000	74.6
All defects (adjusted/crude data <sup>e</sup> )	45		1.39 (1.29–1.50)	.000	72.6
Adjusted data	33		1.36 (1.25–1.47)	.000	65.3
Crude data	27		1.45 (1.33–1.59)	.000	80.7
Subgroup analyses					
Population or Clinic-based data					
Population-based data	26		1.34 (1.24–1.45)	.000	78.9
Clinic-based data	19		1.67 (1.32–2.11)	.031	41.4
No. conceived by ART					
>1,000	23		1.31 (1.21–1.41)	.000	78.6
<1,000	22		1.77 (1.43–2.18)	.008	47.4
IVF or ICSI vs. SC					
IVF vs. SC	16		1.30 (1.17–1.46)	.055	39.1
ICSI vs. SC	15		1.58 (1.27–1.95)	.001	61.6
Systems					
Nervous system	15		2.01 (1.27–3.20)	.000	89.3
Genitourinary system	17		1.69 (1.33–2.15)	.000	86.4
Digestive system	19		1.66 (1.28–2.16)	.000	72.5
Circulatory system	21		1.64 (1.30–2.07)	.000	91.0
Musculoskeletal system	18		1.48 (1.09–2.02)	.000	90.8
Eye, ear, face, and neck	15		1.43 (1.01–2.05)	.000	84.5
IVF vs. ICSI					
All defects (adjusted/crude data <sup>d</sup> )	24		1.05 (0.91–1.20)	.003	50.6
Subgroup of IVF vs. ICSI					
System					
Nervous system	4	0.80 (0.51–1.27)		.534	0.0
Digestive system	8	1.28 (0.90–1.82)		.327	13.2
Genitourinary system	8		1.00 (0.71–1.41)	.073	46.0
Circulatory system	8	0.95 (0.79–1.13)		.170	32.3
Musculoskeletal system	8	0.83 (0.69–1.00)		.495	4.6
Eye, ear, face, and neck	7	1.14 (0.82–1.57)		.755	0.0

Note: ART = assisted reproductive technologies; ICSI = intracytoplasmic sperm injection; SC = spontaneously conceived children.

<sup>a</sup> With fixed-effect model.

<sup>b</sup> With random-effect model.

<sup>c</sup> For heterogeneity test.

<sup>d</sup> Included adjusted or crude RR, if adjusted RR was not given, crude RR was used.

<sup>e</sup> After excluding Sauder et al., 1996.

Wen. ART and the risk of birth defects: a meta-analysis. *Fertil Steril* 2012.

population-based studies (1.34) (heterogeneity test:  $P=.081$ ); and the RR of the groups with sample size less than 1,000 (1.77) was significantly larger than that of the groups with sample size more than 1,000 (1.31) (heterogeneity test:  $P=.009$ ). In addition, subgroup analyses by defect-affected system were all significant, especially the nervous system ( $RR = 2.01$ , 95% CI 1.27–3.20) (Table 1).

Twenty-four studies were for birth defects in children conceived by IVF compared with those by ICSI, involving 46,890 IVF infants and 27,754 ICSI infants. Overall, there is no risk difference for birth defects between IVF and ICSI groups ( $RR = 1.05$ , 95% CI 0.91–1.20), which is consistent with the risk estimates when compared with SC children. The individual point estimates for these studies ranged from 0.33–3.05 (Fig. 3). In subgroup analysis, the difference in risk for musculoskeletal system malformations was approaching significance, but considering multiple comparisons, we believe that it is not reliable evidence.

Then we evaluated publication bias by using funnel plots (Supplemental Figs. 1 and 2, available online) and the Begg's test. The  $P$  value of the Begg's test for the adjusted/crude data

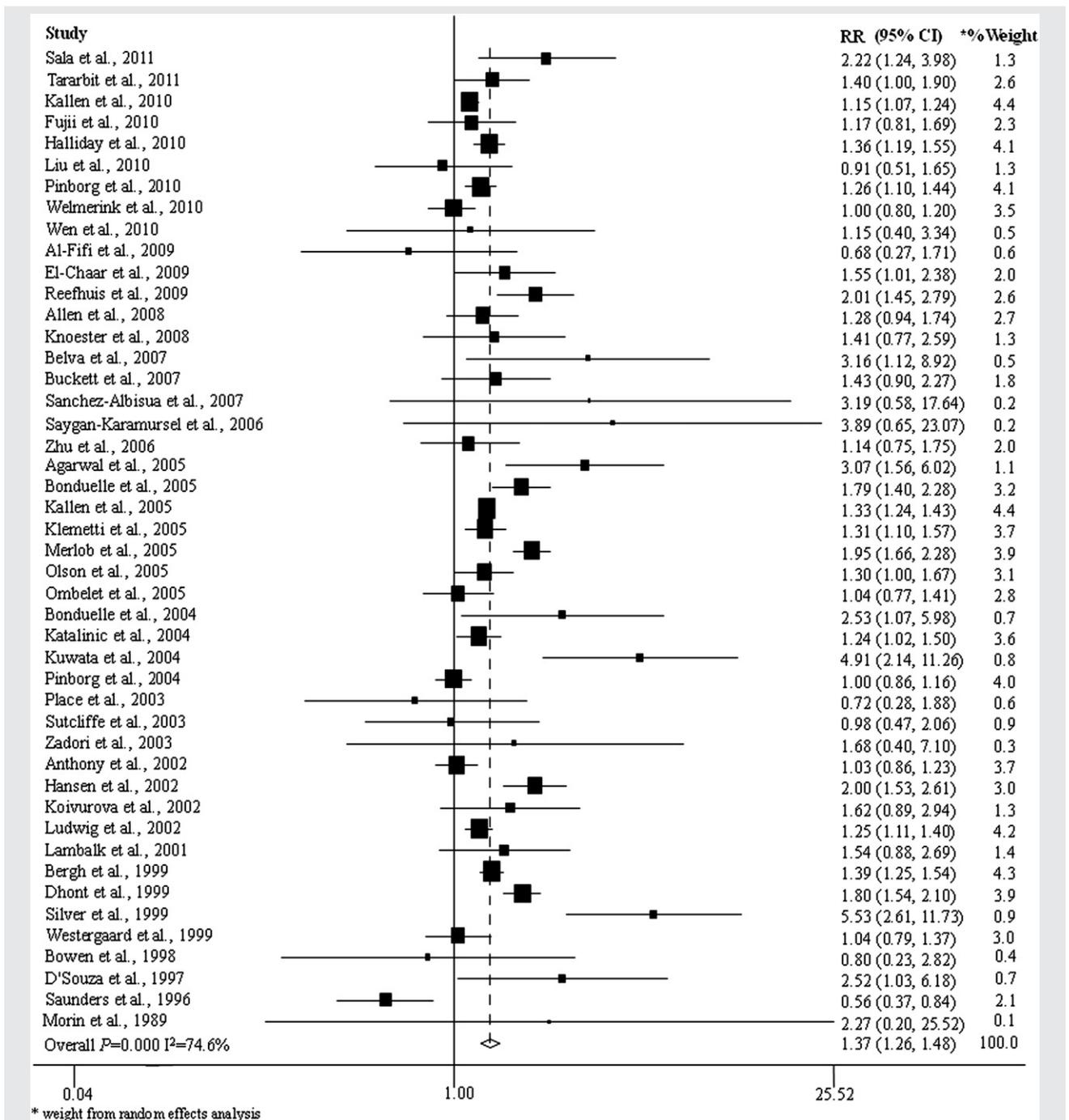
of IVF and/or ICSI versus SC and IVF versus ICSI were 0.293 and 0.552, respectively, indicating that there was no obvious publication bias in our analysis.

## DISCUSSION

This report reviewed and pooled epidemiological data assessing the risk of birth defects after ART and compared the risk difference of birth defects after ICSI and IVF. Our results suggest that there is a significantly increased risk of birth defects in infants conceived by ART, but ICSI did not increase the risk compared with IVF.

According to the source of control, we divided studies into population-based studies and clinic-based studies. The RR of clinic-based group was significantly larger than that of population-based ones. This may be because hospitals do not actively seek birth defect information beyond that obtained at birth and therefore clinic-based control data are likely to underestimate the rate of birth defects. In addition, the RR of the group with a sample size less than 1,000 was significantly larger than that of the group with sample size of

**FIGURE 2**



Individual risk ratio estimates and pooled ratio estimates from the studies relating IVF and ICSI children compared with spontaneously conceived children. Abbreviations as in Fig. 1. \*Weight from random effects analysis.

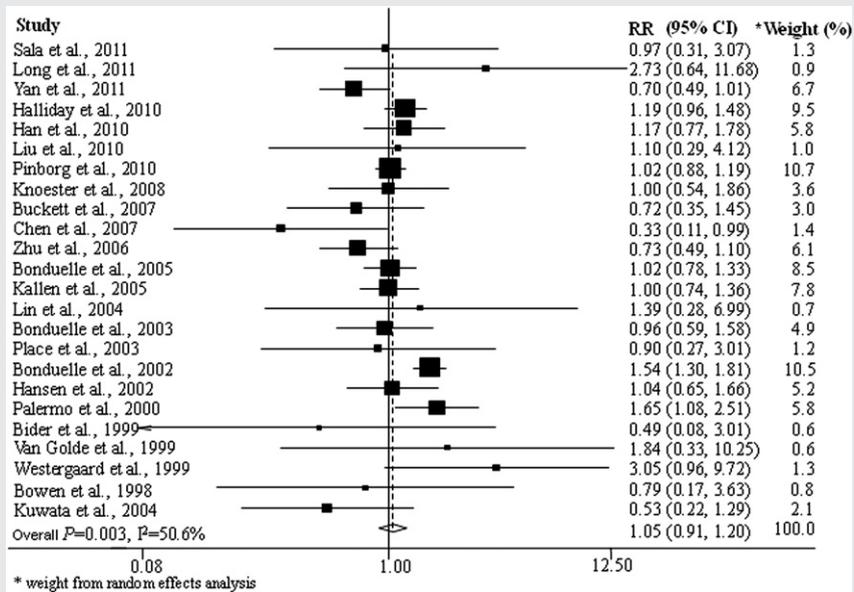
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more than 1,000. Because larger sample studies are more representative and have less bias, the results of studies with larger sample size tended to be more reliable, and were similar to the adjusted RR. Although the difference in the prevalence of defects among the various systems was not significant, the effect of ART on the nervous system is relatively obvious,

compared with the effects on eyes, ears, face, and neck, which may suggest that the earlier developed systems were more sensitive to birth defects by ART.

There is considerable heterogeneity among the 56 included studies. The differences included methodologies for assessing the case and control infants and the extent of

FIGURE 3



Individual risk ratio estimates and pooled risk ratio estimates from studies relating birth defects in children conceived by IVF compared with ICSI. Abbreviations as in Fig. 1. \*Weight from random effects analysis.

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matching for maternal age. There are also many competing risks that could increase the rate: age of mother, factors causing the infertility in the mother or father, prior treatment for infertility, duration of infertility, environmental exposures, risk behaviors such as alcohol and smoking, and the ART procedures themselves. As we can see, the overall effect decreased after adjustment for some variables.

Factors associated with ART that may increase the risk of birth defects include the underlying infertility in the couples seeking treatment, and factors associated with the ART procedures themselves. Some researchers have argued that the excess risk of birth defects found in infants born after ART treatment may be due to the underlying infertility of the couples seeking treatment, rather than the treatments themselves (63, 67). Ericson and Kallen (63) proposed that the excess risk for some specific defects after ICSI may be related to paternal subfertility with a genetic background. A careful analysis of the outcome of singleton pregnancies resulting from IVF versus artificial insemination obtained with or without the use of ovarian stimulatory agents and obtained with or without the use of a semen donor, suggests that female infertility is an important risk factor (67). Thus, the major limitation of the study is that the comparison group for IVF and/or ICSI is SC rather than babies born to infertile couples who conceived without these procedures. The reason why we did not do a meta-analysis with this comparison group is because there are very few such studies in the literature. It has recently been suggested that, to address this question, an appropriate comparison group would include children born to infertile couples who do eventually conceive spontaneously without ART treatment (68).

Taken together, large-scale research on the prevalence of ART-associated birth defects and long-term follow-up of the infants are still essential for the estimation of birth defects risk after ART. In addition, studies of special defects are also needed.

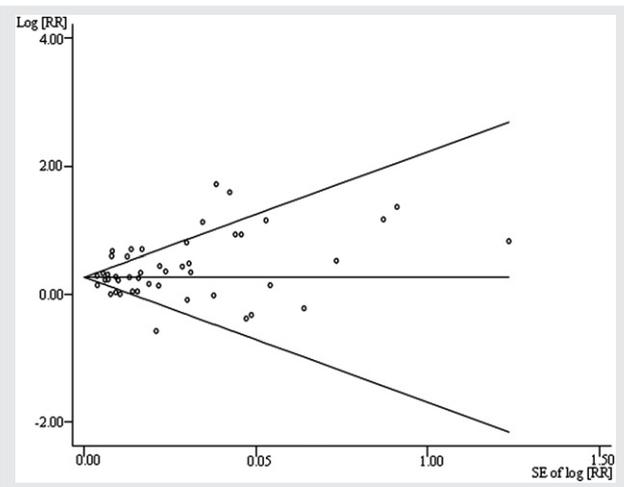
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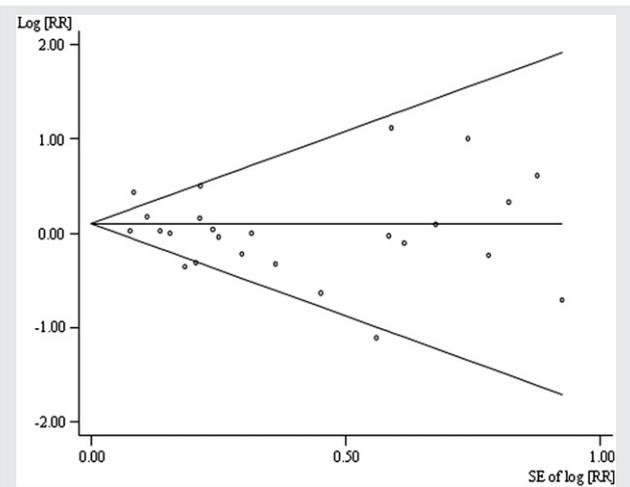
**SUPPLEMENTAL FIGURE 1**



Funnel plot of log (RR) against SE of log (RR) for studies relating birth defects in IVF and/or ICSI children compared with spontaneously conceived children.

*Wen. ART and the risk of birth defects: a meta-analysis. Fertil Steril 2012.*

**SUPPLEMENTAL FIGURE 2**



Funnel plot of log (RR) against SE of log (RR) for studies relating birth defects in children conceived by IVF compared with by ICSI.

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## SUPPLEMENTAL TABLE 1

## Details of studies included in the meta-analysis of effects of IVF and/or ICSI on birth defects.

Author(s), publication year	Location	No. conceived by IVF and/or ICSI	No. conceived by IVF	No. conceived by ICSI	Study evaluated	Adjusted <sup>a</sup> or crude data	ART vs. SC RR <sup>b</sup> (95% CI)	IVF vs. ICSI RR <sup>b</sup> (95% CI)
Bonduelle et al., 2005	Europe	977	437	540	Both	Adjusted	1.79 (1.40–2.28)	1.02 (0.78–1.33)
Tararbit et al., 2011	France	5,493			ART vs. SC	Adjusted	1.40 (1.00–1.90)	
Kallen et al., 2010	Sweden	15,570			ART vs. SC	Adjusted	1.15 (1.07–1.24)	
Reefhuis et al., 2009	USA	9,584			ART vs. SC	Adjusted	2.01 (1.45–2.79)	
El-Chaar et al., 2009	Canada	790			IVF vs. SC	Adjusted	1.55 (1.01–2.38)	
Belva et al., 2007	Belgium	150			ICSI vs. SC	Crude	3.16 (1.12–8.92)	
Olson et al., 2005	USA	1,462			ART vs. SC	Adjusted	1.30 (1.00–1.67)	
Merlob et al., 2005	Israel	1,910			ART vs. SC	Crude	1.95 (1.66–2.28)	
Klemetti et al., 2005	Finland	4,559			IVF vs. SC	Adjusted	1.31 (1.10–1.57)	
Agarwal et al., 2005	Multiple locations	76			ICSI vs. SC	Adjusted	3.07 (1.56–6.02)	
Katalinic et al., 2004	Germany	3,372			ICSI vs. SC	Adjusted	1.24 (1.02–1.50)	
Bonduelle et al., 2004	Multiple locations	300			ICSI vs. SC	Adjusted	2.53 (1.07–5.98)	
Ludwig et al., 2002	Germany	3,372			ICSI vs. SC	Crude	1.25 (1.11–1.40)	
Silver et al., 1999	USA	481			IVF vs. SC	Crude	5.53 (2.61–11.73)	
Dhont et al., 1999	Belgium	5,539			ART vs. SC	Adjusted	1.80 (1.54–2.10)	
Bergh et al., 1999	Sweden	5,856			IVF vs. SC	Adjusted	1.39 (1.25–1.54)	
D'Souza et al., 1997	UK	278			IVF vs. SC	Adjusted	2.52 (1.03–6.18)	
Sala et al., 2011	Italy	225	88	137	Both	Crude	2.22 (1.24–3.98)	0.97 (0.31–3.07)
Pinborg et al., 2010	Denmark	11,233	7,564	3,669	Both	Adjusted	1.26 (1.10–1.44)	1.02 (0.88–1.19)
Halliday et al., 2010	Australia	6,946	3,312	3,634	Both	Adjusted	1.36 (1.19–1.55)	1.19 (0.96–1.48)
Kallen et al., 2005	Sweden	16,280	11,283	4,949	Both	Adjusted	1.33 (1.24–1.43)	1.00 (0.74–1.36)
Kuwata et al., 2004	Japan	232	148	84	Both	Adjusted	4.91 (2.14–11.26)	0.53 (0.22–1.29)
Hansen et al., 2002	Australia	1,138	837	301	Both	Adjusted	2.00 (1.53–2.61)	1.04 (0.65–1.66)
Wen et al., 2010	Canada	1,044			ART vs. SC	Adjusted	1.15 (0.40–3.34)	
Welmerink et al., 2010	USA	2,182			IVF vs. SC	Adjusted	1.00 (0.80–1.20)	
Fujii et al., 2010	Japan	1,408			ART vs. SC	Adjusted	1.17 (0.81–1.69)	
Al-Fifi et al., 2009	Saudi Arabia	253			ICSI vs. SC	Crude	0.68 (0.27–1.71)	
Allen et al., 2008	UK	1,524			IVF vs. SC	Crude	1.28 (0.94–1.74)	
Sanchez-Albisua et al., 2007	Germany	34			ICSI vs. SC	Crude	3.19 (0.58–17.64)	
Saygan-Karamursel et al., 2006	Turkey	274			ICSI vs. SC	Adjusted	3.89 (0.65–23.07)	
Ombelet et al., 2005	Belgium	2,757			ICSI vs. SC	Adjusted	1.04 (0.77–1.41)	
Pinborg et al., 2004	Denmark	3,393			ART vs. SC	Crude	1.00 (0.86–1.16)	
Zadori et al., 2003	Hungary	262			IVF vs. SC	Adjusted	1.68 (0.40–7.10)	
Sutcliffe et al., 2003	Multiple locations	264			ICSI vs. SC	Adjusted	0.98 (0.47–2.06)	
Koivurova et al., 2002	Finland	304			IVF vs. SC	Adjusted	1.62 (0.89–2.94)	
Anthony et al., 2002	Netherlands	4,224			IVF vs. SC	Adjusted	1.03 (0.86–1.23)	
Lambalk et al., 2001	Netherlands	480			IVF vs. SC	Adjusted	1.54 (0.88–2.69)	
Saunders et al., 1996	Australia	314			IVF vs. SC	Adjusted	0.56 (0.37–0.84)	
Morin et al., 1989	USA	83			IVF vs. SC	Adjusted	2.27 (0.20–25.52)	
Liu et al., 2010	China	567	415	152	Both	Crude	0.91 (0.51–1.65)	1.10 (0.29–4.12)
Knoester et al., 2008	Netherlands	87	81	81	Both	Adjusted	1.41 (0.77–2.59)	1.00 (0.54–1.86)
Buckett et al., 2007	Canada	377	217	160	Both	Adjusted	1.43 (0.90–2.27)	0.72 (0.35–1.45)
Zhu et al., 2006	Denmark	6,278	1,483	398	Both	Adjusted	1.14 (0.75–1.75)	0.73 (0.49–1.10)
Place et al., 2003	Belgium	118	52	66	Both	Adjusted	0.72 (0.28–1.88)	0.90 (0.27–3.01)
Westergaard et al., 1999	Denmark	2,245	1,913	180	Both	Adjusted	1.04 (0.79–1.37)	3.05 (0.96–9.72)
Bowen et al., 1998	Australia	173	84	89	Both	Adjusted	0.80 (0.23–2.82)	0.79 (0.17–3.63)

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## SUPPLEMENTAL TABLE 1

Continued.

Author(s), publication year	Location	No. conceived by IVF and/or ICSI	No. conceived by IVF	No. conceived by ICSI	Study evaluated	Adjusted <sup>a</sup> or crude data	ART vs. SC RR <sup>b</sup> (95% CI)	IVF vs. ICSI RR <sup>b</sup> (95% CI)
Yan et al., 2011	China		7,096	3,103	IVF vs. ICSI	Crude		0.70 (0.49–1.01)
Long et al., 2011	China		1,575	388	IVF vs. ICSI	Crude		2.73 (0.64–11.68)
Han et al., 2010	China		4,670	3,837	IVF vs. ICSI	Crude		1.17 (0.77–1.78)
Chen et al., 2007	China		991	283	IVF vs. ICSI	Crude		0.33 (0.11–0.99)
Lin et al., 2004	China		134	185	IVF vs. ICSI	Crude		1.39 (0.28–6.99)
Bonduelle et al., 2003	Belgium		207	439	IVF vs. ICSI	Crude		0.96 (0.59–1.58)
Bonduelle et al., 2002	Belgium		2,295	2,840	IVF vs. ICSI	Crude		1.54 (1.30–1.81)
Palermo et al., 2000	USA		1,796	2,059	IVF vs. ICSI	Crude		1.65 (1.08–2.51)
Van Golde et al., 1999	Spain		132	120	IVF vs. ICSI	Crude		1.84 (0.33–10.25)
Bider et al., 1999	Israel		80	60	IVF vs. ICSI	Crude		0.49 (0.08–3.01)

Note: ART = assisted reproductive technologies; ICSI = intracytoplasmic sperm injection; SC = spontaneously conceived children; Both = studies evaluating both IVF and/or ICSI vs. SC and IVF vs. ICSI; Adjusted<sup>a</sup>: included adjusted and matched data; RR<sup>b</sup>: pooled from adjusted RR or crude RR, if adjusted RR was not given, crude RR substituted for it.

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# Bleeding and spontaneous abortion after therapy for infertility

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**Objective:** To determine the incidence of early-pregnancy bleeding and spontaneous abortion (SAB) after various treatments for infertility and to assess whether bleeding is predictive of SAB.

**Design:** An historic cohort study of women who conceived after various treatments.

**Setting:** Hospital-based private practice.

**Patient(s):** We studied 418 patients in whom 500 consecutive clinical pregnancies occurred.

**Intervention(s):** Patients were grouped according to the method of conception: ovulation induction, IVF, and other. The latter category included interventions not requiring ovulation induction, such as surgery and insemination. A fourth group of subjects who conceived independently of treatment was used as the control.

**Main Outcome Measure(s):** Bleeding and pregnancy outcome (SAB, ectopic pregnancy, or ongoing pregnancy).

**Result(s):** Rates of SAB did not differ among the treatment groups. SAB occurred significantly more often after bleeding than when bleeding did not occur (30.8% versus 19.8%, respectively). Bleeding was predictive of SAB only in patients <35 years old (odds ratio 2.4).

**Conclusion(s):** Infertile women who conceive after reproductive therapy are not at increased risk for SAB compared with women who conceive naturally. There appears to be no association between previous diagnosis or treatment and the occurrence of SAB in previously infertile women. Bleeding is associated with a twofold relative risk of SAB. (Fertil Steril® 2000;74:504–8. ©2000 by American Society for Reproductive Medicine.)

**Key Words:** Bleeding, infertility, pregnancy, spontaneous abortion

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Spontaneous abortion (SAB) occurs in 20%–25% of pregnancies (1, 2). In the setting of previous infertility, SAB is especially troubling to the patient, who typically questions whether the treatment leading to her pregnancy was related in some way to the adverse outcome. Any amount of bleeding that occurs during early pregnancy in these women evokes a similar question. Counseling of patients with a history of infertility who conceive is often required to explain the significance of bleeding and the risk of miscarriage.

Although most reproductive specialists are aware that bleeding during early pregnancy is common and usually does not herald pregnancy loss, there are few data upon which to base this reassurance. Our review of the current literature yielded no statistics on the precise incidence of bleeding in pregnancies after fertility therapy. There are also no data describing

the presence or absence of an association between fertility therapies and subsequent bleeding during pregnancy.

This study was performed to determine the incidence of early-pregnancy bleeding after various treatments for infertility, to compare it with the incidence of bleeding in naturally occurring pregnancies, and to assess whether such bleeding is related to the occurrence of SAB.

## MATERIALS AND METHODS

We performed a retrospective cohort analysis of women who conceived after infertility treatments at Brooklyn IVF from May 1996 through September 1998. We used the patients' medical records to simulate the prospective experience. The cohorts were determined on the basis of exposure to a specific type of treatment for infertility and the presence or

absence of bleeding. The predictor variables were cause of infertility, method of treatment, and bleeding. The outcome of primary interest was pregnancy outcome (SAB, ectopic pregnancy, or ongoing pregnancy).

## Subjects

All patients sought treatment for infertility at Brooklyn IVF, a hospital-based private practice. All patient records are kept in an electronic database (Labsystem; Kretz Consulting, Cabin John, MD) that runs on a Novell network of 30 linked PCs. Every encounter is recorded, including patient telephone calls, which are routed electronically from receptionists to the clinicians responsible for responding. These messages, as well as the responses from the clinicians, become part of the electronic record. Patients' subjective complaints are also recorded at the time of each office visit with the use of an electronic version of the traditional subjective, objective, assessment, and plan protocol.

A list of all patients who conceived during the study period was assembled with the use of data from serum hCG testing at the on-site laboratory. This hormone was assayed with an immunofluorescence technique (3). Levels of hCG of  $\geq 10$  mIU/mL were considered positive. All patient records corresponding to a positive test were reviewed. Patients who conceived before initiating any fertility evaluation or treatment as well as patients with "chemical pregnancies" (transiently positive hCG and no sac detected on ultrasonographic (US) examination) were excluded from the study. Patients who conceived after evaluation and treatment but independent of the treatment given were included in the study as controls. During the study period, all patients were given US examinations at 6 and 8 weeks of pregnancy and were referred out for obstetric care at 12 weeks.

## Definitions

Infertility was defined as the inability to conceive after 12 months of unprotected coitus. Causes of infertility were categorized as follows. Patients were considered to have male factor infertility if they were being treated for a known defect in their husbands' sperm count, motility, or morphology as defined by World Health Organization (4) or the criteria of Kruger et al. (5). Patients were diagnosed with ovulatory dysfunction if they were being treated for anovulation, luteal phase defect, or oligoovulation. Anatomic infertility included women with a diagnosis of tubal occlusion on hysterosalpingogram or with laparoscopic confirmation of adhesive disease. Endometriosis was diagnosed by laparoscopic findings. Cervical factor infertility was diagnosed when repeated, well-timed postcoital testing showed no viable sperm but the partner's semen analysis was normal. Miscellaneous diagnoses included known uterine anomalies, immunologic infertility, advanced maternal age, hyperprolactinemia, and history of infertility after recurrent pregnancy loss. Unexplained infertility was diagnosed when all of the aforementioned causes of infertility had been ex-

cluded. Any patient with more than one known cause of infertility was categorized as having multifactorial infertility.

Using primary modes of treatment exclusively, we identified and labeled three distinct treatment groups as follows. Group I (ovulation induction) consisted of women who conceived after ovulation induction with either clomiphene citrate (CC), gonadotropins, or gonadotropins in combination with a GnRH analog (GnRH-a). The use or nonuse of intrauterine insemination (IUI) did not alter assignment to this group. Group II (IVF) included women who conceived after IVF with or without intracytoplasmic sperm injection. All patients were pretreated with a GnRH-a and gonadotropins with either a long or short protocol (6). Group III (other) included patients who conceived after any type of reproductive surgery, IUI during an unstimulated cycle, or medical treatment, including bromocriptine and P. Group IV (untreated controls) was comprised of women who presented with infertility and were evaluated and/or treated, but who conceived independently of any treatment.

The diagnosis of bleeding during early pregnancy was based upon the patients' subjective complaints and/or physical examination. The outcome of every early pregnancy was known. Outcomes were coded as SAB, ectopic pregnancy, or ongoing pregnancy. Spontaneous abortions included complete, incomplete, and missed abortions. Ongoing pregnancies were defined as those progressing to  $\geq 12$  weeks.

## Database

Data were organized with Microsoft Access (Microsoft, Redmond, WA), a relational database program that can be customized for unlimited data fields. The age and parity of every patient were entered. The primary diagnosis as well as any secondary diagnosis and treatment were entered. We used the age breakdown of  $<35$ ,  $35-39$ , and  $\geq 40$  years to be consistent with the IVF Registry (7). The presence or absence and the severity of bleeding were noted in the database. Mild, moderate, and severe bleeding were combined in our analysis because patients' subjective experience of bleeding could easily affect this classification, and it was not always possible to examine patients at the time of the reported episode.

Fisher's exact test was used for assessing the association of two variables. An exact trend test was used to examine the associations by age group. Multiple logistic regression was performed to analyze the association among several variables. In the regression model, SAB was the outcome and bleeding was the predictor variable; age, treatment, and diagnosis were the covariates. Interactions among bleeding, age, treatment, and diagnosis were examined. Models were assessed for goodness of fit and outliers. Statistical significance was defined as  $P < .05$ , and confidence intervals (CIs) were at the 95% level. Computations were performed with the use of software from StatXact (Cytel Software, Cambridge, MA) and SPSS (Chicago, IL).

**TABLE 1**

Treatments leading to pregnancy, grouped according to age.

Age (y)	Treatment group <sup>a</sup>				Total
	I	II	III	IV	
<35	191 (50.1)	69 (18.1)	44 (11.5)	77 (20.2)	381
35–39	41 (46.6)	19 (21.6)	10 (11.4)	18 (20.5)	88
≥40	8 (25.8)	8 (25.8)	3 (9.7)	12 (38.7)	31
Total	240 (48.0)	96 (19.2)	57 (11.4)	107 (21.4)	500

Note: Values are n (%). Numbers in parentheses indicate percentage of total age group. <sup>a</sup> Group key: I = ovulation induction; II = IVF; III = other; IV = spontaneous conception.

Pezeshki. Bleeding and spontaneous abortion. Fertil Steril 2000.

The analysis focused on risk factors for SAB compared with ongoing pregnancies. Ectopic pregnancies, although shown in the tables for completeness, were not included in the analysis. The implication of failing to find a difference in the frequency of bleeding by treatment group was quantified by estimating the power to detect such a difference given the current sample sizes.

## RESULTS

The study group consisted of 418 patients, among whom 500 clinical pregnancies occurred. The age breakdown was <35 years old (76.2%), 35–39 years (17.6%), and ≥40 years (6.2%). Approximately two thirds of the study population was nulliparous. The diagnostic groups of infertility were as follows: 11.8% male factor, 29.2% ovulatory dysfunction, 12.8% anatomic, 3.6% endometriosis, 4.2% cervical factor, 10.2% miscellaneous, 16.4% unexplained, and 11.8% multifactorial.

Table 1 shows the age distribution of patients in treatment groups I–IV. There were 240 women in group I (ovulation induction), 96 in group II (IVF), 57 in group III (other), and 107 in group IV (untreated).

Table 2 presents the outcomes of pregnancy in the study

**TABLE 2**

Age and pregnancy outcome.

Age (y)	Ongoing	SAB	Ectopic	Total
<35	276 (72.4)	76 (19.9) <sup>a</sup>	29 (7.6)	381
35–39	57 (64.8)	25 (28.4)	6 (6.8)	88
≥40	15 (48.4)	15 (48.4)	1 (3.2)	31
Total	348 (69.6)	116 (23.2)	36 (7.2)	500

Note: Values are n (%).

<sup>a</sup>  $P < .01$ .

Pezeshki. Bleeding and spontaneous abortion. Fertil Steril 2000.

**TABLE 3**

Rate of SAB by maternal age and treatment.

Age (y)	Treatment groups <sup>a,b</sup>			
	I	II	III	IV
<35	36 (18.8)	13 (18.8)	12 (27.3)	15 (19.5)
35–39	11 (26.8)	3 (15.8)	3 (30.0)	8 (44.4)
≥40	4 (50.0)	3 (37.5)	3 (100.0)	5 (41.7)
Total	51 (21.3)	19 (19.8)	18 (31.6)	28 (26.2)

Note: Values are n (%). Numbers in parentheses indicate percentage of total age group who received the indicated treatment and had SAB.

<sup>a</sup> Group key: I = ovulation induction; II = IVF; III = other; IV = spontaneous conception.

<sup>b</sup>  $P =$  not significant for all treatment and age groups.

Pezeshki. Bleeding and spontaneous abortion. Fertil Steril 2000.

population by age group. The overall rate of SAB was 23.2%. SAB occurred significantly less often in women <35 years old than in women in the two older categories (odds ratio [OR] 1.6, 95% CI 0.9–2.7 and OR 3.6, 95% CI 1.7–7.8;  $P_{\text{trend}} < .01$ ). There was no association between the diagnosis for which treatment was given and the occurrence of SAB (data not shown).

Table 3 groups the patients who had SAB by maternal age and treatment. Rates of SAB were not significantly different across treatment groups ( $P \leq .26$ ). Furthermore, the association of SAB with age was consistent among the treatment groups ( $P_{\text{interaction}} \leq .82$ ). Patients in group I (ovulation induction) also were analyzed according to whether they were treated with CC or gonadotropins. There was no statistically significant difference in the rates of SAB between the groups (data not shown).

In Table 4, pregnancy outcome is analyzed by age and the

**TABLE 4**

Early-pregnancy bleeding and pregnancy outcome.

Age (y)	Bleeding	Ongoing	SAB	Ectopic
<35	No	197 (75.8)	39 (15.0)	24 (9.2)
	Yes	79 (65.3)	37 (30.6) <sup>a</sup>	5 (4.1)
35–39	No	37 (63.8)	16 (27.6)	5 (8.6)
	Yes	20 (66.7)	9 (30.0)	1 (3.3)
≥40	No	12 (46.2)	13 (50.0)	1 (3.8)
	Yes	3 (60.0)	2 (40.0)	—
Total	No	246 (71.5)	68 (19.8)	30 (8.7)
	Yes	102 (65.4)	48 (30.8) <sup>b</sup>	6 (3.8)

Note: Values are n (%).

<sup>a</sup>  $P < .01$  compared with SAB rate among women in the same age group with no early-pregnancy bleeding (OR 2.4, 95% CI 1.4–3.98).

<sup>b</sup>  $P < .01$  compared with SAB rate among all women with no early-pregnancy bleeding (OR 1.85, 95% CI 1.18–2.89).

Pezeshki. Bleeding and spontaneous abortion. Fertil Steril 2000.

presence of early bleeding. SAB was seen more commonly after bleeding than when bleeding did not occur (30.8% versus 19.8%, respectively;  $P < .01$ ). Bleeding was associated with an 85% increased risk of subsequent SAB as compared with ongoing pregnancy (OR 1.85, 95% CI 1.18–2.89) after adjusting for age. In analyses stratifying by age, bleeding was predictive of miscarriage in patients <35 years old (OR 2.4, 95% CI 1.4–3.98;  $P < .01$ ) but not in women >35 years old (OR 0.81, 95% CI 0.34–1.89). The attributable risk of SAB, which is the difference in occurrence between women who had bleeding and those who did not, was 15.6% in women <35 years old.

There was no association between the occurrence of bleeding and the diagnosis for which patients were treated (data not shown). By treatment group, bleeding varied from 26.2% among spontaneous pregnancies to 34.4% among patients who had ovulation induction ( $P \leq .52$ ). The power to detect an 8% difference in bleeding, if one existed, by treatment in the population with the present sample size was only 24%.

## DISCUSSION

The initial purpose of this study was to provide information with which to counsel infertile women who conceived after reproductive therapy about their risks of SAB, particularly in the setting of first-trimester bleeding. Women who bled had a higher risk of SAB than women who did not. The frequency of SAB was twice as high when bleeding was encountered. Because bleeding occurred in one third of the women <35 years old, the attributable risk was high (15.6%). Nonetheless, the fact that two thirds of the patients who bled did not lose their pregnancies should provide some reassurance.

The high frequency of bleeding reported by women in this cohort (31.2%) may be related to the composition of the study group (infertility patients) who, after pregnancy is diagnosed, tend to be sensitive to even the smallest amount of staining or spotting. Although bleeding often resolves before an office visit, we were able to track the occurrence of bleeding accurately because our institutional protocol requires that all patient encounters, including telephone calls, be recorded in our electronic patient database.

An additional purpose of this study was to compare rates of SAB in a cohort of previously infertile women who conceived spontaneously or after various treatments for infertility. We found that whether pregnancy occurred spontaneously or through treatment, women who conceived after experiencing infertility had a similar risk of losing the pregnancy before 12 weeks. This risk did not vary by the diagnosis for which the women were treated or by the specific treatment given. Again, these findings should be reassuring to infertile women and their physicians. The rate of SAB detected in this study was similar to that reported in studies of women with normal fertility and subfertility (2, 8). As in

other studies on SAB (9), the risk in our study population was directly related to maternal age.

Although this study is limited by its retrospective design, its inclusion of a relatively large number of patients as well as an untreated control group makes the conclusions more compelling. There is also some internal consistency to the data, in that SAB rates were significantly higher in older women and conceptions in older women were achieved independently of treatment more often than in their younger infertile counterparts (Table 1). The makeup of the study population in terms of age, gravidity, and distribution of diagnoses appears typical of women seeking treatment for infertility.

The finding that bleeding was predictive of miscarriage only in the younger group of women is likely a result of the nature of SAB in older women. Although almost all women with SAB will bleed eventually if untreated, older women with histories of infertility are more likely to have suspicious US examinations at their first prenatal visit. This occurs because of the relatively more common occurrence in this population of chromosomal anomalies as a cause of miscarriage. Earlier diagnosis of SAB and termination of the pregnancy in these women may have reduced the frequency with which bleeding was encountered.

In conclusion, infertile women who conceive after fertility therapy are not at increased risk of SAB compared with those who conceive spontaneously. Overall, their risk of SAB appears to be similar to that of infertile women who conceive without treatment as well as to that of women with normal fertility. There appears to be no association between previous diagnosis or treatment and the occurrence of SAB in previously infertile women. Although bleeding does not herald SAB in most of these women, it does in many, and its occurrence is associated with approximately a twofold risk of SAB compared with women who do not experience bleeding.

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## Cardiometabolic Differences in Children Born After *in Vitro* Fertilization: Follow-Up Study

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**Context:** Increasing evidence suggests that adverse conditions during early prenatal life are associated with cardiometabolic dysfunction in postnatal life. *In vitro* fertilization (IVF) conception may be an early prenatal life event with long-term health consequences.

**Objective:** Our objective was to investigate several cardiometabolic measures in 8- to 18-yr-old IVF singletons and spontaneously conceived controls born from subfertile parents.

**Design and Setting:** This follow-up study was conducted at the VU University medical center, Amsterdam, The Netherlands.

**Participants:** Blood pressure was examined in 225 IVF-conceived children and 225 age- and gender-matched spontaneously conceived control children. Several indicators of insulin resistance were studied in a pubertal subpopulation (131 IVF children and 131 controls).

**Main Outcome Measures:** Blood pressure, fasting glucose, and fasting insulin were determined.

**Results:** Systolic and diastolic blood pressure levels were higher in IVF children than controls ( $109 \pm 11$  vs.  $105 \pm 10$  mm Hg,  $P < 0.001$ ; and  $61 \pm 7$  vs.  $59 \pm 7$  mm Hg,  $P < 0.001$ , respectively). Children born after IVF were also more likely to be in the highest systolic and diastolic blood pressure quartiles (odds ratio = 2.1, 95% confidence interval 1.4, 3.3; odds ratio = 1.9, 95% confidence interval 1.2, 3.0, respectively). Furthermore, higher fasting glucose levels were observed in pubertal IVF children ( $5.0 \pm 0.4$  vs.  $4.8 \pm 0.4$  mmol/liter in controls;  $P = 0.005$ ). Blood pressure and fasting glucose differences could not be explained by current body size, birth weight, and other early life factors or by parental characteristics, including subfertility cause.

**Conclusions:** These findings highlight the importance of continued cardiometabolic monitoring of IVF-conceived children and might contribute to current knowledge about periconceptual influences and their consequences in later life. (*J Clin Endocrinol Metab* 93: 1682–1688, 2008)

According to the “developmental origins of adult disease” hypothesis, adaptive responses to environmental stimuli during critical or sensitive periods in early life may have long-lasting consequences due to permanent reprogramming of physiological, metabolic, and endocrine key systems (1, 2). Specific critical windows in prenatal development for long-term programming of cardiovascular and metabolic dysfunction have

been identified. In rats and sheep, maternal undernourishment solely during either the periconceptual or preimplantation period induced irreversible programming of hypertension and cardiovascular dysfunction among offspring (3–5). Maternal undernutrition during the periconceptual period has also been associated with altered fetal metabolism in sheep (6). Furthermore, animal studies have shown that conditions during assisted

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Abbreviations: BMI, Body mass index; CI, confidence interval; HOMA, homeostasis assessment model; HPA, hypothalamo-pituitary-adrenal; IVF, *in vitro* fertilization; SDS, sd score; VUmc, VU University medical center.

reproductive technologies may interfere with normal programming of early development with subsequent postnatal developmental consequences (7), including aberrant cardiovascular physiology (8).

In humans, little is known about the effects of poor periconceptional and/or preimplantation environment on postnatal cardiovascular and metabolic functioning. Concerns have recently been raised about the children born after subfertility treatment (9). Accumulating evidence suggests that *in vitro* fertilization (IVF) singletons are at increased risk for adverse perinatal outcome (10, 11). It is still unclear whether the IVF process in humans could affect the vulnerable processes occurring during early embryonic development with long-term health consequences. Therefore, we studied postnatal growth and development in 8- to 18-yr-old children born from subfertile parents who were either successfully treated with IVF or conceived spontaneously. The main objective of the present study was to investigate blood pressure and indicators of insulin resistance in IVF and control children.

## Subjects and Methods

### Study population

The OMEGA study is a Dutch retrospective cohort study aimed to examine long-term health effects of hormone stimulation. The cohort consists of 26,428 women diagnosed with subfertility problems in one of the 12 IVF clinics between 1980 and 1995; 19,840 women received IVF treatment, and 6,588 women did not (12–14). Eligible women had not achieved conception after at least 1-yr frequent unprotected intercourse at their first visit to the fertility clinic. Risk factor questionnaires to the women and detailed data collection from the medical records provided information on the children born from the OMEGA participants up to 1996–1997. The questionnaire response rate was 73% among subfertile women with children. The present study was restricted to IVF and spontaneously conceived children born from OMEGA participants who were treated for subfertility in the VU University medical center (VUmc). IVF children born from women treated in the VUmc who did not participate in the OMEGA study were also eligible for recruitment.

### Approach of study subjects

From the 553 eligible singletons born after standard IVF treatment, we invited 95% of IVF children born between 1986 and 1991, 74% of IVF children born between 1992 and 1993, and 41% of IVF children born between 1994 and 1995 to achieve equal representation of all 1-yr

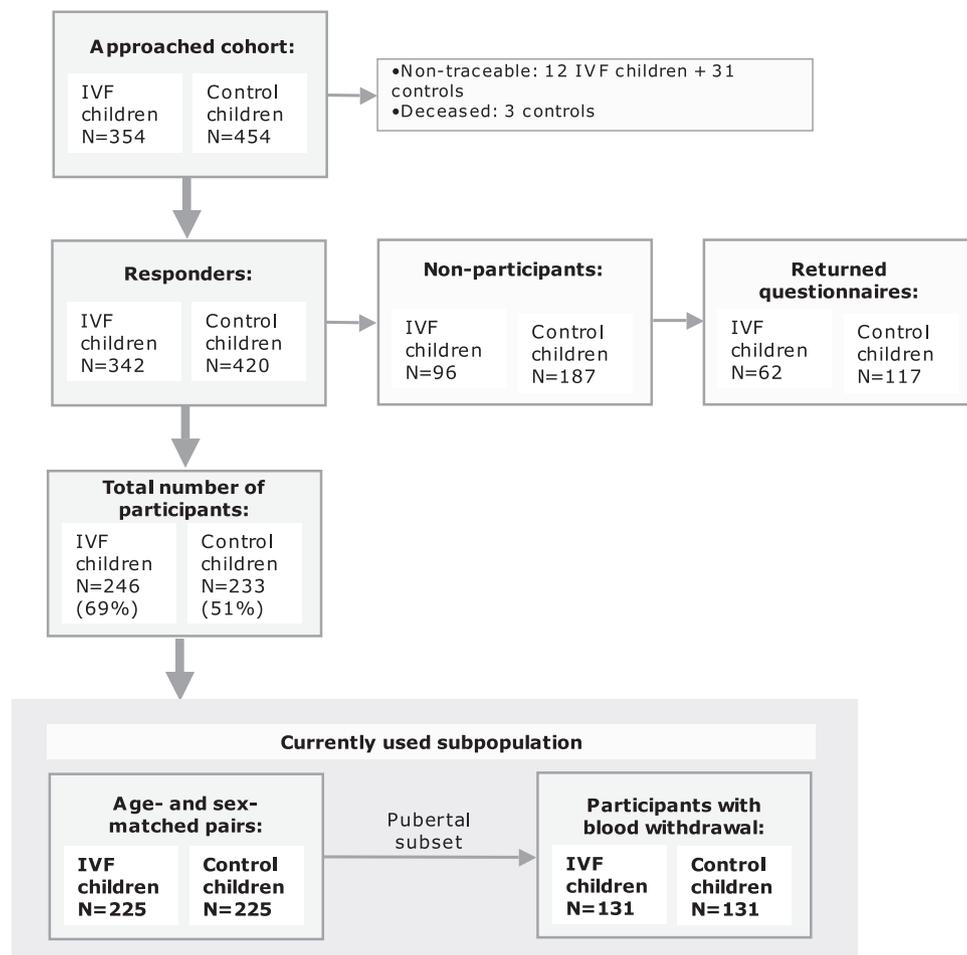


FIG. 1. Overview of the inclusion process.

age categories. For each participating IVF child, we searched one control child of the same gender and similar age ( $\leq 3$ -month age difference) born after spontaneous conception from subfertile parents. In case an approached control child did not want to participate, the control recruitment process was repeated until an appropriate control child was found that did agree to participate. Between 2003 and 2006, 354 IVF and 454 control children and their parents were informed by letter about our study on growth and development of IVF children (Fig. 1). Finally, 69% of the approached IVF children and 51% of the approached controls agreed to participate, resulting in 233 matched pairs. Subsequently, pubertal children were recruited for additional research, including the collection of a fasting blood sample [female criteria of puberty:  $\geq$  stage 2 breast development; male criteria:  $\geq$  stage 2 genital development and/or testis volume  $\geq 4$  ml (15)]. Additional research was restricted to pubertal children only to avoid low participation rates, especially among the youngest children, due to the invasive character of these tests. In total, 80% of the pubertal children underwent a blood withdrawal. All children and their parents gave written informed consent to participate in the study.

Families who refused to participate in the study received a single questionnaire regarding health, education, and other characteristics of the respective child ( $n = 283$ ). A total of 179 families (63%) returned the questionnaire. Nonparticipation analysis yielded no significant differences between participants and nonparticipants regarding children's current height, weight, and body mass index (BMI). Nonparticipating children were older ( $12.9 \pm 2.6$  vs.  $12.0 \pm 2.6$  yr;  $P = 0.002$ ), and their mothers were less often highly educated ( $26$  vs.  $37\%$ ;  $P = 0.015$ ), but these findings were observed in both the IVF and control population.

The study protocol was approved by the ethics committee of the VUmc and the National Medical Ethics Committee known as the "Cen-

trale Commissie Mensgebonden Onderzoek” located in The Hague, The Netherlands.

### Data collection

Systolic and diastolic blood pressure was measured twice at the non-dominant arm in the sitting position using an automatic device with appropriate cuff size (Dinamap PRO 100; Criticon, Munich, Germany). The first measurement was performed after a 30- to 45-min interview and the second measurement within a few minutes after the first one. The mean of these two readings was used in analyses. Body weight and height were assessed to the nearest 0.1 kg and 0.1 cm using an electronic scale (SECA, Hanover, MD) and a stadiometer (Holtain Ltd., Crymych, Dyfed, UK), respectively. Skinfold thickness measurements (triceps, biceps, subscapular, and suprailiac) were collected by a Harpenden caliper (Harpenden, West Sussex, UK). Other body fat measures have been reported elsewhere.

Blood samples were drawn between 0900 and 1000 h after an overnight fast. Fasting glucose and insulin were determined using the Glucoquant Glucose/HK, Roche assay kit (Roche Diagnostics GmbH, Mannheim, Germany) and Bayer/ACS Centaur immunoassay (Bayer Diagnostics, Mijdrecht, The Netherlands), respectively. The glucose to insulin ratio and the homeostasis assessment model (HOMA) were chosen as measures of insulin sensitivity. HOMA insulin resistance and  $\beta$ -cell function were calculated according the original formula (16). Laboratory measurements were performed at the Department of Clinical Chemistry of the VUmc.

Before the follow-up visit in the VUmc, a questionnaire was sent to the parents to gather information on various demographical, lifestyle, and medical factors, including the cause of subfertility, parental education level, maternal smoking during pregnancy, and birth weight and gestational age of the respective child. Maternal BMI and highest level of education completed by either parent were used as indicators of socioeconomic conditions (17). Information about drug use of the child and family history of disease in terms of diabetes type 2, cardiovascular disease, and hypertension among parents and grandparents was obtained by an interview. None of the children used medication that could have affected blood pressure. Birth weight, either extracted from VUmc birth certificates (49%) or outpatient clinic reports (38%), or self-reported by the parents (13%), was expressed as the SD score (SDS) to correct for gestational age and gender (18).

### Statistical analysis

After exclusion of eight matched pairs due to missing blood pressure measurements, data of 225 IVF-control pairs and data of the subsequent pubertal subset consisting of 131 unmatched IVF children and 131 controls were analyzed (Fig. 1). Differences between IVF-control pairs were tested using the paired *t* test for continuous variables and McNemar's test for dichotomous variables. Metabolic data of pubertal IVF and control children were compared after correction for age and gender. Logistic regression analyses were performed to estimate crude odds ratios for being in the highest quartile of several outcome parameters associated with IVF conception. Furthermore, potential confounders of the association between blood pressure and indicators of insulin sensitivity on the one hand and IVF conception on the other hand were examined separately by regression analysis (e.g. gender, current weight, birth weight, gestational age, parity, maternal smoking during pregnancy, parental education, parental age, maternal BMI, subfertility cause, and family history of disease). Factors that changed the crude difference in outcome between IVF and control children with more than 10% were considered as confounders and included in the final regression model. Reported *P* values were based on two-sided tests of significance.

### Results

Perinatal and follow-up characteristics of the study population are shown in Table 1. Birth weight, birth weight SDS, and ges-

tational age were significantly lower in children conceived by IVF compared with controls. Age at follow-up of IVF and control children was  $12.3 \pm 2.6$  yr. Both systolic and diastolic blood pressures were higher in IVF children ( $109 \pm 11$  vs.  $105 \pm 10$  mm Hg in controls,  $P < 0.001$ ; and  $61 \pm 7$  vs.  $59 \pm 7$  mm Hg in controls,  $P < 0.001$ , respectively). Furthermore, IVF children were 2.1 times more likely to be in the highest systolic blood pressure quartile ( $\geq 114.5$  mm Hg) and 1.9 times more likely to be in the highest diastolic blood pressure quartile ( $\geq 65.5$  mm Hg) than controls [highest quartile vs. lowest three quartiles: 95% confidence interval (CI) 1.4, 3.3; 95% CI 1.2, 3.0, respectively]. IVF children had a significantly higher sum of skinfolds compared with controls ( $40.5 \pm 20.4$  vs.  $36.9 \pm 17.5$  mm;  $P = 0.04$ ). In addition, higher fasting glucose levels were observed in IVF children ( $5.0 \pm 0.4$  vs.  $4.8 \pm 0.4$  mmol/liter in controls;  $P = 0.005$ ). IVF-conceived children were 2.5 times more likely to be in the highest fasting glucose quartile ( $\geq 5.2$  mmol/liter) than controls (highest quartile vs. lowest quartile: 95% CI 1.2, 5.2). No significant differences in fasting insulin concentrations, insulin resistance measures, height, weight, and BMI were found between both study groups.

Influences of potentially confounding factors on the difference in blood pressure and fasting glucose levels between IVF children and controls are shown in Table 2. The systolic blood pressure difference was predominantly affected by birth weight, gestational age, and sum of skinfolds, whereas the diastolic blood pressure difference was also influenced by parity. By contrast, subfertility cause was the main factor that substantially changed the fasting glucose difference between IVF and control children. Multivariate regression analysis demonstrated that blood pressure and fasting glucose levels in IVF children remained significantly increased after controlling for the relevant confounding factors simultaneously (Table 3).

### Discussion

This is the first follow-up study investigating blood pressure levels and several indicators of insulin resistance in 8- to 18-yr-old IVF and control children born from subfertile parents. Significant differences in both systolic and diastolic blood pressure, as well as in fasting glucose levels were found among IVF children compared with controls. These differences could neither be explained by current risk indicators, early life factors, nor by parental characteristics, including subfertility cause.

In clinical practice, the 3- to 4-mm Hg higher systolic blood pressure and the 1- to 2-mm Hg higher diastolic blood pressure in IVF children may seem like small increases, but at a population level, these differences might have a major impact on public health. A slight increase in blood pressure is associated with a remarkably increased risk of developing cardiovascular disease. For instance, lowering mean systolic blood pressure in adults by 2 mm Hg corresponds to an 8% reduction in the risk of stroke (19). Furthermore, it cannot be excluded that increased blood pressure after IVF may be amplified throughout life because blood pressure is known to track from childhood into adult life (20).

**TABLE 1.** Perinatal and follow-up characteristics of the study population

	IVF children	Controls	P value
Perinatal characteristics			
No. of subjects	225	225	
Birth weight (kg)	3.22 ± 0.63	3.44 ± 0.54	<0.001
Birth weight SDS <sup>a</sup>	-0.16 ± 1.00	0.09 ± 1.07	0.02
Gestational age (wk)	38.9 ± 2.5	39.6 ± 1.8	0.002
No. of preterm infants (%) <sup>b</sup>	29 (13)	13 (6)	0.01
Anthropometry and blood pressure			
No. of subjects	225	225	
Age (yr)	12.3 ± 2.6	12.3 ± 2.6	0.35
Gender (% male)	49	49	1.00
Height (cm)	156.4 ± 15.0	155.8 ± 15.7	0.39
Body weight (kg)	47.8 ± 16.0	46.7 ± 14.8	0.19
BMI (kg/m <sup>2</sup> ) <sup>c</sup>	19.1 ± 3.6	18.7 ± 3.2	0.25
Sum of skinfolds (mm)	40.5 ± 20.4	36.9 ± 17.5	0.04
Systolic blood pressure (mm Hg)	109 ± 11	105 ± 10	<0.001
Systolic blood pressure: quartiles			
≤99.5 mm Hg	44 (20%)	72 (32%)	0.001
100.0–106.0 mm Hg	50 (22%)	59 (26%)	
106.5–114.0 mm Hg	59 (26%)	53 (24%)	
≥114.5 mm Hg	72 (32%)	41 (18%)	
Diastolic blood pressure (mm Hg)	61 ± 7	59 ± 7	<0.001
Diastolic blood pressure: quartiles			
≤54.5 mm Hg	42 (19%)	63 (28%)	0.01
55.0–59.5 mm Hg	58 (26%)	65 (29%)	
60.0–65.0 mm Hg	56 (25%)	55 (24%)	
≥65.5 mm Hg	69 (31%)	42 (19%)	
Heart rate (beats per min)	74 ± 11	72 ± 11	0.02
Fasting glucose and insulin			
No. of subjects <sup>d</sup>	131	131	
Fasting glucose (mmol/liter)	5.0 ± 0.4	4.8 ± 0.4	0.005
Fasting glucose: quartiles			
≤4.6 mmol/liter	21 (16%)	39 (30%)	0.05
4.7–4.9 mmol/liter	43 (33%)	36 (28%)	
5.0–5.1 mmol/liter	25 (19%)	26 (20%)	
≥5.2 mmol/liter	41 (32%)	30 (23%)	
Fasting insulin (pmol/liter) <sup>a</sup>	47.5 (33.0–69.2)	47.2 (34.2–63.6)	0.58
Glucose to insulin ratio <sup>a</sup>	0.10 (0.07–0.15)	0.11 (0.08–0.14)	0.88
HOMA-insulin resistance <sup>a</sup>	1.8 (1.2–2.6)	1.8 (1.2–2.3)	0.35
HOMA β-cell function <sup>a</sup>	110.4 (77.6–151.4)	117.3 (81.7–164.2)	0.24

Data represent mean ± SD, percentages, or median (25th–75th percentile).

<sup>a</sup> Birth weight SDS is a measure of birth weight corrected for gestational age and gender using a reference population (18).

<sup>b</sup> Premature birth was defined as birth occurring before 37-wk gestation.

<sup>c</sup> BMI was defined as weight divided by height squared.

<sup>d</sup> Metabolic data were only available for children who participated in the pubertal substudy; these unmatched data were corrected for age and gender.

Over the past years, cardiovascular developmental consequences and potentially underlying mechanisms after environmental manipulation during early prenatal development have been documented in both human and animal studies (3, 5, 21, 22). The Dutch famine study demonstrated that exposure to malnutrition during early pregnancy is associated with an increased risk of coronary heart disease in adult life (22). Periconceptional undernutrition has been associated with the precocious activation of the hypothalamo-pituitary-adrenal (HPA) axis (23–26). Gardner *et al.* (27) recently reported minor influences on HPA axis function in young adult sheep after periconceptional undernutrition. It has been suggested that the early activation of the HPA axis may not only lead to inappropriate elevation of prostaglandin levels and early birth but may also be associated with further programming effects due to inappropri-

ate exposure of the fetus to glucocorticoids (28). Other targets, like the renin-angiotensin system and the sympathoadrenal axis, have also been associated with developmental origins of nutritional or other influences on cardiovascular function (29). Due to the complexity of the cardiovascular system, it is unlikely that the relation between periconceptional insults and postnatal cardiovascular dysfunction originates from one single cause. Early prenatal developmental plasticity in relation to environmental stimuli has been reported to lead to changes in fetal development through changes in imprinted gene expression, nutrient and stress-related signaling pathways, or cell cycle and apoptotic rates (9). Further research is necessary to investigate the role of these pathways in the development of cardiovascular dysfunction after periconceptional insults. In addition, in view of the present study, it remains to be elucidated whether increased

**TABLE 2.** Differences in blood pressure (mm Hg) and fasting glucose (mmol/liter) between IVF children and control children after adjustment for current risk indicators, early life factors, and parental characteristics

Model adjustment for the following potential confounders	Systolic blood pressure			Diastolic blood pressure			Fasting glucose		
	Difference (mm Hg)	95% CI	P value	Difference (mm Hg)	95% CI	P value	Difference (mmol/liter)	95% CI	P value
Unadjusted	4.2	2.2–6.2	<0.001	2.3	1.1–3.6	<0.001	0.13	0.04–0.22	0.005
Gender	4.2	2.2–6.2	<0.001	2.3	1.1–3.6	<0.001	0.13	0.04–0.22	0.005
Current risk indicators									
Current weight (kg)	3.9	2.0–5.7	<0.001	2.3	1.0–3.6	0.001	0.13	0.04–0.22	0.006
Current height (cm)	4.2	2.3–6.0	<0.001	2.3	1.1–3.6	<0.001	0.13	0.04–0.22	0.005
BMI (kg/m <sup>2</sup> )	4.0	2.1–5.9	<0.001	2.3	1.0–3.6	0.001	0.13	0.04–0.22	0.007
Sum of skinfolds (mm)	3.7	1.8–5.7	<0.001	2.1	0.8–3.4	0.001	0.13	0.04–0.22	0.007
Early life factors									
Birth weight (kg)	3.5	1.5–5.5	0.001	2.1	0.8–3.4	0.002	0.13	0.03–0.22	0.007
Gestational age (wk)	3.6	1.6–5.6	<0.001	2.1	0.8–3.4	0.002	0.13	0.04–0.22	0.007
Primiparity (Y/N)	3.8	1.7–5.9	0.001	1.8	0.5–3.2	0.009	0.13	0.03–0.23	0.010
Maternal smoking during pregnancy (Y/N)	3.8	1.8–5.8	<0.001	2.2	0.9–3.5	0.001	0.13	0.04–0.22	0.006
Parental characteristics									
Subfertility cause <sup>a</sup>	3.9	1.8–6.0	<0.001	2.2	0.9–3.6	0.001	0.11	0.02–0.21	0.022
Parental educational level <sup>b</sup>	4.1	2.1–6.1	<0.001	2.3	1.0–3.6	<0.001	0.13	0.03–0.22	0.008
Maternal age at follow-up (yr)	4.1	2.2–6.1	<0.001	2.3	1.1–3.6	<0.001	0.13	0.04–0.23	0.005
Paternal age at follow-up (yr)	4.2	2.2–6.2	<0.001	2.3	1.0–3.6	<0.001	0.13	0.04–0.22	0.005
Maternal BMI at follow-up (kg/m <sup>2</sup> )	4.0	2.0–6.0	<0.001	2.2	0.9–3.5	0.001	0.13	0.04–0.22	0.006
Family history of diabetes type 2 (Y/N) <sup>c</sup>	4.3	2.3–6.3	<0.001	2.3	1.0–3.6	0.001	0.13	0.04–0.22	0.006
Family history of hypertension (Y/N) <sup>c</sup>	4.4	2.4–6.5	<0.001	2.4	1.0–3.7	<0.001	0.13	0.04–0.23	0.004
Family history of cardiovascular disease (Y/N) <sup>c</sup>	4.3	2.3–6.4	<0.001	2.3	1.0–3.6	0.001	0.13	0.04–0.23	0.005

Each row represents a separate regression analysis. N, No; Y, yes.

<sup>a</sup> Subfertility was categorized as female subfertility (tubal factors, endometriosis, ovarian disorders, cervical factors, and uterine abnormalities), male subfertility, subfertility caused by both parents, or unexplained subfertility. In 14 cases the cause of subfertility was missing.

<sup>b</sup> Highest level of education completed by either parent was categorized as low (primary school, low occupational training), medium (high school, medium occupational training), and high (high occupational training, university).

<sup>c</sup> Family history of diabetes type 2, hypertension, and cardiovascular disease was considered positive if any of the parents or grandparents was reported to suffer from this type of disease. Data on the family history of disease of 14 children were missing.

blood pressure among IVF children originates from early prenatal life adaptations mediated through neuroendocrinal pathways related to the HPA axis and/or through one of the unidentified mechanisms. However, increased blood pressure levels in IVF children were to a large extent independent of birth weight, suggesting that the underlying mechanisms can modify the cardiovascular system even without affecting size at birth. This is in line with previous studies examining associations between early life factors and blood pressure among offspring (22, 30). It is important to realize that birth weight is just a proxy for fetal growth. Prenatal environmental insults that may affect embryonic and/or fetal growth trajectories can result in altered postnatal physiology without an effect on birth weight (31). Similarly, exposure to early prenatal life effects may even induce

developmental adaptations in organ development and function that are not accompanied by changes in fetal growth characteristics.

Although exposure to adverse prenatal conditions, especially during late gestation, has been linked to decreased glucose tolerance in adults (32), it is unclear whether early prenatal insults in humans can influence postnatal glucose metabolism. Fasting glucose levels studied in the present study are within the normal range, and the difference in fasting glucose levels between pubertal IVF children and controls is small, not accompanied by differences in fasting insulin levels and other related measures. However, in view of the observed differences in blood pressure and body fat composition between IVF children and controls, considerable research is necessary to investigate the hypothesis

**TABLE 3.** Differences in blood pressure (mm Hg) and fasting glucose (mmol/liter) between IVF children and control children after adjustment for confounders: multivariate analysis

Multivariate models	Unstandardized regression coefficient	95% CI	P value
SBP difference (mm Hg) after adjustment for birth weight, gestational age, and sum of skinfolds	3.0	1.1–5.0	0.003
DBP difference (mm Hg) after adjustment for birth weight, gestational age, parity, and sum of skinfolds	1.4	0.03–2.8	0.046
Glucose difference (mmol/liter) after adjustment for subfertility cause	0.11	0.02–0.21	0.02

DBP, Diastolic blood pressure; SBP, systolic blood pressure.

that further changes in glucose metabolism might manifest in later life. Gold standard assessments, *i.e.* euglycemic hyperinsulinemic clamp tests and hyperglycemic clamp tests, will be useful to additionally investigate insulin sensitivity and  $\beta$ -cell capacity in IVF-conceived offspring.

When interpreting our results, the strengths and limitations of our study need to be considered. The strengths of our study include the relatively large study size and the comparison group consisting of spontaneously conceived children born from subfertile parents. Furthermore, we collected various variables related to blood pressure and metabolism that provided the opportunity to examine blood pressure and fasting glucose differences between IVF children and controls while adjusting for these potentially confounding factors. It is possible that we have underestimated the true association between IVF and outcome parameters by adjusting for intermediate factors (*e.g.* sum of skinfolds in case of evaluated blood pressure). In addition, the slight attenuation of the blood pressure differences by adjusting for birth weight and gestational age was to be expected. IVF is known to be associated with lower birth weight and shorter gestational age (11, 33), whereas these factors themselves have been found to increase blood pressure. Another limitation is potential selection bias because our study was based on 56% ( $n = 450$ ) of the total number of subjects approached ( $n = 808$ ). However, nonparticipation analysis yielded no significant differences between participants and nonparticipants in anthropometric measures. The main reason for nonparticipation was unwillingness of the child to participate. In addition, it cannot be excluded that the degree of subfertility in those who conceived after IVF was more severe compared with those who conceived spontaneously and that this difference in subfertility contributed to the physiological abnormalities observed in IVF offspring. Nevertheless, most control mothers who participated in the present study were diagnosed with subfertility in an era (1982–1990) when IVF was not a routine procedure and was not ethically acceptable to many women. Moreover, estimated differences in blood pressure between IVF and control children were hardly affected by adjustment for parental subfertility causes, rendering residual confounding very unlikely. Similarly, the fasting glucose difference between IVF and control children remained statistically significant after adjustment for subfertility cause.

In conclusion, increased blood pressure and fasting glucose levels among IVF children could not be explained by current risk indicators, early life factors, and parental characteristics. Although underlying mechanisms are largely elusive, the periconceptual period of IVF-conceived children might be a critical time window during which cardiometabolic function can be perturbed. Before definitive conclusions can be drawn, our results need to be reproduced by other prospective follow-up studies. Nevertheless, our findings underscore the importance of the continuing worldwide monitoring of postnatal development of IVF children and contribute to the current understanding of periconceptual exposure effects with regard to the development of both short- and long-term consequences in humans.

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# Cerebral Palsy, Autism Spectrum Disorders, and Developmental Delay in Children Born After Assisted Conception

## A Systematic Review and Meta-analysis

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**Objective:** To assess the existing evidence of associations between assisted conception and cerebral palsy (CP), autism spectrum disorders (ASD), and developmental delay.

**Data Sources:** Forty-one studies identified in a systematic PubMed and Excerpta Medica Database (EMBASE) search for articles published from January 1, 1996, to April 1, 2008.

**Study Selection:** Studies written in English comparing children born after assisted conception with children born after natural conception assessing CP, ASD, and developmental delay, based on original data with a follow-up of 1 year or more.

**Main Exposures:** In vitro fertilization (IVF) with or without intracytoplasmic sperm injection or ovulation induction with or without subsequent intrauterine insemination.

**Main Outcome Measures:** Cerebral palsy, ASD, and developmental delay.

**Results:** Nine CP studies showed that children born after IVF had an increased risk of CP associated with preterm delivery. In our meta-analysis including 19 462 children exposed to IVF, we estimated a crude odds ratio of 2.18 (95% confidence interval, 1.71-2.77). Eight ASD studies and 30 studies on developmental delay showed inconsistent results. No studies assessed the risk of CP, ASD, or developmental delay in children born after ovulation induction exclusively.

**Conclusions:** Methodological problems were revealed in the identified studies, and the gaps in our knowledge about the long-term outcomes of children born after assisted conception are considerable, including a lack of information on the long-term consequences of ovulation induction. Possible associations with ASD and developmental delay need assessment in larger studies. Studies on assisted conception and CP from countries outside of Scandinavia are needed, including detailed information on time to pregnancy, underlying cause of infertility, and type of IVF treatment.

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**I**N THIS REVIEW, WE EXAMINED studies on the long-term outcomes of assisted conception, defined as in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) or ovulation induction (OI) with or without subsequent intrauterine insemination (IUI).

In vitro fertilization treatment alone now accounts for an estimated 1% to 4% of births in European countries<sup>1</sup> and 1% of US births.<sup>2</sup> There is a particular obligation to evaluate treatments offered in health care systems to guarantee their safety.

In vitro fertilization is associated with adverse perinatal outcomes such as preterm delivery (PTD, <37 weeks of gestation) and low birth weight (LBW, <2500 g) because of the strong association be-

tween IVF and multiple pregnancies and because even IVF singletons have an increased risk of PTD and LBW compared with naturally conceived (NC) singletons.<sup>3</sup> Likewise, OI leads to more multiple pregnancies than natural conception,<sup>1,4</sup> and children born after OI also have an increased risk of PTD and LBW, as reported in most studies<sup>4-8</sup> but not all.<sup>9,10</sup> Pregnancy with multiples, PTD, and LBW are strongly associated with a range of long-term child health problems<sup>3,11,12</sup> including admission to neonatal intensive care units and prolonged hospitalization,<sup>13</sup> vision impairment,<sup>14</sup> and cerebral palsy (CP).<sup>15</sup> Moderate associations between delivery of multiples, PTD, LBW, advanced parental age, and developmental disabilities such as autism spectrum disorders (ASD) have

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**Table 1. MeSH Terms and Hits in Literature Search<sup>a</sup>**

Exposures and Outcomes	No.	
	MeSH	AllFields
"Cerebral palsy" and		
"Ovulation induction"	1	3
"Fertilization in vitro"	11	24
"Reproductive techniques, assisted"	19	34
"Sperm injections, intracytoplasmic"	3	5
"Insemination, artificial"	0	1
Autism spectrum disorders, "autistic disorder" or "Aspergers syndrome" and		
"Ovulation induction"	0	0
"Fertilization in vitro"	2	2
"Reproductive techniques, assisted"	4	4
"Sperm injections, intracytoplasmic"	1	1
"Insemination, artificial"	0	0
Developmental delay, "developmental disabilities," "child development disorders," or "child development" and		
"Ovulation induction"	7	
"Fertilization in vitro"	70	
"Reproductive techniques, assisted"	93	
"Sperm injections, intracytoplasmic"	33	
"Insemination, artificial"	12	

Abbreviation: MeSH, Medical Subject Headings of the National Library of Medicine.

<sup>a</sup>Limits, January 1, 1996 to October 31, 2007; humans; English language. The articles on autism spectrum disorders all appear in the cerebral palsy search, except for the article by Maimburg. Articles may have appeared in more than 1 search category.

also been reported.<sup>16-23</sup> Advanced parental age is strongly associated with assisted conception. Additionally, possible prenatal hormonal disturbances in autism, eg, elevated levels of prenatal testosterone<sup>24</sup> and lower levels of oxytocin,<sup>25</sup> have been reported and may be linked to reproductive problems.

We conducted a systematic review of the current evidence regarding associations between assisted conception and severe long-term outcomes, specifically CP and ASD. We also reviewed general developmental delay outcomes, as these are often the initiating diagnoses or symptoms.

## METHODS

### LITERATURE SEARCH

We searched PubMed using the Medical Subject Headings of the National Library of Medicine terms presented in **Table 1**. We limited our search to studies reporting human outcomes of assisted conception published from January 1, 1996, through March 31, 2008 in English and including children exposed to assisted conception (IVF, ICSI, IUI, or OI). We performed our search on April 10, 2008. The main outcome measures of interest were CP, ASD, and developmental delay.

### STUDY SELECTION

A total of 130 articles met our initial search criteria. All abstracts were reviewed and 41 articles met the additional inclusion criteria of original data, follow-up time of 1 year or more, and a comparison group of unexposed NC children. Excluded articles are shown in an online appendix (<http://www.nanea.dk/articles/hvidtjorn-2008-review>). We examined the reference lists of all 41 articles eligible for full review, but did not identify any additional articles. We searched EMBASE using

the search terms in Table 1 for CP and ASD, but did not identify any additional articles with original data. The initial screening of abstracts was conducted by an author (D.H.). Each of the articles meeting the final inclusion criteria were reviewed in full by 2 authors (D.H. and 1 coauthor; L.S., D.S., P.T., or B.J.).

Here, we present findings from the reviewed studies on CP, ASD, and developmental delay. For each outcome we discuss the main methodological strengths and limitations of the studies. Because of the elevated risk of multiple births in assisted conception, whenever possible we present the articles' findings for singletons and multiples separately, as well as all births combined, with or without adjusting for PTD.

### META-ANALYSIS

We performed meta-analyses with fixed-effect models using the Mantel-Haenzel method and calculated nonadjusted summary estimates for the CP studies. Several studies included overlapping cohorts; for these we selected the study presenting the most detailed data (the necessary numbers to calculate a weighted summary). Stata software version 10.0 (Stata, College Station, Texas) was used.

We did not perform meta-analyses for the ASD studies reviewed because of the small number of studies within the specific study design types and potential methodological problems in the few studies similarly designed (see below). We did not perform meta-analyses with the developmental delay studies because a wide range of different measurements were used to assess cognitive, motor, and behavioral development. Moreover, even when 2 studies used the same instrument, child age at assessment was often variable, as were sample inclusion and/or exclusion criteria.

Of the 41 included studies, only 2 were case-control studies; the remainder were cohort studies. Nine studies assessed the risk of CP (**Table 2**) and 8 the risk of ASD (**Table 3**). Thirty studies assessed developmental delay based on various standardized scales (**Table 4**). Within all 3 outcomes there were overlapping study populations.

**Table 2. Assisted Conception and Cerebral Palsy Associations by Reference**

Source, y	Country, Cohort	Follow-up		Data Sources		Sample Size (Multiples, %)		Associations, Not Adjusting for Preterm Delivery		
		Design	Duration, y	Assisted Conception	Cerebral Palsy	Exposed	Unexposed	Ratio (95% CI)	Exposed Outcomes	Preterm Delivery
<b>All Children</b>										
Klemetti et al, 2006	Finland, 1996-1999	Retrospective cohort	2	All fertility clinics, IVF	HDR, ICD-10 G80 and child care support register	4527 (35.7)	26 877 (2.2)	OR, 2.92 (1.63-5.26) <sup>a</sup>	17	88% of IVF children with CP
Hvidtjorn et al, 2006	Denmark, 1995-2000	Retrospective cohort	1-7	IVF register, IVF	NRHD, ICD-10 G80.0-83.9	9255 (38.6)	383 919 (2.7)	HRR, 1.61 (1.13-2.30) <sup>b</sup>	40	HRR, 1.07 (0.76-1.52), <sup>b</sup> adjusting for PTD
Källen et al, 2005	Sweden, 1987-2002	Retrospective cohort	1-20	All fertility clinics, IVF	HDR, ICD-9 or -10 CP	16 280	All other births	OR, 1.89 (1.37-2.60) <sup>c</sup>	37	OR, 0.88 (0.46-1.70) <sup>c</sup> in children carried to term
Strömberg et al, 2002	Sweden, 1982-1995	Retrospective cohort	1.5-15	All fertility clinics, IVF	Disability centers' medical records, ICD-10 CP	5680 (43.0)	11 360 (3.9)	OR, 3.7 (2.0-6.6) <sup>d</sup>	31	OR, 2.9 (1.40-6.0), <sup>d</sup> adjusting for PTD
Ericson et al, 2002	Sweden, 1984-1997	Retrospective cohort	1-14	All fertility clinics, IVF	HDR, ICD-9343 or ICD-10 G81	9056	1 408 110	OR, 1.69 (1.06-2.68) <sup>e</sup>	NS	...
<b>Singletons</b>										
Klemetti et al, 2006	Finland, 1996-1999	Retrospective cohort	2	All fertility clinics, IVF	HDR, ICD-10 G80 and child care support register	2911	26 296	OR, 1.15 (0.40-3.27) <sup>a</sup>	4	...
Hvidtjorn et al, 2006	Denmark, 1995-2000	Retrospective cohort	1-7	IVF register, IVF	NRHD, ICD-10 G80.0-83.9	5685	383 919	HRR, 1.28 (0.80-2.03) <sup>b</sup>	NS	HRR, 0.84 (0.43-1.63) <sup>b</sup> singletons carried to term
Lidegaard et al, 2005	Denmark, 1995-2001	Retrospective cohort	1-7	IVF register, IVF	NRHD, ICD-10 G80.0	6052	442 349	Rate ratio, 1.8 (1.2-2.8)	25	...
Strömberg et al, 2002	Sweden, 1982-1995	Retrospective cohort	1.5-15	The National Board of Health and Welfare, IVF	Disability centers' medical records, ICD-10 CP	3228	11 070	OR, 2.8 (1.3-5.8) <sup>d</sup>	12	OR, 2.4 (0.9-6.0), <sup>d</sup> adjusting for PTD
<b>Twins</b>										
Klemetti et al, 2006	Finland, 1996-1999	Retrospective cohort	2	All fertility clinics, IVF	HDR, ICD-10 G80 and child care support register	1616	581	OR, 1.52 (0.43-5.40) <sup>a</sup>	13	...
Hvidtjorn et al, 2006	Denmark, 1995-2000	Retrospective cohort	1-7	IVF register, IVF	NRHD, ICD-10 G80.0-83.9	3570	10 794	HRR, 1.08 (0.57-2.05) <sup>b</sup>	NS	HRR, 0.83 (0.27-2.61) <sup>b</sup> twins carried to term
Pinborg et al, 2004	Denmark, 1995-2000	Retrospective cohort	2-7	IVF register, IVF	NRHD, ICD-10 G80.0-83.8	3393	10 239	OR, 0.83 (0.43-1.67) <sup>f,g</sup>	11	OR, 0.8 (0.4-1.6), <sup>f</sup> adjusting for PTD
Pinborg et al, 2003	Denmark, 1997	Retrospective cohort	3-4	IVF register, IVF	Mother questionnaires and NRHD, ICD-10	472	1132	OR, 0.61 (0.17-2.16) <sup>g</sup>	3	...
Strömberg et al, 2002	Sweden, 1982-1995	Retrospective cohort	1.5-15	The National Board of Health and Welfare, IVF	Disability centers' med records, ICD-10 CP	2060	4120	OR, 0.9 (0.4-1.8) <sup>d</sup>	15	...
<b>Triplets</b>										
Skrablin et al, 2007	Croatia, 1986-2000	Retrospective cohort	2-12	One hospital-assisted conception	Rehabilitation centers, CP diagnosis	68	9	OR, 0.31 (0.15-0.65) <sup>g</sup>	9	...

Abbreviations: CP, cerebral palsy; CI, confidence interval; ICD-8, -9, or -10, *International Classification of Diseases, Eighth, Ninth, or Tenth Revision*; IVF, in vitro fertilization; HDR, Hospital Discharge Register; HRR, hazard rate ratio; NRHD, National Register of Hospital Discharges; NS, not shown; OR, odds ratio; PTD, preterm delivery.

<sup>a</sup>Adjusted for mother's socioeconomic position.

<sup>b</sup>Adjusted for parity, sex, maternal age, and educational level.

<sup>c</sup>Adjusted for year of birth.

<sup>d</sup>Adjusted for sex, year of birth, and birth hospital.

<sup>e</sup>Adjusted for year of birth, maternal age, parity, and smoking.

<sup>f</sup>Adjusted for sex and year of birth.

<sup>g</sup>Calculated from raw numbers presented in the article.

## RESULTS

### CEREBRAL PALSY

#### Generalizability

Results for CP are shown in Table 3. Of the 9 studies assessing the risk of CP in children born after IVF, only 1 was con-

ducted outside of Scandinavia (a Croatian study of triplets).<sup>26</sup> The Scandinavian studies were population-based, diminishing any selection bias. However, the Scandinavian countries are very similar in demographic factors, socioeconomic status, ethnicity (mainly white), and free access to health care (including fertility treatment), and this uniformity might limit extrapolation of the findings to populations of different ethnic profiles, demography, and health care systems.

**Table 3. Assisted Conception and Autism Spectrum Disorders Associations by Reference**

Source, y	Country, Cohort	Follow-up		Data Source		Sample Size (Multiples, %)		Associations	
		Design	Duration, y	Assisted Conception	Autism Spectrum Disorders	Exposed	Unexposed	Ratio (95% CI)	Exposed Outcomes
<b>All Children</b>									
Maimburg and Vaeth, 2007	Denmark, 1990-1999	Case-control		Birth records, assisted conception	DPCRR, ICD-8 299.0 or ICD-10 F84.0	473	473	OR, 0.37 (0.14-0.98) <sup>a</sup>	10
Stein et al, 2006	Israel, 1970-1998	Case-control		Interviews, infertility medical intervention	Autism treatment organization, ICD-8 or DSM III-IV Autism	206	152	OR, 1.91 (0.94-3.88) <sup>b</sup>	29
Klemetti et al, 2006	Finland, 1996-1999	Retrospective cohort	2	All fertility clinics, IVF	HDR, ICD-10 F80-98 and child care support register (more than ASD)	4527 (35.7)	26 877 (2.2)	OR, 1.68 (1.11-2.53) <sup>c</sup>	>ASD
Strömberg et al, 2002	Sweden, 1982-1995	Retrospective cohort	1.5-15	National Board of Health and Welfare, IVF	Disability centers' medical records, ICD-10 infantile autism	5680 (43.0)	11 360 (1.3)	Too infrequent	NS
Ericson et al, 2002	Sweden, 1984-1997	Retrospective cohort	1-14	All fertility clinics, IVF	HDR, ICD-9 and ICD-10 (more than ASD)	9056	1 408 110	OR, 1.35 (0.86-2.11) <sup>d</sup>	>ASD
<b>Singletons</b>									
Klemetti et al, 2006	Finland, 1996-1999	Retrospective cohort	2	All fertility clinics, IVF	HDR, ICD-10 F80-98 and child care support register (more than ASD)	2911	26 296	OR, 1.05 (0.57-1.91) <sup>c</sup>	>ASD
Lidegaard et al, 2005	Denmark, 1995-2001	Retrospective cohort	1-7	IVF register, IVF	DPCRR, ICD-10 F84	6052	442 349	Rate ratio, 1.2 (NS)	13
<b>Twins</b>									
Klemetti et al, 2006	Finland, 1996-1999	Retrospective cohort	2	All fertility clinics, IVF	HDR, ICD-10 F80-98 and child care support register (more than ASD)	1616	581	OR, 3.05 (0.70-13.29) <sup>c</sup>	>ASD
Pinborg et al, 2004	Denmark, 1995-2000	Retrospective cohort	2-7	IVF register, IVF	DPCRR: ICD-10 F84 and F84.5	3393	10 239	OR, 0.82 (0.23-2.95) <sup>b</sup>	3
Pinborg et al, 2003	Denmark, 1997	Retrospective cohort	3-4	IVF register, IVF	Maternal questionnaires and discharge records, ICD-10 F84 and F84.5	472	1132	NA	0

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; DPCRR, The Danish Psychiatric Central Research Register; *DSM-III or -V*, *Diagnostic and Statistical Manual of Mental Disorders* (Third or Fourth Edition); HDR, Hospital Discharge Register; *ICD-8, -9, or -10*, *International Classification of Diseases, Eighth, Ninth, or Tenth Revision*; IVF, in vitro fertilization; NA, not available; NS, not shown; OR, odds ratio.

<sup>a</sup>Adjusted for parity, multiplicity, birth weight, gestational age, birth defect, maternal age, and country of origin.

<sup>b</sup>Calculated from raw numbers presented in the article.

<sup>c</sup>Adjusted for mother's socioeconomic position.

<sup>d</sup>Adjusted for year of birth, maternal age, parity, and smoking.

### Methodological Quality of Included Studies

**Sample Size and Precision.** The Scandinavian studies were based on data from registers comprising health status on the whole population and information on IVF treatments from all fertility clinics retrieved via the unique identification number given to each citizen, resulting in large cohorts reporting fairly precise risk estimates. Risk estimates were less precise in the strata of multiplicity because of reduced sample sizes and lower expected prevalence within the group of singletons only.

**Exposure Data.** One study assessed the risk of CP in triplets born after all types of assisted conception, identified through a single hospital.<sup>26</sup> The remaining 8 studies evaluated children born after IVF using specific population-based data on exposure and identified either

through an IVF register (in Denmark) or through all fertility clinics in the specific country (Sweden or Finland); they likely included practically all children born after IVF. However, no study specifically examined OI and only 1 study specified that pregnancies resulting from OI (OI children) were excluded from the comparison group of children born after natural conception.<sup>27</sup> Thus for most studies, some misclassification of the unexposed group likely occurred, as OI children were counted as NC children. This would presumably lead to bias toward the null hypothesis, as OI is strongly associated with multiple pregnancies.<sup>28,29</sup> In the studies of twins, Pinborg et al<sup>30,31</sup> reported that 17.3% of pregnancies in the unexposed groups were the result of OI.

**Outcome Data.** Cerebral palsy was defined as a diagnosis of *International Statistical Classification of Dis-*

**Table 4. Assisted Conception and Developmental Delay Associations by Reference**

Source, y	Country, Cohort	Follow-up		Exclusions	Data Source		Sample Size (Multiples, %)		Measurement Scale	Associations	Adjusting or Matching
		Design	Duration, y		Assisted Conception	Outcomes	Exposed	Unexposed			
<b>All Children</b>											
Kallen et al, 2005	Sweden, 1987-2002	Retrospective cohort	1-20		All fertility clinics, IVF	HDR, ICD-9 or -10	16280	All other births	Behavioral problems	OR, 1.74 (95% CI, 1.11-2.74)	Maternal age, birth year, parity, and smoking
Leslie et al, 2003	Australia, 1993-1995	Retrospective cohort	5		1 Fertility clinic, ICSI	Psychological examination	97 (23)	110 (13)	WPPSI	NS findings in cognitive development at age 5 y	Parental education and language
Agarwal et al, 2005	Singapore, 1998-1999	Retrospective cohort	2		1 Fertility clinic, ICSI	Parent report and neurological examination	76 (46)	261 (46)	BSID-II, WABS	NS findings in mental and motor development and adaptive behavior at age 2 y	Maternal age, sex, race, plurality, parity, and date of delivery
La Sala et al, 2004	Italy, 1998-2001	Retrospective cohort	2	Congenital abnormalities, cerebral palsy	1 Fertility clinic, ICSI	Psychological examination	50 (40)	51 (24)	BSID-II, Videotaped interacting between parents and child	NS findings in mental development at ages 1 and 2 y, statistically significant lower scores in motor development and in cooperation with parents at age 1 and 2 y	No
Papaligoura et al, 2004	Greece, 1998-2000	Retrospective cohort	1	Born second or later	1 Fertility clinic, ICSI	Psychological examination	34 (23)	29 (21)	BSID	NS findings in mental and motor development at age 1 y	GA, maternal age, and birth weight (g)
Koivurova et al, 2003	Finland, 1990-1995	Retrospective cohort	3		2 Fertility clinics, IVF	Psychological examination	299	558	Psychomotor developmental milestone (modified Bayley scale)	NS findings in psychomotor development at ages 1, 2, and 3 y	Sex, maternal age and occupation, parity, and year of birth
Bowen et al, 1998	Australia, 1993-1995	Prospective cohort	1		1 Fertility clinic, ICSI	Psychological examination	89 (22)	80 (25)	BSID-II	Statistically significant lower scores in mental development at age 1, NS findings in motor development at age 1 y	Maternal age, parity, and multiplicity
Levy-Shiff et al, 1998	Israel	Retrospective cohort	10	PTD < 36	2 Fertility clinics, IVF	Psychological examination, self-report	51	51	WPPSI state-trait anxiety, depression, aggression, and behavior inventories for children	NS findings in cognitive development at ages 9 and 10 y, statistically significant lower scores in behavior development at ages 9 and 10 y	Sex, age, parity, and parental education and social level
D'Souza et al, 1997	Great Britain, 1984-1991	Prospective cohort	4		1 Fertility clinic, IVF (fresh)	Medical examination	278 (46)	278 (0)	Developmental delay (Griffiths score with DQ)	2 IVF multiples, 0 IVF singletons, and 0 NC children had adverse developmental outcome	Sex and father's occupation
<b>Twins</b>											
Minakami et al, 2008	Japan	Prospective cohort	1	Singletons	Assisted conception	Medical examination	136 (100)	72 (100)	Physical and neurological development	Statistically significantly less assisted conception, twins had adverse outcome (death, cerebral palsy, and mental retardation)	
Ito et al, 2006	Japan, 1994-2000	Retrospective cohort, corrected for age	3	Birth weight >1500 g, singletons	1 Fertility clinic, assisted conception	Psychological examination	28 (100)	16 (100)	Kyoto scale of psychological development DQ for posture, cognition, and language	6 IVF and 0 NC twins had borderline or lower total DQ, statistically significantly more IVF twins had borderline or lower DQ for cognition and language, NS findings in DQ for posture at age 3 y	

(continued)

ases, 10th Revision (ICD-10) code G80.0-G83.9, stated as "ICD-10 diagnosis CP," or described in 1 study as "children diagnosed with CP by a pediatric neurologist."<sup>26</sup> Six studies obtained information about CP diagnoses from hospital discharge registers<sup>27,31-35</sup>; the remaining 3 used records from rehabilitation centers<sup>26,36</sup> or questionnaires confirmed by discharge reg-

isters.<sup>30</sup> However, the validity and completeness of the CP diagnoses in hospital discharge registers have been questioned.<sup>37</sup> Klemetti et al<sup>27</sup> supplemented their information on CP diagnosis using registers reporting child care support. Only Strömberg et al<sup>36</sup> obtained the CP diagnoses from the medical records of all disability centers in Sweden.

**Table 4. Assisted Conception and Developmental Delay Associations by Reference (continued)**

Source, y	Country, Cohort	Follow-up		Exclusions	Data Source		Sample Size (Multiples, %)		Measurement Scale	Associations	Adjusting or Matching
		Design	Duration, y		Assisted Conception	Outcomes	Exposed	Unexposed			
Leunens et al, 2008	Belgium, 1993-1995	Retrospective cohort	10	PTD < 32, multiples	1 Fertility clinic, ICSI	Psychological examination	109 (0)	90 (0)	WISC, MABC	NS findings in cognitive and motor development at age 10 y	
Knoester et al, 2007	Netherlands, 1996-1999	Retrospective cohort	5-8	Oocyte or sperm donation, cryopreservation, multiples	1 Fertility center, ICSI	Questionnaires to parents	87 (0)	85 (0)	Child behavior checklist	NS findings in behavioral development at ages 5-8 y, 3/87 (3.4%) of ICSI children had an ASD diagnosis	
Knoester et al, 2007	Netherlands, 1996-1999	Retrospective cohort	5-8	Oocyte/sperm donation, cryopreservation, multiples	1 Fertility center, ICSI	Neurological examination	87 (0)	85 (0)	Neurological parameters, Touwen criteria	NS findings in neuromotor development at ages 5-8 y	Maternal age and parity
Knoester et al, 2007	Netherlands, 1996-1999	Retrospective cohort	5-8	Oocyte/sperm donation, cryopreservation, multiples	1 Fertility center, ICSI	Psychological examination	86 (0)	85 (0)	RAKIT	Statistically significant lower scores in cognitive development at ages 5-8 y	
Sanchez-Albisua et al, 2007	Germany, 1996-2001	Retrospective cohort	1.5 and 3	PTD < 35, multiples, perinatal complications	1 Fertility clinic, ICSI	Parent report, neurological examination	34 (0)	39 (0)	Developmental milestones	No difference in mental and motor development at ages 18 mo and 3 y	GA, age at examination, sex, and management of perinatal complications
Belva et al, 2006	Belgium, 1993-1995	Retrospective cohort	8	PTD < 32, multiples	1 Fertility clinic, ICSI	Neurological examination	150 (0)	147 (0)	Neurological parameters, Touwen criteria	NS findings in motor development at age 8 y except for coordination, ICSI children had statistically significant lower score	Sex, age at examination, and maternal education level
Leuens et al, 2006	Belgium, 1993-1995	Retrospective cohort	8	PTD < 32, multiples	1 Fertility clinic, ICSI	Psychological examination	151 (0)	152 (0)	WISC, MABC	Statistically significant higher scores in cognitive development at age 8 y, NS findings in motor development at age 8 y	Sex, age at examination, and maternal education level
Ponjaert-Kristoffersen et al, 2005	International, 1993-1995	Retrospective cohort	5	PTD < 32, multiples	Multicenter, IVF and ICSI	Psychological examination	ICSI, 511 (0); IVF, 424 (0)	488 (0)	WPPSI, McCarthy Scales of Children's Abilities Motor Scale	NS findings in cognitive and motor development at age 5 y	Sex, maternal education level, and birth order
Ponjaert-Kristoffersen et al, 2004	International, 1993-1995	Retrospective cohort	5	PTD < 32, multiples	Multicenter, ICSI	Psychological examination	300 (0)	260 (0)	WPPSI, PDMS	NS findings in cognitive development at age 5 y, statistically significant lower scores in motor development at age 5 y	Sex, age at examination, and maternal age
Barnes et al, 2004	International	Retrospective cohort	5	PTD < 32, not white, multiples	Multicenter, IVF and ICSI	Parental questionnaires	ICSI, 514 (0); IVF, 424 (0)	488 (0)	The McDewitt and Carey temperament questionnaires, Childs Behavior Checklist	NS findings in behavioral development at age 5 y	Sex, maternal age and education, and parental socioeconomic status
Bonduelle et al, 2004	International	Retrospective cohort	5	PTD < 32, multiples	Multicenter, ICSI	Neurological examination	300 (0)	266 (0)	Walking, running, jumping	12 in each group had walking, running, or jumping disorders at age 5 y	Sex, age at examination, and maternal age
Sutcliffe et al, 2003	International	Retrospective cohort	1	Multiples	Multicenter, ICSI	Medical examination	264 (0)	260 (0)	Griffiths Mental Development Scales	NS findings in mental development at age 1 y	Sex, age, maternal age, and social and educational level

(continued)

**Table 4. Assisted Conception and Developmental Delay Associations by Reference (continued)**

Source, y	Country, Cohort	Follow-up		Exclusions	Data Source		Sample Size (Multiples, %)		Measurement Scale	Associations	Adjusting or Matching
		Design	Duration, y		Assisted Conception	Outcomes	Exposed	Unexposed			
<b>Singletons (continued)</b>											
Place et al, 2003	Belgium, 1998-2000	Retrospective cohort	3 and 5	PTD < 37, multiples, birth weight < 2500 g	1 Fertility clinic, IVF and ICSI	Psychological examination	ICSI, 66 (0); IVF, 52 (0)	59 (0)	The revised Brunet-Lezine scale, WPPSI	NS findings in motor and behavioral development at age 18 mo, NS findings in cognitive development at ages 3 and 5 y	Sex, age, maternal age, and demographic factors
Colpin et al, 2002	Belgium, 1990-1991	Retrospective cohort	8-9	Multiples	1 Fertility clinic, IVF	Questionnaires to parents, teachers	27 (0)	23 (0)	Child Behavior Checklist, Teacher's Report Form	NS findings in behavioral development at age 8-9 y	No
Golombok et al, 2002	International	Retrospective cohort	11-12	Perinatal problems, multiples	Multicenter, IVF	Questionnaires to mothers and teachers, interviews with children	ICSI, 102 (0); IVF, 94 (0)	102 (0)	SDQ, Child and adolescent functioning and environment schedule	NS findings in behavioral development at age 11-12 y	Sex, age, age of mother, social class, and family size
Golombok et al, 2001		Retrospective cohort	11-12	Perinatal problems, multiples	IVF	Maternal interview and questionnaires, child interview, questionnaires to mothers and teachers	34 (0)	38 (0)	The social adjustment inventory for children and adolescents, SDQ	3 IVF and 3 NC showed a psychiatric disorder, NS findings in behavioral development at age 12 y	
Sutcliffe et al, 2001	Great Britain, 1995-1997	Retrospective cohort	1	Multiples	Multicenter, ICSI	Medical examination	208 (0)	221 (0)	Griffiths mental development scales	NS findings in mental development at age 1-2 y	Sex, maternal race, social and educational level
Sutcliffe et al, 1999	Great Britain	Retrospective cohort	1.5	Multiples	Multicenter, ICSI	Medical examination	123 (0)	123 (0)	Griffiths mental development scales	NS findings in mental development at age 17 mo	Sex, maternal race, social and educational level
Golombok et al, 1996	International	Retrospective cohort	5-6	Perinatal problems, multiples	Multicenter, IVF	Questionnaires from mothers and teachers	ICSI, 116 (0); IVF, 111 (0)	120 (0)	Rutter A and B scale	NS findings in behavioral development at ages 5-6 y	Sex, age, age of mother, social class, and family size
Wennerholm et al, 1998	Sweden, 1990-1995	Retrospective cohort	1.5	Stillborn twin, trisomy 13	2 Fertility clinics, IVF, cryopreserved	Medical examination	255 (0)	252 (0)	Psychomotor development	No difference in psychomotor development at age 18 mo	No

Abbreviations: ASD, autism spectrum disorder; BSID, The Bayley Scale of Infant Development; CI, confidence interval; DQ, Developmental Quotient; GA, gestational age; HDR, Hospital Discharge Register; *ICD-9*, *International Classification of Diseases, Ninth Revision*; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; MABC, Movement Assessment Battery for Children; NC, naturally conceived; NS, not significant; OR, odds ratio; PDMS, Peabody Developmental Motor Scales; PTD, preterm delivery; RAKIT, Revised Amsterdam Child Intelligence Test; SDQ, Strength and Difficulties Questionnaires; WABS, Vineland Adaptive Behavior Scale; WISC, Wechsler Intelligence Scale for Children; WPPSI, Wechsler Preschool and Primary Scales of Intelligence.

### All Birth Findings

All studies of singletons and multiples combined found a statistically significant increase in the risk of CP in children born as the result of IVF (IVF children) compared with NC children.<sup>27,32-34,36</sup> However, a disproportionate number of the IVF children were multiples. Odds ratios (OR) ranged from 1.6 to 3.7 in analyses adjusting for various factors other than PTD. The strongest association between IVF and CP (OR, 3.7; 95% confidence interval [CI], 2.0-6.6) was reported by Strömberg et al.<sup>36</sup> A meta-analysis (without overlapping study cohorts) included 19 462 IVF children and demonstrated an increased risk of CP in IVF children (OR, 2.18; 95% CI, 1.71-2.77) (**Figure**).

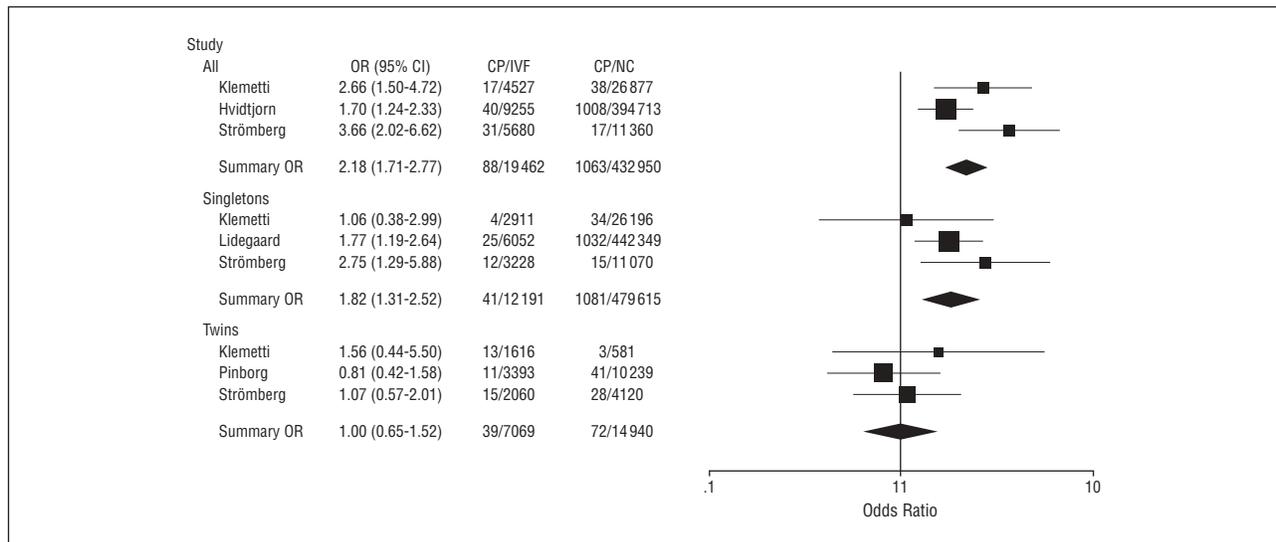
Some studies also presented results from analysis including PTD. Although Strömberg et al found a decrease in the magnitude of the association after adjusting for PTD, it remained strong and statistically significant

(OR, 2.9; 95% CI, 1.4-6.0). Hvidtjørn et al<sup>32</sup> found no association between IVF and CP after adjusting for PTD. In the study by Källen et al,<sup>33</sup> the OR in a stratum of children carried to term was 0.88 (95% CI, 0.46-1.70), and Klemetti et al<sup>27</sup> found that 88% of IVF children with CP were born preterm. Dissimilarities in analytic approach complicate an immediate comparison of these findings; however, it seems clear that the risk of CP in IVF children at least partially operates through PTD.

Only Källen et al<sup>33</sup> had information on time to pregnancy (TTP) and reported no association between IVF and CP after adjusting for TTP.

### Singleton Findings

There was a tendency toward an increased risk for CP in singletons born as a result of IVF (IVF singletons) compared with non-IVF singletons,<sup>27,32,35,36</sup> but not all studies



**Figure.** Meta-analyses of studies assessing the risk of cerebral palsy (CP) in children born as a result of in vitro fertilization (IVF). OR indicates odds ratio; CI, confidence interval; NC, naturally conceived.

reached statistical significance.<sup>27,32</sup> The 2 Danish studies used overlapping cohorts but had different analytical approaches; Lidegaard et al<sup>35</sup> reported a crude rate ratio of 1.8 ( $P < .01$ ) in contrast to the non-statistically significant finding by Hvidtjorn et al<sup>32</sup> who found a hazard rate ratio of 1.28 (95% CI, 0.80-2.03) in an analysis adjusting for sex, parity, maternal age, and educational level. Strömberg et al<sup>36</sup> reported an OR of 2.8 (95% CI, 1.3-5.8) after adjusting for sex, year of birth, and birth hospital. A meta-analysis (without overlapping study cohorts) comprised 12 191 IVF singletons and showed an increased risk of CP in IVF singletons (OR, 1.82; 95% CI, 1.31-2.52) (Figure).

### Multiples Findings

While the effect estimates varied (OR range, 0.6-1.5) of the 5 studies examining CP in twins born as a result of IVF (IVF twins) vs non-IVF twins, confidence limits were wide and overlapping (1.0) in all studies.<sup>27,30-32,36</sup> In contrast, in the study on the risk of CP in triplets, assisted conception had a statistically significant protective effect.<sup>26</sup>

## AUTISM SPECTRUM DISORDERS

### Generalizability

Data regarding ASD can be found in Table 4. All but 1 of the ASD studies (an Israeli case-control study)<sup>26</sup> originated in Scandinavian countries; consequently, they have the same limitations regarding external validity as the CP studies.

### Methodological Quality of Included Studies

**Sample Size and Precision.** The identified ASD studies covered the period from 1970 to 2001. During this period the prevalence of ASD apparently changed considerably from 4 to 5 per 10 000 children to 6 to 7 per 1000 children.<sup>38</sup> It has been questioned whether this increase reflects a true rise in the prevalence of ASD or at least partly reflects changes in diagnostic criteria and in-

creased medical and/or public awareness of these disabilities.<sup>39,40</sup> Given that active monitoring of ASD was not in operation in most areas, the expected prevalence at a particular time and area is not known. If we apply a conservative estimate of about 3/1000 children for the time period of these ASD studies, it would require sample sizes of 5268 exposed and 15 804 unexposed to detect a risk of 2.0 in cohort studies with 80% power and 95% CI (estimations in Calculations in Epi Info; Centers for Disease Control, Atlanta, Georgia). Only 2 studies on multiples and singletons combined and 1 on singletons alone achieved this size.<sup>34,35</sup>

**Exposure Data.** For the cohort studies from Scandinavia, nondifferential misclassification of OI in the unexposed group was likely, as described above for the CP studies.

In the 1 case-control study, information on exposure was retrieved differently, namely from birth records. However, a validation study on information about infertility treatment in birth records indicated low sensitivity overall for fertility treatment reporting and potentially differential reporting. Higher-risk infants such as multiples were more likely than singletons to be correctly reported as conceived after infertility treatment (personal communication, L.S.; February 1, 2008).

**Outcome Data.** Most studies obtained information on outcome from hospital discharge registers<sup>27,31,34,35,41</sup> and others used records from rehabilitation centers<sup>36,42</sup> or questionnaires confirmed by discharge registers.<sup>30</sup> Autism spectrum disorder was defined as a diagnosis of ICD-10 code F84.0, F84.1, F84.5, or F84.9 or ICD-8 and *Diagnostic and Statistical Manual of Mental Disorders* (Third or Fourth Edition) (*DSM III-IV*). Three of the studies used only infantile autism as the study outcome, 3 used ASD, and 2 used an even broader range of psychiatric diagnoses including ASD, complicating the comparison between the studies. Diagnosis was retrieved from Hospital Discharge Registers, from The Danish Psychiatric

Central Research Register, and in 1 case from an autism treatment organization.<sup>42</sup>

**Findings.** Two studies evaluated children born after assisted conception in general,<sup>41,42</sup> while the remaining 6 evaluated IVF using information from all of the fertility clinics in each country. Findings were inconsistent overall and when considering singletons and multiples separately. Only the study by Klemetti et al<sup>27</sup> reported a statistically significant increased risk for a broad range of psychiatric disorders (F80-F98) including ASD in children born after IVF (OR, 1.68; 95% CI, 1.11-2.58); however, they did not provide results for ASD alone.

In contrast, Maimburg and Væth<sup>41</sup> reported a protective effect between assisted conception and infantile autism in their case-control study, including 473 children with infantile autism and 473 matched control children (OR, 0.37; 95% CI, 0.14-0.98) adjusting for several factors including gestational age.

## DEVELOPMENTAL DELAY

### Generalizability

Developmental delay associations can be seen in Table 4. Most studies on developmental delay limited their study participants to children exposed to assisted conception at 1 or 2 fertility clinics, introducing the possibility of selection bias if these clinics were not representative of the entire population. Furthermore, bias might have resulted from differential participation because of substantial nonparticipation in some studies requiring individual examination of the included children. For example, in one of the largest international studies,<sup>43</sup> participation rates varied across countries, with rates from 25% to 96% and differences of participation rates of exposed and nonexposed children up to 50%.

Perhaps the foremost problem with these studies was that 21 of them excluded children with risk factors such as multiplicity, PTD, and neonatal complications a priori to test the possible influence of IVF or ICSI on development apart from these complications. While this was certainly a reasonable method for the stated objective, it severely compromised the value of the studies to inform an increase in general developmental delay in the total population of children born after assisted conception, as one could argue that delivery of multiples and PTD are key factors in the causal pathway.

However, studies on developmental delay were conducted on several continents, ensuring representation of diverse populations.

### Methodological Quality of Included Studies

**Sample Size and Precision.** Except for 1 population-based register study,<sup>33</sup> the studies on developmental delay individually included between 43 and 999 children, with each study sample of insufficient size to identify relatively rare events such as CP or ASD. Many sample sizes were also insufficient to detect moderate differences in the broader child development measures.

**Exposure and Outcome Data.** Exposed children were identified at fertility clinics. Nearly all studies used parental questionnaires and/or some type of individual standardized examination of the child to assess development, but they used different measurement scales. The population-based study used hospital discharge registers and ICD codes.<sup>33</sup> While ascertainment of both exposure and outcome were based on reliable sources and standardized measures, these strengths did not overcome the limitations of possible selection bias due to low participation rates in some studies and selection of specific low-risk children only.

**Findings.** We identified 30 studies assessing developmental delay in children born after assisted conception. Seventeen studies evaluated children born after ICSI solely, 13 also evaluated IVF, and 2 evaluated children born after assisted conception in general. Eight studies reported the number of children with CP in their cohorts and 2 stated the number of children with ASD. The numbers were too small for statistical estimation regarding these conditions, as would be expected in cohorts of fewer than 1000 children. In one study the children identified with CP or other adverse outcomes were excluded before further examination.<sup>44</sup>

Fourteen studies assessed motor development,<sup>43-56</sup> and 2 reported that children born as a result of ICSI (ICSI children) had a statistically significant higher risk of delayed motor development at 1 to 2 or 5 years of age, respectively.<sup>44,57</sup> Eleven studies assessed behavioral development<sup>33,44,46,56,58-64</sup>; 2 reported a statistically significant higher risk of delayed behavioral development in ICSI children aged 1 to 2 years<sup>44</sup> and IVF children aged 9 to 10 years,<sup>58</sup> respectively. The large population-based register study found an increased risk of behavioral problems in children born after IVF (OR, 1.74; 95% CI, 1.11-2.74).<sup>33</sup> Nine studies assessed delay in cognitive development<sup>43,45,51,54,56-58,65,66</sup> and 1 study reported that ICSI children had a statistically significant lower risk of delayed cognitive development at 8 years of age, while another study reported that ICSI children had a statistically significant higher risk of delayed cognitive development at 5 to 8 years of age.<sup>65</sup> Eleven studies assessed delay in mental development,<sup>44,46-50,52,67-71</sup> and 1 found less adverse outcomes in twins born as a result of assisted conception<sup>71</sup> while all other studies on developmental delay described non-statistically significant findings.

## COMMENT

This systematic review included studies assessing the risks of CP, ASD, and developmental delay in children born after assisted conception. Owing to the size of the study cohorts and the concordant results, the CP studies offer persuasive evidence of an increased risk of CP in children born after IVF that is explained in part by an increased risk of PTD. Cerebral palsy is a lifelong condition and a heavy burden on the child, family, and health care system in terms of both personal and economic costs.<sup>72</sup>

In contrast, studies assessing the risk of ASD were inconsistent. This might have been owing to the aforementioned methodological problems in these studies or simply might have reflected a lack of association between assisted conception and ASD. As ASD seems to have a heterogenic etiology, the effect of weak associations will only be apparent in larger samples.<sup>40</sup> Moreover, a possible association between assisted conception and ASD needs examination in studies covering recent time periods with more complete ASD reporting.

Studies on developmental delay following assisted conception mainly included small select groups of low-risk children, and they presented generally non-statistically significant results. Thus, data on larger samples of the full range of children conceived via assisted conception are needed.

We did not identify any studies assessing the risk of CP, ASD, or developmental delay in children born after OI specifically. The lack of evidence on the long-term risks of OI is of concern given associations shown in previous studies between OI and PTD, LBW, and delivery of multiples.<sup>1,4-8</sup>

While the studies offer persuasive evidence of an association between IVF and CP, gaps remain in understanding this relationship. Because the increased risk of CP in children born after IVF seems to operate partly through the causal pathway of IVF, delivery of multiples, and PTD, the extent of the CP risk associated with IVF in a population will likely depend on the rate of IVF multiples. This rate is lower in Scandinavian countries that regulate the number of embryos transferred; eg, in Denmark in 2005 only 1.5% of IVF children were triplets and 32.6% were twins.<sup>73</sup> In contrast, the rate of multiples born after IVF in the United States in 2004 was 50%, with a larger proportion of triplets and longer gestations.<sup>2</sup>

The etiology behind the risk of CP in IVF singletons remains unclear, but 2 Danish studies suggest that the phenomenon of vanishing embryos in early pregnancy might be part of the etiology. Hvidtjorn et al<sup>74</sup> found that 3.9 of 1000 (95% CI, 2.2-5.5/1000) singletons born after transfer of more than 1 embryo had CP, similar to the proportion among twins born after the transfer of 2 embryos (4.4 of 1000 children; 95% CI, 1.9-6.9). Pinborg et al<sup>75</sup> showed that the group of IVF singletons in which a coembryo had vanished before 22 weeks of gestational age had nearly twice the risk of CP (OR, 1.9; 95% CI, 0.7-5.2) compared with IVF singletons originating from pregnancies with only 1 fetus at 8 weeks of gestational age. These findings need further exploration.

We also still need to determine whether the increased risk of CP after IVF is associated with subfertility, type of subfertility, or any specific subtype of IVF. Subfertility has been associated with adverse pregnancy outcomes such as PTD and neonatal death,<sup>76,77</sup> though not by Kapitejn et al.<sup>9</sup> Ericson et al<sup>34</sup> found an increased risk of hospitalization with increasing time to pregnancy. We found only 1 study that adjusted for time to pregnancy<sup>33</sup> and, in doing so, the risk of CP disappeared. The type of subfertility was taken into account in 1 study only, revealing similar risks of CP within the different types, though this was based on small numbers.<sup>32</sup>

While animal studies report long-term adverse effects of urinary gonadotrophins compared with other treat-

ment regimens used in controlled ovarian stimulation,<sup>78,79</sup> only a few studies in this review compared different treatment types. Three studies evaluated possible differences between conventional IVF and ICSI<sup>31-33</sup> and found comparable risks in the 2 groups. One study compared the risk of CP in children born after use of fresh vs frozen embryos and reported a hazard rate ratio of 2.32 (95% CI, 0.80-6.76) in the latter group,<sup>32</sup> but this was based on small numbers.

A comprehensive search in PubMed and EMBASE revealed 130 articles, of which 41 were eligible for review. The main reasons for exclusion of articles were (1) commentary and/or review, (2) different exposure or outcome, and (3) no NC comparison group. Excluded articles are shown in an online appendix. We consider the possible selection bias minimal, as none of the studies excluded fulfilled the a priori criteria.

In summary, this systematic review revealed important gaps in the evidence of long-term outcomes in children born after assisted conception. Possible associations between assisted conception and ASD need assessment in larger studies with well-defined outcomes. Studies on assisted conception and CP from countries outside of Scandinavia are needed as well as studies with detailed information on TTP, underlying causes of infertility, and types of IVF treatment. The long-term outcomes of OI must be addressed. Given the continually increased use of fertility treatments worldwide, studies addressing these very large gaps in the knowledge of the long-term health and development of children born after assisted conception are an important public health objective.

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## Children born after assisted fertilization have an increased rate of major congenital anomalies

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**Objective:** To study the occurrence of major congenital anomalies (CAs) among children born after IVF (IVF, microinjections, and frozen embryo transfers) and after ovulation inductions with or without insemination (other assisted reproductive technologies [ART]).

**Design:** Register-based study.

**Setting:** Data regarding CAs were obtained from the Register of Congenital Malformations.

**Patient(s):** Children from IVF (n = 4,559), children from other ART (n = 4,467), and controls (n = 27,078, a random sample of naturally conceived children) from the Medical Birth Register.

**Intervention(s):** In vitro fertilization and other ART treatment in ordinary practice.

**Main Outcome Measure(s):** Rate of major CAs. Children from IVF and other ART were compared with control children, both overall and by plurality, controlling for confounding factors by logistic regression.

**Result(s):** For IVF children, the adjusted odds ratio (OR) was 1.3 (95% confidence interval [CI], 1.1–1.6). Stratifying by gender and plurality showed that the risk was only increased for boys, and the risk was decreased for multiple IVF girls (OR = 0.5, 95% CI 0.2–0.9). The crude OR of major CA for other ART children was 1.3 (95% CI 1.1–1.5), but adjusted differences by gender and plurality were statistically insignificant.

**Conclusion(s):** In vitro fertilization was associated with an increased risk for major CAs among singleton boys and a decreased risk among multiple girls. The risk after other ART was only slightly increased. (Fertil Steril® 2005;84:1300–7. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** Major congenital anomaly, ART, register-based study

In vitro fertilization and its related procedures—intracytoplasmic sperm injection (ICSI) and frozen embryo transfer (FET)—have become common infertility treatments. For example, in Finland approximately 2.5% of all infants are born as a result of these therapies (1). The exact number of children born after other assisted reproduction technologies (ART), such as ovulation inductions and inseminations, is unknown, but according to our estimation during 1996–1999 2.4% of all infants were born after other ART in Finland.

Some studies (2–9) but not others (10–13) have shown an increase in some congenital anomalies (CAs) among IVF or

ICSI children. Most published studies have had methodological problems, such as small sample sizes, lack of proper controls, and different definitions of CA among IVF and naturally conceived children. In a recent Australian study, the rate of musculoskeletal, cardiovascular, chromosomal, and urogenital defects was increased among IVF children (7). In a small Finnish study, the prevalence of heart malformations was fourfold among IVF infants compared with control infants (8). We found only one study on malformations of children born as a result of other ART (14). There were increased rates of congenital malformations, but these could be mainly explained by maternal characteristics.

In this study, we compared the prevalence of major CAs among IVF and other ART children with that among naturally conceived children, controlling for confounding factors. The data source of CAs was the same for all children—the Finnish Register of Congenital Malformations (RCM)—and we have information regarding the drugs used in the infertility therapy.

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## MATERIALS AND METHODS

The study is based on children born to women having received IVF (IVF, ICSI, and FET) and other ART between 1996 and 1998 in Finland. The women were identified with a predesigned algorithm from the reimbursement files of the Social Insurance Institution (15) and linked to the Finnish Medical Birth Register (MBR); the time difference between the beginning of the last treatment cycle and the birth of the child was used to estimate which infants resulted from IVF or other ART (16).

The MBR includes the mother's and child's unique personal identification numbers and contains information on maternal background and on the infant's outcome until the age of 7 days for all infants born in Finland. The duration and causes of infertility are not registered. The data are collected by delivery hospitals and completed by linkage to the Central Population Register and the Cause-of-Death Register. The quality of the MBR has been found to be high for the variables used in this study (17, 18).

We identified 4,559 IVF and 4,467 other ART children born between October 1996 and September 1999. As controls, 27,078 naturally conceived children (three times the number of cases) were selected randomly from the MBR, excluding children having a note of IVF or other ART in MBR. Children from ICSI ( $n = 861$ ) could be distinguished from IVF children only if the treatment had been given in private clinics because a specific code for ICSI exists only there.

The identified children were linked to the RCM according to the mothers' identification numbers and the children's dates of birth. The RCM collects information on all infants with a CA or birth defect through several data sources, including a form completed by delivery hospitals, neonatal, pediatric, and pathology departments, and cytogenetic laboratories and by linkage to several other nationwide registers. More than 99% of the major CAs are registered before the age of 1 year.

In the RCM a CA has been defined as a major congenital structural anomaly, chromosomal defect, or congenital hypothyroidism involved in a birth. The register records all notified cases, but the physician responsible for RCM routinely classifies congenital anomalies into major, other, and rejected. Rejected anomalies include some minor congenital anomalies, as defined by the European Surveillance of Congenital Anomalies (European Concerted Action on Congenital Anomalies and Twins [EUROCAT]; <http://www.eurocat.ulster.ac.uk>) exclusion list (hereditary diseases, dysfunction of organs or tissues, developmental disabilities, congenital infections, isolated minor dysmorphic features, normal variations, and other less significant anomalies). For this study, the physician reviewed all diagnosis and inclusion criteria without knowing the mode of conception of the children.

The study plan was approved by the National Research and Development Centre for Welfare and Health (STAKES)

research ethics committee. For register linkages, the National Data Protection Authority was consulted and permissions from the register keepers were obtained.

The differences were tested by *t*-test, a test for relative proportions, and a  $\chi^2$  test. The statistical analyses were performed in SAS, version 8 (SAS Institute, Cary, NC). The IVF and other ART children were compared with the control children according to odds ratios (OR) and 95% confidence intervals (CI), stratifying by gender and multiplicity. Twins and triplets were analyzed separately. Differences in age of the mother, parity, socioeconomic position (measured from maternal occupation), and the region of residence were controlled by logistic regression.

In the analysis by organ system, a child appeared more than once if (s)he had more than one major CA affecting different organ systems. Different CAs in the same organ system were calculated as one, but if the child had a major CA both in the urinary and genital system, only one was taken into account by combining these two groups as the "urogenital system." Only major CAs, as defined in the RCM, were included in the analysis, but minor urinary and genital CAs were separately compared between studied groups.

To investigate which of the infertility drugs used in the treatment were related to CAs, a nested case-control design in the IVF and other ART cohorts was used: mothers of children with CAs were compared with mothers of non-malformed children. Drugs used during the last IVF cycle preceding the birth were classified into five groups: GnRH, FSH, hCG, progesterones (Ps) (among IVF women 99% and among other ART women 50% were natural Ps), and estrogens (Es), and the age-adjusted ORs for using at least one of the drugs from the category were calculated.

To estimate the total prevalence of major CAs, we linked the IVF and other ART women to the Register of Induced Abortions, specifying induced abortions performed because of a suspected or confirmed CA. The rates were compared with the national rates per 10,000 births.

## RESULTS

In vitro fertilization and other ART mothers differed from control mothers, and IVF mothers from other ART mothers in regard to most characteristics (Table 1). Multiplicity was much higher in the IVF than in the other ART group, but the number of triplets was the same (16 vs. 17).

Among IVF and other ART children, 51% of reported major CAs had been accepted by the RCM, whereas among control children the proportion was 46%. In total, 195 IVF children (4.3%), 166 other ART children (3.7%), and 787 control children (2.9%) had at least one major CA. The prevalence of a major CA was the same for IVF singletons and IVF multiples but much higher for multiples than for singletons among other ART and control children (Table 2).

**TABLE 1****Characteristics of IVF, other ART, and control mothers and children by multiplicity and gender.**

	IVF	Other ART	Controls
Mothers	n = 3,737	n = 4,188	n = 27,022
Age (y) (mean ± SD) <sup>a</sup>	33.9 ± 4.5	31.2 ± 4.6	29.8 ± 5.3
Age (y) <sup>b</sup>			
<25	2.2	8.3	19.7
25–29	17.3	34.1	32.2
30–34	40.6	37.2	31.4
35–39	30.1	16.4	13.5
40+	9.8	4.0	3.1
Married <sup>c</sup>	76.1	74.8	60.5
Parity <sup>b</sup>			
0	71.7	54.3	38.7
1	21.1	32.4	33.4
2	4.2	9.3	16.4
3+	2.4	3.3	10.1
Missing	0.6	0.7	1.4
Socioeconomic position <sup>b</sup>			
Upper white-collar	26.1	21.2	15.7
Lower white-collar	48.8	47.8	41.3
Blue-collar	12.8	13.9	16.6
Other	12.3	17.2	26.4
Place of residence <sup>b</sup>			
Southern Finland	44.8	38.6	40.6
Western Finland	33.4	38.3	34.4
Eastern Finland	9.9	9.5	10.4
Northern Finland	11.6	13.3	13.9
Missing	0.3	0.3	0.7
Children	n = 4,459	n = 4,467	n = 27,078
Singletons	64.3	87.9	97.8
Girls	32.7	42.8	48.6
Boys	31.6	45.1	49.3
Multiples	35.7	12.1	2.2
Girls	17.6	6.0	1.2
Boys	18.1	6.0	1.0

Note: Values are percentages, unless otherwise noted.

<sup>a</sup>  $P < .001$ ,  $t$ -test.

<sup>b</sup>  $P < .001$  for all comparisons (IVF vs. other ART, IVF vs. controls, and other ART vs. controls),  $\chi^2$  test.

<sup>c</sup>  $P < .001$  (IVF vs. controls and other ART vs. controls), test for relative proportions.

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Boys from IVF, both singletons and multiples, had major CAs more often than IVF girls (Table 3). The same was seen among multiples from other ART.

An increased OR for having any major CA was found in the crude analysis both for IVF and other ART children (Table 3). The adjustment for maternal age or other confounding factors somewhat decreased the ratio for IVF children but not for other ART children. The total risk for singletons was statistically significantly increased and for multiples insignificantly decreased. A significantly increased

OR was found among singleton IVF boys, and a significantly decreased OR for multiple IVF girls. The result did not change after taking into account the confounding factors. In the separate analysis for twins, excluding triplets, the results for IVF girls remained similar (the adjusted OR was 0.31, 95% CI 0.11–0.88).

In the analysis by different organ system, compared with controls, IVF children had a higher risk for CA for most categories. Compared with other ART children, IVF children had more CAs in the categories of “eye, ear, face, and neck,”

**TABLE 2**Prevalence of major congenital anomalies per 10,000 infant by the organ system affected.<sup>a</sup>

	Singletons						Multiples									
	IVF (n = 2,930)		Other ART (n = 3,926)		Controls (n = 26,489)		IVF (n = 1,629)		Other ART (n = 541)		Controls (n = 589)					
	n	/10,000	P <sup>b</sup>	n	/10,000	P <sup>b</sup>	n	/10,000	n	/10,000	P <sup>b</sup>	n	/10,000			
Any	125	427	<.001	138	352	.022	756	285	70	430	.335	27	499	.836	31	526
Central nervous system	9	31	.008	12	31	.003	31	12	9	55	.071	7	129	.006	0	0
Eye, ear, face and neck	12	41	.009	6	15	.693	48	18	5	31	.583	1	18	.952	1	17
Heart	44	150	.042	59	150	.021	287	108	33	203	.791	11	203	.840	13	221
Other circulatory system	6	20	.740	12	31	.088	47	18	2	12	.790	0	0	.338	1	17
Respiratory system	5	17	.284	5	13	.647	27	10	3	18	.496	0	0	.175	2	34
Cleft palate and cleft lip	12	41	.034	14	36	.076	56	21	5	31	.904	0	0	.175	2	34
Digestive system	14	48	.028	16	41	.083	67	25	5	31	.093	4	74	.836	5	85
Urogenital system	35	119	<.001	26	66	.150	129	49	12	74	.789	4	74	.836	5	85
Musculoskeletal system	34	116	.004	30	76	.588	182	69	20	123	.270	6	111	.441	4	68
Skin, hair and nails	1	3	.533	2	5	.757	17	6	2	12	.395	0	0	NA	0	0
Chromosomal anomalies	8	27	.304	7	18	.927	49	18	3	18	.496	2	37	.932	2	34
Other congenital anomalies and the defects	12	41	.171	19	48	.020	71	27	9	55	.237	3	55	.381	6	102

Note: NA = not applicable.

<sup>a</sup> n = number of children. If a child had a major malformation in more than one organ system, the child appears several times in the table. If the malformations affect the same organ system, the child appears only once in the table.

<sup>b</sup> Test for relative proportions, control group as a reference group.

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TABLE 3

Total risk of major congenital anomalies and risk according to organ system affected<sup>a</sup> by gender and multiplicity.

Multiplicity	Group	Risk								
		Girls			Boys			Total		
		n <sup>b</sup>	OR (95% CI)	OR <sup>c</sup> (95% CI)	n <sup>b</sup>	OR (95% CI)	OR <sup>c</sup> (95% CI)	n <sup>b</sup>	OR (95% CI)	OR <sup>c</sup> (95% CI)
Singletons										
Total	Control	348	1.00	1.00	408	1.00	1.00	756	1.00	1.00
	IVF	48	1.23 (0.90–1.66)	0.97 (0.69–1.36)	77	1.79 (1.40–2.30)	1.63 (1.23–2.15)	125	1.52 (1.25–1.84)	1.30 (1.05–1.61)
	Other ART <sup>d</sup>	67	1.34 (1.02–1.74)	1.21 (0.98–1.67)	71	1.16 (0.90–1.50)	1.12 (0.86–1.46)	138	1.24 (1.03–1.49)	1.17 (0.97–1.41)
Heart	Control	128	1.00	1.00	136	1.00	1.00	264	1.00	1.00
	IVF	17	1.17 (0.71–1.95)	1.05 (0.62–1.78)	19	1.30 (0.80–2.11)	1.21 (0.73–2.00)	36	1.24 (0.87–1.75)	1.13 (0.79–1.62)
	Other ART	29	1.57 (1.04–2.35)	1.52 (1.01–2.28)	24	1.17 (0.76–1.81)	1.17 (0.75–1.81)	53	1.36 (1.01–1.83)	1.33 (0.99–1.80)
Urogenital	Control	52	1.00	1.00	80	1.00	1.00	26	1.00	1.00
	IVF	9	1.53 (0.75–3.11)	1.47 (0.70–3.07)	22	2.57 (1.60–4.14)	2.46 (1.49–4.07)	31	2.14 (1.44–3.17)	2.05 (1.36–3.10)
	Other ART	4	0.53 (0.19–1.46)	0.52 (0.19–1.45)	20	1.66 (1.02–2.72)	1.62 (0.99–2.65)	24	1.23 (0.79–1.90)	1.20 (0.78–1.87)
Musculoskeletal	Control	72	1.00	1.00	110	1.00	1.00	182	1.00	1.00
	IVF	11	1.35 (0.72–2.55)	1.26 (0.65–2.44)	23	1.95 (1.24–3.07)	1.75 (1.09–2.81)	34	1.70 (1.17–2.45)	1.55 (1.05–2.27)
	Other ART	12	1.15 (0.62–2.12)	1.11 (0.60–2.05)	18	1.09 (0.66–1.79)	1.04 (0.63–1.72)	30	1.11 (0.76–1.64)	1.07 (0.73–1.58)
Multiples										
Total	Control	18	1.00	1.00	13	1.00	1.00	31	1.00	1.00
	IVF	26	0.55 (0.30–1.02)	0.45 (0.22–0.93)	44	1.13 (0.60–2.14)	1.31 (0.64–2.71)	70	0.81 (0.52–1.25)	0.80 (0.48–1.32)
	Other ART	7	0.44 (0.18–1.08)	0.41 (0.16–1.05)	20	1.59 (0.77–3.26)	1.56 (0.71–3.42)	27	0.95 (0.56–1.61)	0.91 (0.52–1.61)
Total	Control	366	1.00	1.00	421	1.00	1.00	787	1.00	1.00
	IVF	74	1.19 (0.93–1.54)	0.97 (0.73–1.28)	121	1.77 (1.44–2.17)	1.66 (1.31–2.10)	195	1.49 (1.27–1.75)	1.31 (1.10–1.57)
	Other ART	75	1.26 (0.98–1.62)	1.15 (0.89–1.50)	91	1.30 (1.03–1.64)	1.26 (0.99–1.59)	166	1.28 (1.08–1.52)	1.21 (1.02–1.44)

<sup>a</sup> Reference group (OR = 1) = control children. If a child had a major CA in more than one organ system, the child appears several times in the table. If the CAs affect the same organ system, the child appears only once in the table.

<sup>b</sup> n = number of malformed children.

<sup>c</sup> For all major CAs adjusted by age, parity, socioeconomic position, and region, and for some specific anomalies according to organ system adjusted only by age owing to the small number of cases.

<sup>d</sup> One other ART child excluded owing to missing gender status.

**TABLE 4****Major genital anomalies (and all hypospadias) among singleton boys: number and rate per 10,000.**

	IVF (n = 1,440)	Other ART (n = 2,014)	Controls (n = 13,339)
Total			
No.	11	6	15
Rate	76	30	11
<i>P</i> <sup>a</sup>	<.001	.036	
Hypospadias			
No.	7	3	10
Rate	15	7	4
<i>P</i> <sup>a</sup>	<.001	.287	
All hypospadias <sup>b</sup>			
No.	11	8	38
Rate	76	40	29
<i>P</i> <sup>a</sup>	.003	.390	

<sup>a</sup> Test for relative proportions, compared with controls.<sup>b</sup> Also includes glandular hypospadias.Klemetti. Assisted reproduction and congenital anomaly. *Fertil Steril* 2005.

“respiratory system,” “urogenital system,” and “musculoskeletal system.” When stratifying the analysis to singletons and multiples (Table 2), IVF singletons had statistically significantly more major CAs than control singletons in many categories. Among multiples the rates were the opposite: in most categories, IVF multiples had fewer major CAs than control multiples, and none of the differences were statistically significant. Among other ART children, singletons had more and multiples had fewer CAs in most organ systems than control singletons, but the differences were not as clear as that between IVF and control children.

When inspecting the risk according to the organ system affected, by gender and multiplicity, we found a slightly increased OR for major heart anomalies among singleton other ART girls and increased ORs for urogenital and musculoskeletal CAs among singleton IVF boys (Table 3). The results remained the same after adjustment for age. Among IVF singleton boys, major urogenital CAs were more severe than among controls. When we checked for minor urogenital CAs of singleton boys, no reported minor urinary CA was observed. In the separate analysis of urinary and genital CAs, it was found that the increased risk was mainly due to the genital CAs. Hypospadias was the most common diagnosis of these major genital anomalies, and control boys had more minor hypospadias than IVF boys (Table 4). In addition, other ART singleton boys had a higher risk for urogenital CAs. No specific musculoskeletal CA among IVF boys was found.

Out of 861 ICSI children, 40 (4.6%) had one or more CA. The frequency of major CAs was in general as among all IVF children. Because of the small number of cases, a more specific analysis of the ICSI group was not done.

By definition, all IVF mothers were exposed to some infertility drugs, most commonly to P (Table 5). Most mothers were exposed to several drugs. Only Es were used more often by the mothers of malformed than by the mothers of

**TABLE 5****Drugs used by mothers of malformed and nonmalformed children.**

Group	Malformed <sup>a</sup>	Non-malformed	<i>P</i> <sup>b</sup>
IVF <sup>c</sup>	n = 179	n = 4,088	
P	87	88	.736
FSH or hMG	59	63	.241
GnRH	55	62	.050
hCG	17	20	.286
E <sub>2</sub>	20	12	.003
Other ART	n = 166	n = 4,301	
Clomiphene citrate	81	86	.056
P	30	30	.896
FSH or hMG	14	11	.250
hCG	5	4	.598

Note: Values are percentages.

<sup>a</sup> At least one major congenital anomaly.<sup>b</sup> Comparisons of malformed and nonmalformed groups, test for relative proportions.<sup>c</sup> Two hundred ninety-two IVF children are excluded owing to the lack of information on drugs used.Klemetti. Assisted reproduction and congenital anomaly. *Fertil Steril* 2005.

nonmalformed IVF children, but mothers of most malformed children had not received it. Some 27% of the mothers of singleton boys with a genital CA had used Es (vs. 13% of mothers of nonmalformed singleton boys) and 82% P (vs. 82% of mothers of nonmalformed singleton boys). Among other ART children, no differences in the drugs used between malformed and nonmalformed children were found, neither in the use of a natural nor a synthetic progestin.

During the study period, 9 of the 9,175 IVF women (19.7 per 10,000 IVF births) and 8 of the 10,270 other ART women (17.9 per 10,000 other ART births) had an induced abortion owing to the suspected or detected fetal defect. The national rate per 10,000 births in 1996–1998 in Finland was 36.7 (The Finnish Medical Birth Register).

## DISCUSSION

We found increased total rates of major CAs for IVF and other ART singletons. Singleton boys from IVF in particular had more major urogenital and musculoskeletal CAs, and other ART singleton girls had more major heart anomalies. Among multiples, the total risk for a major CA was not increased, and for multiple IVF girls the risk had even decreased.

Can our results be trusted? Our data include most infants born as a result of IVF and other ART in Finland during the study period. The identification was based on drugs used in infertility therapy and reimbursed treatments (only private clinics) (15, 16). It is possible that we missed some women who had received their treatment in the public sector and had used drugs bought and reimbursed before 1996. We have estimated that our data lack approximately 4% of IVF women and 6% of other ART women (15), but the identification of the women was made before pregnancy and is unlikely to relate to the occurrence of major CAs.

Data on major CAs in all three groups came from a routine nationwide register, the RCM, to which information is collected and classified blindly with regard to IVF or ART status. However, we do not know whether physicians who reported CA to the register knew the mode of conception. It could be that IVF children were more carefully examined and/or that CAs for them were more conscientiously reported than those for naturally conceived children. However, the fact that more reports that were rejected by the RCM occurred for control children than for IVF children speaks against this source of bias. Likewise, the checking of minor genital anomalies showed that the number of reported minor CAs of IVF children was smaller than that of controls. One could have expected it to be greater if the IVF and other ART children had been more carefully examined and reported. The types of genital anomalies suggest that classification into major CAs has been clear-cut.

We do not have information regarding induced abortions of the naturally conceived children's mothers or of major

CAs among them. However, the rates of induced abortions due to CAs were so low that they are unlikely to bias the results.

According to previously published studies, twins have more CAs than singletons (19). That was also true among control children in our study but not among IVF children, which is in accordance with the results from a recent Danish study of IVF and ICSI twins, in which no differences in malformation rates between IVF/ICSI and naturally conceived twins were found (20). What, therefore, could explain this discrepancy between multiples and singletons? One explanation could be the fact that many singletons originated from multiple ET and from multiple pregnancy with a higher risk (during the study period 15% of ETs [IVF, ICSI, and FET] were single-embryo transfers, but 88% of live births were singleton births [21]). If two embryos succeed during implantation and develop in assisted reproduction, it can be assumed that conditions have to have been especially favorable.

Another possible explanation is zygosity: monozygotic twins have more malformations than dizygotic, and monozygosity is rarer among twins of assisted reproduction than among naturally conceived twins (1% vs. 30%) (22). Although IVF and other ART increase monozygotic twinning (6, 14), transfer of several embryos causes the majority of IVF twins to be dizygotic. The fact that the CA rate was not smaller among IVF twin boys could result from a higher risk of CA among IVF boys.

Most hormones in IVF treatment are used before pregnancy, and the half-life of most of these drugs is short. However, the duration of active drugs and metabolites in the body and their individual variations are not clear. Some drugs are also used as luteal-phase support during pregnancy. In addition to direct toxic effect, the drugs might have their effect through the mother's hormonal secretion balance. Although the dangers of hormones in early pregnancy have been discussed for decades (23, 24), this has not been the focus when the health effects of IVF have been discussed. We had information regarding fertility drugs (dosages and number of packages) bought, but the exact date and duration of their use was not known. Because the treated women received many and varied medicines during the last cycle, it was not possible to identify any specifically harmful drug.

Our study verified an earlier result of the overall risk for urogenital CAs (7), but ours was too small to study the risk of individual diagnoses, such as the hypospadias previously reported (6, 25). The use of P during IVF treatment has been offered as one explanation for the increased risk of one genital CA, hypospadias (25). Children exposed in utero to E and P or only P were found to have more male genital malformations than nonexposed children (23). However, in our study no difference in P use was found among boys with major genital anomalies and other IVF boys. Instead, E use was more frequent, but most boys with genital CAs were not exposed to it.

Another explanation for the higher rate of male genital anomalies might be the hereditary paternal subfertility associated with ICSI (5). Unfortunately, we could identify ICSI children only when the treatment was given in a private clinic. Because of the possible bias and the small number of children, we did not study specific CAs of ICSI children. Because the genital CAs were more severe among IVF than among control children, the risk for major urogenital CAs could be greater than our results show. The risk was also somewhat increased among other ART boys.

In another Finnish study, IVF children had more heart anomalies than control children (8). This was also true in our study, but the risk was not statistically significant. Rather, it was found among other ART children. This might relate to the use of clomiphene citrate (14). The increased risk for musculoskeletal CAs among IVF children is in accordance with a previous study from Australia (7).

Other than drugs, potential causes for congenital anomalies could include infertility itself, the advanced age of mothers, and factors related to the IVF procedure, such as the freezing and thawing of embryos. We did not have any information about the duration and causes of infertility and could not adjust data for them. The higher age of mothers did not explain the increased risk for major CAs.

In conclusion, our study verifies an increased risk for major CAs among IVF singleton boys and suggests that the risk after other ART is also slightly increased and not explained by those maternal characteristics available in the Finnish MBR. The actual risk is, however, quite small. Because our findings regarding different organ systems are based on small numbers of children, further studies are needed to explain them. It would be important to perform a large follow-up study of IVF and other ART births which includes information on the duration and causes of infertility, exact information regarding maternal drug exposure, and other maternal background characteristics. Meanwhile, the techniques used in IVF and other ART should be considered potentially teratogenic, thus requiring that information be given to the physicians and the public.

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# Clinical and molecular genetic features of Beckwith–Wiedemann syndrome associated with assisted reproductive technologies

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**BACKGROUND:** Beckwith–Wiedemann syndrome (BWS) is a model imprinting disorder resulting from mutations or epigenetic events affecting imprinted genes at 11p15.5. Most BWS cases are sporadic and result from imprinting errors (epimutations) involving either of the two 11p15.5 imprinting control regions (IC1 and IC2). Previously, we and other reported an association between sporadic BWS and assisted reproductive technologies (ARTs).

**METHODS:** In this study, we compared the clinical phenotype and molecular features of ART (IVF and ICSI) and non-ART children with sporadic BWS. A total of 25 patients with post-ART BWS were ascertained (12 after IVF and 13 after ICSI).

**RESULTS:** Molecular genetic analysis revealed an IC2 epimutations (KvDMR1 loss of methylation) in 24 of the 25 children tested. Comparison of clinical features of children with post-ART BWS to those with non-ART BWS and IC2 defects revealed a lower frequency of exomphalos (43 versus 69%,  $P = 0.029$ ) and a higher risk of neoplasia (two cases,  $P = 0.0014$ ). As loss of methylation at imprinting control regions other than 11p15.5 might modify the phenotype of BWS patients with IC2 epimutations, we investigated differentially methylated regions (DMRs) at 6q24, 7q32 and 15q13 in post-ART and non-ART BWS IC2 cases ( $n = 55$ ). Loss of maternal allele methylation at these DMRs occurred in 37.5% of ART and 6.4% of non-ART BWS IC2 defect cases. Thus, more generalized DMR hypomethylation is more frequent, but not exclusive to post-ART BWS.

**CONCLUSIONS:** These findings provide further evidence that ART may be associated with disturbed normal genomic imprinting in a subset of children.

**Key words:** Beckwith–Wiedemann syndrome / imprinting disorder / assisted reproductive technologies / epimutations / loss of methylation

## Introduction

Beckwith–Wiedemann syndrome (BWS) is a congenital overgrowth disorder resulting from altered expression or function of genes within the 11p15.5 imprinted gene cluster. In particular, reduced expression (or less frequently inactivation) of the maternally

expressed growth suppressor *CDKN1C* and/or increased expression of the paternally expressed growth promoter *IGF2* appear to have a major role in the pathogenesis of BWS. Multiple genetic and epigenetic mechanisms including paternal uniparental disomy of chromosome 11p15 may lead to alterations in *CDKN1C* and *IGF2* function resulting in BWS (see Cooper *et al.*, 2005 and references within).

However, the most common mechanism observed in up to 50% of patients is loss of maternal allele methylation (LOM) at a differentially methylated region (KvDMR1) between *CDKN1C* and *IGF2*. KvDMR1 marks an imprinting control centre [imprinting centre 2 (IC2)] and KvDMR1 LOM is associated with loss of maternal allele *CDKN1C* expression, biallelic expression of the untranslated RNA *KCNQ1OT* (usually only expressed from the paternal allele) and, in some cases, biallelic expression (loss of imprinting) of *IGF2* (Lee et al., 1999; Smilnich et al., 1999; Diaz-Meyer et al., 2003). Rarely KvDMR1 LOM may result from a germline maternal allele deletion, but in most KvDMR1 LOM results from an IC2 epimutation (Niemitz et al., 2004).

Although IC2 epimutations represent the most common cause of BWS, little information is available regarding the aetiology of IC2 epimutations. However, we and others have reported an association between BWS and assisted reproductive technologies (ARTs) such as IVF and ICSI (DeBaun et al., 2003; Gicquel et al., 2003; Maher et al., 2003; Halliday et al., 2004). To date, most post-ART BWS children have been found to have KvDMR1 LOM but detailed comparison of the clinical and molecular features of post-ART and non-ART BWS children with IC2 defects has not been undertaken.

## Materials and Methods

### Patients

#### ART group

Twenty-five BWS children born after IVF or ICSI were referred to the West Midlands Regional Genetics Service/University of Birmingham for molecular testing and/or research studies.

#### Non-ART group

Eighty-seven BWS children without a history of ART with KvDMR1 LOM tested at the same laboratory were identified.

The clinical and molecular features of the ART group were compared with the non-ART group. Clinical information was collected by a standard questionnaire, inspection of hospital notes or direct examination.

### Molecular analysis

DNA was extracted from peripheral blood lymphocytes by standard procedures. After the exclusion of paternal isodisomy of chromosome 11p15, KvDMR1 methylation status was performed as previously described with PCR amplification of bisulphite modified DNA and digestion with restriction enzyme *Bst*UI yielding different sized fragments which is separated using ABI377 or 3730 (Cooper et al., 2005). The methylation index is then calculated as the ratio of methylated to unmethylated DNA. In addition, a cohort of 55 BWS IC2 defect patients (including eight post-ART cases), in whom sufficient DNA was available, was analysed for methylation status at up to three additional DMRs at the Transient Neonatal Diabetes Mellitus (TNDM) locus at 6q24 (ZAC), 7q32 (*PEG1*) and the Angelman/Prader-Willi locus at 15q13 (*SNRPN*). The methylation status of the *DLK1*-IG DMR at 14q32 was analysed in patients found to have multi-DMR LOM. Methylation at these DMRs was assessed by methylation-specific PCR (ZAC and *DLK1*), direct sequencing of bisulphite modified DNA (*PEG1*) or pyrosequencing of bisulphite modified DNA (*SNRPN*). The methylation status of these four DMRs were also analysed in a group of 20 normal controls.

### Bisulphite modification

Genomic DNA (2 µg) derived from peripheral blood lymphocytes was bisulphite modified using the EZ DNA Methylation Gold kit (Zymo Research).

### Methylation analysis

#### 6q24 (ZAC) and 14q32 (DLK1) methylation-specific PCR

Methylation status of the TNDM CpG island at 6q24 (ZAC) and *DLK1* IG-DMR at 14q32 were analysed using methylation-specific PCR (MS-PCR) as previously described (Mackay et al., 2005; Temple et al., 2007). The methylated:unmethylated area ratio was calculated for 20 normal controls to establish a normal range to compare the test data. Hypomethylation is defined as a ratio of more than two standard deviations below the mean value.

#### 7q32 (PEG1) bisulphite sequencing

Primers were designed for the analysis of *PEG1* DMR at 7q32 using the Methyl Primer Express software by Applied Biosystems (sense primer 5'-AGTTGGGGTTGTTTTGG-3' and 3' anti-sense primer 5'-TACCAAAATCTAAAAATCCCAATT-3'). This amplified a 264 bp fragment which contains 15 CpGs. PCR was performed with Hot Star *Taq* and buffer (Qiagen) with final concentrations of 0.2 mM dNTP, 2 mM MgCl<sub>2</sub> and 0.2 µM primers with the following cycling conditions: 95° 15' → [95° 20''/56° 10''/72° 10'']<sub>35</sub> → 72° 5'.

#### 15q13 (SNRPN) pyrosequencing

The methylation status at the Prader-Willi/Angelman locus at 15q13 (*SNRPN*) was performed by pyrosequencing using the commercially available PyroMark kit by Biotage according to the manufacturer's protocol.

### Statistical analysis

Fisher exact testing, Wilcoxon–Rank sum, *t*-testing and Kaplan–Meier analysis were used as appropriate. Statistical significance was taken at the 5% level.

## Results

### Patient demographics

In the post-ART group, there were 25 affected children from 23 pregnancies with 10 twins (two affected twin pairs and six twins with no clinical evidence of BWS in their co-twins). In the non-ART group, there were 87 affected children including two twin and one triplet pregnancies (all co-twins and co-triplets were clinically unaffected). There were a total of 14 male and 11 female patients in the post-ART group ( $n = 25$ , mean age 3.4 years) and 42 males and 45 females in the non-ART group ( $n = 87$ , mean age 6 years). In the post-ART group, the mean maternal age was 36.7 years and the mean paternal age was 41.8 years. Except for one patient, all had a molecular genetic diagnosis of BWS and two or more clinical features. The one patient without a molecular abnormality in the post-ART group had features of macroglossia, macrosomia, umbilical hernia, earlobe creases and mild speech and language delay.

## Clinical features of post-ART and non-ART children with BWS<sup>ICD2</sup>

The 25 post-ART cases were conceived by IVF ( $n = 12$ ) or ICSI ( $n = 13$ ). Molecular genetic analysis revealed that 24 of the 25 post-ART children had LOM at KvDMR1 (no molecular cause was found in one post-ART child conceived by IVF).

In view of the known genotype–phenotype correlations of BWS (see Cooper *et al.*, 2005 and references within), we compared the phenotypes of the 24 post-ART BWS children with KvDMR1 LOM to those of the 87 non-ART BWS children with KvDMR1 LOM. The mean methylation index for KvDMR1 in the post-ART and non-ART group were 4.6% (range 0–18) and 7.6% (range 0–13), respectively, with no statistically significant difference ( $P = 0.6$ ). A methylation index of <20% is used as a cut-off point for diagnosis of KvDMR1 LOM. This value is the operational diagnostic threshold used in the diagnostic laboratory at the West Midlands Regional Genetics Service following robust validation comparing normal controls and known positive controls. The frequencies of neonatal hypoglycaemia (ART 44% versus non-ART 50%), macroglossia (90 and 87%), macrosomia (in singleton births) (70 and 79%), ear creases (56 and 65%) and hemihypertrophy (13 and 16%, respectively) were similar. However, facial naevus flammeus was more common in the ART patients (90 versus 46%,  $P = 0.0004$ ) and exomphalos was less common (43 versus 69%,  $P = 0.029$ ).

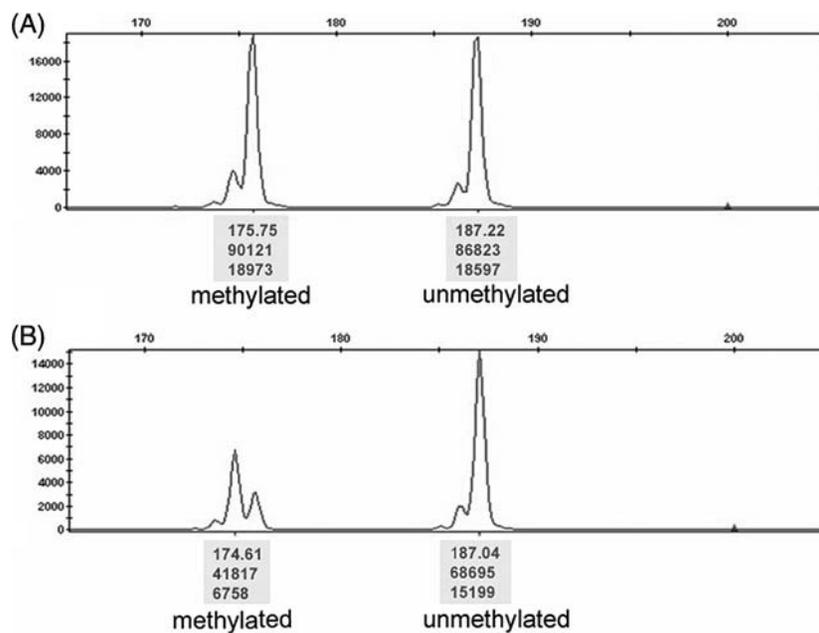
None of the non-ART BWS<sup>ICD2</sup> children (mean age 6.0 years) developed a neoplastic lesion, but two of the post-ART children (mean age 3.4 years) had developed an embryonal tumour. One post-IVF child developed a hepatoblastoma at 8 months and one

post-ICSI child a rhabdomyosarcoma at age 9 months. Kaplan–Meier analysis of tumour risk in the ART and non-ART demonstrated a significantly increased risk of tumours in ART cases (log rank  $\chi^2 = 10.18$ ,  $P = 0.0014$ ).

In the cohort of 13 twins/triplets, the overall incidence of exomphalos was 80% with 50% (five of 10) in the post-ART group and 100% (three of three) in the non-ART group but this did not reach statistical significance. Only one twin in the post-ART group developed a tumour (a rhabdomyosarcoma mentioned in the paragraph above). The co-twin was not clinically affected with BWS.

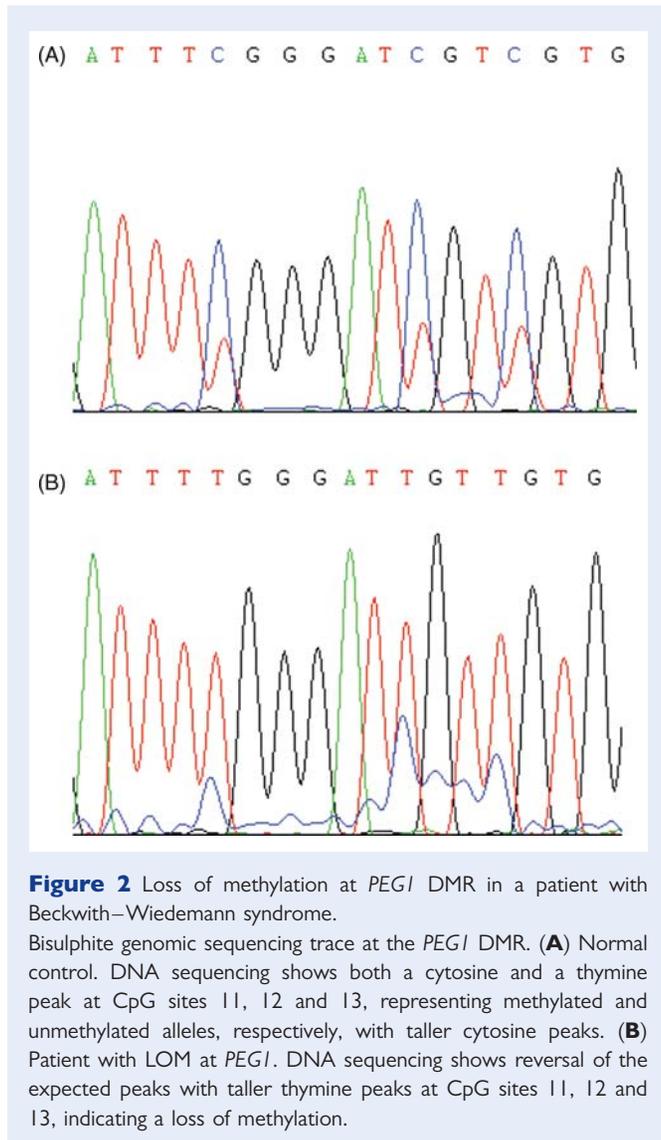
## Methylation profiling of BWS<sup>ICD2</sup> children at ZAC, PEG1, SNRPN and DLK1

Six cases tested had loss of methylation at ZAC (two of 55 tested), PEG1 (four of 55 tested) or SNRPN (one of 55 tested) DMRs (Figs 1–3). In the ART group, three children had additional LOM. One ICSI child demonstrated LOM at both PEG1 and SNRPN. Another ICSI child and one IVF child had single locus LOM at ZAC and PEG1, respectively. In the non-ART group, three children had single locus LOM with two children at PEG1 and one child at ZAC. In two of the three non-ART cases with LOM at other imprinted loci, there were no reports of fertility problems in the parents or the use of ovarian stimulation but we do not have information regarding the use of ovarian stimulation in the third couple apart from the child was conceived naturally. We went on to test the methylation status of the DLK1 IG-DMR in these six cases with hypomethylation and found normal methylation levels. No major phenotypic differences



**Figure 1** Loss of methylation at ZAC DMR in a patient with Beckwith–Wiedemann syndrome.

Electropherogram of amplification products of MS-PCR. The X-axis represents the calculated product size (in bp and also represented as the top number in the box). The Y-axis represents the peak height (bottom number in the box). The methylated to unmethylated ratio was calculated as the area under the curve (middle number in the box) of methylated versus unmethylated amplified products. (A) Normal Control (ratio 1.04), (B) patient with LOM at ZAC (ratio 0.61).



**Figure 2** Loss of methylation at *PEG1* DMR in a patient with Beckwith–Wiedemann syndrome. Bisulphite genomic sequencing trace at the *PEG1* DMR. **(A)** Normal control. DNA sequencing shows both a cytosine and a thymine peak at CpG sites 11, 12 and 13, representing methylated and unmethylated alleles, respectively, with taller cytosine peaks. **(B)** Patient with LOM at *PEG1*. DNA sequencing shows reversal of the expected peaks with taller thymine peaks at CpG sites 11, 12 and 13, indicating a loss of methylation.

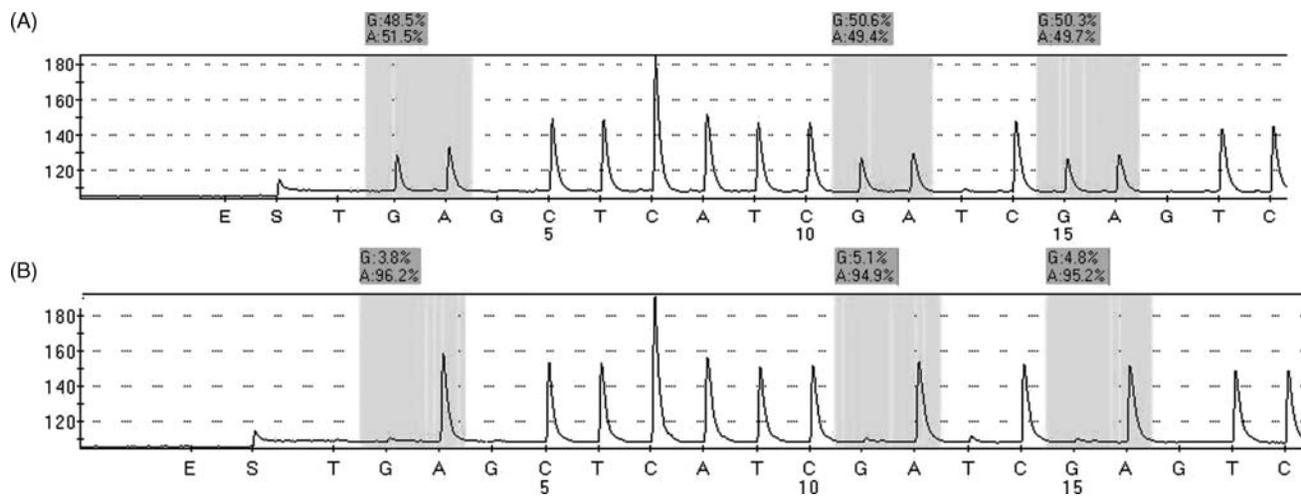
were apparent between children with LOM at additional loci and those without this epigenotype (Table I). DNA from the two children with tumours was not available for analysis. The frequency of additional LOM was significantly higher in the post-ART group than in the non-ART BWS<sup>ICD2</sup> patients (37.5 versus 6.4%,  $P = 0.034$ ). As expected, methylation analysis of the group of normal controls showed no methylation abnormalities at the four loci analysed.

## Discussion

Previously, we reported that six of the 149 UK children with BWS had a history of ART (4% of BWS cases versus 1.2% of the general population,  $P = 0.009$ ) (Maher et al., 2003). Similar findings were also reported from USA and France (DeBaun et al., 2003; Gicquel et al., 2003). A case–control study in an Australian population estimated the risk of BWS after ART is approximately nine times greater than that for natural conceptions (Halliday et al., 2004). In addition, two studies suggested a link between Angelman syndrome and ICSI (Cox et al., 2002; Orstavik et al., 2003). We identified KvDMR1

LOM in 24 of the 25 post-ART BWS patients. Previously, KvDMR1 LOM was described in 11 of the 12 post-ART BWS described by DeBaun et al. (2003) and Gicquel et al. (2003). Thus, a much higher than expected proportion of post-ART BWS patients have KvDMR1 LOM (in unselected up to 50% would be expected) (Cooper et al., 2005). This observation provides further evidence of a causal link between ART and IC2 epimutations. Nevertheless, the precise cause of this association is unclear. Both IVF and ICSI are often undertaken for unexplained infertility and require ovarian stimulation, oocyte collection and *in vitro* culture before the embryos are implanted in the womb. Although ICSI also requires an additional step (direct injection of sperm into the ovum), both IVF and ICSI appear to be associated with an increased relative risk (although the absolute risk is small) of imprinting disorders. Animal studies suggest that *in vitro* embryo culture may be associated with epigenetic alterations and, in particular, the large offspring syndrome in sheep and cattle undergoing ART has phenotypic similarities to BWS and, in some cases, is associated with loss of maternal allele methylation at an *IGF2R* DMR (Reik et al., 1993; Dean et al., 1998; Khosla et al., 2001; Young et al., 2001). However, it has also been suggested that infertility and ovarian stimulation may predispose to epigenetic errors (Ludwig et al., 2005).

Previously, we and others have reported significant differences between Wilms tumour risk and the frequency of exomphalos in children with different molecular subtypes of BWS (Lam et al., 1999; Engel et al., 2000; Bliiek et al., 2001; Gaston et al., 2001; Weksberg et al., 2001; DeBaun et al., 2002; Cooper et al., 2005; Sparago et al., 2007). Thus, Wilms tumour has not been reported in those with IC2 defects or *CDKN1C* mutations but UPD and IC1 defects are associated with a significant risk of Wilms tumour. In contrast, exomphalos is rare in BWS patients with UPD or IC1 defects but is common in those with IC2 defects and *CDKN1C* mutations. Previously, Chang et al. (2005) reported no phenotypic differences between post-ART and naturally conceived BWS patients. However, as almost all post-ART BWS children have IC2 defects, we compared these children to non-ART BWS cases with IC2 defects (and not an unselected group of non-ART BWS). We found that post-ART cases had a significantly lower risk of exomphalos and a higher risk of non-Wilms tumour neoplasia. The increased risk of neoplasia, although statistically significant, is based on only two cases and must be considered a preliminary finding. Hence we hope that this finding will prompt other groups to examine their data to better define the relationship between ART and multilocus hypomethylation in BWS children with non-Wilms tumour neoplasia. We note that a childhood tumour was present in two of the 19 post-ART BWS children reported by Chang et al. (2005). Two of the 11 children with loss of methylation at multiple loci reported by Rossignol et al. (2006) had developed a tumour (a rhabdomyosarcoma and a hepatoblastoma) but neither of these was conceived by ART. The reasons for the phenotypic differences between post-ART and non-ART BWS IC2 defects cases are uncertain. Although it might be suggested that less severe KvDMR1 hypomethylation in post-ART cases might lead to a milder phenotype with a lower incidence of exomphalos, comparison of blood KvDMR1 methylation indices in the two groups did not show any significant difference. However, methylation patterns may differ in different tissues. Nevertheless, such an explanation would not seem to account for the apparent higher risk of neoplasia in post-ART cases. Recently, Rossignol et al. (2006) reported that



**Figure 3** Loss of methylation at *SNRPN* DMR in a patient with Beckwith–Wiedemann syndrome. Reverse strand Pyrosequencing trace. Percentage of methylated cytosines is represented as the percentage of guanine (G) and the percentages of unmethylated cytosines which normally will be represented by thymine is represented by alanine (A) on the reverse strand. (A) Normal control, (B) patient with LOM at *SNRPN*.

**Table 1** Clinical and molecular characteristics of Imprinting Centre 2 defect Beckwith–Wiedemann syndrome patients with additional loss of methylation at other imprinted loci

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Sex	M	F	M	F	M	M
ART	IVF	ICSI	ICSI	No	No	No
Pregnancy	Singleton	Singleton	Twin	Singleton	Singleton	Singleton
Macrosomia	No	NR	No	No	Yes	Yes
Exomphalos	Yes	No	No	Yes	Yes	No
Umbilical Hernia	No	Yes	Yes	No	No	Yes
Macroglossia	No	Yes	Yes	Yes	Yes	Yes
Hemihypertrophy	Yes	No	No	No	No	Yes
Embryonal Tumour	No	No	No	No	No	No
Ear creases	Yes	No	Yes	NR	Yes	Yes
Neonatal Hypoglycaemia	No	Yes	Yes	Yes	Yes	Yes
Facial Naevus Flammeus	Yes	Yes	Yes	No	Yes	Yes
6q24 (ZAC) methylation	Normal	Normal	LOM	Normal	Normal	LOM
MI $N = (0.65 - 1.78)$	1.25	1.23	0.55	1.22	0.93	0.61
7q32 (PEG1) methylation	LOM	LOM	Normal	LOM	LOM	Normal
15q13 (SNRPN) methylation	Normal	LOM	Normal	Normal	Normal	Normal
MI $N = (0.55 - 1.1)$	0.84	0.05	0.98	0.82	0.77	0.89
14q32 (DLK1) methylation	Normal	Normal	Normal	Normal	Normal	Normal
MI $N = (0.5 - 1.4)$	0.91	0.82	0.83	0.85	0.76	0.88
11p15.5 KvDMR1 methylation	LOM	LOM	LOM	LOM	LOM	LOM
MI	0.04	0.0	0.0	0.12	0.0	0.02

M, male; F, female; IVF, *in vitro* fertilization; ICSI, intra-cytoplasmic sperm injection; LOM, loss of methylation, MI, methylation index, NR, not recorded, N, normal range.

BWS children with IC2 defects might also display loss of methylation at other non-11p15.5 imprinting region DMRs. We found significantly higher frequencies of loss of methylation at DMRs unlinked to 11p15.5 in ART cases than in non-ART cases. This contrasts with

the results of Rossignol *et al.* (2006) who found similar rates in both groups, but could be consistent with the hypothesis that differences in phenotype between ART and non-ART IC2 defect BWS patients might be caused by epigenetic differences at non-11p15.5 loci.

Analysis of more extensive cohorts of patients at a larger number of DMRs should provide further information on the relative frequency of hypomethylation at different loci in ART and non-ART BWS patients. Due to the limited amount of DNA available, we only tested the methylation status at the *DLK1*-IG DMR in the six patients with additional loci hypomethylation who are more likely to have a methylation abnormality. In addition, a previous study looking at the methylation status in this paternally methylated DMR in TNDM cases did not find any methylation abnormality at this locus (Mackay et al., 2006).

We did not detect any marked differences between the phenotype of non-ART patients with and without additional DMR hypomethylation (LOM+ cases) but the numbers were small. Clearly, it will be interesting to compare the phenotype of BWS LOM+ children to other groups including the subgroup of patients with TNDM who display maternal allele hypomethylation at multiple loci and who are reported to have some phenotypic differences (including a higher birth weight) from TNDM patients without additional DMR hypomethylation (Mackay et al., 2006). Recently, mutations in the *ZFP57* gene were identified in some TNDM patients with hypomethylation at multiple imprinted loci (Mackay et al., 2008). Finally, our finding that multiple DMR hypomethylation is more frequent in ART cases raises the possibility that some cases of developmental defects or abnormal growth in ART children might be caused by variable combinations of epigenetic alterations at imprinted DMRs.

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## Author's roles

E.R.M., D.L., S.C.B.—Study design.

D.L., E.R.M.—Writing of first draft of manuscript.

D.L., E.R.M.—Data interpretation.

D.L., L.T., F.M.—Molecular genetic analysis.

D.L., G.A.K., S.C.B., E.B., A.F., W.L., C.O., T.C., L.A.B., E.R.M.—

Patient recruitment, clinical information and sample collection.

All authors—Critical appraisal and correction of draft manuscript.

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# Congenital Anomalies in Infants Conceived by Assisted Reproductive Techniques

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## Abstract

**Background:** Many studies show that congenital defects in infants conceived by assisted reproductive techniques (ART) are more than infants of normal conception (NC). The aim of this study is to determine the frequency of congenital anomalies in ART infants from Royan Institute and to compare congenital anomalies between two ART techniques.

**Methods:** In a cross-sectional descriptive study, 400 ART infants from Royan Institute who resided in Tehran were selected by non-random, consecutive sampling. Infants were examined twice (until 9 months of age) by a pediatrician.

Infants' congenital anomalies were described by each body system or organ and type of ART. Data were analyzed by SPSS version 16 and Fisher's exact test.

**Results:** The frequency of different organ involvement in the two examinations were: 40 (10%) skin, 25 (6.2%) urogenital system, 21 (5.2%) gastrointestinal tract, 13 (3.2%) visual, and 8 (2%) cardiovascular system. Major congenital defects in infants conceived by *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were hypospadias, inguinal hernia, patent ductus arteriosus plus ventricular septal defect (PDA + VSD), developmental dysplasia of the hip, lacrimal duct stenosis during the first year of life, hydronephrosis and urinary reflux over grade III, undescending testis, ureteropelvic junction stenosis, and torticoli.

**Conclusions:** Two-thirds of ART infants had no defects. A total of 7% of IVF and ICSI infants had one of the major abovementioned congenital anomalies. This rate was higher than NC infants (2%–3%). There was no difference between the ICSI and IVF group.

**Keywords:** Assisted reproductive techniques, congenital anomalies, infants, intracytoplasmic sperm injection (ICSI), *in vitro* fertilization (IVF)

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## Introduction

Many studies have been performed concerning infants conceived by assisted reproductive techniques (ART). In the general population, 3% of surviving neonates have one major congenital anomaly at birth. Some anomalies are detected during childhood or adolescence. The major cause of congenital anomalies are genetic factors which cause 50% of spontaneous abortions during the first trimester of pregnancy and 5% of neonatal deaths.<sup>1</sup> Some factors probably increase the incidence of congenital anomalies in ART infants. For example, natural selection which occurs in normal conception (NC) pregnancies does not occur in ART fertilization. Changes of hormonal status in the lab during the process of mitosis or myosis may cause chromosomal aneuploidy. Chemical compounds may also induce point mutations during artificial conception.

Although in European countries congenital anomalies of ART infants are recorded and followed, they could not be compared with the incidence of anomaly in the normal population. The reason for higher incidence of congenital anomalies in ART infants

is due to the careful, continuous examination of these infants in comparison with normal infants. Some anomalies such as small umbilical hernias and pigmented skin spots or ear tags, which may not be reported in normal infants, are reported in ART infants.<sup>2</sup>

Many centers that follow up ART infants report a higher incidence of hypospadias and undescended testes in these infants.<sup>3</sup> One of the major biases is careful sonography of these infants during pregnancy. In 1990, one center in the United States reported a high incidence of periventricular cysts in the brain, hydronephrosis and unilateral agenesis of the kidneys in these infants, which could not be detected by physical examination.<sup>4</sup>

In many countries only birth time anomalies are recorded, however many anomalies such as pyloric stenosis appear later.<sup>2</sup>

Another reason for increased reporting of more anomalies in ART infants is the precise reporting of these anomalies in the aborted ART fetus or neonate in comparison to the normal population. We could not compare ART infants with normally conceived infants.<sup>5</sup>

The only comparable group with ART infants are those parents became fertile by other techniques, such as ovulation induction. However there are few studies about these infants.

The other confounding factors are the higher age of these couples. In older mothers (27–28 years old) who request ART,<sup>6</sup> the probability of abortion<sup>7,8</sup> or aneuploidy increases<sup>9,10</sup> and Mendelian mutation incidences increase in ART infants of older fathers.<sup>10–12</sup>

Many studies have reported more congenital anomalies in the ART methods, particularly *in vitro* fertilization (IVF) infants, in comparison to natural conception (NC) infants, which is related

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**Table 1.** Prevalence of organ and system anomalies in ART infants.

ART method	IVF		ICSI		ART	
	Number	Percentage	Number	Percentage	Number	Percentage
Normal	52	67.5	215	66.5	267	66.7
Skin	6	7.8	34	10.5	40	10
Urogenital	6	7.8	19	5.9	25	6.2
Gastrointestinal	3	3.9	18	5.5	21	5.2
Visual	1	1.3	12	3.7	13	3.25
Cardiovascular	3	2.6	5	1.5	8	2
Limbs-bones	2	2.6	6	1.8	8	2
Endocrine	3	3.9	3	0.9	6	1.5
Otolaryngeal	—	—	5	1.5	5	1.2
Blood and lymphatic	1	1.3	3	0.9	4	1
Nervous	—	—	3	0.9	3	0.75
Abnormal (total)	25	32.5	108	33.4	133	33.3
Total	77	19.2	323	80.8	400	100

**Table 2.** Prevalence of major anomalies in ART infants.

ART method	IVF		ICSI		ART	
	Number	Percentage	Number	Percentage	Number	Percentage
Inguinal hernia	1	1.3	4	1.2	5	1.2
Undescended testis	0	0	2	0.6	2	0.5
UPJ stenosis	0	0	1	0.3	1	0.2
Hypospadias	1	1.3	4	1.2	5	1.2
Hydronephrosis and reflux	2	2.6	1	0.3	3	0.7
Severe PDA + VSD	2	2.6	1	0.3	3	0.7
Lacrimal duct stenosis	1	1.3	3	0.9	4	1
DDH	2	2.6	2	0.6	4	1
Torticoli	0	0	1	0.3	1	0.2
Total anomalies	9	11.7	19	5.9	28	7
Total	77	19.2	323	80.8	400	100

**Table 3.** Prevalence of congenital anomalies in IVF and ICSI infants in different countries.

Country	Congenital anomaly in IVF infants (%)	Congenital anomaly in ICSI infants (%)
Iran	5.9	11.7
Belgium <sup>50</sup>	4.2	4.5
Australia <sup>48</sup>	8.6	9
Sweden <sup>46</sup>	8.6	8.1
Norway <sup>51</sup>	3.1	3

to multi-fetal pregnancy and prematurity.<sup>13-18</sup>

In more invasive methods, particularly intracytoplasmic sperm injection (ICSI) in which sperm does not pass its natural way and natural selection deletion does not occur (in an oligospermic man), more chromosomal anomalies have been seen. Other interventions also used in IVF are the gonadotropin stimulator, oocyte aspiration, and culture media, which probably increases the incidence of congenital anomalies.<sup>19,20</sup>

In Iran many infants are conceived by ART, however there are no Iranian studies about congenital anomalies. The aim of this study is the determination of congenital anomalies and distribution rate of organ and system defects of these anomalies in a comparison of infants conceived by different ART methods.

## Materials and Methods

In a cross-sectional descriptive study, 400 ART infants from Royan Institute who were residents of Tehran were examined during 22 month in the Child Health and Development Research Center. This study was approved by the Ethics Committee of the Academic Center for Education, Culture and Research (ACECR) and Royan Institute.

This was a non-random, consecutive sampling due to the limited number of available infants which did not allow us to sample randomly. Infants were examined twice, at birth to 6 months and from 6 to 9 months by a pediatrician. Congenital anomalies of

infants were classified by body organs or limb involvement and divided into 10 groups by ICD-10 (International Classification of Disease) classification. If the anomaly was not exactly diagnosed by physical exam, sonography, radiography or echocardiography were used.

Data were analyzed by SPSS version 16 and Fisher's exact test.

## Results

A total of 208 (52%) boys and 192 (48%) girls were examined. The prevalence of congenital anomalies according to organ or system involvement and ART method are shown in Table 1. Skin anomalies were: 3 (0.75%) hemangioma, 19 (4.7%) umbilical hernia, 7 (1.7%) eczema, 4 (1%) skin hyperpigmentation, 2 (0.5%) semian line, and 5 (1.2%) inguinal hernia. Anomalies seen in the urogenital system were: 1 (0.2%) microlitiasis, 7 (1.7%) hydrocele of the testis, 5 (1.2%) labia adhesion, 1 (0.2%) micro-penis, 2 (0.5%) undescended testes, 1 (0.2%) ureteropelvic junction (UPJ) stenosis, 5 (1.2%) hypospadias, 3 (0.7%) renal reflux and hydronephrosis. Gastrointestinal anomalies were: 1 (0.2%) constipation, 11 (2.75%) gastro-esophageal reflux, 5 (1.2%) prolonged icter, and 4 (1%) thrush.

In the cardiovascular system there were 8 (2%) patent ductus arteriosus ± ventricular septal defect (PDA + VSD); visual anomalies were: 4 (1%) stenosis of the lacrimal duct, 3 (0.75%) conjunctivitis, and 6 (1.5%) strabismus. Orthopedics were: 4 (1%)

developmental dysplasia of the hip (DDH), 1 (0.2%) torticoli, 1 (0.2%) spina bifida, and 2 (0.5%) pes varus.

The otolaryngeal system anomalies were 3 (0.7%) lingual frenulum, 1 (0.2%) hearing loss, and 1 (0.2%) ear deformity. In the blood and lymphatic system there were 1 (0.2%) anemia, 1 (0.2%) lymphadenitis, and 2 (0.5%) G6 PD. Nervous system anomalies included 3 (0.7%) infants with cerebral palsy (CP), and finally the endocrine system anomalies were: 3 (0.7%) hypothyroidism and 3 (0.7%) rickets.

According to ICD-10, hypospadias, inguinal hernia, severe PDA + VSD, stenosis of the lacrimal duct until age one year, urethral reflux more than grade III and hydronephrosis, undescended testis (until one year of age), UPJ stenosis, torticoli, and DDH, which requires surgery are all considered major anomalies. Anomalies in Table 2 are classified according to major anomalies and ART technique.

## Discussion

The major problem in classification of congenital anomalies is the definition of a major anomaly. In this study all anomalies that required surgery, until one year of age or a disturbed function of the organs have been considered major congenital anomalies.

Of ART infants who were examined twice, one-third had congenital anomalies. IVF infants had higher numbers of congenital heart diseases, DDH and hydronephrosis with renal reflux (Table 2).

A higher prevalence of congenital anomalies were seen in the skin (10%), urogenital (6.2%), gastrointestinal (5.2%), visual (3.2%), and cardiovascular systems (2%).

However, the prevalence of inguinal hernia was 1.2%, which was lower than term (3%–5%) or preterm (9%–11%) NC infants.<sup>21</sup> There were no significant differences in the prevalence of inguinal hernias in both groups ( $P = 1$ ).

The prevalence of major anomalies in the urogenital system in IVF infants was 7.8%, whereas for ICSI infants it was 5.9%. In the another study the prevalence of major anomaly of urogenital system were 3.9% and 2.5% (in IVF and ICSI infants, respectively) which was not significant ( $P = 0.45$ ) with each other and other studies (3% in IVF and 5% in ICSI).<sup>22</sup>

Gastrointestinal anomalies were seen in 5.2% of infants; all of which were classified as minor anomalies, the higher rate seen in ICSI infants compared to the IVF group.

Major anomalies of the cardiovascular system were seen in 0.7% of infants, which was similar to the general population (0.8%).<sup>23</sup> However, it was not significantly different between the two groups ( $P = 0.09$ ).

A total of 3.2% of infants had visual anomalies, which was higher in the ICSI group ( $P = 0.57$ ). In one study, this was reported as 0.9%–4.2% in IVF infants<sup>24</sup>; other studies reported no differences between ART infants and the control group.<sup>22,25–28</sup> In the general population, 1.6% of infants needed lacrimal duct stenosis surgery,<sup>29</sup> which was 1% in our study.

There were 2% of infants who had either limb or bone anomalies, which was not different between the ICSI and IVF infants ( $P = 0.17$ ). The prevalence of DDH in our study was 1%, the same as seen in the general population (0.8%–1%).<sup>30</sup>

Congenital anomalies of the otolaryngeal system were seen in 1.2% of infants; sensory hearing loss was 0.2%, which was reported only in ICSI infants and did not differ with respect to

the prevalence of sensory hearing loss in NC (0.5–1 in 1000 infants).

Regarding nervous system defects, there were 3 (0.7%) CP infants conceived by ICSI which was higher than the prevalence in the general population (0.2%).<sup>31</sup> In other studies, the prevalence of CP in ART infants was 4 times,<sup>24</sup> 1.6 times,<sup>32</sup> and 1.8 times<sup>33</sup> more than NC infants. In 5 studies, no significant differences were noted between ART and NC infants in nervous system anomalies.<sup>25,26,34–36</sup> In all studies, the most common reason for CP in these infants was prematurity and low birth weight.<sup>36,37</sup> There was no significant difference in the prevalence of CP in ICSI and IVF infants in our study ( $P = 1$ ).

Congenital hypothyroidism was (0.7%) in our study and 0.1% in Tehran's neonate population.<sup>38</sup> There were no differences between the two groups of ART infants ( $P = 1$ ).

In general, one-third of ART infants had either one minor or major anomaly and 7% had one of the major congenital anomalies, which was higher when compared with the general population (2%–3%).<sup>1,39</sup> This prevalence was similar to a study in Finland (5.5%–6.6%)<sup>40,41</sup> and higher than the prevalence of major congenital anomalies in the Netherlands (2.3%, 3.7%),<sup>42,43</sup> England (4.8%),<sup>44</sup> Australia (4.3%),<sup>45</sup> and Sweden (5%).<sup>46</sup>

In comparison with other studies, Germany (8.6%)<sup>47</sup> and Australia (8.9%),<sup>48,49</sup> had lower prevalences.

In our study the percent of major congenital anomalies among ICSI (5.9%) and IVF (11.7%) infants was not significantly different between the two groups ( $P = 0.08$ ).

In 4 studies on IVF and ICSI infants in other countries, there were no reported significant differences between the two groups regarding major congenital anomalies (Table 3).

In our country, after more than one decade of infants born via ART, there were no studies on congenital anomalies and the comparison to NC infants. The results of two examinations of ART infants until 9 months of age showed that two-thirds were normal, 7% had one major anomaly, which was 3 times more than the general population and there was no significant difference between ICSI and IVF infants.

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## Depressive symptoms during late pregnancy and early parenthood following assisted reproductive technology

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**Objective:** To evaluate the relationship between assisted reproduction technology (ART) and depressive symptoms during late pregnancy and early parenthood.

**Design:** Case-control longitudinal study.

**Setting:** The Center of Reproductive Medicine, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.

**Patient(s):** Women who conceived by ART compared with men and compared with women following spontaneous conceptions.

**Intervention(s):** The sample of 87 subjects, 48 ART (25 mothers, 23 fathers; response rate of 30%) and 39 non-ART mothers were evaluated by the Edinburgh Postnatal Depression Scale (EPDS) at 30–32 weeks of gestation, and at 1 week and 3 months after delivery.

**Main Outcome Measure(s):** Mean scores and prevalence of low scores.

**Result(s):** The main sociodemographic and obstetric characteristics were similar between groups. Edinburgh Postnatal Depression Scale scores were higher in ART women compared with non-ART women during all assessments and higher during the third trimester of pregnancy and at 1 week postpartum compared with ART men. The prevalence of depressed subjects was significantly higher in ART women compared with non-ART women during the antenatal assessment.

**Conclusion(s):** Assisted reproductive technology pregnancies are more frequently associated with depressive symptoms that may persist after delivery, suggesting a greater emotional vulnerability of these women. The risk of depression during and following ART pregnancies needs monitoring to avoid adverse effects of postpartum depression on the mother-infant relationship and infant's psychologic development. (*Fertil Steril*® 2009;91:851–7. ©2009 by American Society for Reproductive Medicine.)

**Key Words:** In vitro fertilization, assisted reproductive techniques, depression, psychology

The World Health Organization estimates that the diagnosis of infertility is constantly increasing, and is found in as many as 20% of couples in the developed Western countries (1). The increasing availability of assisted reproductive techniques (ART) helps in reducing the burden of infertility worldwide. However, at the same time that ART may fulfill the wish of infertile couples via an entirely somatic answer to a subjective problem, it creates risks for a divide between the physiologic and psychologic aspects of procreation. Simply put, ART may potentially cause psychologic problems that are seldom or never encountered during spontaneous conceptions. Moreover, ART may suppress the symptoms of infertility but it does not eliminate them in the sense that it provides a solution to infertility but does not remove the

stigmatization of being infertile. Hart (2) vividly described this situation: “Infertility touches all aspects of a person’s life. It affects how individuals feel about themselves, their relationships, and their life perspective. Stress is only one of a myriad of emotional realities that couples facing infertility deal with, often for extended periods of time.”

Students of this complex issue found it necessary to consider the phenomenon of infertility in terms of a psychophysical interaction, in which neither the psychologic nor the physical events have precedence, but are mutually important and reciprocally influenced by each other. This approach was epitomized by Nunziante Cesàro et al. (3): “the body speaks by means of symptoms ... the body is the scenario that the mind chooses for its representation,” and the biologic processes “are mirrored by those activities which are subconscious fantasies in the mind” (1).

The literature suggests that women who conceived after ART are more emotionally vulnerable and show higher levels of distress compared with their partners as well as compared

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with women who had a spontaneous conceptions (2, 4). The ART women experience more emotional disturbance and manifest depressive and anxiety disorders (5, 6). These disorders may negatively affect early mother–infant interactions and increase the risk of early parenting difficulties as well as problems experienced by the couples (7). Surprisingly, little is known about the course of depressive symptomatology throughout pregnancy and the perinatal period among women and men.

The purpose of this study was to assess depressive symptoms in ART patients, from pregnancy until the 3 months postpartum.

## PATIENTS AND METHODS

### Sample

This present study is part of a large project assessing depression, anxiety, and parental representations during pregnancy following ART, and to assess the psychologic aspects characterizing the transition to parenthood in comparison between ART and spontaneous conceptions. The study has been approved by the respective institutional review boards of the University of Bologna and the Santa Maria Nuova Hospital at Reggio Emilia, Italy. The entire sample comprised 87 subjects: 48 ART patients (25 mothers, 23 fathers) and 39 non-ART mothers. The selection of the ART and non-ART subjects was independently performed. The ART patients were recruited between April 2005 until December 2006 at the Center of Sterility and Fertility, Santa Maria Nuova Hospital, Reggio Emilia, Italy. The inclusion criterion for ART was Italian nationality. We contacted a total of 122 eligible ART couples (61 women and their partners) attending our Center; but only 35 couples and one woman agreed to participate in the study (response rate of 30%). As the research plan included three home visits conducted by the psychologists of the Center, only couples residing in regions near to the hospital (i.e., Lombardy, Veneto, and Marche) were included, for a total of 57 subjects. Dropout rate at each follow-up visit was as follows: 2.8% (men and women) at the first assessment because of preterm birth; 2.8% were lost for follow-up by the second assessment, and 1.4% women and 5.6% men were not reachable for the last follow-up.

For the non-ART sample, we recruited 224 eligible women at our obstetric ward who met the same inclusion criteria as for the ART sample. After matching for plurality, parity gestational age, and mode of delivery, 39 women completed the three assessments. Recruited men were not included in this study because only a few could or wished to participate in the longitudinal study. Controls were not controlled for birth complication or health status of the baby because they were recruited before birth.

### Procedure

Psychologists involved in this project contacted the subjects between 20 and 24 weeks' gestation, explaining the purpose of the longitudinal project and receiving an informed consent

if the subjects agreed to participate in the study. Between 30 and 32 weeks' gestation, each psychologist met the couples at their home, using two self-administered questionnaires: on anxiety (ASQ-IPAT Anxiety Scale) (8) and on depression symptomatology (Edinburgh Postnatal Depression Scale; EPDS) (9), and conducted an interview on parental representations during pregnancy, one on maternal representations (*Intervista per le rappresentazioni materne in gravidanza*, IRMAG) (10) and the other on paternal representations (*Intervista per le rappresentazioni paterne in gravidanza*, Ra.Pa.G) (11). On this occasion, copies of the EPDS and the ASQ-IPAT were given to every parent to be completed during the second assessment.

Within 1 week after birth, the psychologist contacted the subjects by telephone, reminding them to complete the questionnaires and to send them back to the hospital. At 3 months after birth, a final visit was made to the subject's home and the same questionnaires were completed and an interview on maternal and paternal representations after birth was conducted (*Intervista sulle rappresentazioni materne e paterne dopo la nascita*, respectively, the Rap.Ma.N. and Rap.Pa.N) (11). In this study, we report the assessment of depressive symptomatology.

### Instrument

The EPDS is a self-administered 10-item questionnaire, specifically designed for screening of postnatal depressive symptoms in community samples (9). The items investigate the presence and intensity of depressive symptoms during the previous 7 days. Specifically, anhedonia, self-blame, anxiety, fear or panic, inability to cope, difficulty in sleeping, sadness, tearfulness, and thoughts of self-harm were assessed. The items are scored from 0 to 3, producing a total score of 0 to 30. The higher the score, the lower the maternal mood. A cutoff value of 12/13 was established by the original investigators as the most accurate to detect major depressive symptoms, and a cutoff of 9/10 was suggested as more suitable for community screenings and for routine use by primary care workers (9).

This tool is one of the most widely used questionnaires in different countries, although different cutoff scores were chosen according to study design and culture (12). The cutoff value of 8/9 was established in the Italian version by Benvenuti et al. (13) as the most accurate in terms of high sensitivity and adequate positive predictive values for detecting postnatal depressive symptoms in community screenings. However, the sample used in the Italian validation study was of limited size and was not confirmed. Thus, we used the higher cutoff of 12/13 suggested by Cox et al. (9) to obtain more accurate values of sensitivity and specificity.

### Data Analysis

In this study, we used a comparison between ART and non-ART women and between ART women and ART men. We used the chi-square test and Fisher's exact test to compare the frequency of subjects for the levels of sociodemographic

variables and the frequency of depressed subjects at each follow-up assessment. The EPDS scores were compared using the non-parametric Mann-Whitney *U* test.

## RESULTS

### Sample Characteristics

The sociodemographic and obstetric characteristics are shown in Table 1. The ART and non-ART sample were similar for the main variables, and most of the subjects were born in Northern Italy, had a higher level of education, were married, employed, belonged to middle social class, and were Catholic. In addition, ART and non-ART women had similar parity, mode of delivery, and frequency of attendance in antenatal classes (Table 1). ART women were older than non-ART women (35.9 vs. 32.1 years,  $P < .0005$ ) and more frequently lived together with their partner for  $>8$  years (48% vs. 10.3%,  $P = .001$ ). ART women were younger compared with ART men (35.9 vs. 39.5 years,  $P = .004$ ), but otherwise were of similar sociodemographic characteristics (Table 2).

### Depression

ART women exhibited a significantly higher mean EPDS score than non-ART women at the third trimester of pregnancy (7.88 vs. 4.32,  $P < .0005$ ), at 1 week (8.0 vs. 4.89,  $P < .005$ ) and 3 months after birth (5.76 vs. 3.87,  $P < .05$ ; Table 3). Compared with ART men, ART women showed a higher mean EPDS scores both at the third trimester of pregnancy (7.88 vs. 3.86,  $P < .0005$ ) and at 1 week after birth (8.0 vs. 4.5,  $P < .005$ ), but not at 3 months after birth (5.76 vs. 4.32,  $P = .06$ ; Table 3). When the frequency of depressed subjects was assessed, ART women showed significantly higher figures than non-ART women only at the first assessment ( $P = .02$ , Table 3). Compared with ART men, the frequency of depression in ART women was not significantly different (Table 3).

## DISCUSSION

Depressive symptoms are among the most common expressions of psychologic distress during and following ART pregnancies, and our study confirms this prevalence. Generally, we found that maternal mood during pregnancy and at 1 week and 3 months after birth was lower in ART women than in the non-ART group. These results are in conflict with Finish data (14) but confirm previous studies (2, 4), and lead to the hypothesis that the transition to parenthood, intended as the delicate process concerning both the biologic and psychologic “ability to be a parent,” can be affected by the biologic inability to procreate spontaneously. For this reason, the exacerbation of depressive feelings would tend to persist after childbirth. ART women, during pregnancy, can experience more concerns, anxieties, and fears compared with non-ART pregnancies, and the evidence of a healthy baby may only partly reassure them, and hence, they continue to experience depressive symptoms.

When women considered depressed were evaluated, we found a significant difference between ART and non-ART

women only at the third trimester of pregnancy and not postpartum. Specifically, no depressed woman was found in the non-ART group before delivery and 3 months after, whereas the ART group included depressed subjects at every assessment, with the highest rate at the first.

These results have important clinical implications, because depression in pregnancy has often been recognized as one of the most reliable signs to predict postnatal depression (15, 16). It has also been noted that depression in pregnancy can affect the development of the fetus: fetal activity is increased, prenatal growth is delayed, and prematurity and low birth weight occur more often (17, 18). Newborns of depressed mothers then show a physiologic profile that mimics their mothers’ prenatal biochemical/physiologic profile including elevated cortisol, lower levels of dopamine and serotonin, greater relative right frontal EEG activation, and lower vagal tone. Increased prenatal maternal cortisol is the strongest predictor of these neonatal outcomes (17). The higher EPDS scores detected in all assessments, albeit not significant in all assessments, may suggest that the entire ART pregnancy–childbirth event is characterized by a greater vulnerability to depression.

Compared with ART men, ART women also showed a lower mood during pregnancy and just after birth, whereas at 3 months postpartum both experienced similar mood levels. These results were not unexpected, because even in ART condition women generally appear to be more involved in the complexity of parenthood than men, especially during pregnancy. The higher prevalence of depressive symptoms in ART pregnancies, especially in women, raises the problem of postnatal depression (PND), a serious mental health disorder, affecting 10% to 18% of women in Western countries (19, 20). Postnatal depression usually appears between 4 and 12 weeks after birth, but some cases have later onsets, within the first year after birth (21–23). It is well known that PND may adversely affect the family, causing poor maternal functioning, marital conflicts, and depression in the partner (24–26), and especially affecting the mother–infant interactions and relationships (24, 27, 28). It has also been demonstrated that long-term infant and child outcome may be influenced even many years after recovery (29–31). Difficulties in mother–infant interactions in the first months after birth can predict and, partly explain, socioemotional and cognitive deficits often observed in infants of depressed mothers at the end of the first year (32).

Infertility is undoubtedly a social experience, and is culture specific (33). In this study we evaluated Italian women whose attitude toward infertility is expected to be different from that in China (5, 6), where infertility is a hidden problem compared with the Western world. Thus, the generalizations from our study should be interpreted with some caution in countries with a different sociocultural approach to infertility. Another limitation of our study is that the relative low response rate might have introduced some selection bias; however, it is unlikely that responders were more or less depressive than nonresponders.

**TABLE 1****Comparison between ART and non-ART women for the main sociodemographic characteristics.**

	ART	Non-ART	P value
N	25	39	
Mean age (years)	35.9 ± 3.2	32.1 ± 3.6	.0005
Place of birth (Italy)			
North	16 (64)	27 (73)	.36
Central	2 (8)	—	
South	6 (24)	8 (21.6)	
Abroad	1 (4)	2 (5.4)	
Residence			
Northern Italy	24 (96)	39 (100)	.39
Central Italy	1 (4)	—	
Town residence	13 (52)	27 (69.2)	.19
Education			
Primary school	1 (4)	—	.14
Secondary school	3 (12)	8 (20.5)	
High school	9 (36)	21 (53.8)	
University	12 (48)	10 (25.6)	
Profession			
Housewife	—	2 (5.1)	.60
Factory worker	5 (20)	5 (12.8)	
Employee	17 (68)	28 (71.8)	
Professional employee	3 (12)	4 (10.3)	
Social class			
High	4 (16)	7 (19.4)	.91
Middle	16 (64)	23 (63.9)	
Low	5 (20)	6 (16.7)	
Marital status			
Married	22 (88)	31 (79.5)	.52
Cohabiting	2 (8)	7 (17.9)	
Separated/divorced	1 (4)	1 (2.6)	
Co-habiting (yrs)			
0–4	5 (20)	23 (59)	.001
5–8	8 (32)	8 (20.5)	
>8	12 (48)	4 (10.3)	
No reply	—	4 (10.3)	
Religion			
Catholic	23 (92)	36 (97.3)	.45
Atheistic	1 (4)	—	
Buddhist	1 (4)	1 (2.7)	
Parity			
Nulliparous	20 (80)	26 (68.4)	.39
Antenatal class			
Yes	15 (62.5)	24 (66.7)	.79
Vaginal delivery	13 (52)	20 (51.3)	1.00

Note: Data presented as mean ± SD or N (%).  
ART = assisted reproductive technology.

Monti. Depression among ART pregnancies. *Fertil Steril* 2009.

The findings of our study need to be corroborated using larger samples, but the results nonetheless confirm a higher prevalence and intensity of depressive symptoms during ART gestations and thereafter. These observations should alert clini-

cians to detect potential depression in these pregnancies and to offer psychologic consultation, support, and intervention to pregnant women and young mothers following ART to avoid the deleterious effects of depression on the mother and child.

**TABLE 2****Comparison between ART women and men for the main sociodemographic characteristics.**

	Women	Men	P value
N	25	23	
Mean age $\pm$ SD	35.9 $\pm$ 3.2	39.5 $\pm$ 4.7	.004
Place of birth (Italy)			
North	16 (64)	14 (60.9)	.64
Central	2 (8)	1 (4.3)	
South	6 (24)	8 (34.8)	
Abroad	1 (4)	—	
Residence			
Northern Italy	24 (96)	22 (95.7)	1.00
Central Italy	1 (4)	1 (4.3)	
Town residence	13 (52)	12 (52.2)	1.00
Education			
Primary school	1 (4)	—	.52
Secondary school	3 (12)	5 (21.7)	
High school	9 (36)	10 (43.5)	
University	12 (48)	8 (34.8)	
Profession			
Unemployed	—	1 (4.3)	.27
Factory worker	5 (20)	4 (17.4)	
Employee	17 (68)	11 (47.8)	
Professional employee	3 (12)	7 (30.4)	
Social class			
High	4 (16)	6 (30)	.53
Middle	16 (64)	11 (55)	
Low	5 (20)	3 (15)	
Marital status			
Married	22 (88)	20 (87)	.99
Cohabiting	2 (8)	2 (8.7)	
Separated/divorced	1 (4)	1 (4.3)	
Cohabiting			
0–4 years	5 (20)	5 (21.7)	.99
5–8 years	8 (32)	7 (30.4)	
>8 years	12 (48)	11 (47.8)	
Religion			
Catholic	23 (92)	22 (95.7)	.62
Atheistic	1 (4)	—	
Budhistic	1 (4)	1 (4.3)	
Parity			
Nulliparous	20 (80)	18 (78.3)	1.00
Antenatal class			
Yes	15 (62.5)	11 (50)	.55
Mean previous ART attempts	2 $\pm$ 1.9	2 $\pm$ 1.9	1.00

Note: Data presented as mean $\pm$ SD or N (%).

ART = assisted reproductive technology.

Monti. Depression among ART pregnancies. *Fertil Steril* 2009.

**TABLE 3****Mean (SD) EPDS scores and frequencies of depression in ART and non-ART women and in ART women and men.**

	ART women	Non-ART women	P value
N	25	39	
Mean EPDS scores (SD)			
Third trimester of pregnancy	7.88 (3.76)	4.32 (3.12)	< .0005
1 week after birth	8.00 (4.37)	4.89 (4.11)	< .005
3 months after birth	5.76 (4.02)	3.87 (3.59)	< .05
Frequency of depressed subjects, N (%)			
Third trimester of pregnancy	4 (16)	—	< .05
1 week after birth	3 (12)	3 (7.9)	NS
3 months after birth	2 (8)	—	NS
	ART women	ART men	
N	25	23	
Mean EPDS scores (SD)			
Third trimester of pregnancy	7.88 (3.76)	3.86 (3.52)	< .0005
1 week after birth	8.00 (4.37)	4.5 (4.54)	< .005
3 months after birth	5.76 (4.02)	4.32 (4.29)	NS
Frequency of depressed subjects, N (%)			
Third trimester of pregnancy	4 (16)	1 (4.3)	NS
1 week after birth	3 (12)	3 (13)	NS
3 months after birth	2 (8)	1 (4.5)	NS

Note: EPDS = Edinburgh Postnatal Depression Scale.

Monti. Depression among ART pregnancies. *Fertil Steril* 2009.

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# Ectopic Pregnancy Risk With Assisted Reproductive Technology Procedures

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**OBJECTIVE:** To assess the ectopic pregnancy risk among women who conceived with assisted reproductive technology (ART) procedures.

**METHODS:** The ectopic rate for ART pregnancies was calculated from population-based data of pregnancies conceived with ART in U.S. clinics in 1999–2001. Variation in ectopic risk by patient and ART treatment factors was assessed by using bivariate analyses and multivariable logistic regression.

**RESULTS:** Of 94,118 ART pregnancies, 2,009 (2.1%) were ectopic. Variation was observed by procedure type. In comparison with the ectopic rate (2.2%) among pregnancies conceived with in vitro fertilization and transcervical transfer of freshly fertilized embryos from the patient's oocytes (fresh, nondonor IVF-ET), the ectopic rate was significantly increased when zygote intrafallopian transfer (ZIFT) was used (3.6%) and significantly decreased when donor oocytes were used (1.4%) or when a gestational surrogate carried the pregnancy (0.9%). Among fresh nondonor IVF-ET procedures, the risk for ectopic pregnancy was increased among women with tubal factor infertility (odds ratio [OR] 2.0, 95% confidence interval [CI] 1.7–2.4; referent group = ART for male factor), endometriosis (OR 1.3, 95% CI 1.0–1.6), and other non-tubal female factors of infertility (OR 1.4, 95% CI 1.2–1.6) and decreased among women with a previous live birth (OR 0.6, 95% CI 0.5–0.7). Transfer of embryos with an indication of high implantation potential was associated

with a decreased ectopic risk when 2 or fewer embryos were transferred (OR 0.7, 95% CI 0.5–0.9), but not when 3 or more embryos were transferred.

**CONCLUSION:** Ectopic risk among ART pregnancies varied according to ART procedure type, reproductive health characteristics of the woman carrying the pregnancy, and estimated embryo implantation potential.

(*Obstet Gynecol* 2006;107:595–604)

**LEVEL OF EVIDENCE: II-2**

Although population-based estimates of the incidence of ectopic pregnancy among assisted reproductive technology (ART) patients have not been reported in the United States, results of small clinical studies published from 1991 through 1995 suggest that the ectopic rate associated with ART conceptions may be elevated, between 2.2 and 8.6 per 100 pregnancies, compared with the estimated rate of 2.0 per 100 pregnancies for the general U.S. population in 1990–1992.<sup>1–8</sup>

Theoretically, differences between conception via ART and natural conception may affect the risk of ectopic pregnancy. As currently performed, the vast majority of ART procedures involve in vitro fertilization and transcervical embryo transfer (IVF-ET) of multiple embryos.<sup>9</sup> Because neither fertilization nor embryo transfer in IVF-ET involves the fallopian tubes directly, ART might reduce the risk of ectopic pregnancy. Nonetheless, ectopic pregnancies have been documented in IVF-ET cycles, raising questions about the etiology of these ectopic implantations, including whether the transfer of multiple embryos plays a role.

The majority of information on ectopic pregnancies among ART conceptions stems from case reports or case series. The few studies with denominator data were small and thus unable to evaluate sufficiently the risk of ectopic pregnancy by important patient subgroups. Limited data from previous studies suggest

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Data Source: U.S. Assisted Reproductive Technology Surveillance System.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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that tubal factor infertility and prior ectopic pregnancy are associated with an increase in the risk for ectopic pregnancy after ART.<sup>1-3,10,11</sup>

We assessed the incidence of ectopic pregnancy among ART conceptions using data from the large, population-based, U.S. registry of ART procedures. These data also allowed us to perform a more detailed analysis of risk factors for ectopic pregnancy among ART conceptions than has previously been reported.

## MATERIALS AND METHODS

Providers of ART in the United States are required to report data on every procedure to the Centers for Disease Control and Prevention (CDC) annually.<sup>12</sup> Assisted reproductive technology is defined as any treatment where oocytes and sperm are handled outside the body for the purpose of establishing a pregnancy. The Society for Reproductive Technology (SART) compiled a data file from participating ART clinics and shared these data with the CDC (the U.S. ART Registry). Data were abstracted from medical records and included patient demographic characteristics, medical history, and clinical information on the ART procedure and resultant pregnancies. From 92% to 95% of U.S. clinics reported data annually to the U.S. ART Registry.<sup>13</sup>

For this study, we selected procedures performed between January 1, 1999, and December 31, 2001, that resulted in a reported clinical intrauterine, ectopic, or heterotopic pregnancy. In the ART Registry, a *clinical intrauterine pregnancy* was defined as documentation of one or more gestational sacs visible by ultrasound examination; *ectopic pregnancy* was defined as documentation of one or more gestational sacs outside of the uterus; and *heterotopic pregnancy* was defined as a pregnancy that met the criteria for both ectopic and clinical intrauterine pregnancy. Pregnancies reported as *biochemical pregnancies* only (defined in the registry as having an elevated human chorionic gonadotropin [hCG] without a visible gestational sac and no clinical diagnosis of pregnancy) were excluded from the analysis.

From 1999 to 2001, 294,852 ART procedures were reported to the ART Registry. Of these procedures, 94,700 resulted in either an ectopic, heterotopic, or intrauterine pregnancy. From this group of all ART pregnancies, we excluded a small number of pregnancies that resulted from less common treatment options (< 1%). These uncommon options included the following: procedures in which any combination of IVF-ET, gamete intrafallopian transfer (GIFT), or zygote intrafallopian transfer (ZIFT) were used for transfer (n = 176), procedures in which

both frozen-thawed and freshly fertilized embryos were transferred (n = 120), procedures in which embryos from both donor and patient oocytes were transferred (n = 109), and GIFT and ZIFT procedures that involved either donor oocytes or frozen-thawed embryos (n = 170). We also excluded pregnancies for which the improbable transfer of 15 or more embryos was reported (n = 7). The resulting study population thus consisted of 94,118 pregnancies.

We classified a pregnancy as ectopic if it was reported as either ectopic only or heterotopic. We calculated ectopic incidence by dividing ectopic pregnancies by the total number of pregnancies (ectopic + clinical intrauterine pregnancies). Rates were presented as per 100 pregnancies. We also calculated heterotopic incidence rates by dividing the number of pregnancies classified as heterotopic by the total number of pregnancies (ectopic + clinical intrauterine pregnancies).

In our initial analyses, we calculated ectopic rates for pregnancies achieved through various ART procedure types, which we classified according to whether the embryos were fertilized during the current procedure (fresh) or had been fertilized during a previous procedure and frozen until the current procedure (frozen) and by whether the source of the oocyte was the patient (nondonor) or an oocyte donor. We calculated rates for procedures in which a surrogate was used to gestate the pregnancy and for GIFT and ZIFT procedures separately.

Because significant variation in ectopic rates was found across procedure types, these types were not combined for further analysis of risk factors. Instead, more detailed analyses were restricted to pregnancies that resulted from the most common type of ART: IVF with transcervical transfer of fresh nondonor embryos. In all, 69,366 clinical intrauterine and ectopic pregnancies were selected for these analyses (74% of our original study population). Risk factors evaluated included patient age, race/ethnicity, previous births, previous spontaneous abortion, and infertility diagnosis; year of ART procedure; use of intracytoplasmic sperm injection (ICSI) (in which a single sperm is injected directly into an oocyte); use of assisted hatching (in which lasers, chemicals, or other means are used to create an opening in the zona pellucida of the embryo before transfer); number of days in embryo culture; number of embryos transferred; and whether extra embryos were available and cryopreserved for future use.

Although the U.S. ART Registry does not contain data on specific embryo quality assessments, 2 factors have been associated with increased implantation



potential (higher estimated embryo potential): extra embryos available for cryopreservation and the number of days in embryo culture.<sup>14,15</sup> Although our data are observational and not randomized, and thus both of the aforementioned factors are limited by provider and patient choices of specific ART treatment practices, these 2 variables nonetheless have been predictive of success rates in past analyses, independent of patient age or the number of embryos transferred, and thus are likely to be correlated with a higher estimation of embryo implantation potential.<sup>14,15</sup>

During preliminary unadjusted analyses, we initially divided infertility diagnoses into 14 distinct categories. For stratified and multivariable analyses, sample size constraints necessitated that we collapse these categories into 5: male factor, tubal pathology with or without a hydrosalpinx, tubal ligation, endometriosis, and nontubal female factors (which include ovulatory dysfunction, uterine factor, diminished ovarian reserve, immunologic factors, infertility related to chemotherapy or other chronic disease, and unexplained infertility). Pregnancies that met the criteria for only one infertility diagnosis group were assigned to that group. Pregnancies among women meeting the criteria for more than 1 of the 5 diagnosis categories were assigned to the group that had the highest ectopic rate in our initial analyses. That is, the ectopic rate for tubal pathology was greater than that for nontubal female factors, which was greater than that for endometriosis, which was greater than that for male factor, which was greater than that for tubal ligation. As a result of this algorithm, only those in the tubal ligation group had a single diagnosis of infertility.

We assessed simple (unadjusted) associations between ectopic pregnancy risk and each factor of interest with  $\chi^2$  tests. On the basis of epidemiologic studies and/or biologic plausibility, we identified 3 a priori risk factors for ectopic pregnancy: type of infertility diagnosis, number of embryos transferred, and use of assisted hatching. Through our analyses, we determined that type of infertility diagnosis and the number of embryos transferred were potentially important confounders or effect modifiers. We further assessed associations between ectopic pregnancy risk and each of the other factors of interest after stratification on infertility diagnosis and stratification on number of embryos transferred. We also performed multivariable logistic regression to independently assess associations between maternal and ART treatment factors. The model included patient age, prior spontaneous abortions, prior births, infertility diagnosis, use of assisted hatching, use of ICSI, and one

interaction term: estimated embryo implantation potential by number of embryos transferred. Estimated implantation potential was a 4-level categorical variable derived from 2 variables included in the initial (unadjusted) analysis, day of embryo transfer (day 3 versus day 5), and availability of extra embryos that were cryopreserved (yes versus no). Number of embryos transferred was classified as 1–2 versus 3+. The interaction term of implantation potential by number of embryos transferred included 8 levels; the referent group was 3+ embryos transferred with no indication of high implantation potential (day 3 transfer and no extra embryos cryopreserved). The data were analyzed in SAS 9.1 (SAS Institute Inc, Cary, NC). This study was approved by the CDC's Institutional Review Board.

## RESULTS

Of the 94,118 pregnancies included in our final study population, 2,009 were reported as ectopic, 143 of which were heterotopic. Thus, the overall rate of ectopic pregnancy for women undergoing ART procedures in the United States from 1999 to 2001 was 2.1%, and the heterotopic pregnancy rate was 0.15%.

The ectopic rate varied significantly by ART procedure type (Table 1). The ectopic rate among fresh nondonor IVF-ET treatment cycles, the most widely used type of ART, accounting for 74% of the pregnancies, was 2.2%. In comparison, the ectopic rate among fresh nondonor ZIFT procedures was significantly increased, at 3.6%. The ectopic rate was significantly decreased among fresh donor IVF-ET (1.4%) and gestational surrogate procedures (0.9%).

More than half of the pregnancies conceived with fresh nondonor IVF-ET procedures were among women less than 35 years of age (Table 2). Although a large proportion (37.2%) of pregnancies were missing data for race/ethnicity, the most common racial-ethnic group reported was white, non-Hispanic. For a majority of pregnancies, no spontaneous abortions had been reported and no previous births were reported. The 2 most common infertility diagnoses were tubal factor (excluding tubal ligation) (24.6%,  $n = 17,087$ ) and male factor (38.0%,  $n = 26,355$ ) (alone, or in combination with other factors). Assisted hatching was used in 40.8% of the treatments, and ICSI was used in 52.7%. Nearly three fourths of the pregnancies resulted from embryos cultured for 3 days and 17.5% from embryos cultured for 5 days (corresponding with the blastocyst stage). Although the number of embryos transferred varied widely, 70.5% of pregnancies involved the transfer of 3 or more embryos. More than one third of the women who became pregnant



**Table 1. Incidence of Ectopic Pregnancy by Type of ART Procedure, United States, 1999–2001**

ART Procedure	No. Total Pregnancies	No. Ectopic Pregnancies	Ectopic Rate (Ectopic/Total Pregnancies) (%)	OR	95% CI
Fresh nondonor IVF-ET	69,366	1,553	2.2	Reference	
Fresh nondonor GIFT	534	13	2.4	1.09	0.63–1.89
Fresh nondonor ZIFT	797	29	3.6	1.65	1.13–2.40
Fresh donor IVF-ET	10,400	149	1.4	0.63	0.54–0.75
Thawed nondonor	9,374	215	2.3	1.03	0.89–1.18
Thawed donor	2,332	38	1.6	0.72	0.52–1.00
Gestational surrogate*	1,315	12	0.9	0.4	0.23–0.71

ART, assisted reproductive technology; OR, odds ratio; CI, confidence interval; IVF-ET, in vitro fertilization–embryo transfer; GIFT, gamete intrafallopian transfer; ZIFT, zygote intrafallopian transfer; fresh nondonor, freshly fertilized embryos from the patient's oocytes; fresh donor, freshly fertilized embryos from donor oocytes; thawed nondonor, thawed embryos from the patient's oocytes; thawed donor, thawed embryos from donor oocytes.

\* All procedures in which a gestational surrogate carried the pregnancy were analyzed as a separate procedure group. Because of sample size constraints, these were not further subdivided as fresh, frozen, nondonor, donor, IVF, GIFT, or ZIFT.

after IVF-ET had extra embryos available for cryopreservation.

In unadjusted analyses, we observed significant variation in ectopic rate by prior births, infertility diagnosis, use of ICSI, and number of embryos transferred (Table 3). Compared with ectopic rates among couples with male factor infertility, ectopic rates among women with tubal pathology (with/without hydrosalpinx), uterine factor, endometriosis, diminished ovarian reserve, infertility relating to immunologic factors, chemotherapy or other chronic disease, unexplained infertility, multiple female and male factors with tubal pathology, or multiple female factors (with or without tubal pathology) were each significantly increased. For example, women diagnosed with tubal pathology with hydrosalpinx had a 4.2% ectopic rate, more than 2.5 times higher than the rate among couples diagnosed with male factor infertility only. Women treated with ART because of a tubal ligation only had the lowest ectopic rate of all infertility diagnoses (1.0%). Use of ICSI was associated with a decreased ectopic rate. The transfer of 3 embryos or 4 or more embryos was associated with an increased ectopic rate.

Because a disproportionate number of pregnancies conceived using ICSI were among couples with male factor infertility (a group at relatively low risk for ectopic pregnancy), the apparent protective effect initially observed with use of ICSI disappeared after stratification and adjustment for infertility diagnosis (odds ratio [OR] 0.94, 95% confidence interval [CI] 0.8–1.0). There was no difference in the risk for ectopic pregnancy among ICSI and non-ICSI pregnancies within specific infertility diagnosis strata.

Our initial analyses indicated that women with a race/ethnicity other than white non-Hispanic were at a greater risk for ectopic pregnancy than white (non-

Hispanic) women, but this association varied according to infertility diagnosis (Table 4). Race/ethnicity other than non-Hispanic white was associated with increased ectopic rates among women seeking ART for either male factor infertility or endometriosis. In contrast, there was no variation by race/ethnicity among women diagnosed with tubal pathology or nontubal female factors. The results for the tubal ligation group are based on a very small sample and thus were not appropriately powered to assess statistical significance. Also, it is important to note that 37% of the data for race/ethnicity were missing and thus were not included in these analyses.

The association between ectopic pregnancy risk and embryo implantation potential, based on the 2 indicators we were able to assess, number of days in embryo culture and availability of extra embryos for cryopreservation, varied according to the number of embryos transferred. The ectopic risk among pregnancies in which 3 or more embryos had been transferred was 2.4–2.5%, whether or not there was an indication of higher embryo implantation potential. However, when only 1–2 embryos were transferred, ectopic rates varied according to embryo implantation potential indicators: 2.2% with neither indicator present, 1.6% when extra embryos had been available and cryopreserved, 1.4% when embryos were cultured for 5 rather than 3 days, and 1.4% when both of these conditions were met. These latter 3 rates were significantly ( $P < .05$ ) different from the referent group, 3+ embryos transferred with neither indication for higher implantation potential.

The unadjusted results (Table 3) for the risk of ectopic pregnancy according to prior births, prior spontaneous abortions, year of current ART procedure, and use of assisted hatching, were not appreciably altered by stratification and adjustment for infer-



**Table 2. Percentage Distribution of Study Population by Patient and ART Treatment Factors for Fresh Nondonor IVF-ET Treatment Procedures, United States, 1999–2001 (N = 69,366)**

	n	%
<i>Patient Factors</i>		
<b>Age (y)</b>		
< 30	10,690	15.4
30–34	27,456	39.6
35–37	16,464	23.7
38–40	10,929	15.8
41–43	3,520	5.1
44+	307	0.4
<b>Race/ethnicity</b>		
White (non-Hispanic)	37,161	53.6
Black (non-Hispanic)	1,772	2.6
Asian	1,981	2.9
Hispanic	2,588	3.7
Other	59	0.1
Ethnicity status missing or unknown	25,805	37.2
<b>Prior spontaneous abortions</b>		
0	49,850	71.9
1	12,878	18.6
2+	6,638	9.6
<b>Prior births</b>		
0	50,932	73.6
1	13,605	19.7
2+	4,664	6.7
<b>Infertility diagnosis</b>		
Male factor	14,926	21.5
Tubal pathologies		
Tubal pathology with hydrosalpinx	1,042	1.5
Other tubal pathology	8,700	12.5
Tubal ligation	2,047	3.0
Endometriosis	5,794	8.4
Other female factors of infertility		
Ovulatory dysfunction	4,368	6.3
Uterine factor	589	0.9
Diminished ovarian reserve	1,649	2.4
Unexplained infertility	7,712	11.1
Other infertility factors*	3,342	4.8
Multiple factors of infertility		
Multiple female + male (with tubal pathology)	2,908	4.2
Multiple female + male (without tubal pathology)	8,521	12.3
Multiple female (with tubal pathology)	4,437	6.4
Multiple female (without tubal pathology)	3,331	4.8
<i>ART treatment factors</i>		
<b>Year of ART procedure</b>		
1999	19,848	28.6
2000	23,028	33.2
2001	26,490	38.2
<b>Use of assisted hatching</b>	28,290	40.8
<b>Use of ICSI</b>	36,546	52.7
<b>Number of days of embryo culture</b>		
0–1	592	0.9
2	3,424	4.9
3	49,676	71.6
4	1,820	2.6
5	12,146	17.5
6	1,708	2.5
<b>Number of embryos transferred†</b>		
1–2	20,477	29.5
3	26,382	38.0
4+	22,507	32.5
<b>Extra embryos available/cryopreserved</b>	26,426	38.1

ART, assisted reproductive technology; IVF-ET, in vitro fertilization with transcervical embryo transfer; ICSI, intracytoplasmic sperm injection.

\* Other infertility factors: infertility related to immunologic factors, chemotherapy, or other chronic disease.

† Distribution of embryos transferred among the 22,507 procedures with 4 or more embryos transferred was as follows: 4 embryos (67%), 5–6 embryos (30%), 7–8 embryos (3%), and more than 8 embryos (< 1%).



**Table 3. Ectopic Pregnancy Rates by Patient and Procedure Characteristics for Fresh Nondonor IVF-ET Procedures, United States, 1999–2001**

	No. Total Pregnancies	No. Ectopic Pregnancies	Ectopic Rate (%)	Unadjusted OR	95% CI*
<i>ART Patient Characteristics</i>					
<b>Age (y)</b>					
< 30	10,690	223	2.1	Reference	Reference
30–34	27,456	607	2.2	1.06	0.91–1.23
35–37	16,464	359	2.2	1.05	0.89–1.23
38–40	10,929	265	2.4	1.16	0.97–1.39
41–43	3,520	92	2.6	1.25	0.99–1.59
44+	307	7	2.3	1.09	0.52–2.30
<b>Prior spontaneous abortions</b>					
0	49,850	1,112	2.2	Reference	Reference
1	12,878	277	2.2	0.96	0.85–1.10
2+	6,638	164	2.5	1.11	0.94–1.30
<b>Prior births</b>					
0	50,932	1,245	2.4	Reference	Reference
1	13,605	249	1.8	0.75	0.65–0.86
2+	4,664	57	1.2	0.50	0.38–0.65
<b>Infertility diagnosis</b>					
Male factor	14,926	235	1.6	Reference	Reference
Tubal factor					
Tubal pathology with hydrosalpinx	1,042	44	4.2	2.68	1.96–3.68
Other tubal pathology	8,700	264	3.0	1.93	1.62–2.29
Tubal ligation	2,047	20	1.0	0.62	0.39–0.98
Endometriosis	5,794	127	2.2	1.39	1.12–1.72
Other female factors of infertility					
Ovulatory dysfunction	4,368	78	1.8	1.13	0.88–1.46
Uterine factor	589	21	3.6	2.26	1.46–3.51
Diminished ovarian reserve	1,649	44	2.7	1.69	1.23–2.33
Unexplained infertility	7,712	167	2.2	1.38	1.13–1.67
Other factors†	3,342	85	2.5	1.62	1.26–2.06
Multiple factors of infertility					
Multiple female + male (with tubal pathology)	2,908	90	3.1	1.97	1.55–2.50
Multiple female + male (without tubal pathology)	8,521	163	1.9	1.22	1.00–1.48
Multiple female (with tubal pathology)	4,437	142	3.2	2.03	1.65–2.50
Multiple female (without tubal pathology)	3,331	73	2.2	1.39	1.07–1.81
<i>ART treatment factors</i>					
<b>Year of ART procedure</b>					
1999	19,848	453	2.3	Reference	Reference
2000	23,028	503	2.2	0.96	0.84–1.09
2001	26,490	597	2.3	0.99	0.88–1.11
<b>Use of assisted hatching</b>					
No	41,076	925	2.3	Reference	Reference
Yes	28,290	628	2.2	0.99	0.89–1.09
<b>Use of ICSI</b>					
No	32,816	817	2.5	Reference	Reference
Yes	36,546	736	2.0	0.81	0.73–0.89
<b>Number of embryos transferred</b>					
1 to 2	20,477	358	1.8	Reference	Reference
3	26,382	601	2.3	1.30	1.14–1.48
4+	22,507	594	2.6	1.51	1.33–1.72

IVF-ET, in vitro fertilization with transcervical embryo transfer; OR, odds ratio; CI, confidence interval; ART, assisted reproductive technology; ICSI, intracytoplasmic sperm injection.

\* Chi-square test was used to assess variations in ectopic pregnancy rates across patient and treatment factor categories.

† Other infertility factors: infertility related to immunologic factors, chemotherapy, or other chronic disease.

tility diagnosis and number of embryos transferred (data not shown).

The final logistic regression model included ma-

ternal age, prior spontaneous abortions, prior births, infertility diagnosis, use of assisted hatching, and use of ICSI as independent variables. Additionally, we



**Table 4. Risk of Ectopic Pregnancy by Race/Ethnicity and Infertility Diagnosis\***

	Ectopic Rate (%)			
	Race/Ethnicity Other Than White Non-Hispanic <sup>†</sup>	White Non-Hispanic	Unadjusted OR	95% CI
Total N (43,561)	n = 6,400	n = 37,161		
Male factor (n = 8,974)	2.2	1.4	1.58	1.04–2.43
Tubal pathology (with or without hydrosalpinx) (n = 10,692)	3.4	3.4	0.99	0.76–1.290
Tubal ligation (n = 1,511)	1.5 <sup>‡</sup>	0.9	1.80	0.61–5.31
Endometriosis (n = 5,269)	3.7	2.2	1.75	1.06–2.88
Nontubal female factors (n = 17,115)	2.6	2.2	1.18	0.88–1.57

OR, odds ratio; CI, confidence interval.

\* Pregnancies that met the criteria for more than 1 of the 5 categories were assigned to the category that had the highest ectopic rate in our initial analyses: the ectopic rate for tubal pathology > nontubal female factors > endometriosis > male factor > tubal ligation.

<sup>†</sup> Includes women who are Hispanic, non-Hispanic black, Asian, or Native American. Women missing data on race/ethnicity were excluded from analyses.

<sup>‡</sup> Fewer than 10 ectopic pregnancies among women in this group.

included an interaction term constructed by using number of embryos transferred and estimated embryo implantation potential (we combined 2 factors into a single estimate of embryo implantation potential: days in embryo culture and extra embryos cryopreserved). Race/ethnicity was excluded from the final logistic regression model because of the large amount of missing data. The results of the logistic regression analysis were consistent with the results of the stratified analyses (Table 5). Women with tubal pathology (with or without a hydrosalpinx) were 2.0 times more likely to have an ectopic pregnancy than women treated with ART because of male factor infertility (95% CI 1.7–2.4). Endometriosis conferred a modest risk (OR 1.3, 95% CI 1.0–1.6). Nontubal female factors also conferred a modest risk (OR 1.4, 95% CI 1.2–1.6). Having a previous birth was protective (OR 0.6, 95% CI 0.5–0.7). The transfer of 2 embryos or fewer was protective among 3 subgroups of women with at least one indication of higher embryo implantation potential (ORs 0.6–0.7). Maternal age, prior spontaneous abortions, use of assisted hatching, and ICSI were not significant predictors of ectopic pregnancy.

## DISCUSSION

The ectopic pregnancy rate reported here (2.1%) for women who conceived with ART in the United States from 1999 to 2001 was below the range of rates reported from previous clinical studies of ART patients (2.2–8.6%) and comparable with the estimated ectopic rate among conceptions in the general U.S. population, (2.0%).<sup>1–8</sup> The rate of heterotopic pregnancy among ART users was 0.15%.

Our results suggest that 3 main factors are impor-

tant in assessing the risk of ectopic pregnancy among ART users: the specific type of ART procedure, the reproductive health characteristics of the woman carrying the pregnancy, and the estimated embryo implantation potential.

Our observation of a significant increase in the risk of ectopic pregnancy after ZIFT, in comparison with fresh nondonor IVF-ET procedures, is perhaps somewhat intuitive because embryos are transferred into the fallopian tubes in ZIFT. However, we did not observe this effect with GIFT procedures. Additionally, we lacked the data to explore the ZIFT association by the location of ectopic pregnancy and the side of the embryo transfer. Limited sample size also precluded analysis of specific patient or treatment risk factors among ZIFT pregnancies.

Ectopic pregnancy risk was associated with several measures of women's reproductive health status. Women with tubal factor infertility had a 2-fold increase in risk, and women with nontubal female factors of infertility or endometriosis had a 30–40% increased risk of ectopic pregnancy. It is possible that women classified as only having infertility related to nontubal female factors might nonetheless have had tubal pathology that was not diagnosed or reported. Dubuisson et al<sup>1</sup> reported tubal damage among women with ectopic pregnancies who had used IVF because of endometriosis or unexplained infertility. Likewise, our finding of an increased ectopic pregnancy risk among women with race/ethnicity other than non-Hispanic white (with a diagnosis of endometriosis or male factor infertility) might be related to tubal pathology if tubal pathology in these women were less likely to be diagnosed or reported.

Women who had a prior birth, a group that has



**Table 5. Factors Associated With Ectopic Pregnancy Among Pregnancies Conceived Using Fresh, Nondonor IVF-ET, Results of Multivariable Logistic Regression Analyses, United States, 1999–2001\***

Patient and Procedure Characteristics	AOR	95% CI
Age (y)		
< 35	Reference	Reference
35+	1.05	0.94–1.18
Prior spontaneous abortions		
No	Reference	Reference
Yes	0.94	0.83–1.06
Prior births		
No	Reference	Reference
Yes	0.62	0.54–0.72
Infertility diagnosis <sup>†</sup>		
Male factor	Reference	Reference
Tubal pathology (with or without hydrosalpinx)	2.01	1.68–2.41
Tubal ligation	0.90	0.55–1.46
Endometriosis	1.30	1.04–1.62
Nontubal female factors	1.38	1.16–1.63
Use of assisted hatching		
No	Reference	Reference
Yes	0.96	0.86–1.08
Use of ICSI		
No	Reference	Reference
Yes	0.91	0.81–1.02
Number of embryos transferred by factors related to estimated embryo implantation potential		
3+ embryos transferred		
Day 3 culture and no extra embryos cryopreserved	Reference	Reference
Day 3 culture and extra embryos cryopreserved	1.04	0.91–1.20
Day 5 culture and no extra embryos cryopreserved	1.04	0.79–1.36
Day 5 culture and extra embryos cryopreserved	0.96	0.69–1.34
1–2 embryos transferred		
Day 3 culture and no extra embryos cryopreserved	0.93	0.76–1.12
Day 3 culture and extra embryos cryopreserved	0.67	0.52–0.87
Day 5 culture and no extra embryos cryopreserved	0.56	0.41–0.77
Day 5 culture and extra embryos cryopreserved	0.55	0.43–0.71

IVF-ET, in vitro fertilization and transcervical embryo transfer; ICSI, intracytoplasmic sperm injection; AOR, adjusted odds ratio.

\* The multivariable logistic model adjusted for all factors listed in the table.

<sup>†</sup> Pregnancies that met the criteria for more than 1 of the 5 categories were assigned to the category that had the highest ectopic rate in our initial analyses: the ectopic rate for tubal pathology > nontubal female factors > endometriosis > male factor > tubal ligation.

demonstrated capacity to have an intrauterine pregnancy, were less likely to have an ectopic implantation in the index pregnancy for this study. Further, the decreased risk in ectopic pregnancy among gestational carriers supports the contribution of host characteristics to the risk of ectopic pregnancy during ART.

In this study, women who had embryos with at least one indication of higher estimated embryo implantation potential were at decreased risk of ectopic pregnancy if 2 or fewer embryos were transferred. The apparent benefit, however, was not evident when 3 or more embryos were transferred. The protective effect we observed among procedures using donor oocytes further supports the hypothesis that embryo implantation potential is associated with risk because, in most clinics, oocyte donors are young women

without an indication of infertility. The underlying mechanism for these findings related to embryo characteristics requires further study. Although prior studies suggested that chromosomal abnormalities may play a role in the etiology of ectopic pregnancy, results across studies are inconsistent, and both positive and negative studies were limited by small sample sizes.<sup>16–18</sup>

A recent study suggested that the assisted hatching procedure was associated with an increased risk of ectopic pregnancy.<sup>19</sup> Our findings do not support that conclusion, but the ART Registry does not include data to separately assess specific types of assisted hatching procedures.

The primary strength of our study was the ability to investigate potential ectopic pregnancy risk factors with a large, population-based sample. Most previous



studies of ectopic pregnancy were clinic based, and the results may not be generalizable. Our large sample of ART pregnancies also allowed us to assess the risk of ectopic pregnancy among several ART subgroups. Although the sample size for individual subgroups varied, for most, our results were stable, as evidenced by the narrow confidence limits around our measures of associations.

Even though we report a lower ectopic pregnancy rate than previous ART studies, our results are nonetheless consistent with these prior reports. All suggest that the condition of the fallopian tubes is central to a woman's risk for ectopic pregnancy.<sup>1-7,11</sup> Many previous studies were published in the early 1990s, when tubal factor infertility was the major indication for the use of IVF-ET.<sup>20</sup> In these studies, 43–74% of clinical pregnancies were among patients with a diagnosis of tubal factor infertility, whereas in our study, only 24.6% of clinical pregnancies were among patients with a tubal factor diagnosis (alone or in combination with other factors, excluding tubal ligation), as ART is now more commonly used among couples diagnosed with nontubal infertility disorders.<sup>1,6,7</sup> Indeed, in our population, the ectopic rates of pregnancies among patients with a sole diagnosis of tubal pathology (4.2% and 3.0% for tubal pathology with and without a hydrosalpinx, respectively) fall within the range of ectopic rates observed in earlier ART studies (2.2–8.6%).<sup>1-7</sup>

The major limitation of this study is that the ART Registry lacks specific clinical data pertaining to embryo factors, transfer techniques (including ultrasound-guided embryo transfer), or history of ectopic pregnancy. Therefore, we were unable to explore underlying mechanisms for the associations reported here.

Another potential concern was the accuracy and completeness of ectopic pregnancy reporting in the ART Registry. However, we found that no single clinic accounted for a disproportionate number of ectopic pregnancies and that ectopic pregnancies were diagnosed and reported by most clinics (data not shown). In addition, ART treatment outcome data for 1999–2001 were validated by trained ART clinicians during on-site medical record reviews at 5–10% of ART clinics included in the ART Registry and were found to be highly concordant with the data in the medical record (< 1% misreporting rate). Finally, because women undergoing ART are routinely monitored by the ART provider with pregnancy testing and ultrasonography, we assume that this surveillance system, based on ART providers, is sensitive and specific at capturing ectopic pregnancies. Even

though *ectopic pregnancy* was narrowly defined in the ART Registry as the documentation of one or more gestational sacs outside the uterus, we assume that ART providers would likely have recorded an ectopic pregnancy even if it was identified by a means other than ultrasound examination (eg, the absence of an intrauterine sac with a hCG level above the discriminatory zone with a clinical history consistent with ectopic pregnancy). Our analysis of the distribution of pregnancies across clinics indicated that, not only was the reporting of ectopic pregnancies evenly distributed, but reported biochemical pregnancies were also distributed evenly (most clinics reporting ectopic pregnancy were also reporting biochemical pregnancies). Thus, the data suggest clinics were distinguishing these 2 types of pregnancy, rather than select clinics conservatively reporting their ectopic pregnancies as biochemical. However, because we do not have data on whether some women may have been diagnosed with an ectopic pregnancy after leaving the care of their ART provider, we cannot discount the possibility that some ectopic pregnancies were missed.

This study demonstrated that the transfer of 2 or fewer embryos with higher estimated implantation potential was protective against ectopic pregnancy. Further research into the relationship between embryo implantation potential and ectopic pregnancy might be able to identify the mechanism behind this reduction in risk. Also, as new technologies in ART become available, their potential impact on ectopic pregnancy should be investigated.

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# Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8–18 years of IVF children and spontaneously conceived controls born to subfertile parents

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**BACKGROUND:** Little is known about post-natal growth in IVF offspring and the effects of rates of early post-natal growth on blood pressure and body fat composition during childhood and adolescence.

**METHODS:** The follow-up study comprised 233 IVF children aged 8–18 years and 233 spontaneously conceived controls born to subfertile parents. Growth data from birth to 4 years of age, available for 392 children ( $n = 193$  IVF,  $n = 199$  control), were used to study early post-natal growth. Furthermore, early post-natal growth velocity (weight gain) was related to blood pressure and skinfold measurements at follow-up.

**RESULTS:** We found significantly lower weight, height and BMI standard deviation scores (SDSs) at 3 months, and weight SDS at 6 months of age in IVF children compared with controls. Likewise, IVF children demonstrated a greater gain in weight SDS ( $P < 0.001$ ), height SDS ( $P = 0.013$ ) and BMI SDS ( $P = 0.029$ ) during late infancy (3 months to 1 year) versus controls. Weight gain during early childhood (1–3 years) was related to blood pressure in IVF children ( $P = 0.014$  systolic, 0.04 diastolic) but not in controls. Growth during late infancy was not related to skinfold thickness in IVF children, unlike controls ( $P = 0.002$  peripheral sum, 0.003 total sum). Growth during early childhood was related to skinfold thickness in both IVF and controls ( $P = 0.005$  and 0.01 peripheral sum and  $P = 0.003$  and 0.005 total sum, respectively).

**CONCLUSIONS:** Late infancy growth velocity of IVF children was significantly higher compared with controls. Nevertheless, early childhood growth instead of infancy growth seemed to predict cardiovascular risk factors in IVF children. Further research is needed to confirm these findings and to follow-up growth and development of IVF children into adulthood.

**Key words:** blood pressure / body composition / growth patterns / growth velocity / IVF

## Introduction

According to the 'developmental origins of adult disease hypothesis', many adult diseases are thought to be the long-term consequence

of programming during early life (Barker, 1995; Bateson *et al.*, 2004). Exposure to environmental insults at critical windows during various stages of prenatal development may induce structural and functional adaptations. It is well recognized that these adaptations

may provide a short-term survival benefit, but eventually lead to an increased risk of chronic diseases, including type 2 diabetes and cardiovascular disease, in later life (Barker, 2004).

Over the last years, numerous epidemiological studies indicated that the link between impaired prenatal development and cardiovascular morbidity in adult life is substantially modified by early post-natal growth (Eriksson *et al.*, 1999; Forsen *et al.*, 1999; Huxley *et al.*, 2000). Individuals exposed to adverse prenatal conditions seem to be more susceptible to cardiovascular disease and type 2 diabetes if they 'catch-up' in weight during early post-natal life. Associations between rapid early post-natal growth and several cardiovascular risk factors, like blood pressure and fat mass, have also been described in children and adolescents (Ong *et al.*, 2000; Horta *et al.*, 2003; Ekelund *et al.*, 2006).

Today, reproductive technologies including IVF are used all over the world to treat subfertility. The number of IVF-conceived children is steadily growing with approximately 1–3% of the current births in developed countries being established after IVF (Maher, 2005). However, concerns that IVF conception may influence prenatal development with long-lasting consequences have been increasingly expressed (Painter and Roseboom, 2006). Numerous studies have reported increased risks of low birthweight and preterm birth among IVF pregnancies (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004). Furthermore, we previously demonstrated that IVF-conceived children and adolescents are at increased risk of higher blood pressure levels and altered body composition (Ceelen *et al.*, 2007, 2008). Little is known, however, about post-natal growth among IVF offspring, and the relationships between early post-natal growth and blood pressure and body fat composition are also still elusive.

Therefore, in the present study, we addressed several early post-natal growth parameters of IVF and spontaneously conceived control children from subfertile parents from birth to 4 years of age. Furthermore, we investigated the associations of growth velocity during infancy and/or early childhood in relation to blood pressure and body fat composition in 8–18-year-old IVF children and controls. In line with numerous studies examining the relationship between post-natal growth and blood pressure and body fat, weight gain was used as an early post-natal growth velocity parameter (Ong *et al.*, 2000; Law *et al.*, 2002; Horta *et al.*, 2003; Euser *et al.*, 2005; Hemachandra *et al.*, 2007).

## Subjects and Methods

### Study population

This study is part of a follow-up study investigating post-natal growth and development in children and adolescents aged 8–18 years old born from subfertile parents who were either successfully treated with IVF or conceived spontaneously, as described previously (Ceelen *et al.*, 2007, 2008). Families with a singleton child born after IVF treatment performed in the VU University medical center (VUmc) in Amsterdam, the Netherlands, were invited by mail to participate in the study. Spontaneously conceived children born from parents who previously visited the Department of Gynecology of the VUmc with fertility problems (i.e. no conception after at least 1 year of frequent unprotected intercourse at the time of their first visit to the fertility clinic) were used as controls. For each participating

IVF child, a control child of same gender and similar age ( $\leq 3$  month's age difference) was identified. If a matched control child did not want to participate, the control recruitment process was repeated until an appropriate control child was found that did agree to participate. Between March 2003 and March 2006, 69% of the 354 IVF children and 51% of the 454 controls who were approached agreed to participate, resulting in 233 matched IVF-control pairs.

### Data collection

Anthropometric measurements of all participating children were obtained during a visit to the VUmc to evaluate their growth and development. Shortly before the hospital visit, a questionnaire was sent to the parents to gather information on various demographic, lifestyle and medical factors including cause of subfertility, parental education level and birthweight and gestational age of the respective child. In addition, parents were asked to bring the original post-natal growth chart of their child to the hospital visit. In the Netherlands, virtually all children undergo regular periodic health examinations by health professionals of the Municipal Health Services during the first years of life. Some parents fulfilled the request to send a copy in case they forgot to bring the growth chart. In total, post-natal growth charts of 394 children were provided by the parents. Post-natal growth data were only used for statistical analysis when the child had been measured more than three times between birth and the age of 4 years. No differences in birthweight or socioeconomic status were found between children with ( $n = 392$  across age groups) versus without (sufficient) post-natal growth data ( $n = 74$ ). Children without sufficient data were more often born preterm (16 versus 8%,  $P = 0.04$ ), but these findings were observed in both the IVF and control population. Post-natal growth measurements were expressed as standard deviation score (SDS) using the 1997 Dutch growth standards (Fredriks *et al.*, 2000).

During the hospital visit at follow-up in the VUmc, blood pressure was measured twice at the non-dominant arm in the sitting position using an automatic device with appropriate cuff size (Dinamap PRO 100, Criticon, Munich, Germany). Skinfold thickness measurements (triceps, biceps, subscapular and supra-iliac) were collected in triplicate by means of a Harpenden caliper. Birthweight, either extracted from VUmc birth certificates (49%) or outpatient clinic reports (38%), or self-reported by the parents (13%), was expressed as SDS to correct for gestational age and gender (Niklasson *et al.*, 1991). Gestational age was obtained using parental recall. Mean age ( $\pm$  SD) at follow-up of IVF children and controls was  $12.2 \pm 2.6$  years. No differences in pubertal stage according to Tanner were found between IVF and control children (prepubertal: 27 versus 29%; postpubertal: 21 versus 18%, respectively) (Tanner and Whitehouse, 1976). Twins were not eligible to participate in the study. The study protocol was approved by the ethics committee of the VUmc and by the National Medical Ethics Committee known as the 'Centrale Commissie Mensgebonden Onderzoek' located in The Hague, the Netherlands. All participating children and their parents gave written informed consent.

### Statistical analysis

Post-natal growth from shortly after birth up to 4 years of age of IVF children and controls was compared by means of general estimation

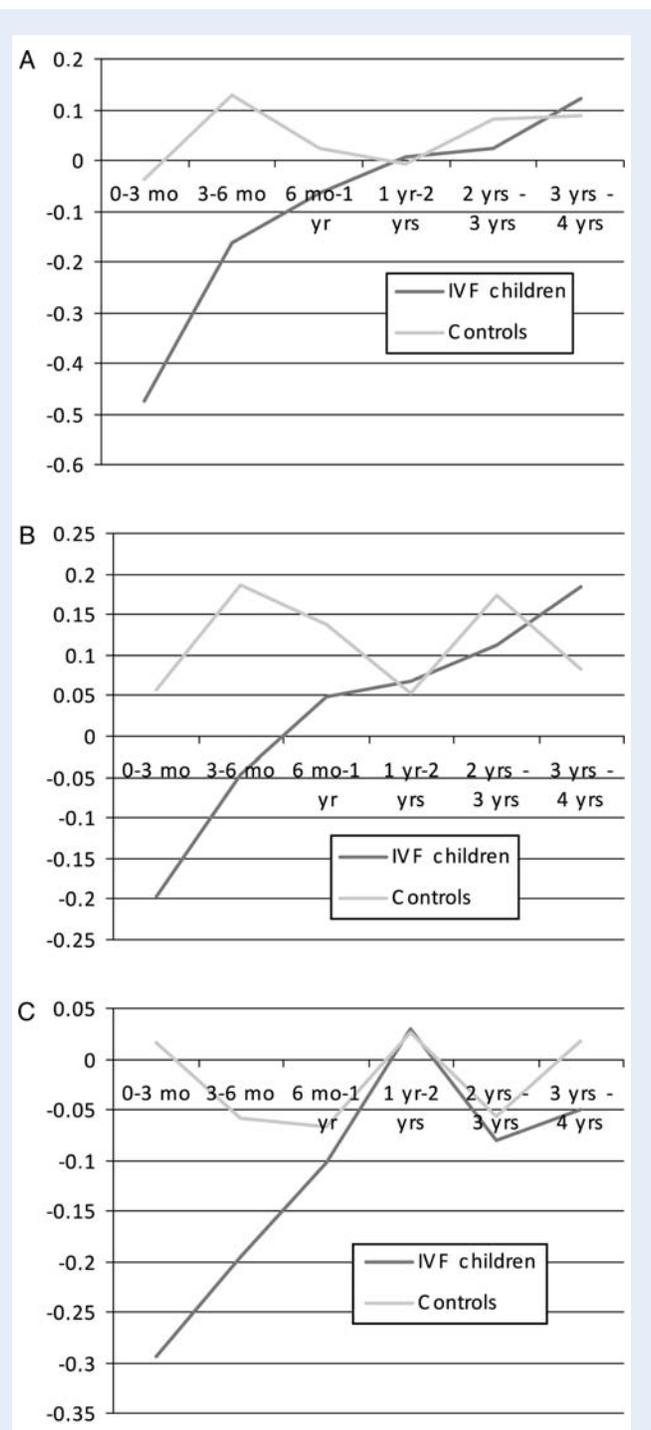
equation (GEE) analyses. This regression technique adjusts for dependency of several measurements within one individual and is capable of dealing with missing data (Twisk, 2004). Post-natal growth of children was also cross-sectionally analyzed at 3 months, at 6 months, at 1 year of age and during the second and third year of life. Weight gain during late infancy (weight SDS at 1 year of age minus weight SDS at 3 months) and during early childhood (weight SDS at 3 years of age minus weight SDS at 1 year) were calculated. In addition to these calculations using the Dutch growth standards, weight gain during early infancy was examined (weight SDS at 3 months minus birthweight SDS). Subsequently, for each of the three periods under examination, weight gain was divided into tertiles of the distribution, as done by Oren *et al.* (2004). The lowest, middle and highest tertiles of weight gain were used as measures for slow ('decelerated'), constant and rapid ('exaggerated') growth during early life. Differences in blood pressure and body fat measures (cardiovascular parameters) at follow up between IVF children with exaggerated growth and IVF children with decelerated growth were compared. To disentangle the effects of perinatal outcome and early post-natal growth on these cardiovascular parameters, additional analyses were performed to correct for birthweight, gestational age and body size at follow-up. The square root of height was used as a measure of body size as suggested by Vanitallie *et al.* (1990). Similar analyses were performed for the control population. Total sum of skinfolds and sum of peripheral skinfolds were not normally distributed and therefore were log-transformed before analysis. A  $P$ -value  $<0.05$  was considered to be statistically significant.

## Results

### Growth characteristics of the study population

Birthweight, birthweight SDS and gestational age were significantly lower in children conceived by IVF than in controls ( $3.2 \pm 0.6$  versus  $3.4 \pm 0.6$  kg,  $P < 0.001$ ;  $-0.15 \pm 1.00$  versus  $0.08 \pm 1.08$ ,  $P = 0.025$ ;  $38.9 \pm 2.5$  versus  $39.5 \pm 1.8$  weeks,  $P = 0.004$ , respectively). Likewise, significantly more IVF children were born prematurely, i.e.  $<37$  weeks of gestation (13 versus 6% in the control group,  $P = 0.015$ ) and had a low birthweight, i.e.  $<2500$  g (11 versus 3.5% in the control group,  $P = 0.004$ ). IVF children had an average of  $16 \pm 4$  growth measurements shortly after birth up to the fourth year of life compared with  $14 \pm 4$  growth measurements in controls ( $P = 0.002$ ). GEE analyses revealed significant differences in post-natal growth parameters between IVF and control children during the first months of life (Fig. 1). Weight SDS and height SDS was significantly lower in IVF children than in controls during the first 6 months of life (0–3 months: weight SDS difference:  $P = 0.001$ ; height SDS difference:  $P = 0.039$ ; 3–6 months: weight SDS difference:  $P = 0.005$ ; height SDS difference:  $P = 0.028$ ). BMI SDS was significantly lower among IVF children compared with control children during the period shortly after birth and 3 months ( $P = 0.004$ ).

Comparison of growth measurements at the different cross-sectional moments demonstrated significant differences in weight SDS, height SDS and BMI SDS at 3 months, and weight SDS at 6 months of age between IVF and control children (Table 1). Gain in weight, height and BMI during late infancy was significantly higher in



**Figure 1** Postnatal measurements of weight ( $n = 5380$ , **A**), height ( $n = 4559$ , **B**) and BMI ( $n = 4540$ , **C**) of 193 IVF and 199 control children.

IVF children as compared with controls (respectively  $P < 0.001$ ,  $P = 0.013$  and  $P = 0.029$ ). No significant differences were found in weight gain during early infancy and weight, height or BMI gain during early childhood between IVF and control children.

Subsequently, we related weight gain during early infancy, late infancy and early childhood to blood pressure levels and skinfold thickness measurements at follow-up in IVF and control children (Tables II

**Table 1** Growth during infancy and early childhood of 193 IVF children and 199 controls, all born to subfertile parents: cross-sectional data

		IVF population	Control population	P-value
Weight	N			
Weight SDS 3 months	388	-0.18 ± 1.17	0.17 ± 1.05	0.002
Weight SDS 6 months	384	-0.13 ± 0.98	0.08 ± 0.86	0.027
Weight SDS 1 year	382	-0.03 ± 0.93	-0.03 ± 0.86	1.0
Weight SDS 2 years	308	0.06 ± 0.90	0.07 ± 0.95	0.9
Weight SDS 3 years	289	0.13 ± 0.91	0.02 ± 0.92	0.3
Height	N			
Height SDS 3 months	381	-0.06 ± 1.09	0.17 ± 1.17	0.045
Height SDS 6 months	379	0.02 ± 1.08	0.20 ± 0.97	0.095
Height SDS 1 year	380	0.07 ± 1.06	0.08 ± 0.97	1.0
Height SDS 2 years	297	0.11 ± 1.01	0.22 ± 1.04	0.3
Height SDS 3 years	284	0.16 ± 1.05	0.06 ± 0.99	0.4
BMI	N			
BMI SDS 3 months	355	-0.22 ± 1.08	0.01 ± 1.02	0.041
BMI SDS 6 months	373	-0.24 ± 0.98	-0.12 ± 0.95	0.2
BMI SDS 1 year	376	-0.02 ± 0.96	-0.03 ± 0.94	1.0
BMI SDS 2 years	292	-0.01 ± 0.93	-0.08 ± 1.05	0.5
BMI SDS 3 years	280	-0.003 ± 0.87	-0.05 ± 0.95	0.7
Weight gain				
Δ weight SDS 0–0.25 year	388	-0.04 ± 1.15	0.15 ± 1.06	0.08
Δ Weight SDS 0.25–1 year	379	0.15 ± 0.94	-0.20 ± 0.86	<0.001
Δ Weight SDS 1–3 years	285	0.15 ± 0.72	0.11 ± 0.67	0.6
Height gain				
Δ Height SDS 0.25–1 year	371	0.15 ± 0.84	-0.07 ± 0.82	0.013
Δ Height SDS 1–3 years	280	0.10 ± 0.79	0.01 ± 0.78	0.3
BMI gain				
Δ BMI SDS 0.25–1 year	343	0.21 ± 1.03	-0.04 ± 1.02	0.029
Δ BMI SDS 1–3 years	280	0.02 ± 0.92	0.03 ± 0.99	0.9

Continuous variables were analyzed using Student t-test. SDS: standard deviation score, 0–0.25 year = early infancy, 0.25–1 year = late infancy, 1–3 years = early childhood.

and III). It was demonstrated that IVF children with rapid weight gain during late infancy did not differ with regard to blood pressure and sum of skinfolds at follow-up from IVF children with slow weight gain during late infancy. In contrast, rapid weight gain among controls in late infancy was associated with significantly increased skinfold thickness compared with controls with slow growth. Furthermore, rapid weight gain during early childhood in IVF children appeared to be related to higher blood pressure levels at follow-up, independently of birthweight, gestational age and height at follow-up, but not in controls. Weight gain during early infancy was not related to blood pressure or skinfold thickness in IVF children and controls. In both IVF and control children, rapid growth during early childhood was related to significantly higher sum of skinfolds at follow-up.

## Discussion

This study is the first to examine growth velocity during early post-natal life in relation to cardiovascular risk factors in 8–18-year-old

IVF and control children. IVF children had lower birthweight and showed a significantly greater gain in weight SDS, height SDS and BMI SDS during late infancy as compared with controls. Interestingly, exaggerated weight gain during late infancy was associated with increased skinfold thickness at follow-up in controls but not in IVF children. However, rapid weight gain during early childhood was related to higher blood pressure levels at follow-up, independent of birthweight, gestational age and body size at follow-up, among IVF children in contradiction to controls. Early childhood weight gain appeared to correlate with follow-up skinfold thickness in both IVF and control children.

In the present study, weight, height and BMI of IVF children were significantly lower shortly after birth compared with controls. Approximately 6 months after birth, the anthropometric differences observed between the IVF and control newborns were no longer present. Our findings are in line with several other studies during the past years that have addressed post-natal growth of IVF children. Normal weight and height parameters in IVF children ranging from 1 to 13 years were

**Table II** Weight gain during early infancy ( $\Delta$  weight SDS 0–0.25 year), late infancy ( $\Delta$  weight SDS 0.25–1 year) and early childhood ( $\Delta$  weight SDS 1–3 years) in relation to blood pressure at follow-up in IVF and control children, all born to subfertile parents

	Systolic blood pressure (mmHg)				Diastolic blood pressure (mmHg)			
	IVF children	P-value <sup>#</sup>	Controls	P-value <sup>#</sup>	IVF children	P-value <sup>#</sup>	Controls	P-value <sup>#</sup>
$\Delta$ Weight SDS 0–0.25 year								
Lowest tertile	109 $\pm$ 11	0.8	106 $\pm$ 10	0.6	61 $\pm$ 8	0.3	59 $\pm$ 7	0.5
Middle tertile	108 $\pm$ 11		104 $\pm$ 9		62 $\pm$ 7		59 $\pm$ 6	
Highest tertile	111 $\pm$ 11		105 $\pm$ 11		61 $\pm$ 6		58 $\pm$ 6	
$\Delta$ Weight SDS 0.25–1 year								
Lowest tertile	110 $\pm$ 13	0.4	104 $\pm$ 10	0.5	61 $\pm$ 6	0.6	59 $\pm$ 6	0.4
Middle tertile	106 $\pm$ 10		104 $\pm$ 10		60 $\pm$ 8		58 $\pm$ 6	
Highest tertile	111 $\pm$ 10		107 $\pm$ 12		62 $\pm$ 7		59 $\pm$ 7	
$\Delta$ Weight SDS 1–3 year								
Lowest tertile	106 $\pm$ 11	0.014	104 $\pm$ 12	0.6	59 $\pm$ 6	0.04	59 $\pm$ 7	0.2
Middle tertile	109 $\pm$ 9		103 $\pm$ 11		62 $\pm$ 8		58 $\pm$ 6	
Highest tertile	112 $\pm$ 13		108 $\pm$ 10		62 $\pm$ 8		61 $\pm$ 7	

<sup>#</sup>Lowest versus highest tertile after adjustment for birthweight, gestational age, gender, age at follow-up and height at follow-up.

**Table III** Weight gain during early infancy ( $\Delta$  weight SDS 0–0.25 year), late infancy ( $\Delta$  weight SDS 0.25–1 year) and early childhood ( $\Delta$  weight SDS 1–3 years) in relation to skinfold thickness at follow-up in IVF and control children, all born to subfertile parents

	Peripheral sum of skinfolds (mm)				Total sum of skinfolds (mm)			
	IVF children	P-value <sup>#</sup>	Controls	P-value <sup>#</sup>	IVF children	P-value <sup>#</sup>	Controls	P-value <sup>#</sup>
$\Delta$ Weight SDS 0–0.25 year								
Lowest tertile	20.4 $\pm$ 9.5	0.5	21.1 $\pm$ 10.3	0.9	37.8 $\pm$ 18.3	0.4	39.3 $\pm$ 20.3	0.8
Middle tertile	23.4 $\pm$ 11.2		19.0 $\pm$ 8.2		42.0 $\pm$ 22.5		35.1 $\pm$ 16.5	
Highest tertile	20.9 $\pm$ 9.3		19.2 $\pm$ 8.0		38.6 $\pm$ 18.3		36.7 $\pm$ 16.3	
$\Delta$ Weight SDS 0.25–1 year								
Lowest tertile	20.3 $\pm$ 8.1	0.3	18.1 $\pm$ 8.2	0.002	36.9 $\pm$ 15.7	0.3	33.5 $\pm$ 15.6	0.003
Middle tertile	21.5 $\pm$ 11.5		19.7 $\pm$ 9.7		38.7 $\pm$ 22.2		37.5 $\pm$ 20.0	
Highest tertile	22.6 $\pm$ 10.4		22.1 $\pm$ 8.4		42.1 $\pm$ 20.8		41.8 $\pm$ 17.2	
$\Delta$ Weight SDS 1–3 year								
Lowest tertile	18.9 $\pm$ 7.6	0.005	17.3 $\pm$ 6.1	0.01	34.5 $\pm$ 15.0	0.003	31.7 $\pm$ 12.1	0.005
Middle tertile	19.7 $\pm$ 8.5		17.9 $\pm$ 7.0		34.8 $\pm$ 14.7		33.3 $\pm$ 14.1	
Highest tertile	24.9 $\pm$ 12.9		22.8 $\pm$ 12.0		46.7 $\pm$ 25.8		44.3 $\pm$ 23.9	

<sup>#</sup>Lowest versus highest tertile after adjustment for birthweight, gestational age, gender, age at follow-up and height at follow-up.

reported previously (Brandes et al., 1992; Saunders et al., 1996; Olivennes et al., 1997; Wennerholm et al., 1998; Place and Englert, 2003; Bonduelle et al., 2005; Kai et al., 2006; Knoester et al., 2008). Only one Finnish population-based cohort study reported that growth in IVF singletons was still behind the controls at the age of 3 years despite a catch-up growth during the first year of life (Koivurova et al., 2003).

We hypothesize that the exaggerated growth of IVF children during infancy, specifically observed between 3 and 12 months after birth, is a physiological and compensatory process to promote the restoration of

the infants' genetic growth trajectory after a period of prenatal growth restraint due unfavorable environmental conditions. This concept is supported by a recent study examining post-natal changes in body fat measures in infants who previously experienced fetal growth retardation (Beltrand et al., 2009). Catch-up growth appeared to correlate to the fetal growth pattern itself, irrespective of birthweight, and not to hyperphagia during infancy. Furthermore, growth velocity returned to physiological values when body composition was restored. An alternative explanation could be that the catch-up growth in IVF children during late infancy is primarily dependent upon post-natal

nutritional environment. Adaptations to adverse conditions during early prenatal life may predispose to rapid post-natal weight gain in a more favorable post-natal environment. Furthermore, it should be explored why IVF children seem to catch-up in weight during late infancy and not directly after birth.

To our knowledge, it is currently unknown during which phase of prenatal life growth retardation in IVF children is caused and, importantly, which factors are responsible. As longitudinal information on fetal growth is rarely available, for most studies, including ours, birthweight is often the only feasible measure. Nevertheless, it is important to emphasize that environmental stimuli may affect embryonic and/or fetal growth trajectories without an effect on birthweight (Bloomfield *et al.*, 2006). It remains to be established if parental characteristics, technical aspects of IVF treatment or a combination of these are involved in the induction of these aberrant prenatal growth patterns and subsequent accelerated post-natal growth.

Currently, there is still debate as to whether there are critical time windows during early post-natal life that are important in determining later blood pressure and body composition. Infancy is often hypothesized to represent an important period, because it is a time of extremely rapid growth, particularly for those infants who experienced fetal growth restriction (Belfort *et al.*, 2007). In the present study, rapid weight gain during late infancy was associated with higher body fat measures in controls. These findings are in accordance with studies linking rapid weight gain during infancy to an increased risk for obesity in childhood and young adulthood (Ong *et al.*, 2000; Stettler *et al.*, 2003). Several cohort studies demonstrated a positive association between weight gain in the first year of life and later blood pressure (Forsen *et al.*, 1998; Jarvelin *et al.*, 2004). However, despite the significantly faster growth during late infancy observed among IVF children as compared with controls, weight gain during infancy was not associated with blood pressure or skinfold measurement at ages 8–18 years in IVF children. Our data emphasize that the catch-up growth during infancy is not accompanied by detrimental consequences on blood pressure and body composition during late childhood and adolescence in IVF offspring. IVF children may follow an optimal pathway of healthy catch-up growth during infancy: such pathways have recently been suggested, although they are considered to be rare or narrow, in view of the accumulating body of evidence for detrimental effects of rapid catch-up growth in general (Ong and Loos, 2006; Druet and Ong, 2008). A recent study supporting this concept showed that rapid weight gain in fetal growth restricted infants promoted the restoration of body size and fat stores without detrimental consequences at 1 year of age on body composition or metabolic profile (Beltrand *et al.*, 2009). On the other hand, rapid weight gain was not related to blood pressure in our controls, and this lack of association has been reported by others (Law *et al.*, 2002). Conflicting results regarding the relationship between rapid weight gain in infancy and blood pressure could occur as a result of differences in characteristics of the study subjects or method of analysis (Law *et al.*, 2002).

Our findings highlight the critical influence of early childhood, rather than infancy, on weight gain in IVF children. Significant associations between childhood weight gain and systolic blood pressure at follow-up were found in IVF children, irrespectively of perinatal outcome and body size at follow-up. Such associations were not found in the control children. Our findings indicate that particularly growth during early childhood programs later systolic blood pressure

in IVF offspring. We propose that IVF children could show different types of rapid growth during early post-natal life, with distinct health effects. Different processes may be involved during rapid weight gain in infancy and early childhood. It is important to realize that catch-up growth is not always similar to weight gain (Hindmarsh, 2004; Beltrand *et al.*, 2009). Catch-up growth indicates weight gain appropriate for height gain, whereas excessive weight gain as such does not necessarily mirror changes in height. This distinction could explain some discordant results on the relationship between growth measurements during early post-natal life and subsequent metabolic risk (Beltrand *et al.*, 2009). The mechanisms linking early childhood growth to later cardiovascular risk factors are not yet known, and will likely include interactions between antenatal growth restraint, post-natal nutrition and genetic factors (Druet and Ong, 2008). Further research is necessary to find out whether these aspects could explain the association between rapid weight gain during early childhood and higher blood pressure at age 8–18 years in IVF children, or whether other mechanisms are involved.

One of the strengths of this study lies in the selection of the control children, which were born from parents previously diagnosed with subfertility. To adequately examine post-natal growth and development in IVF children, an appropriate comparison group of children who were conceived without use of assisted reproduction technology was needed. It is generally known that IVF parents differ from the general reproductive population in terms of age, parity and other important characteristics. Specifically, there are several studies indicating that subfertility itself, independent from the mode of conception, is a risk factor for adverse neonatal outcome and pregnancy complications (Basso *et al.*, 2003; Wang *et al.*, 2004; Romundstad *et al.*, 2008). Studies which compare growth and development of IVF children to spontaneously conceived children from fertile couples should always keep in mind a potential effect of the underlying subfertility in the IVF group (Knoester *et al.*, 2008). To avoid confounding related to these known differences, comparison with children born to subfertile parents after spontaneous conception was preferred. Our study sample, based on availability of sufficient early growth measurements and follow-up measurements, did not differ regarding birthweight from the larger study cohort. However, there appeared to be a significant difference in the number of children born preterm. This phenomenon was found in both the IVF and the control population. Therefore, it is unlikely that the comparisons performed between IVF and control children in the present study have been confounded, but it might have implications for the generalizability of our findings. Body fat composition differs between prepubertal and postpubertal children, and between boys and girls. One could argue whether the combined analysis of pre- and postpubertal children as well as boys and girls is appropriate. Our previous study (Ceelen *et al.*, 2007) demonstrated that differences in body fat composition between IVF and control children could not be explained by pubertal stage or gender. Therefore, data regarding blood pressure and body fat of children aged 8–18 years were combined in the present study. As previously discussed, it has to be taken into account that especially peripheral adipose tissue was increased in IVF children, although increased risk for cardiovascular health problems has been indicated to be primarily linked to a central body fat deposition (Ceelen *et al.*, 2007). However, in view of our other findings demonstrating higher blood pressure and fasting glucose levels in IVF children

compared with controls (Ceelen et al., 2008), continued body fat monitoring in IVF offspring is of great importance. Lastly, we might have underestimated the true link between early post-natal growth and cardiovascular risk factors at follow-up among IVF children by correcting for birthweight, gestational age and body size at follow-up. IVF is known to be associated with lower birthweight and shorter gestational age (Helmerhorst et al., 2004; Jackson et al., 2004), although these factors themselves have been found to relate to early post-natal growth, blood pressure and body fat composition.

In conclusion, late infancy growth velocity of IVF children was significantly higher compared with controls. However, growth during this period has not been associated with an adverse cardiovascular profile at follow-up in IVF children. In contrast, weight gain during early childhood seems to predict cardiovascular risk factors in IVF children. These results suggest that caution in promoting excessive early childhood growth among IVF children is necessary. Preventive health strategies might be useful in the future to moderate early post-natal growth of IVF children by taking into account the possible benefits of early growth, such as improved cognition, as well as the potential harms to cardiovascular and metabolic health. However, as this study is the first to examine the associations of early post-natal growth in relation to blood pressure and body fat composition in IVF and control children, our results first need to be reproduced by other prospective follow-up studies.

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## Health of children born after ovulation induction

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**Objective:** To study the health of children born after ovulation induction (OI).

**Design:** Nationwide register-based study.

**Setting:** The OI children were followed up to the age of 4 years and compared with other children.

**Patient(s):** The OI children (N = 4,467). Two control groups: all other children (excluding children born after IVF, N = 190,398) and a random sample of those children (n = 26,877).

**Intervention(s):** Ovulation induction treatment in ordinary practice.

**Main Outcome Measure(s):** Mortality rates and adjusted odds ratios for perinatal outcomes, hospitalizations, health-related benefits, and long-term medication use.

**Result(s):** A total of 12% of OI and 2% of control children were multiples. Even after stratifying for multiplicity and adjusting for the available confounding factors (region, smoking, maternal age, socioeconomic position, and parity for perinatal health and mother's socioeconomic position for other indicators), most indicators showed worse health among OI children compared with control children. The OI children had poorer perinatal health and more episodes of long hospitalization than the control children. Singleton OI children had more long-term illnesses in childhood, as measured by child disability allowance, long-term medication use, and hospital care episodes.

**Conclusion(s):** Either OI treatment or the reasons for the treatment increase the risk of health problems in early childhood. (Fertil Steril® 2010;93:1157–68. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Ovulation induction, perinatal health, childhood health, morbidity, register-based study

Ovulation induction (OI) has been a common infertility treatment for many decades, even after the introduction of IVF in the late 1970s. Ovulation induction and ovarian (hyper)stimulation are two methods of hormonal treatment used in ovarian stimulation (1), and both can be combined with intrauterine insemination (IUI). In Finland, an estimated 2.5% of infants in the late 1990s were born after OI (2). More recent data do not exist, because the proportion of OI children is not registered. This figure is the same as for Denmark in 2002 of 2.3% (3).

Regardless of the wide and long-term use of OI, very little is known of the health of children born after it. Studies on perinatal health show that OI children are more often premature and/or of low birth weight, and/or were more often

treated in the neonatal intensive care unit than naturally conceived children (4–9); in all studies some kind of hormonal stimulation was used with or without IUI.

Results on congenital anomalies are inconclusive. Three relatively small-sized studies reported no statistically significant difference in the occurrence of congenital anomalies between OI and control children (6, 10, 11). However, two larger studies showed an increased risk for congenital anomalies (12, 13), but the risk was partly explainable by plurality (12) or underlying infertility or its determinants (13).

Sufficiently large long-term follow-up studies beyond the perinatal period are lacking. This article reports the health effects for OI children up to 4 years of age, separately for singletons and multiples, based on a combination of data from several population-based registers.

### MATERIALS AND METHODS

This study is based on children whose mothers were treated with OI (including here ovulation induction and low stimulation with or without insemination) in 1996 to 1998 in Finland (12, 14–16). The OI women were identified with a pre-designed algorithm from the drug reimbursement files of the Social Insurance Institution (SII) (14). The children born after OI (N = 4,467) and their perinatal health were obtained from the Finnish Medical Birth Register (MBR) (12, 15,

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Some results of this study have been presented at The Nordic Meeting on Register-based Health Research, Helsinki, Finland, September 22–23, 2005.

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16) using women's personal identification (ID) numbers and the children's dates of birth as the linkage keys. The quality of the MBR is high for the variables used in this study (17).

The identified children were linked to four other nationwide registers by children's ID numbers: the Cause-of-Death Register, the Hospital Discharge Register (HDR), the Care Register for Social Welfare, and reimbursement files of health-related social benefits from the SII.

As controls, two groups of children were selected from the MBR: all children other than OI and IVF children who have been conceived during the same time period ( $N = 190,398$ ) and a random sample among the live borns in the first control group (size of the group = 26,877). The second control group was made to reduce the workload caused by large registry linkages, and its size was threefold the number of IVF and OI children in the cohort. Stillbirths were excluded.

Information on use of health care services during the pregnancy and child birth and on infant outcomes, first including all children and then including first births only, were gathered from the MBR. As infant health outcomes we used very low/low birth weight (under 1,500/2,500 g), very preterm/preterm birth (under 32/37 weeks), low 1-minute Apgar scores (0–6), treatment in an intensive care/neonatal surveillance unit, need for respiratory treatment, hospitalization of the child 7 or more days after birth, and perinatal mortality.

Information on all hospitalizations (hospital episodes with information on admission and discharge date and diagnosis according to 10th revision of the International Classification of Diseases, ICD-10 codes) before the age of 4 years were received from the 1996 to 2003 HDR. From the Care Register for Social Welfare we received information on numbers of OI children having at least one period of care in social institutions up to the end of 2004, most often because of severe intellectual disabilities. We compared the rates of institutionalized OI children to national rates of children born in 1997 to 1998, excluding the number of children born after IVF and OI. Information on both child disability allowance and long-term medication use (excluding cow's milk intolerance) in 1996 to 2001 before the age of 2 years was gathered from the SII. From the Cause-of-Death Register we received the number and causes of deaths of all children before the age of 2 years in 1996 to 2001.

Finally, we combined information from the different data sources for eight diseases or conditions (Table 4). We calculated the number of children who had used services up to age of 2 years recorded in the three registers; the registers defined diseases and conditions by the ICD-10 classification.

The differences between the OI and control groups were tested with a chi-square test,  $t$  test for relative proportions, and with logistic regression analyses, adjusting for available background characteristics (region, smoking, maternal age, marital status, socioeconomic position at birth, and parity for perinatal outcomes and socioeconomic position for hospi-

talizations, reimbursement of long-term medication, child disability allowance, and for the combined analysis). The adjustment for other factors was not possible because of the small number of cases. The socioeconomic position of the women was defined by using their own occupation at delivery. All analyses were also made separately for singletons and multiples.

The study plan was approved by the STAKES research ethics committee. For register linkages, the National Data Protection Authority was consulted and permissions from the register keepers were obtained.

## RESULTS

Of the 4,467 OI children 490 (11%) were twins and 51 (1.1%) were from triplet pregnancies. Among the 190,398 control children 4,143 (2.2%) were twins and only 39 were triplets (<0.01%). OI mothers were somewhat older, more often married, and from a higher socioeconomic position than the other mothers (Table 1). They were less frequently smokers and more often primipara.

Compared with other mothers, OI mothers were more often hospitalized during pregnancy, gave birth by Caesarean section, and had long hospital stays after the delivery (Table 2). Adjustment for mothers' background characteristics did not change the results. Results for singletons were similar than for all OI children. For OI multiple pregnancies, a statistically significant higher risk was found only for hospitalization during pregnancy.

The indicators of perinatal health showed worse health for OI children (Table 2). The perinatal health of OI singletons was worse than that of control singletons. Among OI multiples, risks were increased less and only the differences for low birth weight, special care, and respiratory treatment were statistically significant. When the analysis was restricted to twins, the statistical significance disappeared: the adjusted odds ratios (95% confidence interval [CI]) were 0.96 (0.78–1.18) for low birth weight rate, 1.09 (0.89–1.38) for special care, and 1.23 (0.87–1.74) for respiratory treatment.

No statistically significant difference in the rate of stillbirth was found among OI children compared with control children (4.3/1,000 vs. 3.9/1,000). The main causes of stillbirths were related to conditions originating in the perinatal period (chapter P in the ICD-10 classification). The three most common causes of death were P95 (fetal death of unspecified cause), P20 (intrauterine hypoxia) and P50 (fetal blood loss). For live births, the total mortality rate up to age of 2 years was 1.8-fold higher among OI children compared with control children (7.2/1,000 vs. 4.1/1,000,  $P = .001$ , test for relative proportions), but this was explained by plurality. Among singletons, the main causes were congenital anomalies and conditions originating in the perinatal period. Among multiples the main causes were conditions originating in the perinatal period.

**TABLE 1****Mother's background characteristics by plurality and group (ovulation induction and control mothers).<sup>a</sup>**

	All			Singleton births			Multiple births		
	OI (n = 4,188)	Controls (n = 188,298)	P value	OI (n = 3,926)	Controls (n = 186,216)	P value	OI (n = 262)	Controls (n = 2,084)	P value
Maternal age at delivery, y (%) <sup>b</sup>									
Mean ( $\pm$ SD) <sup>c</sup>	31.2 $\pm$ 4.6	29.7 $\pm$ 5.3	< .001	31.2 $\pm$ 4.6	29.7 $\pm$ 5.3	< .001	30.6 $\pm$ 4.3	30.5 $\pm$ 5.2	.719
<25	10.6	22.4		10.6	22.4		11.1	17.4	
25–29	36.2	33.6		36.1	33.7		38.2	31.7	
30–34	35.6	29.4		35.6	29.4		35.5	33.2	
35–39	14.4	12.2		14.5	12.2		13.7	15.4	
40 or more	3.1	2.3	< .001	3.2	2.3	< .001	1.5	2.3	.015
Marital status (%) <sup>b</sup>									
Married or cohabiting	93.8	87.5		93.8	87.5		93.9	86.4	
Single	5.3	10.6		5.3	10.6		5.3	11.3	
Unknown	0.9	1.9	< .001	0.9	1.9	< .001	0.8	2.4	.003
Socioeconomic position (%) <sup>b</sup>									
Upper white-collar	20.6	15.1		20.7	15.1		18.7	17.0	
Lower white-collar	47.3	40.6		47.3	40.6		48.1	40.3	
Blue-collar	14.0	17.0		14.0	17.0		15.3	16.1	
Others	11.3	18.5		11.2	18.5		12.2	18.2	
Unknown	6.8	8.9	< .001	6.8	8.9	< .001	5.7	8.4	.030
Smoked during pregnancy (%) <sup>d</sup>	6.7	14.8	< .001	6.7	14.8	< .001	5.3	16.6	< .001
First birth (%) <sup>d</sup>	54.7	39.5	< .001	54.4	39.5	< .001	58.1	35.6	< .001

Note: OI = ovulation induction.

<sup>a</sup> Control group consisting of all other mothers (excluding mothers of IVF children) whose children were fertilized in the same time period as OI children.

<sup>b</sup> For chi-square tests between OI and controls.

<sup>c</sup> For t-test in comparisons between OI and controls.

<sup>d</sup> For t-test for relative proportions in comparisons between OI and controls.

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TABLE 2

Proportions (%) and odds ratios<sup>a</sup> (95% confidence intervals) of treatments and infant outcomes, by plurality and group.

	Total			Singletons			Multiples		
	No. or proportion OI	Controls	OR (95% CI)	No. or proportion OI	Controls	OR (95% CI)	No. or proportion OI	Controls	OR (95% CI)
N									
Deliveries, n	4,188	188,298		3,926	186,216		262	2,084	
Infants, n	4,467	190,398		3,926	186,216		536	4,182	
Mother, %									
Hospital treatment during pregnancy	31.8	20.6	1.75 (1.64–1.88)	29.4	20.2	1.61 (1.50–1.72)	66.4	54.2	1.61 (1.21–2.15)
Hospitalization after delivery ≥ 7 days	8.7	4.5	1.49 (1.32–1.67)	6.9	4.2	1.22 (1.07–1.39)	40.5	31.7	1.16 (0.84–1.60)
Caesarean section	22.4	15.3	1.34 (1.25–1.45)	20.9	15.0	1.25 (1.15–1.35)	45.8	41.8	1.09 (0.83–1.43)
Infant, %									
Very preterm (<32 gw)	2.3	0.9	2.40 (1.95–2.96)	1.2	0.8	1.52 (1.13–2.04)	9.7	7.0	1.24 (0.89–1.72)
Preterm (<37 gw)	11.2	5.5	2.03 (1.84–2.24)	6.4	4.7	1.32 (1.15–1.50)	45.4	42.2	0.95 (0.78–1.15)
Birth weight <1,500 g	2.4	0.8	2.79 (2.28–3.41)	1.3	0.7	1.78 (1.33–2.38)	10.6	7.4	1.27 (0.92–1.73)
Birth weight <2,500 g	9.9	4.0	2.55 (2.30–2.82)	4.7	3.2	1.43 (1.23–1.67)	47.9	39.2	1.23 (1.01–1.49)
Apgar score 0–6	6.5	4.4	1.36 (1.20–1.54)	5.2	4.2	1.11 (0.97–1.28)	16.5	12.5	1.20 (0.93–1.57)
Special care <sup>b</sup>	15.1	8.2	1.81 (1.67–1.97)	10.9	7.6	1.35 (1.22–1.50)	45.7	36.0	1.27 (1.05–1.54)
Respiratory treatment	2.6	1.1	2.29 (1.88–2.78)	1.5	0.9	1.46 (1.11–1.91)	10.5	6.7	1.40 (1.02–1.92)
Hospitalization ≥ 7 d	12.8	6.4	1.88 (1.72–2.07)	8.5	5.8	1.34 (1.19–1.50)	44.0	37.6	1.05 (0.86–1.28)
Perinatal mortality	0.8	0.6	1.33 (0.95–1.85)	0.5	0.5	0.87 (0.55–1.37)	3.1	2.9	1.05 (0.61–1.79)

Note: OR = odds ratio; CI = confidence interval; OI = ovulation induction.

<sup>a</sup> Adjusted for mother's region, smoking, age, marital status, previous births, socioeconomic position.

<sup>b</sup> Treatment in intensive care unit or in newborn surveillance unit. Reference group (OR = 1) = controls.

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**TABLE 3**

**Raw proportions (%) of children and adjusted odds ratios<sup>a</sup> (95% confidence intervals) of having any period of child disability allowance or any long-term medication use until the age of 2 years, by plurality and group.**

	All			Singletons			Multiples		
	No. or proportion		OR (95% CI)	No. or proportion		OR (95% CI)	No. or proportion		OR (95% CI)
	OI	Controls		OI	Controls		OI	Controls	
No. of children	4,448	26,877		3,912	28,296		536	581	
Any child disability allowance	12.7	9.9	1.37 (1.24–1.51)	12.4	9.5	1.35 (1.21–1.50)	14.6	13.1	1.14 (0.81–0.61)
Any long-term medication use <sup>b</sup>	3.8	2.8	1.37 (1.15–1.62)	3.7	2.8	1.34 (1.11–1.61)	4.9	4.5	1.08 (0.62–1.89)

Note: OI = ovulation induction; OR = odds ratio; CI = confidence interval.

<sup>a</sup> Adjusted for mother's socioeconomic position. Reference group (OR = 1) = controls.

<sup>b</sup> Reimbursement for cow's milk or soy milk intolerance were excluded.

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According to the Care Register for Social Welfare Institutions, 2.9 out of 1,000 OI children had had at least one period of institutional care at social welfare institutions. For other children born in 1997 and 1998, the rate was similar, at 2.7 per 1,000 children.

Up to the age of 2 years, a larger proportion of OI children and separately OI singletons received child disability allowance than controls (Table 3). This was also true when inspected by plurality, but only the results for singletons remained statistically significant. The most common reasons (by ICD-10 classification) for receiving child disability allowance were the same for OI and control singletons: diseases of the skin and subcutaneous tissue, the respiratory system, eyes and ears. For multiples the most common reasons included, in addition, certain conditions originating in the perinatal period. OI children (in total and singletons separately) had an increased risk for long-term medication use during the first 2 years of life. Among OI and control children the most common reasons for using medication were the same: asthma and epilepsy.

When combining information from different data sources up to the age of 2 years, all OI children had an increased risk for cerebral palsy, allergy, asthma, and diarrhea (Table 4). With the exception of asthma, this was found also for multiples, but all differences were statistically nonsignificant.

Up to 4 years of age a somewhat larger proportion of OI children were hospitalized, OI children had more often long hospital episodes (7 days or more), and their length of episodes per child was longer than control children (Table 5). For all ages OI children had more hospital episodes than control children, but the difference was most prominent during infancy.

The most common diagnoses recorded as the reason for hospitalization for singletons were diseases of the respiratory system, conditions originating from the perinatal period, infectious, and parasitic diseases, and diseases of the eyes and ears. For multiples, the most common hospitalization diagnoses were conditions originating from the perinatal period. Compared with control children, the risk for being hospitalized was increased among OI singletons for 12 of 16 categories of diseases (according to ICD-10 grouping), even after adjusting for the mother's socioeconomic position. For example, OI had twofold risk for diseases of the circulatory system, neoplasms, and for diseases of the nervous system. Similar increased risks were found also for multiples, but the differences were statistically nonsignificant with the exception of an excess risk of having problems originating from the perinatal period (1.78, 1.47–2.15). In almost every category the proportion of hospitalized children was higher among multiples than among singletons (data not shown).

In the subanalysis for first births, the results were mainly similar to the results for all children: among singleton births, the risk for very preterm birth was not more statistically significant and among multiples none of the risks of perinatal

**TABLE 4**

Raw proportions (per 1,000) of children and adjusted odds ratios<sup>a</sup> of having an allergic or chronic disorder or a common condition (ICD-10 codes) until the age of 2 years, by plurality and group (by any available data source).

	All			Singletons			Multiples		
	No. or proportion OI	Controls	OR (95% CI)	No. or proportion OI	Controls	OR (95% CI)	No. or proportion OI	Controls	OR (95% CI)
Number of children	4,448	26,877		3,912	26,296		538	581	
Cerebral palsy (G80) <sup>b</sup>	3.1	1.4	2.36 (1.27–4.38)	2.6	1.3	2.09 (1.03–4.26)	7.5	5.2	1.23 (0.27–5.55)
Epilepsy (G40–G41) <sup>b</sup>	3.1	2.5	1.26 (0.71–2.25)	3.3	2.5	1.34 (0.74–2.44)	1.9	3.4	0.48 (0.04–5.33)
Behavioral disorders (F80–F98) <sup>b,d</sup>	5.8	4.1	1.45 (0.94–2.22)	6.1	4.1	1.52 (0.97–2.37)	3.7	3.4	0.98 (0.14–6.98)
Diabetes (E10) <sup>c</sup>	0.4	0.5	0.84 (0.19–3.72)	0.5	0.5	1.01 (0.23–4.52)	0.0	1.7	NA
Asthma (J45–J46) <sup>c</sup>	35.7	28.1	1.28 (1.07–1.52)	35.3	27.8	1.28 (1.06–1.54)	39.2	43.0	0.93 (0.51–1.70)
Allergy (L20–L23, L27, L50) <sup>c</sup>	66.5	53.8	1.23 (1.08–1.40)	68.0	54.0	1.25 (1.09–1.43)	56.0	46.5	1.20 (0.70–2.05)
Pneumonia (J12–J18) <sup>c</sup>	12.8	11.4	1.11 (0.84–1.48)	12.3	11.4	1.06 (0.78–1.44)	16.8	8.6	1.92 (0.63–5.82)
Diarrhea (A08–A09) <sup>c</sup>	49.5	38.6	1.30 (1.12–1.61)	45.5	38.1	1.20 (1.02–1.42)	78.4	60.2	1.31 (0.82–2.09)

Note: OI = ovulation induction; CI = confidence interval; OR = odds ratio; HDR = hospital discharge register.

<sup>a</sup> Adjusted for mother's socioeconomic position.

<sup>b</sup> Data sources: the HDR and child-disability allowance.

<sup>c</sup> Data sources: the HDR, long-term medication and child-disability allowance.

<sup>d</sup> Disorders of psychological development and behavioral and emotional disorders.

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TABLE 5

## Use of hospital services until the age of 4 years among OI and control children, by plurality and group.

	Total		Singleton		Multiple	
	OI (n = 4,448)	Controls (n = 189,656)	OI (n = 3,912)	Controls (n = 185,530)	OI (n = 536)	Controls (n = 4,126)
Use of hospital services						
Total number of hospital episodes	4,216	136,782	3,459	131,459	757	5,323
Hospitalized children, %	40	33	38	32	55	49
OR (95% CI) <sup>a</sup>	1.40 (1.32–1.49)	1.00	1.30 (1.22–1.39)	1.00	1.32 (1.10–1.58)	1.00
Time in hospital per child, d <sup>b</sup>	4.6	2.7	3.5	2.6	12.6	9.9
Proportion of long hospital episodes (⇒ 7days), % <sup>c</sup>	14	11	12	10	28	24

Note: OI = ovulation induction; OR = odds ratio; CI = confidence interval.

<sup>a</sup> Adjusted for mother's socioeconomic position. Reference group (OR = 1) = controls.

<sup>b</sup> For t-test:  $P < .001$  in all comparisons.

<sup>c</sup> For t-test of relative proportions:  $P < .001$  for OI in total and OI singletons and  $P < .05$  for OI multiples compared to controls.

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outcomes were statistically significant (Table 6). However, perinatal mortality of OI children reached a borderline significance. Furthermore, some of the increased risks for childhood morbidity (Table 6), long-term medication use, and child disability allowance (data not shown) could be found also among OI singleton births.

Compared with our previous results of IVF children (16) OI children had better perinatal health than IVF children (Appendix). However, some health indicators in the early childhood showed worse health among OI than among IVF children.

## DISCUSSION

Most indicators showed worse health effects among OI children compared with control children, even with stratifying for multiplicity. They had poorer perinatal health and more periods of long hospitalization than control children. Singleton OI children had more long-term illnesses in childhood. The occurrence of less-serious diseases cannot be estimated from the registers, because the use of outpatient care is not registered unless it includes an operation.

Possible sources of bias in our study could be identification and misclassification of children, the quality of the registers used in the study, different thresholds in hospital admission between the groups, use of nonregistered health care services, or parents' ability to apply for available health-related benefits (16). In brief, the number of missing

or misclassified children is not likely to be large (14–16). Once a reimbursement application for a used service has been made, it is correctly recorded by SII. For prescribed drugs, reimbursement is made in pharmacies at the time of purchase, and information transferred electronically to SII. There is no information of whether OI drugs were actually used, but for subfertile women having a child soon after buying the drug, the use is very likely. Proportion of OI women in the control groups (drugs bought abroad or reimbursement not wished for) is very small among the large number of women.

Most data on perinatal health received from the MBR (17, 18) and data on deaths are reliable. Other outcome measures depend on service use (using the purchased and reimbursed drugs, seeking care or an application for benefits); technically the registers are considered to be of good quality (19).

It is possible that parents of OI children searched care more or less frequently than other parents. Care-seeking is likely to depend on the socioeconomic position of the parents. Our results were adjusted for socioeconomic position, but there may have been some residual confounding.

Because OI children did not have an increased rate of hospitalizations in all 16 categories of diseases, it is unlikely that varying concern of parents or lower threshold for hospitalization could solely explain the higher frequency of visits; the higher frequency very likely reflects higher morbidity among singleton OI children.

TABLE 6

Proportions (%) and odds ratios<sup>a</sup> (95% confidence intervals) of perinatal outcomes, and rates (1/1,000) and odds ratios<sup>c</sup> (95% CI) of childhood outcomes among OI children of primiparous women, by plurality and group.

	Total			Singletons			Multiples		
	Proportion or rate			Proportion or rate			Proportion or rate		
	OI (n = 2,431)	Controls (n = 74,062)	OR (95% CI)	OI (n = 2,122)	Controls (n = 72,600)	OR (95% CI)	OI (n = 309)	Controls (n = 1,462)	OR (95% CI)
Perinatal outcomes, % <sup>a</sup>									
Very preterm (<32 gw)	2.6	1.1	2.44 (1.86–3.15)	1.2	0.9	1.30 (0.87–1.95)	12.3	10.7	1.26 (0.84–1.89)
Preterm (<37 gw)	12.6	6.6	1.99 (1.75–2.25)	7.0	5.7	1.21 (1.02–1.44)	51.1	52.5	0.89 (0.68–1.15)
Birth weight <1500 g	3.1	1.0	3.12 (2.44–3.99)	1.4	0.8	1.76 (1.21–2.56)	14.6	11.6	1.37 (0.94–1.99)
Birth weight <2500 g	11.9	5.1	2.47 (2.18–2.82)	5.5	4.2	1.32 (1.09–1.60)	56.3	54.0	1.06 (0.82–1.38)
Apgar score 0–6	8.1	5.7	1.39 (1.20–1.62)	6.4	5.5	1.11 (0.93–1.33)	20.3	16.6	1.20 (0.86–1.67)
Special care <sup>b</sup>	16.7	10.0	1.72 (1.54–1.92)	11.8	9.3	1.25 (1.09–1.43)	49.8	46.2	1.06 (0.82–1.38)
Respiratory treatment	3.2	1.3	2.40 (1.95–3.14)	1.7	1.1	1.46 (1.04–2.06)	13.6	9.9	1.46 (0.95–2.09)
Hospitalization ≥ 7 days	14.8	8.3	1.77 (1.58–2.00)	10.0	7.5	1.26 (1.09–1.46)	48.2	51.0	0.83 (0.63–1.08)
Perinatal mortality	0.9	0.6	1.56 (1.02–2.38)	0.4	0.6	0.76 (0.39–1.47)	4.5	3.7	1.43 (0.74–2.74)
Childhood outcomes, 1/1,000 <sup>c</sup>									
Cerebral palsy (G80) <sup>d</sup>	4.1	1.6	2.67 (1.16–5.55)	3.3	1.5	2.50 (1.00–6.22)	9.9	9.8	1.01 (0.17–6.08)
Epilepsy (G40–G41) <sup>d</sup>	3.7	2.6	1.46 (0.68–3.13)	3.8	2.5	1.53 (0.68–3.40)	3.3	4.9	0.57 (0.04–9.24)
Behavioral disorders (F80–F98) <sup>d</sup>	5.8	5.0	1.10 (0.61–2.00)	6.1	4.9	1.20 (0.65–2.22)	3.3	9.8	0.29 (0.03–3.19)
Diabetes (E10) <sup>e</sup>	0.8	0.5	1.83 (0.35–9.60)	0.9	0.5	2.06 (0.39–10.81)	0.0	0.0	NA
Asthma (J45–J46) <sup>e</sup>	31.4	20.7	1.56 (1.19–2.04)	29.8	20.7	1.48 (1.11–1.97)	42.8	24.5	1.92 (0.66–5.57)
Allergy (L20–L23, L27, L50) <sup>e</sup>	64.1	53.0	1.20 (0.99–1.44)	65.2	53.4	1.21 (1.00–1.47)	55.9	34.3	1.55 (0.63–3.85)
Pneumonia (J12–J18) <sup>e</sup>	10.3	8.3	1.21 (0.77–1.91)	9.9	8.4	1.14 (0.70–1.85)	13.2	0.0	NA
Diarrhea (A08–A09) <sup>e</sup>	50.0	40.4	1.27 (1.03–1.56)	43.5	39.5	1.12 (0.88–1.41)	95.4	88.2	1.16 (0.60–2.19)

Note: OR = odds ratio; OI = ovulation inductions; CI = confidence interval. Reference group (OR = 1) = controls.

<sup>a</sup> Adjusted for mother's region, smoking, age, marital status, socioeconomic position.

<sup>b</sup> Treatment in intensive care unit or in newborn surveillance unit.

<sup>c</sup> Adjusted for mother's socioeconomic position.

<sup>d</sup> Data sources: the HDR and child-disability allowance.

<sup>e</sup> Data sources: the HDR, long-term medication and child-disability allowance.

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In Finland, no private hospitals for children exist, but in 2005 about 28% of children up to 4 years of age had visited private physicians (SII, unpublished data, 2005). If OI children were treated more or less frequently in private care instead of hospital care, then a bias would result. Our results were adjusted for socioeconomic position, and it is unlikely that use of OI would otherwise relate to the use of private services. We have also no reason to believe that OI parents' would be more or less able or interested to apply for available benefits for long-term medication or child disability allowance (16).

Our study confirms earlier findings of the poorer perinatal health of OI singletons (5, 6, 8) and increased risk for preterm (9) or very preterm birth and low birth-weight of OI multiples (8), although not all our results were statistically significant because of the low number of multiples in our study.

The long-term health of OI children was worse than that of other children. According to our previous results OI children in total had a slightly increased risk for congenital anomalies, but after stratifying the analysis to multiplicity and gender the risks were not more statistically significant (12). We could find no other previous study examining the long-term health of OI children. The poorer long-term health of OI children can be partly explained by the poorer perinatal health.

Although perinatal health of OI singletons was worse than that of control singletons, it was not as poor as the perinatal health of IVF singletons (16), which is in accordance with other previous findings (6, 9). However, this was not systematically so after the perinatal period. Some indicators of childhood morbidity, such as the use of long-term medication and payment of child disability allowance, as well as some typical childhood illnesses (asthma, allergy, and diarrhea) even showed worse health among OI singletons compared with IVF singletons. No differences were found in the use of institutional care.

Potential reasons for the poorer health of OI children include infertility itself, infertility treatments (ovulation induction and/or IUI), varying background characteristics, including mother's health, mother's varying behavior during pregnancy, multiplicity, zygosity, and "vanishing twins"; singletons originating from twin pregnancies had a higher risk for preterm birth and low birth weight compared with singletons originated from singleton gestations (20). Low pregnancy-associated plasma protein levels as an indicator of poorer placental function in the first trimester can also have a role in poorer outcome of children born after infertility treatments (21, 22).

Infertile women even without infertility treatments have had a higher risk for adverse birth outcomes (23). We did not have any information on the reason or length of infertility. Experience from a Swedish study (24) suggests that if we had been able to adjust for the number of years of maternal infertility, the overall risk for hospitalization might have been smaller. It is difficult to separate the effect of infertility from the effect of infertility treatments on the infant

outcomes in nonexperimental study designs. The risk for poor infant outcomes among infertile or subfertile couples has increased from nontreatment groups to low-technology treatment groups, being highest in high-technology treatment groups (6, 7, 9, 23), which suggests that infertility is unlikely to be the sole cause for problems. The reason for the poorer outcome in the high-technology treatment groups can also be because of their poorer reproductive prognosis and longer duration of infertility compared with the patients in the low-technology treatment groups.

In terms of OI, possible origins for problems could be the insemination technique, the medication used in ovulation induction, or hyperstimulation. We could not analyze separately OI with and without IUI, because IUI is not registered with a specific code in the files of the SII. The evidence on the association between the use of CC and infant outcomes (25) is still lacking. The evidence on the use of gonadotrophins combined with IUI suggests that one effect might be lower birth weights of infants (26, 27). We had detailed information on the drugs purchased for use in OI, but the exact date and duration of their use was not known. Because many women bought both CC and gonadotrophins, it was not possible to identify which drug may carry specifically harmful effects. The question of hormonal stimulation of ovaries and the outcomes of OI children remains open.

We adjusted the results for background variables available from registers, but residual differences may have remained. Perinatal outcomes for OI children were adjusted for smoking, but otherwise we had no information on mothers' health behavior during pregnancy.

The health of OI multiples was much worse than that of singletons, as found earlier (8), and risks for many health indicators were increased, although not statistically significantly because of the low number of multiples. Zygosity plays a significant role when the health of OI multiples are compared with the health of other multiples. In general, monozygotic twins have had poorer perinatal outcomes than dizygotic twins. After adjusting for zygosity, OI twins have had a slightly increased risk for preterm birth (9). We did not have data on zygosity, but 51% of OI and 30% of control twins were opposite-gender twins, which suggested that more OI children were dizygotic. Neither did we have information on vanishing twins.

Decreasing the multiplicity among OI children is more complicated than reducing the multiplicity in IVF, which can be made by favoring single-embryo transfers. OI has been considered—even combined with IUI—as a lighter and less invasive than IVF, but particularly the number of oocytes cannot be fully controlled in OI. The epidemic of multiple births may continue despite single embryo transfer because of the wide use of ovulation induction drugs (9). Strategies for decreasing multiplicity in OI could be weight reduction in obese women before treatment, adequate monitoring, strict cancellation criteria, aspirations of excess follicle, favoring natural cycle IUI, use of clomiphene citrate

instead of gonadotrophins, and when gonadotrophins are used to favor low-dose protocols (1). Infertile couples should be informed about the risk of multiplicity after OI and risks related to multiple pregnancies and births.

The way to improve the health of singletons is more problematic because we do not know the reasons for poorer health outcomes. Reducing multiplicity may reduce the instances of vanishing twins and thereby also slightly improve the health of singletons. Further initial and follow-up studies are needed to explain the poorer health of OI singletons and to examine the health of OI children from 4 years and upward.

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**Comparison of OI and IVF children.**

Number of Infants	Total				
	No. or proportion			OR (95% CI)	
	IVF 4,559	OI 4,467	Controls 190,398	IVF	OI
Perinatal outcomes, <sup>a</sup> %					
Very preterm (<32 gw)	4.7	2.3	0.9	4.45 (3.80–5.21)	2.40 (1.95–2.96)
Preterm (<37 gw)	23.6	11.2	5.5	4.43 (4.10–4.77)	2.03 (1.84–2.24)
Birth weight <1,500 g	4.2	2.4	0.8	4.19 (3.55–4.95)	2.79 (2.28–3.41)
Birth weight <2,500 g	19.8	9.9	4.0	4.77 (4.40–5.18)	2.55 (2.30–2.82)
Apgar score 0–6	8.8	6.5	4.4	1.68 (1.50–1.87)	1.36 (1.20–1.54)
Special care <sup>b</sup>	23.0	15.1	8.2	2.71 (2.52–2.92)	1.81 (1.67–1.97)
Respiratory treatment	4.3	2.6	1.1	3.61 (3.08–4.24)	2.29 (1.88–2.78)
Hospitalization ≥ 7 days	23.8	12.8	6.4	3.42 (3.08–4.24)	1.88 (1.72–2.07)
Perinatal mortality	1.3	0.8	0.6	1.85 (1.40–2.44)	1.33 (0.95–1.85)
Childhood outcomes (ICD-10), <sup>c,d</sup> 1/1,000					
Cerebral palsy (G80) <sup>e</sup>	3.8	3.1	1.4	2.92 (1.63–5.26)	2.36 (1.27–4.38)
Epilepsy (G40–G41) <sup>e</sup>	3.3	3.1	2.5	1.33 (0.76–2.34)	1.26 (0.71–2.26)
Behavioral disorders (F80–F98) <sup>e</sup>	6.6	5.8	4.1	1.68 (1.11–2.53)	1.45 (0.94–2.22)
Diabetes (10) <sup>f</sup>	0.9	0.4	0.5	1.57 (0.51–4.84)	0.84 (0.19–3.72)
Asthma (J45–J46) <sup>f</sup>	30.3	35.7	28.1	1.08 (0.90–1.30)	1.28 (1.07–1.52)
Allergy (L20–L23, L27, L50) <sup>f</sup>	59.9	66.5	53.8	1.07 (0.94–1.23)	1.23 (1.08–1.40)
Pneumonia (J12–J18) <sup>f</sup>	9.9	12.8	11.4	0.85 (0.62–1.17)	1.11 (0.84–1.48)
Diarrhea (A08–A09) <sup>f</sup>	44.2	49.5	38.6	1.17 (1.00–1.37)	1.30 (1.12–1.51)
Hospitalization, <sup>f,g</sup> %	40.0	40.0	33.0	1.40 (1.31–1.48)	1.40 (1.32–1.49)
Any long-term medication use, <sup>d,h</sup> %	3.3	3.8	2.8	1.18 (0.98–1.41)	1.37 (1.16–1.62)
Any child disability allowance, <sup>d,h</sup> %	10.6	12.7	9.9	1.11 (1.00–1.23)	1.37 (1.24–1.51)

**Note:** Proportions (%) or rates (1/1,000) and odds ratios (95% confidence Intervals [CI]) of perinatal outcomes, childhood outcomes, hospitalization, and long-term medication use and any child disability allowance by plurality. HDR = hospital discharge register; OR = odds ratio; CI = confidence interval; OI = ovulation induction. Reference group (OR = 1) = controls.

<sup>a</sup> Adjusted for mother’s region, smoking, age, marital status, previous births, socioeconomic position.

<sup>b</sup> Treatment in intensive care unit or in newborn surveillance unit.

<sup>c</sup> Having an allergic or chronic disorder or a common condition (ICD-10 codes) until the age of 2 years by any available data source.

<sup>d</sup> Adjusted for mother’s socioeconomic position.

<sup>e</sup> Data sources: the HDR and child-disability allowance.

<sup>f</sup> Data sources: the HDR, long-term medication and child-disability allowance.

<sup>g</sup> Use of hospital services until the age of 4 years.

<sup>h</sup> Having any period of child disability allowance or any long-term medication use until the age of 2 years.

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Continued.

			Singletons		Multiples				
No. or proportion			OR (95% CI)		No. or proportion			OR (95% CI)	
IVF 2,930	OI 3,926	Controls 186,216	IVF	OI	IVF 1,629	OI 536	Controls 4,182	IVF	OI
2.0	1.2	0.8	2.06 (1.56–2.71)	1.52 (1.13–2.04)	9.6	9.7	7.0	1.26 (0.99–1.60)	1.24 (0.89–1.72)
9.5	6.4	4.7	1.72 (1.51–1.96)	1.32 (1.15–1.50)	49.2	45.4	42.2	1.06 (0.93–1.21)	0.95 (0.78–1.15)
1.9	1.3	0.7	2.17 (1.64–2.88)	1.78 (1.33–2.38)	8.2	10.6	7.4	0.95 (0.74–1.22)	1.27 (0.92–1.73)
6.5	4.7	3.2	1.60 (1.37–1.87)	1.43 (1.23–1.67)	43.7	47.9	39.2	0.92 (0.81–1.06)	1.23 (1.01–1.49)
5.6	5.2	4.2	1.07 (0.91–1.26)	1.11 (0.97–1.28)	14.5	16.5	12.5	1.10 (0.90–1.33)	1.20 (0.93–1.57)
12.6	10.9	7.6	1.36 (1.21–1.53)	1.35 (1.22–1.50)	42.1	45.7	35.0	1.04 (0.91–1.19)	1.27 (1.05–1.54)
2.0	1.5	0.9	1.76 (1.34–2.31)	1.46 (1.11–1.91)	8.4	10.5	6.7	1.19 (0.93–1.53)	1.40 (1.02–1.92)
10.8	8.5	5.8	1.43 (1.26–1.61)	1.34 (1.19–1.50)	47.4	44.0	37.6	1.02 (0.88–1.17)	1.05 (0.86–1.28)
0.9	0.5	0.5	1.32 (0.88–1.98)	0.87 (0.55–1.37)	2.0	3.1	2.9	0.73 (0.47–1.13)	1.05 (0.61–1.79)
1.4	2.6	1.3	1.15 (0.40–3.27)	2.09 (1.03–4.26)	8.0	7.5	5.2	1.52 (0.43–5.40)	1.23 (0.27–6.55)
3.4	3.3	2.5	1.39 (0.71–2.71)	1.34 (0.74–2.44)	3.1	1.9	3.4	0.95 (0.18–5.01)	0.48 (0.04–5.33)
4.1	6.1	4.1	1.05 (0.57–1.91)	1.52 (0.97–2.37)	11.1	3.7	3.4	3.05 (0.70–13.29)	0.98 (0.14–6.98)
1.0	0.5	0.5	1.98 (0.56–7.07)	1.01 (0.23–4.52)	0.6	0.0	1.7	0.28 (0.02–4.50)	NA
26.5	35.3	27.8	0.95 (0.74–1.20)	1.28 (1.06–1.54)	37.1	39.2	43.0	0.93 (0.57–1.51)	0.93 (0.51–1.70)
61.8	68.0	54.0	1.10 (0.94–1.30)	1.25 (1.09–1.43)	56.3	56.0	46.5	1.25 (0.80–1.96)	1.20 (0.70–2.05)
9.6	12.3	11.4	0.81 (0.55–1.20)	1.06 (0.78–1.44)	10.5	16.8	8.6	1.26 (0.46–3.49)	1.92 (0.63–5.82)
35.4	45.5	38.1	0.94 (0.76–1.15)	1.20 (1.02–1.42)	60.0	78.4	60.2	1.04 (0.69–1.58)	1.31 (0.82–2.09)
34.0	38.0	32.0	1.12 (1.04–1.21)	1.30 (1.22–1.39)	50.0	55.0	49.0	1.07 (0.95–1.20)	1.32 (1.10–1.58)
2.9	3.7	2.8	1.03 (0.82–1.30)	1.34 (1.11–1.61)	4.1	4.9	4.5	0.95 (0.59–1.52)	1.08 (0.62–1.89)
10.5	12.4	9.5	1.10 (0.97–1.25)	1.35 (1.21–1.50)	10.8	14.6	13.1	0.81 (0.62–1.10)	1.14 (0.81–1.61)

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# Health of Children Born as a Result of In Vitro Fertilization

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## ABSTRACT

**OBJECTIVE.** The purpose of this study was to use nationwide registries to examine the health of children up to 4 years of age who were born as a result of in vitro fertilization.

**METHODS.** Children born after in vitro fertilization ( $N = 4559$ ) from 1996 to 1999 were monitored until 2003. Two control groups were selected from the Finnish Medical Birth Register as follows: all other children (excluding children born after ovulation induction) from the same period ( $N = 190\,398$ , for study of perinatal health and hospitalizations) and a random sample of those children ( $n = 26\,877$ , for study of health-related benefits). Mortality rates and odds ratios for perinatal outcomes, hospitalizations, health-related benefits, and long-term medication use were calculated.

**RESULTS.** Although the health of most in vitro fertilization children was good, such children had more health problems than other children. A total of 35.7% of in vitro fertilization children and 2.2% of control children were multiple births, and the health of multiple births was worse than that of singletons. Perinatal outcomes of in vitro fertilization children were worse and hospital episodes were more common than among control children. Risks for cerebral palsy and psychological and developmental disorders were increased. Among in vitro fertilization singletons, worse results for perinatal outcomes and hospitalizations, but no increased risk for specific diseases, were found. The health of in vitro fertilization multiple births was comparable to the health of control multiple births.

**CONCLUSIONS.** Reducing the number of transferred embryos would improve the health of in vitro fertilization children. Additional studies are needed to explain the poorer health of in vitro fertilization singletons, as well as follow-up studies to examine the health of in vitro fertilization children from 4 years onward.

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### Key Words

in vitro fertilization, perinatal health, morbidity, multiplicity, registry-based study

### Abbreviations

CP—cerebral palsy  
IVF—in vitro fertilization  
HDR—Hospital Discharge Register  
MBR—Medical Birth Register  
SII—Social Insurance Institution  
OR—odds ratio  
CI—confidence interval  
ICD-10—*International Classification of Diseases, 10th Revision*

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**I**N VITRO FERTILIZATION (IVF) (including intracytoplasmic sperm injections and frozen embryo transfers) is a common infertility treatment. In Finland, currently ~2.5% of infants are born as a result of IVF,<sup>1</sup> with women <40 years of age being able to receive 2 to 5 IVF treatment cycles within the public sector<sup>2</sup> while paying a small fee for visits. Approximately 60% of all IVF services are supplied by private clinics, with no strict age limit. Private physicians' charges and drug costs are partly reimbursed by the Social Insurance Institution (SII). Usually, the pharmacies and IVF clinics take care of billing for the reimbursements. Approximately 76% of total IVF costs (visits, examinations, treatments, and drugs) are covered in the public sector and 50% in the private sector, with the rest being paid by women (R.K., T.S., M.G., and E.H., unpublished data, 2006).

The perinatal health of IVF singletons has been reported as being worse than that of naturally conceived singletons,<sup>3-5</sup> with more-recent studies also showing an increased risk for preterm birth and/or low birth weight for twins.<sup>6,7</sup> However, studies of the long-term health of IVF children are few, and their results are conflicting.

According to previous small cohort studies, the morbidity rates, growth, and development of IVF children are similar to those of control children (as reviewed by Koivurova<sup>8</sup>). Although the health of children is mainly good, large studies are needed to clarify potential health problems. Registry-based studies allow for large sample sizes and are published regularly from the Nordic countries.<sup>9-17</sup> In the case of IVF children, research has found an excess use of hospital services, long hospitalizations, and increased risk for infections, epilepsy, and tumors,<sup>10</sup> asthma,<sup>10,17</sup> cerebral palsy (CP),<sup>10,11,14</sup> sleep disturbances,<sup>14</sup> convulsions, behavioral problems, and accidents,<sup>17</sup> and congenital malformations.<sup>9,10,15-17</sup> However, some of those studies were based on early IVF experience and concentrated on specific diagnoses, hospital care utilization, or singletons/twins only or did not consider multiplicity.

Results on the perinatal health of IVF twins are controversial, whereas data on the long-term health of IVF children are sparse. For this reason, our aim was to perform a large, thorough, up-to-date, registry-based study of the health of IVF children up to 4 years of age, separately for singletons and multiple births, by using several population-based registries.

## METHODS

### Identification of IVF Children

The study is based on children born to women who received IVF between 1996 and 1998 in Finland. The women were identified, with a predesigned algorithm, from the reimbursement files of the SII.<sup>18</sup> Data on children born as a result of IVF treatment ( $N = 4559$ ) and their perinatal health were obtained from the Finnish

Medical Birth Register (MBR)<sup>15,19</sup> by using women's personal identification numbers and the children's dates of birth as the linkage keys. The MBR also includes the children's unique identification numbers. It contains information on maternal backgrounds and on infant outcomes until the age of 7 days for all infants born in Finland. The data are collected by delivery hospitals and are completed by linkage to the Central Population Register and cause-of-death statistics (compiled by Statistics Finland). The identified children were linked to 4 other nationwide registries through the children's identification numbers, namely, cause-of-death statistics, the Hospital Discharge Register (HDR) (hospital episodes, diagnoses, ie, *International Classification of Diseases, 10th Revision* [ICD-10] codes, and dates of admissions and discharges), the Care Register for Social Welfare (episodes in institutional care), and health-related social benefits from the SII (reimbursements for long-term medication use and child disability allowance).

### Control Groups

As control groups, 2 groups of children were selected from the MBR. The first control group consisted of all children other than IVF children or those born as a result of ovulation induction ( $N = 190\,398$ ) who had been conceived during the same period (1996-1998). The second control group ( $n = 26\,877$ ) was a random sample of the first control group, selected to reduce the workload caused by large registry linkages in the SII, and was used to study the benefit payments from the SII and for the combined analysis.

### Data Collection

The number of deaths of all children from 1996 to 2001 until the age of 2 years was obtained from cause-of-death statistics. We grouped the causes of deaths (given as ICD-10 codes) into 4 categories, namely, conditions originating from the perinatal period, congenital malformations, other medical causes, and deaths from external causes.

The HDR collects information on inpatient care and visits to outpatient clinics involving surgical or other procedures. The HDR gathers information on diagnoses (ICD-10 codes) and dates of admissions and discharges. The diagnoses include the main diagnosis and 2 secondary diagnoses for each episode. All hospitalizations until the children were 4 years of age were studied (1996-2003).

The Care Register for Social Welfare collects information on care episodes in social institutions, such as institutions for people with intellectual disabilities. For this study, we received information on the numbers of IVF children having  $\geq 1$  period of institutional care up until the end of 2004. We compared the rates of institutionalized children with the national rates for children born

in 1997 or 1998, excluding the numbers of children from IVF or ovulation induction.

The SII grants child disability allowances for families who have a disabled or chronically sick child needing continuous help and surveillance at home. A child's parents applying for benefits are required to supply recent medical documents. The register of child disability allowances contains information on start and end dates, type (temporary or permanent), level (normal, increased, or special), and diagnoses. The special refund category covers ~50 chronic diseases, entitling patients to receive higher reimbursements of long-term medication costs. Among children, the most common diseases in the special refund category are asthma, epilepsy, diabetes mellitus, and rheumatoid arthritis. The data on special refunds included the start and end dates of entitlement periods and the reasons. Information on both child disability allowance and long-term medication use was gathered from 1996 to 2001 (ie, until the children were 2 years of age).

### Data Analyses

A comparison was made between control and IVF mothers in their utilization of health care services during the pregnancy and child birth (hospitalization during the pregnancy, cesarean section, and hospitalization of  $\geq 7$  days after delivery) and in infant outcomes, first including all children and then including first births only. As health outcomes, we used very low birth weight (<1500 g), low birth weight (<2500 g), very preterm birth (<32 weeks), preterm birth (<37 weeks), low 1-minute Apgar scores (scores of 0–6), treatment in an ICU or neonatal surveillance unit, need for respiratory treatment, hospitalization of the child for  $\geq 7$  days after birth, and perinatal death.

All inpatient hospital episodes until 2 years and 4 years of age were collected separately from the HDR. The total number of hospital episodes, the length of the episodes, and the number of hospitalized children were determined. We grouped diagnoses (ICD-10 codes) into 16 categories. The 2 categories of "symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified" (codes R00–R99) and "factors influencing health status and contact with health services" (codes Z00–Z99) were combined and renamed as "other." Both main and secondary diagnoses were taken into account. If the child was hospitalized more than once because of the same diagnosis, then only the first hospitalization was included.

We calculated the numbers of IVF and control children who had received  $\geq 1$  child disability allowance period or reimbursement for long-term medication. The most common reasons for child disability allowance and reimbursement were counted and IVF children were compared with naturally conceived children. Finally, we combined information from the different data

sources and calculated the number of children who had used services, according to any of the data sources, because of an allergic and chronic disorder and common infection-like allergy (ICD-10 codes L20–L23, L27, and L50), asthma bronchial (ICD-10 codes J45 and J46), CP (ICD-10 code G80), epilepsy (ICD-10 codes G40 and G41), diabetes mellitus (ICD-10 code E10), diarrhea (ICD-10 codes A08–A09), pneumonia (ICD-10 codes J12–J18), or disorders of psychological development and behavioral and emotional disorders usually occurring in childhood and adolescence (ICD-10 codes F80–F98).

### Statistical Analyses

The differences between the IVF and control groups were first tested with a  $\chi^2$  test and *t* test for relative proportions and with logistic regression analysis, adjusting for available background characteristics. For perinatal outcomes, these characteristics were county, smoking, maternal age, socioeconomic position, and previous births. The socioeconomic position of the women was defined by using their own occupation when delivering, which is collected routinely from maternity hospitals and classified automatically by the MBR into 5 categories according to the national classification compiled by Statistics Finland, that is, upper white-collar workers, lower white-collar workers, blue-collar workers, others (entrepreneurs, students, pensioners, unemployed women, and women with an unclassified position), and unknown position.<sup>20</sup> All analyses were made separately for singletons and multiple births. Two logit models were used, namely, an ordinary logit model in which all children were assumed to be independent and an additional model created by using the iterative, generalized, least-squares method, in which siblings born in the same delivery were assumed to be dependent.

### Research Ethics and Data Protection

The study plan was approved by the National Research and Development Centre for Welfare and Health research ethics committee (September 18, 1998). For register linkages, the National Data Protection Authority was consulted, and permissions were obtained from the registry keepers.

### RESULTS

Of the 4559 IVF children, 34.7% were twins and 1.1% were triplets. Among the 190 398 control children, 2.2% were twins and only 13 sets were triplets (<0.01%). IVF mothers were older, more often married, and from a higher socioeconomic position than other mothers (Table 1).

Compared with other mothers, IVF mothers received more hospital care during pregnancy and more cesarean sections (Table 2). Adjustment for mothers' background characteristics did not change the results. Inspection of singletons and multiple births separately showed that

**TABLE 1 Mothers' Background Characteristics According to Group and Plurality for IVF and Control Mothers**

	Total Births			Singleton Births			Multiple Births		
	IVF (n = 3737)	Control (n = 188 298)	P	IVF (n = 2930)	Control (n = 186 216)	P	IVF (n = 807)	Control (n = 2084)	P
Maternal age at delivery									
Mean ± SD, y <sup>a</sup>	33.9 ± 4.5	29.7 ± 5.3	<.001	34.1 ± 4.6	29.7 ± 5.3	<.001	33.1 ± 4.3	30.5 ± 5.2	<.001
Age group, %									
<25 y	2.9	22.4		2.6	22.4		4.1	17.4	
25–29 y	20.1	33.6		18.8	33.7		24.5	31.7	
30–34 y	41.2	29.4		41.2	29.4		41.5	33.2	
35–39 y	41.2	12.2		28.3	12.2		25.3	15.4	
≥40 y <sup>b</sup>	8.1	2.3	<.001	9.1	2.3	<.001	4.6	2.3	<.001
Marital status									
Married or cohabiting	95.3	87.5		95.4	87.5		94.9	86.4	
Single	3.9	10.6		3.9	10.6		4.0	11.3	
Missing information <sup>b</sup>	0.8	1.9	<.001	0.8	1.9	<.001	1.1	2.4	<.001
Socioeconomic position, %									
Upper white-collar	25.1	15.1		25.5	15.1		23.7	17.0	
Lower white-collar	48.5	40.6		48.2	40.6		49.9	40.3	
Blue-collar	13.0	17.0		13.0	17.0		12.6	16.1	
Others	8.0	18.5		8.0	18.5		8.1	18.2	
Unknown <sup>b</sup>	5.4	8.9	<.001	5.3	8.9	<.001	5.7	8.4	<.001
Smoked during pregnancy <sup>c</sup>	6.6	14.8	<.001	6.6	14.8	<.001	6.6	16.6	<.001
First birth <sup>c</sup>	72.2	39.5	<.001	72.0	39.5	<.001	72.9	35.6	<.001

The control group consisted of all other mothers whose children were fertilized in the same time period as IVF children.

<sup>a</sup> For *t* tests in comparisons between IVF and control subjects.

<sup>b</sup> For  $\chi^2$  tests in comparisons between IVF and control subjects.

<sup>c</sup> For tests for relative proportions in comparisons between IVF and control subjects.

**TABLE 2 Raw Proportions and Adjusted ORs of Pregnancy and Birth Treatments and Infant Outcomes Among IVF Mothers and Infants, Compared With Other Mothers and Infants**

	Total Births			Singleton Births			Multiple Births		
	No. or Proportion		OR (95% CI)	No. or Proportion		OR (95% CI)	No. or Proportion		OR (95% CI)
	IVF	Control		IVF	Control		IVF	Control	
Deliveries, <i>n</i>	3737	188 298		2930	186 216		807	2084	
Infants, <i>n</i>	4559	190 398		2930	186 216		1629	4182	
Mother, %									
Hospital treatment <sup>a</sup>	43.0	20.6	2.61 (2.43–2.79)	36.4	20.2	1.99 (1.84–2.16)	66.9	54.2	1.51 (1.24–1.85)
Hospitalization of ≥7 d <sup>b</sup>	16.8	4.5	2.33 (2.11–2.57)	9.6	4.2	1.23 (1.07–1.41)	46.8	31.7	1.04 (0.83–1.30)
Cesarean section	35.8	15.3	1.95 (1.81–2.10)	30.4	15.0	1.51 (1.39–1.65)	55.5	41.8	1.24 (1.03–1.50)
Infant, %									
Very preterm (<32 wk)	4.7	0.9	4.45 (3.80–5.21)	2.0	0.8	2.06 (1.56–2.71)	9.6	7.0	1.26 (0.99–1.60)
Preterm (<37 wk)	23.6	5.5	4.43 (4.10–4.77)	9.5	4.7	1.72 (1.51–1.96)	49.2	42.2	1.06 (0.93–1.21)
Birth weight of <1500 g	4.2	0.8	4.19 (3.55–4.95)	1.9	0.7	2.17 (1.64–2.88)	8.2	7.4	0.95 (0.74–1.22)
Birth weight of <2500 g	19.8	4.0	4.77 (4.40–5.18)	6.5	3.2	1.60 (1.37–1.87)	43.7	39.2	0.92 (0.81–1.06)
Apgar score of 0–6	8.8	4.4	1.68 (1.50–1.87)	5.6	4.2	1.07 (0.91–1.26)	14.5	12.5	1.10 (0.90–1.33)
Special care <sup>c</sup>	23.0	8.2	2.71 (2.52–2.92)	12.5	7.6	1.36 (1.21–1.53)	42.1	35.0	1.04 (0.91–1.19)
Respiratory treatment	4.3	1.1	3.61 (3.08–4.24)	2.0	0.9	1.76 (1.34–2.31)	8.4	6.7	1.19 (0.93–1.53)
Hospitalization of ≥7 d	23.8	6.4	3.42 (3.08–4.24)	10.8	5.8	1.43 (1.26–1.61)	47.4	37.6	1.02 (0.88–1.17)
Perinatal death	1.3	0.6	1.85 (1.40–2.44)	0.9	0.5	1.32 (0.88–1.98)	2.0	2.9	0.73 (0.47–1.13)

ORs were adjusted for mother's county, smoking, age, marital status, parity, and socioeconomic position. The reference group (OR = 1) was the control group.

<sup>a</sup> During pregnancy.

<sup>b</sup> After delivery.

<sup>c</sup> Treatment in ICU or in newborn surveillance unit.

this difference was partly, but not totally, explained by IVF children more often being twins.

Similarly, the indicators of perinatal health showed much worse health of IVF children, which was explained partly by plurality. The perinatal health of IVF

multiple births was comparable to that of control multiple births; the risk for very preterm birth was increased but not statistically significantly.

Stillbirths were more common among IVF children in total, compared with other children in total (7.2 cases

per 1000 vs 3.9 cases per 1000;  $P < .001$ ), and among IVF singletons, compared with control singletons (6.5 cases per 1000 vs 3.7 cases per 1000;  $P = .014$  in a test for relative proportions), but not separately for multiple births. The main causes of stillbirths were conditions originating in the perinatal period (for example, placental infarction, extreme immaturity, and abruptio placentae).

The total mortality rate up to the age of 2 years was twofold higher among IVF children, compared with control children (9.0 deaths per 1000 and 4.1 deaths per 1000, respectively). Among singletons, rates of deaths after birth until the age of 2 years were similar in all groups of children; the main causes were congenital malformations (2.4 cases per 1000 among IVF children and 1.4 cases per 1000 among control children) and conditions originating in the perinatal period (for example, extremely low birth weight and respiratory distress syndrome; 1.4 cases per 1000 and 1.3 cases per 1000, respectively). The main causes among multiple births were the same as those among singletons (malformations: 11.2 cases per 1000 and 4.6 cases per 1000; perinatal causes: 11.2 cases per 1000 and 14.8 cases per 1000, respectively), and no significant differences between the groups were found.

According to the Care Registry for Social Welfare Institutions, 2.2 IVF children per 1000 had had  $\geq 1$  period of institutional care at a social welfare institution. For other children born in 1997 to 1998, the rate was 2.7 per 1000 children. Institutional care does not include children taken into children's homes or custody but refers mainly to mentally handicapped children who stayed in an institution for people with intellectual disabilities.

Until the age of 2 years, larger proportions of IVF children and IVF singletons received child disability allowances, compared with control children (Table 3). The most common reasons (according to ICD-10 classification) for receiving child disability allowances were the same for IVF and control singletons, namely, diseases of the skin and subcutaneous tissue, diseases of the respiratory system, and conditions involving the eyes and

ears. For multiple births, the most common reasons included, in addition, certain conditions originating in the perinatal period. No statistically significant differences in long-term medication use were found between IVF and control children.

When information from different data sources until the age of 2 years was combined, it was found that IVF children, singletons and multiple births taken together, had a threefold increased risk of CP and more often had disorders of psychological development or behavioral and emotional disorders, compared with control children (Table 4). This was not the case when IVF singletons and multiple births were considered separately. Of the infants with CP, 88% were preterm.

Up to the age of 4 years, a larger proportion of IVF children were hospitalized, IVF children more often had long hospital episodes, and the average length of their episodes was greater, compared with control children (Table 5). IVF children had somewhat more hospital episodes than control children at all ages, but the difference was clearest during infancy.

Compared with control children, the risk of being hospitalized was increased among IVF children for many categories of diseases (according to ICD-10 grouping), even after adjustment for the mother's socioeconomic position (data not shown). The risk among IVF singletons was increased statistically significantly for perinatal problems (ICD-10 codes P00–P96; odds ratio [OR]: 1.76; 95% confidence interval [CI]: 1.54–2.01), congenital malformations (codes Q00–Q99; OR: 1.45; 95% CI: 1.20–1.75), and problems of the genitourinary system (codes N00–N99; OR: 1.40; 95% CI: 1.11–1.77) and decreased for diseases of the respiratory system (codes J00–J99; OR: 0.86; 95% CI: 0.76–0.97). IVF multiple births had increased risk for hospitalization because of diseases originating from the perinatal period (OR: 1.34; 95% CI: 1.18–1.53) and "other" diagnoses (codes R00–R99 and Z00–Z99; OR: 1.27; 95% CI: 1.09–1.48) and decreased risk for hospitalization because of diagnoses in the categories of eye and ear (codes H00–H95; OR: 0.77;

**TABLE 3** Raw Proportions of Children and Crude and Adjusted ORs (and 95% CI) of Having Any Child Disability Allowance Period or Any Long-Term Medication Use Until the Age of 2 Years

	Total		Singleton		Multiple	
	IVF (n = 4527)	Control (n = 26 877)	IVF (n = 2911)	Control (n = 26 296)	IVF (n = 1616)	Control (n = 581)
Any child disability allowance						
Proportion, %	10.6	9.5	10.5	9.5	10.8	13.1
Crude OR (95% CI)	1.13 (1.02–1.25)	1.00	1.13 (0.99–1.28)	1.00	0.81 (0.61–1.08)	1.00
Adjusted OR (95% CI)	1.11 (1.00–1.23)	1.00	1.10 (0.97–1.25)	1.00	0.81 (0.62–1.10)	1.00
Any long-term medication use <sup>a</sup>						
Proportion, %	3.3	2.8	2.9	2.8	4.1	4.5
Crude OR (95% CI)	1.18 (0.98–1.41)	1.00	1.03 (0.82–1.29)	1.00	0.91 (0.57–1.45)	1.00
Adjusted OR (95% CI)	1.18 (0.98–1.41)	1.00	1.03 (0.82–1.30)	1.00	0.95 (0.59–1.52)	1.00

The ORs were adjusted for mother's socioeconomic position. The reference group (OR = 1) was the control group.

<sup>a</sup> Reimbursements for cow's milk or soy milk intolerance were excluded.

**TABLE 4 Raw Proportions of Children and Adjusted ORs of Having an Allergic or Chronic Disorder or a Common Infection (ICD-10 Codes) Until the Age of 2 Years, From Any Available Data Source**

	Total		Singleton		Multiple	
	IVF (n = 4527)	Control (n = 26 877)	IVF (n = 2911)	Control (n = 26 296)	IVF (n = 1616)	Control (n = 581)
CP (code G80) <sup>a</sup>						
Proportion, cases per 1000	3.8	1.4	1.4	1.3	8.0	5.2
OR (95% CI)	2.92 (1.63–5.26)	1.00	1.15 (0.40–3.27)	1.00	1.52 (0.43–5.40)	1.00
Epilepsy (code G40–G41) <sup>b</sup>						
Proportion, cases per 1000	3.3	2.5	3.4	2.5	3.1	3.4
OR (95% CI)	1.33 (0.76–2.34)	1.00	1.39 (0.71–2.71)	1.00	0.95 (0.18–5.01)	1.00
Behavioral disorders (code F80–F98) <sup>a,c</sup>						
Proportion, cases per 1000	6.6	4.1	4.1	4.1	11.1	3.4
OR (95% CI)	1.68 (1.11–2.53)	1.00	1.05 (0.57–1.91)	1.00	3.05 (0.70–13.29)	1.00
Diabetes mellitus (code E10) <sup>b</sup>						
Proportion, cases per 1000	0.9	0.5	1.0	0.5	0.6	1.7
OR (95% CI)	1.57 (0.51–4.84)	1.00	1.98 (0.56–7.07)	1.00	0.28 (0.02–4.50)	1.00
Asthma (code J45–J46) <sup>b</sup>						
Proportion, cases per 1000	30.3	28.1	26.5	27.8	37.1	43.0
OR (95% CI)	1.08 (0.90–1.30)	1.00	0.95 (0.74–1.20)	1.00	0.93 (0.57–1.51)	1.00
Allergy (code L20–L23, L27, L50) <sup>b</sup>						
Proportion, cases per 1000	59.9	53.8	61.8	54.0	56.3	46.5
OR (95% CI)	1.07 (0.94–1.23)	1.00	1.10 (0.94–1.30)	1.00	1.25 (0.80–1.96)	1.00
Pneumonia (code J12–J18) <sup>a</sup>						
Proportion, cases per 1000	9.9	11.4	9.6	11.4	10.5	8.6
OR (95% CI)	0.85 (0.62–1.17)	1.00	0.81 (0.55–1.20)	1.00	1.26 (0.46–3.49)	1.00
Diarrhea (code A08–A09) <sup>a</sup>						
Proportion, cases per 1000	44.2	38.6	35.4	38.1	60.0	60.2
OR (95% CI)	1.17 (1.00–1.37)	1.00	0.94 (0.76–1.15)	1.00	1.04 (0.69–1.56)	1.00

ORs were adjusted for mother's socioeconomic position.

<sup>a</sup> Data sources: the HDR and child-care support.

<sup>b</sup> Data sources: the HDR, long-term medication use, and child-disability allowance.

<sup>c</sup> Disorders of psychological development and behavioral and emotional disorders.

**TABLE 5 Use of Hospital Services Until the Age of 4 Years Among IVF and Control Children, According to Multiplicity**

Use of Hospital Services	Total		Singleton		Multiple	
	IVF (n = 4527)	Control (n = 189 656)	IVF (n = 2911)	Control (n = 185 530)	IVF (n = 1616)	Control (n = 4126)
Total no. of hospital episodes	4397	136 782	2281	131 459	2116	5323
Hospitalized children, %	40	33	34	32	50	49
OR (95% CI)	1.40 (1.31–1.48)	1.00	1.12 (1.04–1.21)	1.00	1.07 (0.95–1.20)	1.00
Time in hospital per child, d	6.3	2.7	3.8	2.6	10.8	9.8
Proportion of long hospital episodes (≥7 d), %	20	11	14	10	28	24

ORs were adjusted for mother's socioeconomic position. The reference group (OR = 1) was the control group.

95% CI: 0.63–0.95) and the respiratory system (OR: 0.74; 95% CI: 0.63–0.87). Otherwise, their outcomes were comparable to those of control multiple births. However, in almost every category the proportion of hospitalized children was higher among multiple births than among singletons.

In the subanalysis for first births, the results were mainly similar to the results for all children; although IVF children in total had increased risk for asthma (adjusted OR: 1.39; 95% CI: 1.08–1.79), the risk for mothers' long hospital stay for IVF singletons (OR: 1.17; 95% CI: 0.89–1.58) and the risk for cesarean sections for multiple births (OR: 1.19; 95% CI: 0.92–1.53) were not statistically significantly increased. In addition, IVF mul-

iple births had statistically significantly decreased risk for low birth weight (adjusted OR: 0.78; 95% CI: 0.65–0.93).

There were no differences in the results of the 2 logit analyses (an ordinary logit model and an additional analysis using the iterative generalized least-squares method; see Methods). For some rare outcomes, adjustment for mother's socioeconomic position was not possible in the additional analysis because of small numbers.

## DISCUSSION

We found an increased burden of disease associated with IVF, with poorer perinatal health, higher mortality rates, increased risk for hospitalization and CP, and longer

hospital episodes. This burden depended in part on higher twin rates among IVF children. However, the burden of disease resulted not only from the greater number of twins but also from the poorer health of singletons, compared with naturally conceived singletons. Increased morbidity was attributable not to any specific disease but rather to small increases in many groups of diseases. In general, the health of IVF multiple births was comparable to that of other multiple births.

Are the results reliable? IVF children were identified on the basis of drugs used, laboratory and radiologic examinations, and infertility treatment procedures. We might have missed some IVF children, who would therefore be included in the control group. However, the number of missing children cannot be large<sup>18,19</sup> and would not affect the results. The data on deaths and perinatal health received from the MBR<sup>21,22</sup> are reliable. However, other outcome measures depend on service utilization (seeking care or applying for benefits); technically, the registers are considered to be of good quality.<sup>23</sup>

The occurrence of less-serious diseases and cases cannot be estimated from these registries, because the use of outpatient care is not registered. Our results might be biased by different thresholds for hospital admissions between IVF and control children. IVF parents, who were more often first-time parents, might have been more worried, which might have led more easily to hospital care and also longer hospital stays. It might also be that IVF children were examined more carefully by physicians, compared with naturally conceived children, if the mode of conception was known to the physicians. However, because IVF children did not have an increased rate of hospitalizations in all categories of diseases and because adjustment for parity and socioeconomic position and a subanalysis of first births did not change our results, it is unlikely that the anxiousness of parents, more-careful examinations, or lower thresholds for hospitalization alone could explain the greater frequency of visits. Rather, the greater frequency likely reflects higher morbidity rates among singleton IVF children. Furthermore, rates of almost every outcome studied were quite similar between IVF multiple births and control multiple births.

In Finland, most health care is public, financed by taxes. Private health care is covered by the national social security system, but some children are covered by additional voluntary private insurance. No private hospitals for children exist but, in 2005, ~28% of children up to 4 years of age used private (outpatient) physicians (Social Insurance Institution of Finland, unpublished data, 2005). It is possible that, in the case of small surgical procedures, private specialist outpatient care competes with hospital outpatient clinics. If IVF children were treated more or less frequently in such private care, then a bias would result.

In Finland, health-related social benefits (child care allowance and reimbursement for long-term medication use) must be applied for. It might be that some parents are more capable of applying for the benefits. Because the adjustment for socioeconomic position did not change the results, however, there is no reason to assume that parents of IVF children with a higher socioeconomic position would receive benefits more easily than parents of control children. Informing and advising parents on these benefits is part of routine clinical practice. In addition, reimbursed diseases for long-term medication use are defined clearly, and recent medical documents are needed for receipt of both child disability allowance and support for long-term medication use. Child disability allowance is based on ICD-10 classifications and long-term medication support on defined diagnoses; therefore, it can be assumed that these are relevant in estimating disease occurrence.

Our study confirms earlier findings of poorer perinatal health,<sup>3-5,8</sup> greater numbers of hospitalizations,<sup>9</sup> and increased risk for congenital anomalies<sup>15,16,24</sup> for IVF singletons, compared with naturally conceived singletons. Perinatal problems had a significant role also in hospitalizations; diseases originating from the perinatal period represented one of the most common diagnoses leading to hospitalization, among both singletons and multiple births. IVF multiple births had worse perinatal health than did IVF singletons, but IVF and control multiple births were similar with respect to perinatal health, which is largely in accordance with an earlier study (except for the finding in that study of an increased risk of admittance to a NICU and more-common longer hospitalizations after the birth).<sup>25</sup> In contrast, a recently published Belgian study found an increased risk for preterm birth also among IVF twins, compared with naturally conceived twins, which was largely explainable by the first birth of IVF women.<sup>7</sup> In accordance with the study by Pinborg et al,<sup>13</sup> we did not find any excess use of hospital services among IVF multiple births.

In addition, our study confirms earlier results of higher mortality rates,<sup>8</sup> greater numbers of hospitalizations,<sup>10,17</sup> and increased risks for behavioral problems,<sup>17</sup> CP,<sup>11,14</sup> and infections<sup>10</sup> among IVF children overall. In accordance with an earlier Finnish study based on both outpatient and inpatient visits,<sup>8</sup> we found a slightly but not statistically significantly increased risk for diarrhea; contrary to that study, however, we did not find an increased risk for pneumonia.

Unlike previous studies,<sup>11,14</sup> we did not find an increased risk for CP or sleeping disturbances among IVF singletons. In our study, the excess risk for CP was mainly explainable by multiplicity. In the study by Strömberg et al,<sup>11</sup> the main reasons for the increased risk for CP were prematurity and the multiple pregnancy rate. A recent Swedish study could not confirm the increased risk for CP; the risk disappeared after adjust-

ment for confounders.<sup>17</sup> Furthermore, we could not find increased risk for epilepsy, tumors, or asthma among IVF children in total, as found earlier in Sweden.<sup>10</sup> However, increased risk for epilepsy was not found in the recent Swedish study.<sup>17</sup>

A few previous studies reported about childhood morbidity for IVF multiple births. In 2 studies, no differences in neurologic sequelae were found.<sup>11,12</sup> In our study, no increased risk for any disease among IVF multiple births was found. In general, however, IVF multiple births had higher childhood morbidity rates than did IVF singletons.

We could not find any other study examining long-term medication use, child disability allowance, and utilization of institutional care among IVF children. A slightly increased risk for child disability allowance among IVF children in our study was explainable by multiplicity, whereas no statistically significant differences in the utilization of long-term medication therapy and institutional care between the groups were found.

Potential reasons for the poorer perinatal health of IVF children include infertility itself,<sup>26–29</sup> infertility treatments, and varying health behavior during pregnancy. Among IVF singletons, the main cause of poorer perinatal health has been suggested to be infertility itself, because of the higher incidence of preterm birth and low birth weight also among infertile women without treatment and women with infertility treatments other than IVF.<sup>30</sup> Some modification in the gestational process induced by IVF and intracytoplasmic sperm injection has been suggested,<sup>31</sup> as well as so-called vanishing twins (singletons originating from twin pregnancies).<sup>32</sup> It has also been found that the risk for preterm birth increases with low-technology treatments, compared with natural pregnancy, and increases further with high-technology treatments.<sup>33</sup>

Zygosity plays a significant role when the health of IVF multiple births are compared with the health of other multiple births. In general, monozygotic twins have poorer perinatal outcomes than dizygotic twins. A larger proportion of twins are dizygotic among medically assisted pregnancies (30%), compared with naturally conceived pregnancies (1%).<sup>34</sup> This can partly explain the results of the similar outcomes of multiple births in studies unable to take zygosity into account. In our study, 50% of IVF twins and 30% of control twins were opposite-gender twins, which suggested that more IVF children were dizygotic.

During the 1990s, the perinatal health of IVF children improved in Finland, mainly because of a decrease in higher-order multiple births.<sup>35,36</sup> Because so many IVF pregnancies in the late 1990s were still multiple births, the health of IVF children in total was worse than that of naturally conceived children, with increased risks for CP and developmental and psychological problems. The best way to improve the health of IVF children is to favor

single-embryo transfers. The way to improve the health of singletons is more problematic, because we do not know the reasons for the findings. Sufficiently large follow-up studies that consider the health of IVF children from 4 years onward are needed.

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*Pediatrics* 2005;116;190

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## Hormonal Effects in Infants Conceived by Assisted Reproductive Technology

Patricia Martin Rojas-Marcos, MD\*; Raphael David, MD‡; and Brenda Kohn, MD‡

**ABSTRACT.** *Objective.* The purpose of this report is to describe 7 infants conceived by assisted reproductive technology (ART) who presented with breast development and/or pubic hair. The clinical presentation in these infants raises awareness that an altered intrauterine hormonal milieu may impact the fetal and infant stages of children conceived by ART.

*Methods.* Between May 2001 and April 2004, 7 children between the ages of 5 and 21 months conceived by ART were referred by their pediatricians to the Division of Pediatric Endocrinology at the New York University School of Medicine for evaluation of possible precocious puberty. Patients were evaluated for the possibility of centrally mediated precocious puberty and pseudoprecocious puberty, with a possible ovarian or adrenal origin.

*Results.* Endocrine evaluation in all patients indicated sex-steroid and hormone levels in the prepubertal range; pelvic sonography confirmed prepubertal ovaries with unstimulated uteri. Clinical follow-up of our patients thus far has not revealed progression of breast development, pubarche, or elevation in sex steroids.

*Conclusions.* It is well established that the developing endocrine system in the fetus and maturation of endocrine-control systems are influenced by hormone concentrations in the fetus. Whether ART alters the intrauterine hormonal milieu for the growing fetus conceived by ART is as yet unknown and is an area of ongoing investigation. Patients conceived through ART, including our patients who presented with hormonal manifestations, will need to be monitored throughout childhood and into adolescence and adulthood to determine if any perturbation exists on the timing of puberty and later fertility. *Pediatrics* 2005;116:190–194; *prenatal care, prenatal exposure, puberty, early-onset puberty, in vitro fertilization.*

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ABBREVIATIONS. ART, assisted reproductive technology; IVF, in vitro fertilization; FSH, follicle-stimulating hormone; LH, luteinizing hormone; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; r-FSH, recombinant follicle-stimulating hormone; r-hCG, recombinant human chorionic gonadotropin; H-P-G, hypothalamic-pituitary-gonadal.

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With the development of new techniques in assisted reproductive technology (ART), progress has been made in the ability of infertile couples to conceive a child.

Data from the American Society of Reproductive Medicine's national in vitro fertilization (IVF) registry indicate that in the year 1999, a total of 88 077 cycles of assisted reproductive treatment led to 21 904 deliveries, with the birth of 30 967 neonates.<sup>1</sup> This represents a success rate (deliveries per transfer) of 30.5%, an increase from 17% in 1992. This increase is related to the higher rate of successful implantations as well as an increase in the number of multiple gestations. At present, ART is responsible for 1% to 2% of births in the United States.<sup>1</sup>

Numerous studies have explored the type and incidence of ART-related side effects in offspring. Included among the most common adverse effects are low birth weight ( $\leq 2500$  g) among term (risk ratio: 2.6; 95% confidence interval: 2.4–2.8) and preterm (risk ratio: 1.3; 95% confidence interval: 1.2–1.4) singleton infants conceived by ART.<sup>2</sup> This increased risk persists after adjustment for maternal age and parity, gestational age at delivery, multifetal reduction procedures, and cause of infertility. Although several studies do not report an increased risk of congenital malformations in children conceived through ART,<sup>3,4</sup> others have shown that the prevalence of  $\geq 1$  major birth defects by the age of 1 year is twice as high in infants conceived by ART.<sup>5</sup> In a Beckwith-Wiedemann registry of 65 children with this syndrome, 3 infants (5%) had been conceived by IVF, indicating a higher-than-expected rate (0.8%).<sup>6</sup>

Initial studies did not demonstrate an increase in cancer risk among children conceived by ART.<sup>7,8</sup> More recently, several reports (including a study from the Netherlands<sup>9</sup>) conclude that the relative risk of retinoblastoma is significantly higher.

There are a range of possible factors associated with ART treatment that may contribute to potential adverse outcomes. These factors include the relatively advanced age of infertile couples seeking ART, the underlying causes of their infertility, the medications used to induce ovulation or to maintain the pregnancy in its early stages, and the ART procedures themselves.

The purpose of this report is to describe 7 infants born by ART who presented with breast development and/or pubic hair. The data available to the authors for this report were obtained through direct patient care and retrospective chart review.

## METHODS

### Analyses

All patients underwent baseline hormone analysis. Patients who demonstrated both breast development and pubic hair were evaluated for the possibility of sexual precocity. In these patients, tests to determine the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and dehydroepiandrosterone sulfate (DHEA-S) and ovarian sonography were performed (patients 1–4). Patients presenting primarily with pubic hair (patients 5–7) were evaluated as well for causes of hyperandrogenism (eg, congenital adrenal hyperplasia). DHEA-S, serum testosterone, and 17-hydroxyprogesterone were performed in these patients (Table 1). In addition, a left-hand- and wrist-for-skeletal-age test was performed for each patient.

Laboratory analysis was performed at Quest Diagnostics (Teterboro, NJ) for patients 1, 3, and 5. Cortisol, estradiol, DHEA-S, and testosterone were measured by chemiluminescence; tests for 17-hydroxyprogesterone,  $\Delta$ 4-androstenedione, and estrone levels were performed by radioimmunoassay. LH and FSH measures were performed by microenzyme immunoassay. For patients 2, 4, 6, and 7, laboratory analysis of androgen levels was performed in-house. Radioimmunoassays for testosterone, 17-hydroxyprogesterone, and DHEA-S levels were performed in our laboratory using a well-described methodology.<sup>10</sup> Radioimmunoassays for testosterone and 17-hydroxyprogesterone were performed after purification by Celite chromatography. The intra- and interassay coefficients were 6% and 11%, respectively, for testosterone and 11% and 15%, respectively, for 17-hydroxyprogesterone. DHEA-S, however, was immunoassayed without purification.

Adrenocorticotrophic hormone-stimulation testing of patient 7 was performed by using cosyntropin 0.25 mg (Amphastar Pharmaceuticals, Inc, Rancho Cucamonga, CA) administered intravenously at 0 minutes. Serum sampling was performed at 60 minutes for serum testosterone and 17-hydroxyprogesterone.

Assessment of skeletal age was performed by well-established methods.<sup>11</sup> Pelvic and adrenal sonography were performed at the Division of Pediatric Radiology at the New York University School of Medicine for all patients except patient 1, who underwent the procedure at Lenox Hill Hospital (New York, NY).

With the exception of 2 patients (patients 2 and 4), who have been evaluated recently, all patients have been seen in follow-up consultation for a period of 3 months to as long as 20 months.

### Patient Population

Between May 2001 and April 2004, 7 children between the ages of 5 and 21 months who were conceived by ART were referred by their pediatricians to the Division of Pediatric Endocrinology at the New York University School of Medicine for evaluation of breast development and/or pubic hair. Six patients were female, and 1 was male. The characteristics of the sample, including the clinical findings, are presented in Table 2.

All of the mothers had been treated with recombinant FSH (r-FSH) followed by recombinant human chorionic gonadotropin (r-hCG) and progesterone by established ART protocols.<sup>12–15</sup> With the exception of families 4 and 5, verbal reporting by the families indicated that maternal anovulation or an inability to conceive spontaneously prompted medical therapy with ART. The family of patient 5 underwent ART and treatment of paternal oligospermia. In addition to standard maternal hormonal therapy (for IVF), the father of patient 5 was treated with Teslac, clomiphene, and indomethacin. Patient 4 was conceived by IVF using a sperm donor. Family history for all patients was negative for congenital adrenal hyperplasia, polycystic ovary syndrome, or precocious puberty. All of the mothers denied using medication for systemic or endocrine disorders during the pregnancy.

Patients 1 and 2 were born at 7 months of gestation and had birth weights of 3.5 and 1.81 kg, respectively. Patients 3, 4, 6, and 7 were born at term and had birth weights that were appropriate for gestational age. The genetic backgrounds of the families were diverse, with no predominance within the patient population. Patient 5 was the 8-month product of a set of fraternal twins that was originally a quadruple pregnancy. According to the mother, the male twin was growing normally, with no evidence of pubarche.

On physical examination, all subjects were healthy appearing infants. Patients 1 through 4 presented with bilateral, well-developed breast tissue and pubic hair (Tanner stages II–III). Patients 5 and 6, both female, presented with Tanner III pubic hair and minimal breast tissue. There was no clitoral enlargement or pos-

TABLE 1. Laboratory Data

No	LH* (FSH+), mIU/mL	E2† (E1§), pg/mL	DHEA-S   ( $\Delta$ 4¶), $\mu$ g/dL (ng/dL)	Testosterone, ng/dL#	17OHP** (CORT), ng/dL ( $\mu$ g/dL)	Ovarian†† Ultrasound, cm	Bone Age, mo (CA $\pm$ SD)
1	<0.3 (2.3)	13 (—)	38 (23)	—	—	Left: 1.6 $\times$ 0.9 $\times$ 1.2; Right: 2.2 $\times$ 1.2 $\times$ 0.9; adrenals normal	9 (8 $\pm$ 1.4)
2	1 (16)	15 (<15)	12 (—)	—	—	Left: 1.2 $\times$ 0.4 $\times$ 0.8; Right: 1.1 $\times$ 0.6 $\times$ 0.7; adrenals normal	21 (15 $\pm$ 3)
3	<1 (5)	21 (—)	9 (11)	7	24 (—)	Left: 1 $\times$ 1.9 $\times$ 0.7; Right: 0.7 $\times$ 1.4 $\times$ 0.8; adrenals normal	21 (16 $\pm$ 3)
4	<1 (4)	7 (—)	5 (—)	10	—	Left: 0.9 $\times$ 0.5 $\times$ 0.6; Right: 0.9 $\times$ 1.2 $\times$ 1.0; adrenals normal	12 (10 $\pm$ 2.1)
5	—	12 (—)	<3 (—)	—	—		
6	—	—	21 (—)	<4	<20 (3.81)	—	6–9 (6 $\pm$ 2.1)
7	—	—	32 (—)	19 (39)††	221§§ (—)	—	7 (5 $\pm$ 2.1)

— indicates that the testing was not performed. CORT indicates cortisol; NB, newborn; CA, chronologic age; AP, antero-posterior.

\* LH: 0.02–7.0 mIU/mL (2 weeks to 11 months); 0.02–0.03 mIU/mL (12 months to 8 years).

† FSH: 0.16–4.1 (NB male: 1 year); 0.24–14.2 (NB female: <2 years); FSH 0.26–3.0 (male: 12 months to 8 years); 1.0–4.2 (female: <2–8 years).

‡ Estradiol (E2): <24 pg/mL (12 months to 8 years).

§ Estrone (E1): <15 pg/mL (2 weeks to 8 years).

|| DHEA-S: <5–57  $\mu$ g/dL (6 months to 6 years).

¶  $\Delta$ 4-androstenedione ( $\Delta$ 4): 6–68 pg/mL (6 months to 8 years).

# Testosterone: 75–400 ng/dL (NB male); 3–10 ng/dL (NB female); 3–10 ng/dL (male/female: >7 months to 8 years).

\*\* 17-Hydroxyprogesterone (17-OHP): <106 ng/dL (6 months to 8 years).

†† Ovarian volume (sagittal  $\times$  AP  $\times$  transverse  $\times$  0.52). Unstimulated: <1.2 cm<sup>3</sup>.

‡‡ Baseline/post-adrenocorticotrophic hormone.

§§ Post-adrenocorticotrophic hormone.

**TABLE 2.** Clinical Data

No.	Age, mo	Gender	Gestational Age, mo	Weight/Length, Percentile	Clinical Findings on Day of Consultation		Findings at Birth	Clinical History
					Thelarche	Pubarche		
1	8	Female	7	95%/95%	Firm nodular glandular tissue; areola: <1.5 cm	Long, curly terminal hair; TIII	Presence of breast tissue and pubic hair at birth	—
2	15	Female	7	<5%/25%	Round and firm bilateral tissue; (right: 5 × 4 cm; left: 4 × 4 cm)	Minimal, pale-white hairs <1 cm in length; TII	Vaginal adhesions	At 10 mo: presence of breast tissue
3	16	Female	Term	25%/25%	Round and firm bilateral tissue (6-cm diameter); areola: 1 cm	Barely visible, short, pale hairs; TII	Presence of breast tissue at birth	—
4	21	Female	Term	75%/75%	Round and firm bilateral tissue (left: 4 cm; right: 3 cm)	Short, dark hair; TII	Presence of breast tissue at birth	At 6 mo: presence of pubic hair
5	10	Female	8	<5%/25%	Bilateral glandular tissue (2.5-cm diameter); areola: <1.5 cm	Sparse, long, and curly terminal hair; TIII	Uncertain	At 6 mo: presence of breast tissue and pubic hair.
6	6	Female	Term	75%/90%	Barely palpable right breast tissue; no left breast tissue	Curly, long terminal hair on labia; multiple, barely visible hair along the mons; TIII	Presence of galactorrhea; no breast tissue	At 6 mo: presence of pubic hair
7	5	Male	Term	75%/75%	—	Curly, long terminal hair along the scrotum; testes: 1.5-mL volume.	Uncertain	Uncertain

T indicates Tanner stage; —, clinical onset at birth.

terior labial fusion in the female patients. Patient 7, a male infant, presented with terminal pubic hair along the scrotum but no gonadal or genital enlargement.

Review of the linear growth trajectories indicated that all term infants were growing consistently along their centile channels. Patients 1, 2, and 5, who had been born prematurely, were demonstrating catch-up growth consistent with growth patterns of prematurity.

## RESULTS

Results are presented in Table 1. Levels of LH, FSH, estradiol, DHEA-S, testosterone,  $\Delta$ 4-androstenedione, 17-hydroxyprogesterone, and estrone were in the prepubertal range in all patients. Pelvic sonography indicated the appearance of prepubertal ovaries (ovarian volumes measuring <1.2 cm<sup>3</sup>) and a nonstimulated uterus in all females. The skeletal ages were within  $\pm$ 2 SD for chronologic age in all patients (Table 1). During the follow-up visits, there was no evidence of pubertal progression or linear growth acceleration in any of the patients.

## DISCUSSION

We present data on 7 patients conceived by ART, ages 5 to 21 months, referred for endocrine evaluation of possible precocious puberty and who presented with breast development and/or pubic hair. Patients who demonstrated both breast development and pubic hair were evaluated for the possibility of sexual precocity. Patients presenting primarily with pubic hair were evaluated as well for causes of hyperandrogenism (eg, congenital adrenal hyperplasia). Endocrine evaluation in all patients indicated hormonal levels in the prepubertal range; pelvic sonography confirmed prepubertal ovaries with unstimulated uteri. Clinical follow-up of our patients did not reveal progression of the breast development or an increase in pubarche. Linear growth velocity continued along prior centile channels.

Our patients were unusual in their clinical presentation. In those infants conceived by ART who were referred for evaluation of breast development, the size and maturation of breast glandular tissue exceeded that generally seen in our patient population with isolated thelarche, a benign but not well-explained condition seen in normal infants that may result from a delay in the transition from the active fetal hypothalamic-pituitary-gonadal (H-P-G) axis to the quiescent prepubertal H-P-G axis. The degree of breast tissue seen in our ART patients, either isolated or in association with pubic hair, raised the possibility of precocious puberty, either centrally mediated or of ovarian or adrenal origin.

By clinical history, it was apparent that the 1 common link among this group of patients was that they had all been conceived by ART. All mothers had received r-FSH, r-hCG, and progesterone as defined by standard ART protocols.<sup>12-16</sup> Review of the literature regarding the pharmacokinetics and pharmacodynamics of r-FSH and r-hCG suggests that these compounds alone cannot be the cause of the clinical findings. Additionally, the timing of administration of both medications for ovulation induction renders it unlikely that there may be a direct effect of these drugs on the fetal H-P-G axis.<sup>16</sup>

We reviewed the literature on the possible relation between ART procedures and the appearance of estrogen and/or androgen effect on offspring. Recent data indicate that maternal serum and amniotic fluid levels of  $\beta$ hCG are elevated in pregnancies conceived after ART.<sup>17,18</sup> In addition, ART twin pregnancies have higher levels of  $\beta$ hCG than are seen in spontaneous twin pregnancies. The increase in  $\beta$ hCG is seen even in pregnancies after spontaneous frozen-embryo transfer. Thus, the increase in  $\beta$ hCG seems not to be related to superovulation hormonal therapy.<sup>17,18</sup> Although we cannot exclude other factors, we suspect that the elevation of maternal  $\beta$ hCG seen in ART may play an etiologic role. It is known that placental  $\beta$ hCG induces maternal and fetal adrenal steroidogenesis of DHEA.<sup>18,19</sup> DHEA-S is then converted to  $\Delta$ 4-androstenedione in the placenta. DHEA-S and  $\Delta$ 4-androstenedione are the major precursors for placental estrogen production.<sup>18,19</sup> Elevated levels of DHEA and  $\Delta$ 4-androstenedione are metabolized to estrogens and androgens that may impact the developing fetus.<sup>20–22</sup> We speculate that elevated levels of estrogen and androgens in utero may directly mediate the development of breast tissue and pubic hair and may further alter the maturation of the H-P-G axis in the developing fetus.

Although the etiology of the elevation in  $\beta$ hCG in ART pregnancies is uncertain, it is known that maternal  $\beta$ hCG levels are higher in twin pregnancies than in singleton pregnancies. Therefore, it is conceivable that, in our patient population, multiple embryo implantations early in gestation could have been a factor in increasing maternal  $\beta$ hCG levels and altering the maternal-fetal hormonal milieu. We should note, however, that 1 study did not find any elevation consistent with the number of implantation and posterior multifetal reduction.<sup>23</sup> Additionally, the impact of artificial fertilization or the embryo culture and the effect of progesterone on the fetus are also unknown.

The impact on the developing fetus of an altered hormonal milieu is unclear. It is known that fetal life is an early and important stage in the development of the H-P-G axis that culminates in adult life with the achievement of full sexual maturation and fertility. In both humans and primates, the fetal hypothalamic gonadotrophin-releasing hormone pulse generator is operant in the fetus by the end of the first trimester. Studies suggest that hormonal imprinting or programming occurs in fetal life during a critical period of maturation<sup>24</sup> and may modulate gene expression and nuclear and/or plasma membrane receptors.<sup>25</sup> Evidence exists to indicate that prenatal androgens program the timing of neuroendocrine puberty in sheep; the higher the dose of prenatal testosterone, the earlier the initiation of the pubertal LH rise.<sup>26</sup> It has been shown that estrogen administration to pregnant rats during the last third of gestation produces cryptorchid male offspring and may suppress spermatogenesis permanently in adult males. Additionally, perinatal estrogen administration to the developing female rodent produced long-term effects including persistent vaginal cornification, hyperplastic vaginal lesions, and cervicovaginal cancer; syn-

thetic nonsteroidal estrogens (diethylstilbestrol) had similar effects.<sup>25,27</sup>

Whatever the mechanisms, the developing endocrine system in the fetus and maturation of endocrine-control systems are influenced by the hormone concentrations in the fetus. Patients conceived through ART, including our patients who presented with hormonal manifestations, will need to be monitored throughout childhood and into adolescence and adulthood to determine what impact, if any, exists on the timing of puberty and, later, fertility.

We recognize the limitations of our study. Our data represent a compilation of our experience in ART-conceived infants referred for evaluation of clinical signs of precocious puberty. Although we postulate that elevation in maternal  $\beta$ hCG may play a critical role in the development of a hormonal effect in these infants, serial maternal  $\beta$ hCG levels are not available for our study. Additionally, the number of ART-conceived infants presenting with clinical signs of puberty is extremely small when compared to the hundreds of infants conceived by ART within our referral area. Well-controlled, prospective studies to include fetal ovarian sonography, hormonal levels in amniotic fluid (when available), and careful ongoing evaluation of the ART-conceived infant will provide additional insight into the mechanism of this entity in infants conceived by ART.

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## PARENTS WRONGFULLY ACCUSED OF CHILD ABUSE CANNOT SUE DOCTORS

“Parents who have been wrongly accused of harming their children cannot sue doctors or social workers for negligence in carrying out investigations into child abuse, the United Kingdom’s highest court ruled. . . . The law lords dismissed appeals by parents in three test cases from earlier judgments that had struck out their claims. In a judgment anxiously awaited by pediatricians, the lords ruled by a majority of four to one that child protection professionals owe a duty of care to the child alone and not to the parents who may suffer as a result of an investigation that is negligent.”

Dyer C. *BMJ*. April 30, 2005

**Editor’s Note: Laws, such as this, were passed in the 1970s in the US. They work!**

Noted by JFL, MD

## *In vitro* babies – medical and legal aspects: a European and North American perspective

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### Abstract

On the basis of observations in Europe and North America, this review will focus on Assisted Reproductive Technologies (ART) and their impact on pregnancy outcomes; in particular multiple births, prematurity, and their impact on birth defects. In Europe, since 1985 this experience has been somewhat different from that in North America due not only to the differing populations, cultures, religious perspectives, but also to the rapid implementation of medical technologies, relative freedom from governmental regulation, as well as the different forms of payment for medical care that exist between the two continents. This review will focus on the impact of ART on the complications of pregnancy, multiple gestations and prematurity, and will evaluate the required process and content of informed consent surrounding ART from the legal perspective. Issues related to complications resulting from the use of ART from the perspective of neonatal care providers will be highlighted as well as its impact on the health care system in both regions. Given the impact of ART on both sides of the Atlantic, we propose that governments, as well as professional organizations – including reproductive specialists, neonatologists, and health economists – recommend that a legal limitation on the number of embryo transfers be imposed and that embryo transfer restrictions be coupled with reimbursement for ART services. We suggest that reproductive rights should not be infringed but that greater concern for and monitoring of the safety of both mothers and their newborns be undertaken by various professional organizations and governments in Europe and North America. We also propose systematic centralized reporting of the effectiveness of each form of ART, along with any associated complications, and that ART babies be carefully monitored for birth defects and imprinting disorders on both continents.

**Key words:** Assisted Reproduction Technologies (ART), prematurity, multiple gestation, imprinting disorders, birth defects, newborn safety, congenital malformation, ART outcomes

With more than a 30 year history, *in vitro* fertilization and other forms of assisted reproductive technologies have expanded and gained widespread use in Europe and North America. However, multiple ethical and legal controversies exist in many European countries. There has been limited legal regulation of these expanded practices (e.g. IVF, ICSP, donor oocytes, number of embryos transferred etc.) that constitute the assisted reproductive technologies (ART) in current use. The Council of Europe meeting held in Oviedo in 1997 gathered participants from 47 countries and created the “Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Bio-

logy and Medicine: The Convention on Human Rights and Biomedicine” more widely known as the “European Bioethical Convention”. This convention outlined the basic standards on the most controversial aspects of ART. The major concerns were focused on the prohibition of the creation of human embryos for research purposes, the prohibition of embryo preselection for the selection of gender, and the prohibition of interference with the human genome for purposes other than prophylactic, therapeutic or diagnostic (<http://conventions.coe.int/Treaty/html/164.htm>). The provisions of the Convention were only a “recommendation” and therefore required ratification by members of the Council of Europe, which

were to commit themselves to enact these provisions into their own legal frameworks to provide for legal authority within each respective country.

Currently, 22 countries have ratified the guidelines of the Convention (<http://www.prawoimedycyna.pl/index.php?str=artykul&id=250>). The remaining countries have distanced themselves from endorsement of the major guidelines of the Convention and have determined their own legal regulations. In some instances their decisions were based on the European Union directives concerning the conduct of research with reproductive cells and human embryos created during *in vitro* fertilization (Directive 2004/23 – Official Journal of the European Union). Only 5 European countries have not enacted legal regulations concerning ART, i.e. Poland, Andorra, Cyprus, Romania and Serbia. The legislative framework of each country is different in respect to, among other things, the number of embryos created, the limitations on use for other than reproductive purposes, the legal status of the embryo, the possibilities for embryo selection, and the use of embryos not transferred for human reproduction. Other issues that have been addressed include the availability of ART to unmarried women, homosexual couples, and the issue of who pays for these procedures – individuals or national health systems within these countries. As a result, legislation has varied throughout Europe from the very liberal with few restrictions as in the United Kingdom or parts of Scandinavia, to the more highly restrictive policies in Germany, Italy, Sweden, and Belgium.

In practice, these different legal restrictions and the variable controls placed on ART make it difficult to standardize the quality of services concerning the securing and use of human cells, tissues or organs, the organizational requirements, or the capacity to provide these procedures to those desiring them. In the absence of such standards, it has been difficult to thoroughly analyze the results of ART as it relates to effectiveness, and rates of success by procedure, and to review the number of primary and repeated procedures, and possible complications to both women and, very importantly, infants conceived using ART. In addition, the financial cost to families and the burden to national health budgets in many countries are difficult to compare. Furthermore, the use of ART by citizens of one country traveling to a neighboring or distant country (“medical tourism”) has been difficult to monitor. As a result, legal authorities in

several countries have suggested that a common policy on ART be developed for use throughout Europe.

The European Society of Human Reproduction and Embryology (ESHRE) has supported the concepts of Nobel Laureate Dr. Robert Edwards regarding the stimulation of research and standardization of ART procedures. ESHRE has also made efforts to monitor and to some degree “control” the “*in vitro* market” throughout Europe. The major aim of this review is to evaluate the medical services offered as a part of ART, and to provide a broader examination of the effectiveness and success rates of individual centers. Such analyses provide information regarding the effectiveness of individual ART procedures as well as complications occurring in the perinatal period concerning the mother, the embryo, and the newborn, and the later influences on the health and well-being of the mother and the child delivered as a result of ART or artificial insemination.

Although the population of Europe exceeds 600 million, being some 15% of the global population, approximately 60% of the world’s centers for ART and artificial insemination are located within Europe. Thus, an analysis of such an extensive number of centers and their datasets should stimulate the formation of a standardization of protocols in terms of “outcome reporting” or assessment of differential “success rates”, and provide for a central reporting mechanism for complications to both mothers and infants. A secondary goal is to provide a comparison of the costs of ART throughout the continent. Such a central database, similar to those developed in the U.S. by the Centers for Disease Control and Prevention, would ultimately provide important data for families seeking ART services, health-policy authorities within governments, and officials responsible for financing health care delivery throughout European states. These goals need to be implemented as soon as possible.

Data from the 1997 ESHRE report involving 18 countries and 482 fertility centers, the majority of which had implemented internal registries, have been incorporated into the 2005 ESHRE report that now includes 30 countries and 923 centers, and serves as the major repository of ART data within Europe. Initially, the reported data included ART procedures such as IVF, ICSI, information on the use of donor oocytes, transfer and storage of frozen embryos, the effectiveness of ART expressed as ultrasound confirmed pregnancy, the number of live births, and the age distribution of mothers.

However, over time these datasets were expanded to include more procedures including insemination with semen from the spouse/partner (IUI-H) or from a donor (IUI-D). Recently obtained additional information on genetic pre-implantation diagnoses (PGD) is now also included.

In 1997 more than 200,000 procedures were reported to the ESHRE. These data documented 18,899 gestations from which 24,283 children were born. The percentage of single births was 70.7 after IVF, and 71.7 after ICSI. Twins occurred in 25.8% of pregnancies resulting from IVF and 25.2% of pregnancies occurring after ICSI. In 3.6% of pregnancies after IVF triplets resulted, and 2.9% occurring after ICSI, with quadruplets occurring in 0.2% of gestations resulting from IVF and 0.1% after ICSI. Based on complete data from 10 countries, it has been extrapolated that for every one million of Europe's population there were on average 765 ART procedures resulting in 1.33% of all births per year. By 2005 the number of registered cycles had reached 320,000, of which 36% resulted from IVF with the remaining from ICSI of the pregnancies, with the result that 78.2% of women gave birth to singletons, 21% had twins, and 0.8% resulted in triplets.

With recent recommendations calling for a reduction in the number of multi-fetal pregnancies and the risks associated with them, there has been an apparent trend to reduce the number of embryos transferred. While in 1997 only 11.5% of pregnancies resulted from single embryo transfer (SET), double embryo transfer (DET) occurred in 39% of cases, while in 38.4% three embryos were transferred, and in 14.3% three or more embryos were transferred. By 2005 SET took place in 20% of procedures in Europe with the greatest number occurring in Sweden (i.e. 65%). However, of all transfers in Europe, DET represented 56% of these procedures, and triple embryo transfer occurred in 21%. Three or more embryo transfers occurred in only 2% of procedures performed. The effectiveness of ART measured using an ultrasound confirmed pregnancy and live-birth differed by ART technique. While *in vitro* fertilization resulted in 26.1% pregnancies with 20.9% live-births, with ICSI there was 26.4% pregnancies resulting in 21.5% live-births. By 2005, ART had risen to 1115 procedures per 1 million of population with 30.3% of pregnancies resulting from IVF and 30.9% from ICSI. Thus, despite the rapid growth in the number of procedures performed, and the overall effectiveness of these procedures in Europe, this was less than the growth in U.S. where 42% of wo-

men receiving ART had a confirmed pregnancy with some 27% of U.S. women having a live-birth (Nygren and Nyboe Andersen, 2001; de Mouzon et al., 2010).

The importance of having a European registry is to persuade and assist many fertility centers that offer ART to actively report to a central database for meaningful comparisons of efficacy and safety, as well as the "quality" of various providers. This is especially important for those countries that do not maintain a registry of mothers and the offspring conceived using ART (including Poland). While the European monitoring system included 30 countries in 2006, there remain significant differences in the scope and use of ART, the proportion of methods used in some countries, differences in the effectiveness of each method, and the age distribution of mothers seeking services at individual centers as well as the number of single or multiple births that result from ART. While a central professional registry has limitations, including incomplete data and the lack of reporting from several countries or indeed incomplete recording of complications occurring in either women or their children, the database has provided useful information. However, this information could be improved substantially.

After the initial euphoria of the late 1970s and early 1980s and the increase in the number of infants born using ART technologies, it was also noticed that untoward and unwanted complications were occurring in some women and in the infants born after ART. Some of these problems were not diagnosed until later childhood. Thus, any risk assessment must include not only complications occurring or resulting from pregnancy after ART, but also the prevalence of prematurity and multiple gestations, and also the health status of the infants born, and malformations identified in the neonatal period.

According to the majority of reports, the risk of spontaneous abortion after ART does not exceed the total population risk and approximates 15% (<http://www.uptodate.com/store>). The ESHRE report from 2006 showed that ART was associated with a pregnancy loss of 9 percent (de Mouzon et al., 2010). It is unclear whether the number of pregnancies lost presented in the ESHRE report also included spontaneous abortions, as well as medical termination of pregnancies. As shown in the MOSAIC project (Models of Organizing Access to Intensive Care for the Very Preterm Baby), based on an analysis of data concerning all births between 23 to 31 weeks gestation from 10 regions in 9 European count-

ries, termination of pregnancy occurred in 0.5-17.6% of all pregnancies depending on the region. The most common reason given for termination in all countries was for congenital anomalies (80% of cases) (Papiernik, 2008). Taking in account that pregnancies resulting from ART have a 40% higher risk of certain malformation than in the general population, information on birth defects in the population of children born after IVF needs to be completed in order to have combined data on the prevalence of specific birth defects, spontaneous abortions, terminations of pregnancy, need for fetal reduction, as well as live births. It is noteworthy that pregnancies resulting from ART double the risk of ectopic pregnancy compared to those conceived naturally, and increase the risk of heterotrophic pregnancies (1 per 100 with ART versus 1 per 30,000 naturally conceived) (<http://www.uptodate.com/store>).

Maternal complications after ART include ovarian hyperstimulation syndrome, a larger percentage of ovarian torsion, preeclampsia, pregnancy induced hypertension, diabetes, vaginal bleeding, placenta previa and premature rupture of the fetal membranes (Kallen et al., 2005; Finnstrom and Kallen, 2011). Newborn complications are related to the higher frequency of multiple gestations, prematurity, low birth weight, congenital malformations, chromosomal anomalies, and other genetic diseases. Neonatal death rates after ART are three fold higher than among infants conceived naturally (Basso and Olsen, 2005; Helmehorst et al., 2004). The majority of these complications are related to the significantly increased number of multiple gestations resulting from ART procedures where some 25% of all pregnancies are twin pregnancies. Indeed 40% of all infants born after ART are from multiple gestations (Land and Evers, 2003). These results are comparable to those reported in the U.S., where the number of twin gestations increased to over 40% following ART, while, disturbingly, the number of higher order multiples (three or more) increased 5-fold (Reynolds et al., 2003).

Multiple pregnancies associated with ART may result from ovarian hyperstimulation as well as the transfer of more than SET. These multiple pregnancies also pose risks to the mother, the fetus, and the newborn. For example, among mothers at risk with multiple pregnancies there is an increased risk of cardiovascular disease, hematologic disorders, preeclampsia, diabetes during pregnancy, postpartum bleeding with the require-

ment for either surgical intervention or transfusion of blood products. Newborns delivered after multi-fetal gestation constitute 14% of all premature births and 21% of infants born with low birth weight (Blickstein and Keith, 2002; Blickstein, 2002). Prematurity and low birth weights are associated with a number of well established complications that impact on the child not only during the newborn period and early childhood, but (indeed) throughout their entire life. Premature infants present a higher occurrence of respiratory illnesses, mostly respiratory distress syndrome, but also the chronic lung disease – bronchopulmonary dysplasia. In addition, prematurity is too frequently associated with an increased risk of necrotizing enterocolitis, renal disease, and damage to the central nervous system. About 70% of all deaths occurring during the perinatal period result from premature birth, and the later development of these infants as children (especially those resulting from multiple births) are a larger burden to their parents and the health care system than infants born closer to term and as single live born infants. Of major concern is the high rate of cerebral palsy (an 8 fold increase) from twin births compared to singleton births, and especially as in the case of the demise of one twin the rate of cerebral palsy increases even 15 fold (Cook et al., 2011).

There also exist unique risks to monozygotic pregnancies or those resulting from the split of one embryo into two genetically identical embryos. This risk primarily occurs when the division takes place 4 days after fertilization to form monozygotic, monochorionic twin pairs. These twins are associated with more frequent placental pathology especially those associated with abnormal vascular connections in the placenta that connect the circulatory systems, and thus the cardiovascular systems, of each twin. This disorder is termed “twin-to-twin transfusion syndrome”. Twin-to-twin transfusion results in the exchange of blood from one twin (the donor) to the other twin (the recipient). The donor twin develops anemia, hypoxemia, and intrauterine growth restriction, while the recipient twin shows symptoms of cardiac overload resulting from hypervolemia, and polycythemia (often with thrombo-embolic phenomena) (Denbow et al., 2000; Revinis and Johnson, 1994). Pregnancies with monozygotic twins also pose a greater than normal risk of malformations compared to dizygotic twins. Furthermore, in monozygotic twin pregnancies there is a characteristic group of disorders occurring

during the first 4 weeks after fertilization and associated with blastogenesis associated birth defects, which include neural tube defects, defects in the gastrointestinal tract specifically tracheoesophageal fistulae, and anal atresia (Halliday et al., 2010). While the frequency of monozygotic twin pregnancies is relatively stable in the general population (0.4%), among those receiving ARTs the rate is 1.4%. Although the mechanism causing the division of a single zygote to divide into two embryos is currently unknown, in ART the risk of monozygotic twin pregnancy is increased and is most likely related to the stimulation of ovulation and micromanipulation of the zona pellucida during the *in vitro* fertilization procedure (Engmann et al., 2001).

In addition to complications arising in mothers, complication rates for infants born after ART are primarily related to the high rate of multiple gestation pregnancies generated by ART and their high rates of prematurity. Steps must be taken to minimize multiple pregnancies primarily by reducing the number of embryos transferred to a single embryo and by reducing the hormonal ovarian stimulation used for oocyte retrieval to improve their outcomes. While in the early 1980s multiple embryos were transferred to improve the effectiveness of ART, an initial limit was placed to no more than three. In 1991, in accordance with the recommendations of the Human Fertilisation Embryology Authority (HEFA), in Great Britain a three embryo transfer limit was established. However, the work of Engmann et al. (Engmann et al., 2001) found that the effectiveness of ART prior to and after the imposition of the three embryo limit was accompanied by no change in the number of multiple gestation pregnancies that occurred – ~30%. One of the reasons for this high rate of multiple pregnancy was the use of excessive ovarian stimulation in hormone treatments to the prospective mother, and the maternal age (older women had more embryos transferred with a tendency of 3 or more). On the other hand, the increase in the number of births after using ART primarily resulted from the selection of “higher quality” embryos by embryologists who evaluate those “created” during IVF or ICSI. The next step was to reduce the number of embryos transferred from two (DET), and now according to current recommendations to only one embryo or SET. The practice of restriction to SET is currently the standard in 5 European countries (<http://www.eshre.eu/ESHRE/English/Guidelines-Legal/ESHRE-Position-Papers>).

Swedish infertility specialists have followed the recommendation for SET very closely with the aim of reducing multiple pregnancies. For over 25 years there has been an obligation to report all ART procedures, and from the mid 1990s the policy of SET has been the Swedish standard. This had led to the impressive result of reducing the number of multiple births from 30% in 1991 to 6% in 2006 (Nygren et al., 2001; Nygren and Nyboe Andersen, 2001; Nygren and Nyboe Andersen, 2002; de Mouzon et al., 2010). In 2011, Swedish scientists published the results of 25 years of analyses in using ART procedures, and their influence on the health of women and their offspring. Two time periods, 1982-1991 and 2001-2006, were compared (Kallen et al., 2005; Finnstrom and Kallen, 2011). The major difference in practice was the number of embryos transferred with 70% being SET in the latter time period versus 9% in the former period. Other changes occurred such as the increase in ICSI frequency that occurred in the latter period contrasting with classical *in vitro* fertilization, as well as a general lowering of the age of women seeking infertility treatment.

Thanks to this spectacular reduction in the number of multiple pregnancies from 30% in 1997 to 6% in 2006 (Nygren et al., 2001; Nygren and Nyboe Andersen, 2001; Nygren and Nyboe Andersen, 2002; de Mouzon et al., 2010) the percentage of complications occurring in both the mother and the newborn have been substantially reduced; however, this population still carries a higher risk than the general population. The frequency of premature birth in 2006 was 10.9% for ART infants compared to 6% of the general population conceived naturally, with the risk of prematurity in singletons of 7.5% compared to a population risk of 5.1%. Low birth weight occurred in 7.5% of ART infants compared to 2.1% of those conceived naturally. There remained a greater risk of intraventricular hemorrhage, neonatal seizures, and respiratory distress requiring mechanical ventilation among infants conceived using ART compared to those conceived naturally. Congenital malformation risk, primarily associated with disorders of the cardiovascular system, musculo-skeletal system, was increased overall, and there was a small decrease in neural tube disorders, esophageal atresia, or cardiac septal disorders compared to the earlier period with multiple embryo transfer. There was also a decrease in cleft palate, imperforate anus, and hypospadias during the later period. Long-term complica-

tions including cerebral palsy, developmental disorders, lower visual acuity, and asthma were diagnosed more frequently among infants delivered after ART than those born after natural conception. During the compared periods no differences in outcomes have been documented between ICSI or *in vitro* fertilization. However, there continues to be an increase in the risk of preeclampsia, premature rupture of the membranes, prematurity, and more caesarean sections among women receiving ART (Kallen et al., 2010).

The issues identified in Europe have also been identified in North America although magnified in terms of the disregard for professional standards in the case of the “Octomom”, and the creation of surrogate pregnancies in which there is a paid surrogate womb for incubation of embryos that have been created by other means. It has recently been documented that some of these women are being sent to Europe (Ukraine specifically and perhaps other European countries) for donated embryo transfer after ovarian stimulation and then completion of the pregnancy within the U.S. with subsequent “selling” of babies to vulnerable parents. Complicating the North American experience is the fact that most health insurance policies, and many publicly paid plans (Medicaid in the U.S.) do not cover ART procedures, and in Canada only Quebec provides government paid but albeit limited ART services.

In the United States, Assisted Reproductive Technologies (ART) are responsible for 16.2% of twin deliveries and 38.3% of triplet deliveries (Wright et al., 2008). ART has been implicated in contributing disproportionately to the failure to achieve significant reductions in premature births. ART is available not only for infertile couples, but also for women seeking pregnancy without a male partner, and to men who choose to have a child through the use of surrogate gestational carrier who agrees to carry a pregnancy using either her own egg(s), or an egg/eggs obtained from another woman. In 1905, in an era preceding ART by seven decades, the United States Supreme Court found, in *Jacobsen v Massachusetts*, that limitations on personal liberty for the purposes of the public health were permissible. The Court held that “persons and property are subjected to all kinds of restraints and burdens, in order to secure the general comfort, health, and posterity of the State; of the perfect right of the legislature to do which no question ever was, or upon acknowledged general principles ever can be

made, so far natural persons are concerned” (United States Supreme Court, 1905). In 1977 the United States Supreme Court ruled that the decision to bear children is constitutionally protected, and thus ART differs from other areas of medicine (United States Supreme Court, 1977). President Carter appointed an Ethics Advisory Board that issued a report in 1979 suggesting that a permanent board should be appointed to monitor ART. Their recommendations were never implemented.

In 1992, Congress enacted the Fertility Clinic and Success Rate and Certification Act that required the Secretary of Health and Human Services, through the Centers for Disease Control and Prevention, to develop a model program for the certification of embryo laboratories. This was to be carried out voluntarily by interested States. Thus, voluntary reporting by Fertility Clinics was established, but no specific mandate for reporting was established. Beginning in 1997, the Centers for Disease Control and Prevention (CDC) issued 12 annual reports detailing the clinical outcome of ART programs (<http://www.cdc.gov/ART/>). To comply with the law, the CDC established a quality control program that reviews the incoming, voluntarily submitted data and performs annual site visits. Rather than individual physician performance, clinic-specific pregnancy and live birth success rates based on ART modality and maternal age are collected. Information collected includes live birth rate per initiated ART cycle, per oocyte retrieval, and per embryo transfer. The CDC also reports on the accreditation status of attendant embryo laboratories and the identity of non-reporting programs (Adashi and Wyden, 2011). While these reports may be used as “quality indicators” of clinic performance, “chief among those improvements is the incidence of multiple births, an outcome driven by the number of embryos transferred in the course of an ART cycle.” A comparison of the U.S. national patterns of practice in 1997 with those in 2008 establishes that the percentage of cycles entailing the transfer of 3 or more embryos decreased from 83% to 35% (the CDC report). Unfortunately, 8% of ART programs in the U.S. do not share data with the CDC, and it is unclear whether attendant quality improvements are due to changed behavior on the part of empowered consumers, or responsive clinicians, or both (Ferris and Torchiana, 2010).

In 2002 the President’s Council on Bioethics issued its recommendations for ART, including continued industry self-regulation and federally funded longitudinal

studies (President's Council on Bioethics, Reproduction and Responsibility: The Regulation of New Biotechnologies) (President's Council on Bioethics, Reproduction and Responsibility: The Regulation of New Biotechnologies, 2002). Arthur Caplan, a noted medical ethicist, proposed that the U.S. Food and Drug Administration should exercise greater control over all new forms of ART; and insurance companies and third-party payers "should pay only for those programs accredited by the American Society for Reproductive Medicine, who are in full compliance..." with the society's guidelines and existing laws. In addition, the National Conference of Commissioners on Uniform State Laws was charged to review the current fragmented state laws and develop model legislation to systematize, as far as possible, legal approaches among states (Caplan, 2007).

In the U.S. the practice of medicine is governed by the 50 states with multiple and diverse regulations affecting reproductive medicine. This has led to growing commercial business entities because of limited regulation. Because ART focuses on babies, and pregnancy is often the result of *in vitro* fertilization and the creation of embryos, as well as private recruitment of "surrogate mothers", it has proven impossible for the U.S. government to regulate reproductive medicine. Legislators and public health policy makers tread lightly where the rights to privacy and reproductive liberty may have a constitutional basis (Ouellette et al., 2005). Furthermore, the use of the internet, and especially "social websites", have promoted "trade" in surrogacy internationally, making national regulation very difficult. Audi and Chang described the current lack of regulation on ART and reproductive medicine specifically: "with an international network of surrogate mothers and egg and sperm donors, a new industry is emerging to produce children on the cheap and outside the reach of restrictive laws" (Audi and Chang, 2010). George Annas has written elegantly that "If the medical community cannot control assisted reproductive procedures that require the application of medical skills, an unregulated market will determine the price, place, and manner in which human sperm, ova, embryos, and services of surrogate mothers will be made available as well as how family relationships with the resulting babies be will structured". He further observed that the "global baby" has arrived in practice, but neither the legal theory nor medical ethics has kept pace with the globalization of human reproduction (Annas, 2011).

In June 2008 Dr. Michael Kamrava, a California fertility specialist, performed six embryo transfers into Nadya Sulemann and she gave birth to octuplets (the "Octomom"). International attention was focused on the lack of adherence to professional guidelines and lack of governmental regulation of ART and California was labeled "the Wild West" of fertility medicine (Reynolds et al., 2003). On 22 December 2009, the Medical Board of California filed an accusation against Kamrava accusing him of violating Business and Professions codes of "gross negligence, and repeated negligent acts, when he repeatedly transferred an excessive amount of embryos into a patient resulting in an octuplet pregnancy". Further, he never recommended or referred the patient to a mental health professional, although she was single and already had six children. He was also accused of having maintained inadequate records. This initial accusation was amended to further charge him with "incompetence" in his treatment of other patients. On June 2011 the Medical Board revoked his medical license ([http://www.mbc.ca/gov/board/media/releases\\_2011\\_06\\_01\\_kamrava.html](http://www.mbc.ca/gov/board/media/releases_2011_06_01_kamrava.html)). Upon appeal to the Courts, a judge refused to reinstate the medical license in December 2011 ending Dr. Kamrava's medical practice in California (<http://www.sacbee.com/2011/12/15/4125570/bid-rejected-to-reinstate-octomom.html>). This lapse of professional judgment, disregard of professional guidelines, and placing both the mother and eight infants at risk of subsequent disability, incurring substantial costs to the health care system at public expense, stimulated additional calls for government regulation. In addition, recent legal cases involving attorneys arranging for surrogates to have embryos implanted in Ukraine with baby selling activities to vulnerable parents in the U.S. have prompted greater legal oversight of these activities. Dr. Kamrava's decision to disregard professional guidelines while endangering the life of the mother and the eight resulting infants has been the subject of both medical and legal discussion and publications calling for further regulation.

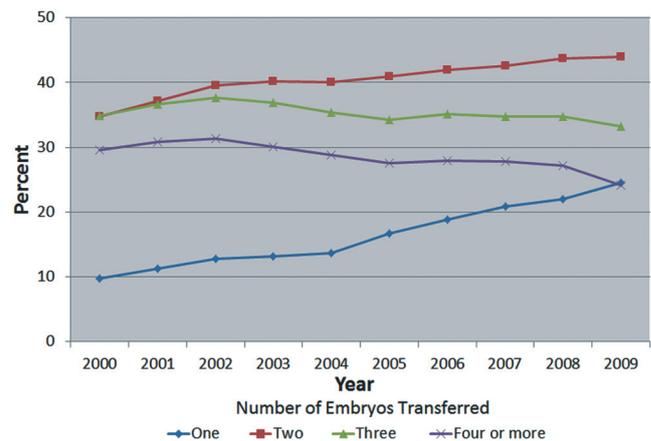
#### **Multiple births and prematurity: ART in the U.S.A.**

One impact of ART in the U.S., is that the twin birth rate rose 2% in 2009, to 33.2 twins per 1000 total births. This was another "high" for the nation according to the Centers for Disease Control (Schieve et al., 2002), and far greater than the "natural" rate of twinning. In-

deed, the rate of twin births has climbed 76% since 1980, and 47% since 1990. Triplet and higher order multiple birth rate also increased in 2009 to 153.5 per 100,000 live births, and the triplet or greater multiples rose more than 400% during the 1980s and 1990s, peaking at 193.5 in 1998. In 2009, 5905 triplets, 355 quadruplets, and 80 quintuplets and higher-order multiples were born. This pronounced increase in twin and triplet or higher order multiples during the 1980s and beyond has been associated with older maternal age at childbirth and the expanded use of fertility-enhancing therapies (Hamilton et al., 2011; Wilcox et al., 1996). A recent decline in the rise of triple or higher multiple births may have been influenced by guidelines from the American Society for Reproductive Medicine. These were first issued in 1998 and revised in 2009 and were perhaps intended to reduce the incidence of higher-order multiple gestations. Refinements in assisted reproductive technology procedures have also have been influential, however (Reynolds et al., 2003; Jain et al., 2004).

Maternal age has been rising in the U.S. over the past two decades. Between 2008 and 2009, birth rates declined among women of all age groups under 40 years of age. Although the birth rate for teenagers fell among women aged 15-19 years, the 2009 rate of 39.1 per 1000 women was the lowest reported in nearly the last seven decades. Among women between 20-24 years of age, the birth rate of 96.3 per 1000 women in 2009 was the largest decline since 1973 (Stern et al., 2007), while for women 25-29 the rate was 110.5 per 1000 women a rate that has declined since 1990. For women 30-34 the birth rate has increased from the 1976 to 2007 figure and was 97.7 births per 1000 women in 2009, while in those aged 35-39 the rate is 46.5 per 1000 women which is the second year of decline (2008 and 2009) since a steady increase from 1979 (see Table 1).

Among women, in their 40-44 years of age, the birth rate was 10.1 live births per 1000 women, the highest birth rate reported since 1967, although this rate has been rising steadily over the last 10 years from 7.4 in 1999 (Martinez et al., 2011). The increase in birth rates for women aged 35 and over during the last 20 years has been linked, in part, to the use of fertility-enhancing therapies or ART (National health statistic report, No XX, 2011). In 2009 the American Society of Reproductive Medicine in 2009 issued additional guidelines on the number of embryos transferred (McLernon et al.,



**Fig. 1.** U.S. Data from Assisted Reproductive Technology, CDC, 2009. Percentage of transfers that resulted in live births using fresh non-donor eggs or embryos, by number of embryos transferred, U.S. 2000-2009 (with permission)

2010; Chandra and Stephen, 2010) – Figure 1, and later in 2011 offered an additional “Committee Opinion: Elective Single Embryo Transfer.” The major conclusions provided by them are as follows: “elective single embryo transfer should be offered to patients with a good prognosis and to recipients of embryos from donated eggs. *In vitro* fertilization centers should promote elective single embryo transfer, when appropriate, through provider and patient education, and improvements in embryo selection should further increase the application of elective single embryo transfer”, along with several additional statements highlighting the results of single embryo transfer compared to double embryo transfer in well-controlled, nonrandomized trials and clinical reports, and noting the improved success of cryo preserved embryos using current technologies (The Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology, 2009).

A meta-analysis of randomized trials, comparing a single with a double embryo transfer confirms that a single embryo results in a higher chance of delivering a term singleton live birth compared with a double embryo transfer ([http://www.asrm.org/uploadedFiles/ASR<](http://www.asrm.org/uploadedFiles/ASR%2009)). Furthermore, this analysis indicates that “although this strategy yields a lower pregnancy rate than a double embryo transfer in a fresh *in vitro* fertilization (IVF) cycle, this difference is almost completely overcome by an additional frozen single embryo transfer cycle” (Balaban et al., 2008). Sweden has mandated the single

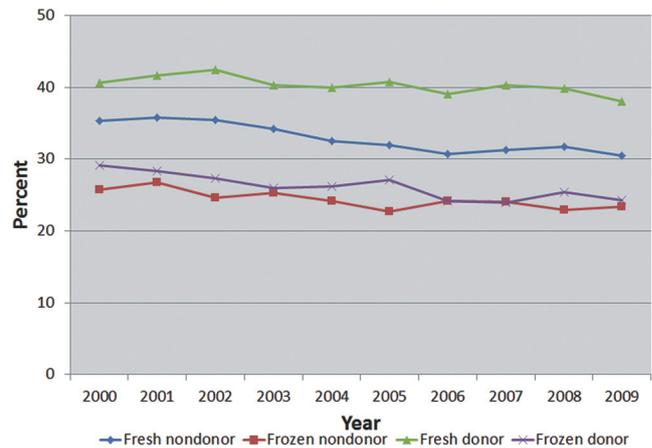
**Table 1.** National summary data from the U.S. of pregnancy success rates – 2009

Pregnancy success rates	Age of woman (years)				
	< 35	35-37	38-40	41-41	43-44+
Fresh embryos from nondonor eggs					
Percentage of cycles with live births*	41.2	31.6	22.3	12.4	4.9
Percentage of transfers resulting in singleton live births	30.9	27.0	21.8	14.0	6.6
Percentage of pregnancies with twins	33.4	27.4	21.5	13.4	7.5
Percentage of pregnancies with triplets or more	2.7	3.6	3.5	2.9	0.8
Frozen embryos from nondonor eggs					
Percentage of transfers resulting in live births	35.2	30.4	25.9	21.9	15.1

\*A multiple infant birth is counted as one live birth, +includes > 44 years because previous data show that patient age does not materially affect success with donor eggs (data from the Centers for Disease Control, 2009 Assisted Reproductive Technology: Success Rates National Summary and Fertility Clinic Reports extracted from page 91, with permission).

embryo transfer protocol, permitting strictly defined exceptions (mother’s age for whom double embryo transfer can be considered and embryo quality) with an overall proportion of deliveries per cycle of 21.9% (McLernon et al., 2010; Chandra and Stephen, 2010). In a commentary regarding the meta-analysis performed by McLernon and his coworkers (2012), Templeton, cite single embryo transfer rates that vary around the world with Australia, Japan, Sweden, Finland, Belgium and the Netherlands at the top of the list whereas the US at the bottom. He summarizes that “Doctors managing infertile couples are no longer entitled to take risks with the health of the next generation” (Nyboe Andersen et al., 2008).

The greater vulnerability and the compromised outcome of multiple birth gestations compared to singleton gestations is illustrated in Figure 2 (adapted from CDC report 2009) which depict that 35% of triplet births were born weighing < 1500 grams compared to 10% of twins and 1% of singletons. Since the average twin weigh 960 grams less than the average singleton at birth, triplets typically weigh about 50% of singletons. What is more concerning is that a shorter gestational age at birth and a smaller size of multiple births create an 8-fold increased risk of dying with the first month of life (cited by CDC 2009 report from the National Center for Health Statistics, unpublished data from the 2007 period linked birth/infant death data set. 2011). Neurodevelopmental outcomes of twins and higher order multiples show poorer cognitive outcomes for twins and that these differences persist even when confounders are taken into



**Fig. 2.** U.S. Data from Assisted Reproductive Technology, CDC, 2009. Percentage of ART cycles that resulted in multiple-infant live births by type of ART cycle, U.S. 2000-2009 (with permission)

account (Templeton, 2010). Cerebral palsy rates are considerably higher in twins, especially with the death of a co-twin (Cook, 2010).

Commenting on rising rates of preterm birth in the U.S., Lantos and Lauderdale (Ong et al., 2006) specifically focus on the observation that more women are delaying childbearing until they are 30-40 years of age and that these older women have high rates of infertility (Lantos and Lauderdale, 2011), leading to the use of ovarian stimulation drugs and IVF (Van Noord-Zaadstra et al., 1991). These treatments result in higher rates of multiple pregnancies and preterm birth. Although infants conceived using ART account for only 1% of all births in the US, the proportion of twin and triple or

more multiple birth attributable to ART is 16 and 44%, respectively (Chandra and Stephen, 1998). Lantos and Lauderdale reflect that the recent drops in preterm birth rates in the U.S. “may reflect an equilibrium, in which advances in prenatal diagnosis, obstetric care of high-risk pregnancies, and neonatal intensive care, along with a new steady state in the demographics of childbearing, and more careful use of assisted reproductive technologies all combine to lead to an optimum balance between reproductive freedom, obstetrical intervention, and perinatal outcomes. “Reports regarding excess premature deliveries following ART, even among singleton deliveries, have been observed in Australia and New Zealand, along with the well-documented increase in preterm infants among twin births (Reynolds et al., 2003; [http://www.preru.unsw.edu.au?PRERUWeb.nsf/reources/ART\\_2005\\_06/\\$file/art11.pdf](http://www.preru.unsw.edu.au?PRERUWeb.nsf/reources/ART_2005_06/$file/art11.pdf)). In a comparison of selected twin deliveries with and without ART from Massachusetts, U.S., restricted to mothers of increased socioeconomic status, private health insurance and intermediate/plus prenatal care, twins resulting from ART were less likely than non-ART to be very preterm, <1500 grams birth weight, or die prior to hospital discharge (Vitthala et al., 2009).

Following IVF, more fetuses are occasionally discovered than the number of fertilized embryos inserted. Pregnancies resulting from ART procedures carry a two-fold or higher risk for producing monozygotic twins, however, this risk varies based on the ART techniques performed. Higher rates of twinning have been reported with other ART forms such as assisted hatching (0.7%); ovulation induction (1.2%); blast transfer (1.7%); and frozen embryo transfer (3.0%) ([http://www.preru.unsw.edu.au?PRERUWeb.nsf/reources/ART\\_2005\\_06/\\$file/art11.pdf](http://www.preru.unsw.edu.au?PRERUWeb.nsf/reources/ART_2005_06/$file/art11.pdf)). ART is associated with significantly higher rates of dizygotic twin gestations as well as higher order of multiples gestations than expected from “natural” conception. Dizygotic twins comprise 95% of twin gestations arising from ART, a much higher percentage than dizygotic twins from “natural” conceptions, and more than 50% of these twin gestations are delivered prior to term, along with higher order multiple birth(s) contribute disproportionately to the need for neonatal intensive care services (Cowan and Demmer, 2007). Data reveal an increased perinatal mortality risk among singletons associated with ART (odds ratio [OR] = 2.19 (95% CI = 1.68-2.98). Meta-analysis have demonstrated that singletons

associated with IVF have an increased risk of preterm birth (OR = 1.95 CI = 1.73-2.20), low birth weight (R = 1.77, 95% CI = 1.4-2.22), very low birth weight (OR = 2.7, 95% CI = 2.31-3.14), and intrauterine growth restriction (OR = 1.6, 95% CI = 1.26-2.04) (Martin et al., 2007). Each of these added risks often requires intensive care, and thus contributes to resource utilization and hospital days. *In vitro* fertilization also increases the risk of cerebral palsy due to its association with preterm birth (among both singletons and multiples), although not necessarily because of IVF (Jackson et al., 2004). The financial burden incurred by these infants is enormous, and beyond the scope of this report.

ART is also associated with a higher risk of antepartum hemorrhage than that seen in the overall population. Pregnancies resulting from ART also are significantly more likely to be associated with pre-eclampsia (4.9% vs 2.6%), abruptio placenta (1.1% vs 0.6%), placenta previa (1.0 vs 0.3%), hyperemesis gravidarum, anemia and postpartum hemorrhage. Induction of labor is more common (OR = 2.1, 95% CI = 1.8-2.4) as well as an instrumented delivery (OR = 2.2, 95% CI = 1.8-2.6). Many infants are more likely to require neonatal personnel for delivery room care. Neonatal intensive care unit (NICU) admission occurs more often (OR = 2.04, 95% CI = 1.23-3.38) following conception with ART (Hvidtjorn et al., 2006; Thomon et al., 2005). Thus the impact of ART for a specific NICU is related to the proportion of birth resulting from ART in a specific region and specific ART modalities used.

Although the correlation between multiple gestation and adverse perinatal outcomes has been well established, most women in the U.S. who choose to use ART elect for multiple embryo transfer. This decision appears to be motivated by a number of factors, including the relatively high cost of ART procedures, which may not be covered by health insurance, and their desire to optimize their chance for pregnancy during a single treatment (Adler-Levy et al., 2007). It is possible that multiple pregnancies may be the preferred outcome for some women with infertility as a survey of fertility clinic patients in the U.S. found that 20% of women desired multiple gestations in preference to a single birth (Boulet et al., 2008). Nonetheless, it is clear that relative to singleton pregnancies, twin pregnancies, and higher multiples, even more so, face significantly higher risks of perinatal and infant morbidity, regardless of whether

ART is the method of conception (Wright et al., 2008). Janvier and coworkers (Janvier et al., 2011) point out “it is clear that physicians performing IVF are aware of these risks, but remain willing to perform procedures that increase the risks to mothers and babies.” She and her co-authors continue to say that “we think that there are a number of reasons for this ethically unusual situation,” including a “perverse economic incentive – for both patients and their providers.” Shah and McCrary (Shah and McCrary, 2010) echo these concerns and proposed “stricter regulation” that could promote patient and family welfare while producing healthier babies and maintaining fertility rates.” ART techniques led to the phenomenon of fetal reduction. Limiting the number of implanted embryos would reduce the scale of this phenomenon.

### **Assisted reproduction in Canada**

Universal public funding for ART is not available in Canada, and thus it is estimated that only 15% of couples affected by infertility can afford IVF or other ART procedures (Nisker, 2008). Only Quebec provides reimbursement for the cost of up to two cycles of IVF treatment (Janvier et al., 2011). A law regulating ART and issues surrounding surrogacy, egg, sperm, and embryo donation was regulated by the Assisted Human Reproduction Act 2004 (<http://www.cam.oo/prg/em/ca/laws/stat/sc-2004/latest/sc-2004-c-2.html>). This law lists prohibited activities related to the purchase of donor gametes, sperm, and compensation surrogates. Health Canada indicates “This legislation has three objectives: It prohibits human cloning and other unacceptable activities and seeks to protect the health and safety of Canadians who use ART procedures.” It further ensures that ART-related research is carried out to find treatments for infertility and “takes place within controlled environments” ([www.hc-sc.gc.ca](http://www.hc-sc.gc.ca)). The legislation established Assisted Reproduction Canada, an agency to implement and enforce this law which licenses all individuals who undertake any controlled activity (those who work with human embryos), and governs the clinical and research activities of medically-assisted human reproduction, and identifies activities that are either prohibited or subject to regulation. This law was challenged, in the Supreme Court of Canada, by the Attorney General of Quebec, joined by the Attorneys General of New Brunswick,

Saskatchewan, and Alberta. The Canadian Supreme Court’s majority held that the regulation of reproductive medicine is a provincial power and that it is not constitutional when conducted by the federal government unless it involves prohibited criminal activities (cloning, germline genetic engineering, and commercialization of human gametes and surrogate pregnancy) while IVF and noncommercial gamete donation and surrogacy are under provincial jurisdiction (Supreme Court of Canada Assisted Human Reproduction Act 2010 SCC 61, 2010). In addition, criminal law prohibitions of the federal act were held that neither physicians nor health care institutions require special licensure to carry out research for medical purposes in assisted reproduction.

Two Canadian technology assessments in 2006 ([http://www.health.gov.on.ca/english/providers/program/ontac/tech/reviews/pdf/rev\\_ivf101906.pdf](http://www.health.gov.on.ca/english/providers/program/ontac/tech/reviews/pdf/rev_ivf101906.pdf)) and 2009 (<https://www.ihsc.on.ca/programs/infertility>) reported IVF and multiple gestation pregnancies. In 2009, the Institute of Health Economics at/in Canada (Anderson and Yan, 2009) sought to clarify the cost burden of multiple pregnancies on health resources and the potential cost impact of ART in Alberta. The 2009 report cites evidence that reducing the number of embryos transferred per IVF cycle to a single embryo transfer reduces the number of multiple births, health complications in the newborn, and the cost associated with multiple gestations. The report found that transferring a single embryo was less costly and just as effective as transferring two embryos for women of 37 years of age or younger. For older women, single embryo transfer was less effective than transferring two embryos, and subsequently, more IVF cycles were required to achieve comparable birth rates. The evidence cited in this report found that reimbursing IVF procedures which transfers fewer embryos was associated with a decrease in the number of multiple births and health services expenses. A greater number of single embryo transfer cycles may be required to produce equivalent results. Janvier et al. (Janvier et al., 2011) reported that if a policy allowing a universal single embryo transfer versus two transfers in as many as one-third of women were in effect for Canada, there would be a saving in the use of NICU resources by 5424 to 7529 fewer NICU patient days of assisted ventilation, and from 35,219 to 42,488 total patient days in an NICU to the Canadian healthcare system. In addition, such

a policy would spare 30-40 deaths per year, 34-46 severe intraventricular hemorrhages, and 13-19 retinal surgeries for retinopathy of prematurity if the rates experienced, at the rates of multiple gestations experience, at the Royal Victoria Hospital in Montreal were extrapolated to all of Canada. These authors also believe that transfer of more than a single embryo is “ethically questionable” and argue that physicians should be restrained from so doing by governmental regulation.

### Birth defects

The relationship between ART procedures and birth defects is less clear, and certainly less disclosed (Johnson et al., 2006; Reefhuis et al., 2009). Hansen and co-workers reported that infants conceived by employing ART were more than twice as likely as naturally conceived infants to have a major birth defect (Hansen et al., 2002). Publications from the late 1990s dismissed the increased risk estimates of birth defects because they failed to reach statistical significance. Furthermore, sample sizes were small and no appropriate controls were available (Van Steirteghem, 1998; Kurinczuk and Bower, 1997). In recent years extensive studies and meta-analyses have been conducted utilizing central data banks such as the facility in Western Australia to determine the rate of birth defects occurring with ART (Hansen et al., 2005). National healthcare registries reporting malformations do not exist in the U.S. and data on ART-related births and outcomes has previously been estimated from voluntary fertility clinic reports (Green, 2004). One recent U.S. study accessed data from the National Birth Defects Prevention Study, a population-based, multicenter, case-control study of birth defects and found that malformations occur more often among infants conceived with ART (Reefhuis et al., 2009) with odds ratios from 2.1 to 4.5 for various major malformations. With larger subject numbers and better controls it is clear that there is an increased risk of malformations with ART (Davies et al., 2012; Wen et al., 2012; Reefhuis et al., 2009; Hansen et al., 2002; Bergh et al., 1999; Wennerholm et al., 2000; Katalinic et al., 2004; Olson et al., 2005). Current results are highly suggestive of increased rates of septal heart defects, cleft lip (with or without cleft palate), esophageal atresia, anorectal atresia, and hypospadias (Table 2) (adapted from Paulson R. *Pregnancy outcome after assisted reproductive*

*technology*. In: *UpToDate*, ed. Basow D.W., UpToDate, Waltham, MA, 2012).

The increased risk regarding birth defects could be conveyed as an increased incidence above the standard risk. Alternatively this event rate difference may be explained as the “number needed to harm” or event rate difference/1. Given a baseline prevalence of birth defects of 2-3% and an increased odds of 2.0, the number of children that are conceived by ART for one additional child to be born with a birth defect is between 33 and 50 (Hansen M. et al., 2005). As assisted reproduction becomes more utilized (and more reimbursed) there is a growing concern that the rates of major birth defects will also rise. Major birth defects that are most often associated with ART include septal heart defects, esophageal atresia, anorectal malformation, and hypospadias (Reefhuis et al., 2009). These defects require major surgical interventions and represent a major burden not only to the infant but also to the health care system. At what level of increased risk is there a duty of the medical profession to advocate for the yet unborn baby? Is a two-fold increased risk of birth defects an acceptable risk of ART? There is a concern that the impact of ART on major malformations and birth defects may be either downplayed or not revealed in the informed consent process. (Birth defects and ART are discussed in detail in the following manuscript).

### Imprinting disorders

Genomic imprinting is an epigenetic process that allows some genes to be expressed from only one parental allele while silencing the other parental allele. Specific disorders including Beckwith-Wiedemann Syndrome (BWS), Angelman Syndrome (AS) and others, have been associated with ART. Publications from Europe, the U.S., and Australia have suggested an association between ART and BWS demonstrating more than 90% of infants with BWS who were born after ART had an imprinting defect. Although limited by their relative rarity and study design, multiple studies from around the globe suggest an association between imprinting disorders and ART, and specifically hypomethylation of maternal allele (Owen and Segars, 2009). Due to the variation of ART protocols and limitations in sample size as well as numerous confounders it has been difficult to rigorously relate an association between imprinting disorders and any

**Table 2.** Risk of birth defects in neonates conceived by artificial reproductive technologies and of specific birth defects 2000-2009

Author	Year	ART	Control	Results	Odds ratios	95%CI
Wennerholm et al.	2000	ICSI	Gen Pop	rate of defects	1.75	1.19-2.58
Katalinic et al.	2004	ICSI	NC	rate of defects	1.24	1.02-1.5
Hansen et al.	2002	ICSI IVF	NC NC	rate of defects	2.2	1.3-3.3
				rate of defects	2.6	1.7-3.0
Olson et al.	2005	IVF	NC	rate of defects	1.3	1.0-1.67
Reefhuis et al.	2009	IVF or ICSI	NC	septal heart defects ASD or ASD + VSD	2.7	1.6-4.8
				cleft lip with or without cleft palate	2.0	1.0-4.0
				esophageal atresia	6.8	2.8-15.5
				anorectal atresia	3.4	1.2-8.3
				hypospadias	4.6	2.0-10.8

ICSI – intracytoplasmic sperm injection, IVF – in vitro fertilization, NC – natural conception, ASD – atrial septal defects, VSD – ventricular septal defects, Gen Pop – general population

specific ART procedure, and researchers and clinicians have speculated that the increased risk of an imprinting disorder may be due to underlying infertility, ovulation induction with or without IVF or intracytoplasmic sperm injection procedures (Manipalviratn et al., 2009). While the exact mechanism remains to be determined, those undergoing ART need to have disclosure regarding these concerns (Owen and Segars, 2009). (Imprinting disorders are more completely discussed in the following article).

**Informed consent and ART**

In none of the publicly available consent forms regarding ART, except for the potentially misleading information about the risk of birth defects or vanishing twin, or imprinting disorders, few consent documents outline the risks to the prematurely born infant including frequent neonatal morbidities, prolonged NICU stay or neuro-developmental handicap or the financial and emotional impact on parents. Recognition that informed consent of highly technical information and the possibility of adverse pregnancy and infant outcomes requires a substantial time commitment on physician’s part; nonetheless, remains a duty for the ethical and legal practice of medicine. The American Society for Reproductive Medicine has provided “A Guide for Patients” (e.g. Third Party Reproduction). Other professional societies related to ART provide websites that carefully describe many of the procedures and technical aspects of ART that should assist couples in reaching informed decision-

making; however, this information, while useful, does not provide physician- or clinic-specific informed consent.

Professional guidelines and legal doctrine require informed consent that is critical for patient information as well as determination of whether medical malpractice or battery has been committed. Adequate consent requires that patients (and others affected by the procedure) be fully informed about the risks and benefits as well as alternatives of the procedure (Kindregan and McBrien, 2005). A woman and generally her spouse (partner) should have their witnessed signatures on a document that clearly states the nature of the procedure, specific qualifications and nuances related to the procedure, and further explains procedural complications and their impact on the patient. Physician’s failure to provide information needed for consent and warn the patient of the potential risks may give rise to legal claims against him/her. Usually specific to the ART, physician may breach their duty to a patient in the course of an ART procedure by failure to provide current information needed to adequately explain the risks to the patient, failure to obtain consent of all interested persons, failure to obtain written consent as required by state statute (often requiring an impartial witness or notary), and failure to warn of the risks associated with ART to the mother, the couple, and potential children. Robertson suggests that “there may be a duty to avoid harm in cases of multiple gestation because some of the children born may have been better off if fewer siblings were

born in the same IVF cycle” (Robertson, 2004). Indeed, legal opinions recommend that physicians have a duty to inform the patient when risk of a procedure or outcome exceeds that of an ordinary occurrence or risk.

In 1998, a task force in New York revealed wide variability in the information provided in consent documents. It was found that many consent forms do not mention or explain the known or potential risk associated with the drugs used for ovarian stimulation. While most consent forms indicate that ART may result in multiple gestation, a significant number does not mention that multiple gestations entail considerable risk. Few consent forms mention the possibility of fetal reduction that may be recommended in the event of a high-order multiple. The New York task force concluded: “The process of obtaining informed consent to assisted reproduction is seriously deficient. There is considerable evidence that physicians provide incomplete or misleading information about the benefits and risks, particularly the risks associated with multiple gestation” ([http://www.health.ny.gov/regulations/task\\_force/reports\\_publications/execsum.html](http://www.health.ny.gov/regulations/task_force/reports_publications/execsum.html)). In February 2008, the American Bar Association published a model Act Governing Assisted Reproductive Technology, which also highlights the importance of fully informing the donor, the patient, and any other interested parties of all of the risks associated with ART and multiple pregnancies ([www.abanet.org/family/committees/artmodelact.pdf](http://www.abanet.org/family/committees/artmodelact.pdf)).

A review of certain publicly available consent documents from North Carolina, New York, Georgia, Utah and California illustrate a wide variation regarding the content, specific information, and a description of the risks to the mother and infant from ART. In some states, informed consent on the psychological impact of assisted reproduction including emotional, social, and relationship factors must also be documented, while in other states consent forms are broadly phrased to include “assisted reproduction technologies and techniques”. Some consent forms are highly specific with separate consents for stimulation of ovulation, IVF, intracytoplasmic sperm injection, fresh embryo transfer, embryo cryopreservation, assisted hatching, frozen embryo transfer consent, anonymous donor egg use, irrevocable consent regarding donated embryos, and intrauterine insemination (<http://www.infertilityspecialist.com>).

Some clinics also specifically document the number of embryos desired by the couple (even against medical

advice), and others seek consent regarding the disposition of embryos in the case of patient or spousal death, death of the couple, and even divorce. Although a comparison of available consent documents is beyond this review, it is noteworthy that few consent documents comment on the increased risks of birth defects among offspring. Indeed global statements are made that state that “human data suggest that IVF does not increase the risk of congenital anomalies (birth defects) in the resultant offspring” (<http://www.infertilityspecialist.com>). In terms of choices regarding the number of eggs to be fertilized and the number of embryos to be transferred (or discarded), one consent document reports A) limit fertilization to 3 ova, and transfer all resulting embryos to the uterus, B) fertilize all ova obtained and transfer the best 1 to 3 embryos that result, if embryos remain [in] the laboratory after transfer to the uterus has been completed, we [parents] request that they 1) be discarded, 2) donate them for research or other purposes, 3) undergo cryopreservation for the potential future intrauterine transfer (<http://healthcare.utah.edu/ucrm/forms.php>).

In contrast, a Canadian consent form “The Informed Consent Booklet for Assisted Reproductive Techniques: Adverse Effects and Risks” (April 9, 2010) from the Ottawa Fertility Center, in Ottawa, Canada provides an extensive list of the benefits and risks of the ART use in their clinic that provides an extensive summary of multifetal gestations and “unknown long-term risk of these treatments including that that could occur in subsequent generations” ([www.conceive.ca](http://www.conceive.ca)). (Informed Consent Booklet for Assisted Reproductive Techniques (ART)-Adverse Effects and Risks, April 9, 2010, [www.conceive.ca](http://www.conceive.ca) (accessed 1/21/2012)). In terms of potential impact on the infant, although the risk of prematurity is mentioned, generally these consent documents state that the risk of birth defects is not higher than that among infants conceived naturally, few mention imprinting disorders, and virtually none explains the risks of being born prematurely in terms of prolonged NICU stay or impact on long-term development.

## Summary

This analysis of the current ART practices in Europe and North America provides justification for the establishment of regulations as well as professional guidelines that:

- 1) Reduce the number of implanted embryos which may lead to a reduction in neonatal morbidity and mortality.
- 2) Efforts to provide a more accurate and population-wide data base to estimate the implications for neonatal use of ART, including IVF and ICSI and other procedures on rates of birth defects and imprinting disorders including the number of spontaneous miscarriages, termination of pregnancy, fetal reduction procedures performed, as well as the number of live births per cycles of ART are needed.
- 3) There is a critical need to compare the economic implications (costs/profits from the procedures and costs for the treatment of these children born as result of ART) within Europe and North America and the cost burden towards health insurance plans or taxpayers within a country.
- 4) There is a need for a greater public education regarding the burden and benefits of ART on maternal and infant health, both during the neonatal period and thereafter.
- 5) There should be a requirement for longitudinal developmental follow-up of children born after ART as well as their offspring to determine the possible epigenetic influences of these procedure(s) in subsequent generations.
- 6) Payment for ART services within a country need to be estimated in order to adhere with professional guidelines and/or governmental regulation.
- 7) While honoring the concept of reproductive liberty and individual rights in choosing ART, for example over adoption of children already born, to create a family, the societal impact of ART needs to be evaluated in terms of family well-being and adjustment.
- 8) The concept of surrogacy (especially paid surrogacy for an unrelated couple) needs to be carefully evaluated by governmental authorities in terms of whether the infants delivered by a non-citizen will have “citizenship” bestowed on the basis of the genetic parentage versus gestational “parentage” or, in the case of donated embryos, citizenship of the parents assuming the care of the infants born from these gestations.
- 9) “Medical Tourism” couples or women for the purpose of undergoing ART should be highly discouraged and available only when such services are not provided within their own country.
- 10) The use of Natural Procreative Technology (Napro-technology) should also be considered by couples with infertility diagnosis, and treatment of conditions resulting in infertility which may be corrected to restore normal reproductive function.
- 11) Advances should continue to improve current ART procedures focused on incubation techniques that do not expose the developing embryo to hypo- or hyper-methylation conditions that might stimulate epigenetic alterations in gene expression.

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# In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods

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**Objective:** To compare infant outcome after different IVF techniques.

**Design:** A register study in Sweden of IVF infants compared with all infants born.

**Setting:** National health registers.

**Patient(s):** We studied 16,280 IVF infants, 30% of whom were conceived by intracytoplasmic sperm injection (ICSI).

**Intervention(s):** None.

**Main Outcome Measure(s):** Multiple births, infant sex, preterm birth, low birth weight, and small for gestational age among singletons, mortality, low Apgar score, neonatal diagnoses.

**Result(s):** Twinning was less frequent after frozen standard IVF (18.1%) and after ICSI (21.8%) than after fresh standard IVF (24.4%). The male/female ratio was significantly increased in infants conceived after standard IVF. No significant differences were seen between singleton infants conceived after different IVF methods with respect to preterm birth, low birth weight, or infant mortality, with the possible exception of frozen standard IVF, for which some of these rates were lower than after fresh standard IVF. Infants born after ICSI had an indicated lower risk of respiratory problems than infants born after standard IVF.

**Conclusion(s):** Little difference in outcome was seen after different IVF methods. The differences observed might be due to dissimilar characteristics of the treated women (e.g., because ICSI was mainly used in connection with male infertility). (Fertil Steril® 2005;84:611–7. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** IVF, ICSI, multiple birth, preterm birth, mortality, neonatal diagnoses

The first infant in the world born after IVF was reported in 1978 (1), the technique of intracytoplasmic sperm injection (ICSI) was introduced in 1992, and the first pregnancy from a cryopreserved embryo occurred in 1993. Most studies have shown that infants born after IVF have a poorer perinatal outcome than naturally conceived infants (e.g., 2–6) although this seems largely to be due to multiple births and to confounding from maternal characteristics (2).

With the advancement and increasing use of new variants of IVF technique, the question arises whether these entail different hazards to the infant. Special interest has been paid to the ICSI technique when the suggested natural selection of sperm is bypassed. As reviewed by Retzlöff and Hornstein (7), no certain differences in congenital malformation rates between infants born after standard IVF or after ICSI have been demonstrated. The authors also point out the difficulties in conducting such analyses, notably when comparisons with naturally conceived infants are made.

For still more specific IVF techniques (e.g., ICSI with epididymal or testicular sperm), few data are available, and it is difficult to draw any firm conclusions.

In the present study, we used a large database of deliveries in Sweden after IVF and made comparisons with all infants born in Sweden, taking various putative confounders into consideration. The data set used includes that presented earlier (2), which contained infants born up to and including 1995 (n = 4,517) and relatively few ICSI infants (7.2%). Data for 1996–2001 have now been added (total n = 16,280, 30% ICSI). The study focuses on the risk for multiple birth, preterm birth among singletons, mortality, and neonatal diagnoses. In other communications, further safety aspects on IVF will be discussed, including the risk for congenital malformations.

## MATERIALS AND METHODS

In vitro fertilization procedures have been performed in Sweden in 17 laboratories in hospitals or private clinics. The first child born after IVF was born in 1982. The National Board of Health and Welfare requested information from these laboratories on all women who had gone through IVF and were known to have given birth or who had a pregnancy with an unknown outcome. Each woman was identified with her personal identification number (PIN), which every person living in Sweden possesses and which is extensively

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used in society and in all health care. A file was prepared with the PIN, a code for the laboratory where IVF was performed, year and month of ET, and the particular IVF method used. All IVF procedures performed before April 1, 2001 were included.

Infants born after IVF from 1982 to 2001 were identified by creating links between the above-mentioned file with the Medical Birth Register (8). This register covers nearly all deliveries in Sweden (a few percent are missing) and is based on copies of medical documents from antenatal care, delivery, and pediatric examination of the newborn. A current evaluation of the content of the register is available (9). The following variables were collected from the Medical Birth Register: year of birth, maternal age, parity, smoking in early pregnancy (reported at the first antenatal care visit, usually before the end of the first trimester), number of infants in birth, pregnancy duration, infant birth weight, Apgar score at 5 minutes, infant survival. Deaths after discharge from the neonatal unit were identified with linkage to the Cause of Death Register (10).

Neonatal diagnoses were obtained from the Medical Birth Register but were also identified (from 1987 onwards) from discharge diagnoses when the infant was hospitalized during the first week of life. Such discharge diagnoses were obtained from the Hospital Discharge Register (11) and were linked with use of the infant PIN. In spite of these efforts, some neonatal diagnoses are probably missing, but it is unlikely that this circumstance is linked to the presence or absence of IVF. Small for gestational age was based on the birth weight for gestational week graphs obtained from the Medical Birth Register (12).

Among live-born infants, certain neonatal diagnoses were studied: cerebral hemorrhage, convulsions, respiratory problems, and sepsis. These conditions were defined by the International Classification of Diseases (ICD) codes in the registers.

Cerebral hemorrhage was defined as 772.0 in ICD-8, 767.0 and 772.1-2 in ICD-9, and as P10, P11.0-P11.2, and P52 in ICD-10. (Sweden began using ICD-9 in 1987 and ICD-10 in 1997.) Neonatal convulsions were defined as 780.2 in ICD-8, 779.0 in ICD-9, and P90 in ICD-10. Respiratory problems were defined as 776.0-2 and 776.6-8 in ICD-8, 768.5-768.7, 769, and 770 in ICD-9, and P22-P23 in ICD-10. Neonatal sepsis was defined as 038 in ICD-9 and as A40, A41, and P36 in ICD-10. The ICD-9 has a poor ability to identify neonatal sepsis because no specific code is available among the codes for perinatal conditions.

Infants born after IVF and identified in the Medical Birth Register were compared with all infants born in Sweden during 1981–2001 and recorded in that register. A few percent of all infants born, according to the vital statistics kept by Statistics Sweden, were not registered in the Medical Birth Register. This can also be true for one of the infants in a multiple birth.

From an ongoing registration of all IVF procedures performed in Sweden (without individual identification), we estimated the number of transferred embryos in different types of IVF techniques. These data were available only for the years 1994–2000 (year of transfer).

### Statistical Analysis

Statistical analysis was performed with the Mantel-Haenszel technique with adjustment for various putative confounders, as further specified. A risk was expressed as an odds ratio (OR) with its 95% confidence interval (95% CI) and was estimated with a test-based method (according to Miettinen). Two adjusted ORs were compared with two-tailed *z*-tests, with the  $\chi^2$  values from the adjusted ORs.

## RESULTS

### In Vitro Techniques Used

A total of 13,241 pregnancies were analyzed, with 16,280 infants registered. The distribution according to the IVF method used is shown in Table 1. Figure 1 shows the number of deliveries after standard IVF and ICSI, according to year of birth.

### Multiple Births

Among the 13,241 deliveries, 3,006 were twin deliveries (23%). The frequency of monozygotic twins can be estimated with Weinberg's law, assuming that the number of dizygotic twin pairs is twice the number of unlike-sex twin pairs. Among the twin pairs, the sex of both twins was known in 2,676 pairs, and among them, 1,412 were

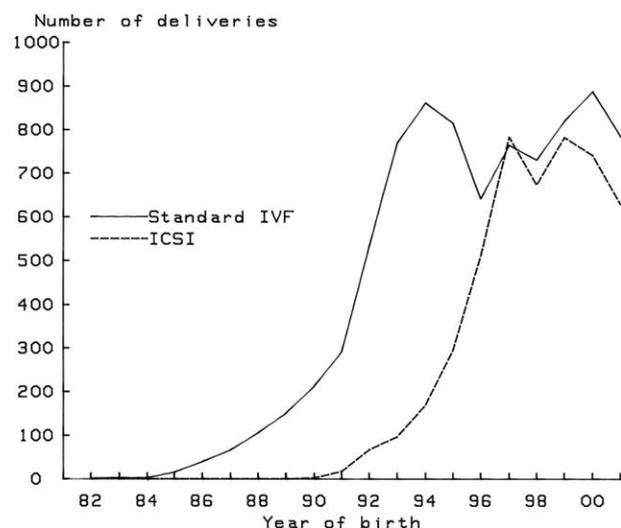
**TABLE 1**  
Number of deliveries and infants, according to IVF technique used.

IVF method	Deliveries	Infants
Standard IVF		
Stimulated, fresh	8,067	10,116
Unstimulated, fresh	103	112
Frozen	890	1,055
Total	9,060	11,283
ICSI		
Ejaculated sperm	3,549	4,248
Epididymal sperm	109	146
Testicular sperm	126	151
Frozen ejaculated	305	343
Frozen other	28	33
Frozen unspecified	38	43
Total	4,155	4,955
Other or unspecified	26	33
Grand total	13,261	16,280

*Källén. Infant outcome after IVF. Fertil Steril 2005.*

**FIGURE 1**

Annual number of deliveries after standard IVF and intracytoplasmic sperm injection (ICSI) in Sweden.



Källén. Infant outcome after IVF. *Fertil Steril* 2005.

like-sexed and 1,264 unlike-sexed. The estimated number of monozygotic twin pairs was thus 148. When this was compared with the rate among all births after adjustment for year of birth and maternal age, we found a threefold increase in the risk of monozygotic twinning (OR = 2.99, 95% CI 2.54–3.52).

The twinning rate after fresh standard IVF was 24.4%, that after frozen standard IVF 18.1%, that after fresh ICSI 22.1%, and that after frozen ICSI 11.38%. With fresh standard IVF used as reference, the OR (after adjustment for year of birth and maternal age) was significantly low for all the other groups: frozen IVF 0.69 (95% CI 0.58–0.83); fresh ICSI 0.87 (95% CI 0.79–0.97); frozen ICSI 0.49 (95% CI 0.37–0.67). For monozygotic twinning,

there were no significant differences between the different IVF techniques.

Data from the specific reporting of all IVF procedures to the National Board of Health (see Material and Methods) showed that among all standard IVF with fresh embryos (1994–2000), 10.7% transferred one embryo, 80.1% two, and 9.2% three. Similar figures were seen for standard IVF with frozen embryos: 10.6%, 81.3%, and 8.1%, respectively. For ICSI with fresh embryos, the percentages were 16.5, 71.6, and 11.7, respectively, and for ICSI with frozen embryos 19.6, 71.9, and 8.5, respectively.

The OR for transferring two embryos, with standard fresh IVF used as a reference and adjusting for year of transfer and IVF clinic, was 0.96 (95% CI 0.89–1.03) for standard frozen IVF, 0.53 (95% CI 0.49–0.58) for fresh ICSI, and 0.43 (95% CI 0.38–0.48) for frozen ICSI. The corresponding ORs for the transfer of three embryos was 1.04 (95% CI 0.93–1.17) for frozen IVF, 0.75 (95% CI 0.65–0.88) for fresh ICSI, and 0.52 (95% CI 0.43–0.62) for frozen ICSI. There is thus no significant difference between fresh and frozen standard IVF. For fresh and frozen ICSI, the ORs differ, but this difference might be random: the OR for the transfer of two embryos at frozen ICSI (with fresh ICSI used as a reference) was 0.90 (95% CI 0.78–1.02) and for three embryos was 0.87 (95% CI 0.70–1.09).

### Infant Sex

Sex was not known for 35 infants (28 of them born in singleton births). The population male/female ratio was 1.06 for singletons and 1.03 for infants born in multiple births. A significantly high ratio was found among infants born after standard IVF (1.13, 95% CI 1.09–1.17) and a significantly low ratio (0.94, 95% CI 0.89–1.00) for infants born after ICSI. There was no statistically significant difference between infants born as singletons or in multiple births within standard IVF or ICSI ( $\chi^2 = 0.22, P = .64, .33, \text{ and } .57$ , respectively).

**TABLE 2**

Preterm birth (<37 weeks), low birth weight (<2,500 g), and low Apgar score (<7 at 5 minutes) among singletons born after IVF, according to IVF technique.

IVF method	Preterm birth		Low birth weight		Low Apgar score	
	OR	95% CI	OR	95% CI	OR	95% CI
Standard fresh IVF	1.00	Reference	1.00	Reference	1.00	Reference
Standard frozen IVF	0.69	0.50–0.95	0.49	0.02–0.75	0.26	0.09–0.78
Fresh ICSI	0.96	0.80–1.15	0.97	0.79–1.19	1.05	0.73–1.50
Frozen ICSI	0.97	0.55–1.38	0.99	0.59–1.66	0.98	0.42–2.31

Note: Odds ratios with 95% CIs adjusted for year of birth, maternal age, parity, smoking, and years of involuntary childlessness.

Källén. Infant outcome after IVF. *Fertil Steril* 2005.

TABLE 3

## Deaths in infants conceived after IVF, compared with all infants born.

Adjustments made	Stillbirth		Neonatal death		Stillbirth and infant death	
	OR	95% CI	OR	95% CI	OR	95% CI
Year of birth	1.37	1.10–1.71	2.92	2.36–3.61	1.72	1.48–1.99
+Maternal age, parity, smoking	1.06	0.85–1.33	2.71	2.18–3.37	1.49	1.28–1.73
+Known childlessness	1.04	0.75–1.45	1.38	0.96–2.00	1.13	0.90–1.43

Källén. Infant outcome after IVF. *Fertil Steril* 2005.

### Preterm Birth, Low Birth Weight, and Small for Gestational Age Among Singleton Births

Among the 10,088 IVF singleton births, information on gestational duration existed for 10,062. Preterm birth (<37 completed weeks) occurred in 964 infants (9.6%), compared with 5.3% among all singleton births. Preterm birth before 32 completed weeks occurred in 192 (1.9%) of the singleton infants born after IVF, compared with 0.7% among all singleton births.

There were 10,004 IVF singleton infants with a known birth weight (99%). Among them, 733 (7.3%) had a birth weight of <2500 g and 184 (1.8%) had a birth weight of <1500 g. The corresponding percentages among all infants born were 3.5% and 0.6%, respectively.

Small for gestational age could be studied in 9,983 infants (97%). Small for gestational age occurred in 507 (5.1%), compared with 2.8% among all births.

With fresh standard IVF used as a reference, no clear-cut difference in the risk for preterm birth or low birth weight among singletons could be seen after other IVF techniques (Table 2). The ORs for preterm birth, low birth weight, and low Apgar score were, however, significantly low among infants conceived after frozen standard IVF.

### Low Apgar Score

Apgar score <7 at 5 minutes among live-born infants occurred in 421 among 15,965 IVF infants with known Apgar score (2.6%) and among 1.3% in the population. This difference was to some extent due to the high rate of multiple births after IVF. Among singleton infants, 1.8% had a low Apgar score, compared with 1.3% in the population. The OR adjusted for year of birth was 1.29 (95% CI 1.11–1.50) for singleton infants, but after additional adjustment for maternal age, parity, known years of involuntary childlessness, and maternal smoking, the OR decreased to 0.77 (95% CI 0.62–0.97). For multiple births, the corresponding adjusted OR was 0.85 (95% CI 0.67–1.07).

When singleton infants born after different IVF techniques were compared, with fresh standard IVF used as a

reference (Table 2), a significantly low OR was seen after frozen standard IVF.

The remaining outcomes were studied in all infants (including those in multiple births).

### Infant Mortality

Deaths before 1 year of age occurred in 195 children (81 stillborn), that is, 1.2%; 1.3% among infants born after standard IVF and 0.9% after ICSI. Table 3 compares mortality in IVF children with that in the population. A markedly increased death risk existed, but it was to a very large extent explained by confounding. A nearly significant increased risk remained only for neonatal deaths. There were no statistically significant differences between mortality according to IVF method used, but the OR for frozen standard IVF was nonsignificantly low (0.57, 95% CI 0.28–1.16).

### Neonatal Diagnoses

Cerebral hemorrhage was recorded in 40 IVF infants (2.5 per 1,000), whereas the population rate was 1.12 per 1,000. Table 4 shows an increased OR for all IVF infants, but when singleton IVF infants were compared with all singleton infants no significantly increased risk was seen, and the same was true when comparing multiple birth after IVF and all multiple birth infants (OR = 0.79, 95% CI 0.35–1.79 and OR = 1.31, 95% CI 0.88–1.95, respectively). The increased risk for all IVF infants was thus due to the high rate of multiple births. No significant difference in risk was seen between infants born after standard IVF or ICSI.

Neonatal convulsions (Table 4) were reported among 45 IVF infants (2.8 per 1,000), whereas the population rate was 1.56 per 1,000. The risk for convulsions was increased among IVF infants, but in a comparison between singleton infants, the increase in risk was not significant (OR = 1.36, 95% CI 0.93–1.97) and in a comparison of infants born in multiple births, no increased risk was seen (OR = 0.81, 95% CI 0.44–1.48). The total effect was thus mainly due to a high rate of multiple births. There was no difference in risk between infants born after standard IVF or ICSI.

TABLE 4

## Risk for neonatal complications among infants born after IVF.

Study group	Number of IVF infants	OR	95% CI
Cerebral hemorrhage	40	3.35	2.48–4.53
Neonatal convulsions	45	1.39	1.02–1.89
Respiratory problems	1,388	2.51	2.37–2.65
Mechanical ventilation	77	2.72	2.18–3.40
Use of CPAP	317	3.38	3.03–3.76
Neonatal sepsis	144	1.48	1.23–1.78

Note: Odds ratios with 95% CIs, adjusted for year of birth, maternal age, parity, and smoking habits.

Källén. Infant outcome after IVF. *Fertil Steril* 2005.

Among infants born after IVF, 1,388 had diagnoses representing respiratory problems (8.5%, compared with 2.99% among all infants born). A significantly increased risk was seen (Table 4). The risk was slightly lower for singleton births (OR = 1.26, 95% CI 1.15–1.43) than for multiple births (OR = 1.29, 95% CI 1.19–1.41), but the two ORs did not differ significantly ( $z = 0.35$ ,  $P = .38$ ). The risk was significantly higher for standard IVF (OR = 2.68, 95% CI 2.12–2.86) than for ICSI (OR = 2.03, 95% CI 1.81–2.27,  $z = 4.2$ ,  $P < .001$ ).

To further evaluate the severity of respiratory problems, an analysis was made of mechanical ventilation and of continuous positive airway pressure (CPAP) (Table 4). Mechanical ventilation use was recorded for 77 IVF infants (0.47%), compared with 0.17% among all infants born. The risk for IVF infants in multiple births was lower (OR = 0.97, 95% CI 0.71–1.32) than that for singletons (OR = 1.42, 95% CI 0.95–2.12), but this might be random ( $z = 1.48$ ,  $P = .13$ ), and the same was true for infants born after ICSI (OR = 2.27, 95% CI 1.44–3.54) compared with infants born after standard IVF (OR = 2.85, 95% CI 1.44–3.54,  $z = 0.86$ ,  $P = .28$ ).

Use of CPAP was recorded for 317 IVF infants (1.9%) and in 0.40% among all infants born. The risk was higher for singleton IVF infants (OR = 1.49, 95% CI 1.19–1.85) than for IVF infants born in multiple births (OR = 1.17, 95% CI 1.00–1.36), but this might be random ( $z = 1.76$ ,  $P = .08$ ). The risk was significantly higher for infants born after standard IVF (OR = 4.35, 95% CI 3.85–4.91) than after ICSI (OR = 3.02, 95% CI 2.43–3.76,  $z = 2.82$ ,  $P = .01$ ).

Neonatal sepsis (Table 4) was recorded for 144 infants, practically all born after the introduction of ICD-10 in Sweden. For the period 1996–2001, 1.3% of the IVF infants had a diagnosis of sepsis, compared with 0.4% among all infants born. The total risk for neonatal sepsis was increased, but when singleton IVF infants were compared with other singleton infants and IVF infants born in multiple births were compared with other infants born in multiple births, no increased risk was found. The total increase was thus an

effect of the high rate of multiple births. The risk after ICSI (OR = 1.34, 95% CI 1.01–1.79) was lower than after standard IVF (OR = 1.55, 95% CI 1.22–1.97,  $z = 2.87$ ,  $P = .001$ ).

To separate the effect of IVF method and number of infants in birth on neonatal morbidity, a further analysis was made, comparing standard IVF and ICSI infants after adjustment for number of infants in birth and comparing twins with singleton infants after adjustment for IVF method. The only difference between ICSI and standard IVF that approached, but did not reach, statistical significance was for respiratory problems (OR = 0.88, 95% CI 0.76–1.02) and use of CPAP (OR = 0.80, 95% CI 0.58–1.09). With the exception of neonatal convulsions, twinning was a significant risk factor for all diagnoses, with ORs varying from 2.15 for neonatal sepsis to 5.39 for CPAP.

## DISCUSSION

This was a population-based, large-scale study, with outcome data obtained from a national health register.

The strongest negative effect on perinatal outcome after IVF is associated with multiple births. The twinning rate obviously depends strongly on the number of embryos transferred, which changed over time, differed between IVF centers, and might also differ with IVF method used. We showed that, after adjustment for year of transfer and IVF center, there was no difference in the rate of transfer of more than one embryo if fresh or frozen standard IVF was used, and the slightly lower rate of transfer of more than one embryo after frozen ICSI compared with fresh ICSI could be random. We found a lower twinning rate after frozen embryos compared with fresh embryos in both standard IVF and ICSI, which was thus not explained by differences in number of embryos transferred but might mirror different embryo viability. The lower rate of twinning after ICSI compared with standard IVF can, however, be explained by the lower rate of transfer of more than one embryo.

The earlier known increase in monozygotic twinning after IVF (13–20) was confirmed. In the present study, the risk

increase was estimated to be approximately threefold, with no detectable variation between IVF methods used. The explanation for the increase in monozygotic twinning after IVF is not clear. Manipulation of the zona pellucida has been suggested, but ovarian stimulation might also be a factor. A 1.7-fold increased risk of monozygotic twinning was observed in a study of infants born after ovarian stimulation without IVF (21).

As expected, the male/female ratio after ICSI was close to 1.0. No selection according to content of a sperm Y or X chromosome is likely to occur. Actually, the sex ratio was significantly below 1.0, which could indicate a prenatal selection against male embryos. It is interesting that the sex ratio after standard IVF was actually higher than the population sex ratio. A similarly high sex ratio has previously been found (22, 23). It might be expected that the in vitro situation would less strongly favor the light Y-chromosome-carrying sperm compared with what might occur under natural circumstances, but apparently the opposite was true. A similar finding was made previously for 5-day ET but not for 3-day ET (23). Only a few blastocyst transfers were included in our material.

The rate of prematurity among singleton infants born after IVF was nearly twice that of all singleton infants, but to a large extent this increase is due to characteristics of the women who underwent IVF, mainly maternal age and parity distribution but also length of involuntary childlessness (2). Involuntary childlessness even in the absence of IVF has an impact on prematurity rate (24, 25). Taking these factors into consideration, in addition to year of birth, only small differences were seen between different IVF methods regarding the risk of preterm birth or low birth weight among singleton infants.

After frozen standard IVF, there is a tendency for a better outcome for these variables, as well as for low Apgar score. This might be an expression of a selection process. Access to frozen pre-embryos is typical for patients from whom a large number of eggs were obtained. In the rather small sample of infants born after fresh epididymal ICSI, the risk for preterm birth and low birth weight seemed to be increased, but this observation must be confirmed through independent studies.

The increased occurrence of low Apgar score in the IVF sample is an indicator of an increased risk for death and for cerebral palsy (26).

No significant differences in infant survival could be seen in relation to IVF technique. However, the conclusions are hampered by low numbers of deaths in many subgroups, which means that only large groups could be compared.

The high rate of multiple births and the increased risk of prematurity and low birth weight will affect the risk for severe complications among infants born. The adverse effects on the infants born were also apparent in the neonatal diagnoses studied: cerebral hemorrhage, neonatal convulsions, and respiratory problems including use of mechanical

ventilation or CPAP, and sepsis. The strongest risk increase was found for cerebral hemorrhage, followed by respiratory problems, sepsis, and neonatal convulsions. Numbers of infants with these complications were low, except for respiratory problems. To a large extent, the increased risks seemed to be due to the high rate of multiple births, but for respiratory problems and sepsis it could be shown that both for singletons and multiple births an increased risk was seen after IVF when comparison was made with all births (singletons or multiple births, respectively). A similar finding was described from a comparison of twins born after IVF with spontaneous twins (27). This is a reasonable consequence of the increased occurrence of preterm birth and low birth weight.

In conclusion, no major differences in outcome can be found that could be attributed to the specific IVF technique used. A lower rate of twinning after frozen standard IVF than after fresh standard IVF is probably due to a lower vitality of the former pre-embryos. On the other hand, preterm birth seemed to occur less often after frozen than after fresh standard IVF. Infants born after ICSI were less likely to have a diagnosis of a respiratory problem than infants born after standard IVF. This is partly a consequence of the lower twinning rate but could also be the result of differences in the composition of the groups of women treated, given that the major reason for ICSI is male subfertility.

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### **In Vitro Fertilization May Increase the Risk of Beckwith-Wiedemann Syndrome Related to the Abnormal Imprinting of the *KCNQ1OT* Gene**

*To the Editor:*

“Parental imprinting” refers to an epigenetic marking of genes that results in monoallelic expression. This phenomenon plays a critical role in embryogenesis and development. The epigenetic modification of the genome involves methylation changes and the remodeling of chromatin-associated proteins (Li 2002). Imprints are established during the development of germ cells, and the reprogramming of imprinting occurs within the first days after fertilization (Reik and Walter 2001). The alteration of normal imprinting patterns is implicated in a number of human genetic diseases. Among them, the Beckwith-Wiedemann syndrome (BWS [MIM 130650]) is an overgrowth syndrome secondary to the dysregulation of the imprinted 11p15 region (Maher and Reik 2000). Numerous mechanisms are involved in BWS, and ~70% of cases of BWS are related to epigenetic abnormalities at the 11p15 locus, mostly demethylation of the *KvDMR1* region of the *KCNQ1OT* (previously called “*LIT1*”) gene (MIM 604115) (Engel et al. 2000; Blik et al. 2001; Gaston et al. 2001; Weksberg et al. 2001; DeBaun et al. 2002). *KCNQ1OT* encodes a noncoding antisense transcript within intron 10 of the *KCNQ1* gene (MIM 192500) (Lee et al. 1999; Mitsuya et al. 1999; Smilnich et al. 1999) and might be involved in the regulation of parental imprinting of the centromeric domain of the 11p15 region (Fitzpatrick et al. 2002).

In sheep and cattle, epigenetic abnormalities have been shown to be involved in large offspring syndrome (LOS) (Young et al. 1998). Affected animals exhibit various phenotypes, including large size at birth. In both species, the syndrome is caused by the in vitro exposure of embryos, between fertilization and the blastocyst stage, to various unusual environments. LOS is related to the loss of imprinting of the *IGF2* receptor gene (MIM 147280), which ensures internalization and degradation of *IGF2* and displays an antiproliferative function (Young et al. 2001). In vitro preimplantation procedures in mice are also responsible for overgrowth, owing to the abnormal ex-

pression of various imprinted genes, particularly the genes located at distal chromosome 7 (*h19* [MIM 103280] and *igf2* [MIM 147470] genes), orthologous to the human 11p15 region (Humpherys et al. 2001; Rideout et al. 2001). In humans, a case of BWS was recently described after in vitro fertilization (IVF) (Olivennes et al. 2001). Moreover, two recent papers (DeBaun et al. 2003; Maher et al. 2003) described an increase in prevalence of assisted reproductive technologies (ART) in patients with BWS. De Baun et al. (2003) reported a sixfold increase (4.6% vs. 0.76% in the general population) and showed that four of the six patients for whom DNA was available exhibited an isolated demethylation of *KvDMR1* in the *KCNQ1OT* gene. Maher et al. (2003) reported a threefold increase (4% vs. 0.997% in the general population) and demonstrated that two of the six patients on whom molecular analysis could be done also exhibited an isolated demethylation of *KvDMR1*.

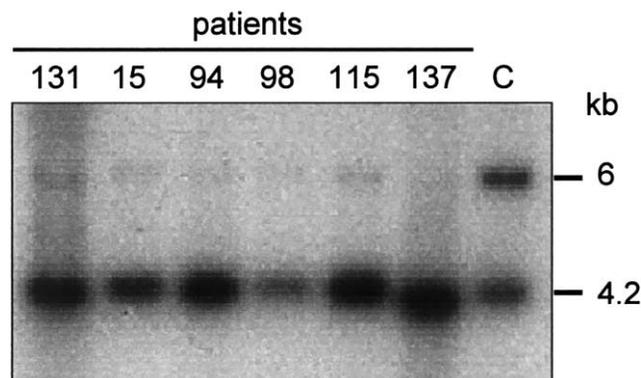
Our department is a reference center in France for molecular diagnosis of BWS, and patients are referred from various medical departments (neonatology, pediatrics, genetics, and fetopathology). We studied a series of 149 patients referred for overgrowth syndromes and diagnosed as BWS, since all of them exhibited genetic or epigenetic defects at the 11p15 locus. According to the inclusion criteria described elsewhere (Gaston et al. 2001), 102 patients exhibited a complete form of BWS, and 47 exhibited an incomplete form of BWS. The techniques used to analyze the 11p15 region have been described elsewhere (Gaston et al. 2000, 2001). Epigenetic changes concerned 104 (70%) patients, most of whom ( $n = 90$ ) exhibited a loss of *KvDMR1* methylation. Fourteen patients (9.4%) exhibited isolated hypermethylation of the *H19* gene. Forty-two patients exhibited a genetic defect: 11p15 uniparental disomy ( $n = 35$ ; 23.5%) and germline *CDKN1C* (MIM 600856) mutation ( $n = 7$ ; 4.7%). Three patients (2%) had a chromosomal abnormality.

Six of the 149 patients were born following ART. Of note, these six patients exhibited the same epigenetic abnormality (isolated demethylation of *KvDMR1* with a demethylation index varying 72%–100%) (fig. 1). All of them were sporadic cases, and one was a DZ twin. The clinical features of these patients and the procedures of ART used for their conception are summarized in table 1. As shown in table 1, the phenotypes of patients

**Table 1****Clinical Characteristics of the Six Patients with BWS Born Following ART**

	CHARACTERISTICS OF PATIENT						CHARACTERISTICS OF OTHER PATIENTS WITH DEMETHYLATION OF KvDMR1 ( <i>n</i> = 84)	<i>P</i> <sup>a</sup>
	15	94	98	115	131	137		
ART procedure:								
Sperm	Ejaculated	Ejaculated	Ejaculated	Ejaculated	Ejaculated	Ejaculated		
ICSI	Yes	No	No	No	No	Yes		
Frozen embryo	No	No	No	Yes	No	No		
Day of transfer	Day 2	Day 3	Day 2	Day 2	Day 2	Day 5 <sup>b</sup>		
Phenotype:								
Sex	F	F	M	F	M	F	42F/42M	
Delivery (weeks)	40	33.5	38.5	37	20 <sup>c</sup>	32/DZ twin <sup>d</sup>		
Macrosomia	Yes	Yes	Yes	Yes	Yes	No	72.3%	NS <sup>e</sup>
Birth weight (g)/Birth length (cm)	4090/51.5	2770/48.5	4460/53.5	4400/55	3/480	1765/43		
Macroglossia	Yes	Yes	Yes	Yes	Yes	Yes	96.4%	NS
Organomegaly	No	No	No	Liver	Pancreas	No	48.7%	NS
Abdominal wall	No	Exomphalos	No	Exomphalos	No	Exomphalos	72.3% <sup>f</sup>	NS
Hemihyperplasia	No	No	No	No	No	No	26.9%	NS
Ear abnormalities	No	No	Yes	No	No	No	68.9%	<i>P</i> = .02
Hypoglycemia	Yes	No	No	Yes	...	No	45.6%	NS
Facial naevus	Yes	No	No	Yes	No	Yes	54.5%	NS
Other	Macrocephalia, cystic fibrosis	Developmental delay, pyelic dilatation	Inguinal hernia		Adrenal cytomegaly, placental chorioangioma			

<sup>a</sup>  $\chi^2$  test.<sup>b</sup> Transfer of three embryos, two at the morula stage and one at the blastocyst stage.<sup>c</sup> Spontaneous abortion.<sup>d</sup> DNA from the normal twin was not available.<sup>e</sup> NS = not significant.<sup>f</sup> 43.4% exomphalos, 24.1% umbilical hernia, 4.8% diastasis recti.



**Figure 1** Methylation analysis of KvDMR1 in liver tissue (patient 131) and leukocytes (patients 15, 94, 98, 115, and 137) from the six patients with BWS born after ART and in leukocytes from a normal control (C). Genomic DNA was digested with *Bam*HI and the methylation-sensitive enzyme *Not*I. Digested samples were subjected to electrophoresis in a 0.7% agarose gel, blotted onto Hybond XL membranes, and hybridized with the HLHAY79 KvDMR1 probe corresponding to EST 68627 (ATCC; Manassas). The upper band (6 kb) is methylated and corresponds to the maternal allele. The lower band (4.2 kb) is unmethylated and corresponds to the paternal allele.

born after ART were not different from phenotypes of the other patients ( $n = 84$ ) with isolated demethylation of KVDMMR1, with the exception that only one patient born after ART exhibited ear abnormalities. These children were issued from various ART procedures: classical IVF, intracytoplasmic sperm injection (ICSI), embryo freezing, and transfer on day 2, day 3, and day 5. More recent procedures, like ICSI (two of six patients) or blastocyst transfer (one of six patients), did not prevail over other techniques. The representation of ART (4%) in our series is three times higher than that in the general population (1.3%), according to the national report of the French Ministry of Health (9,930 of 770,000 live births resulting in 1,999 from IVF, ICSI, or frozen embryo transfer). On the basis of this report, we would have expected 1.94 of the 149 patients with BWS to be born as a result of ART. To test the significance of this difference of frequencies, we used the Fisher's exact test ( $P = .01$ ) as well as the Poisson approximation (two-tailed  $P = .018$ ; 95% CI 1.5–8.7). Strength of the association between exposure to ART and risk of BWS is expressed by an odds ratio of 3.2 (95% CI 1.4–7.3). This rate is the same as that described by Maher et al. (2003) but lower than that described by DeBaun et al. (2003), which addressed a prospective study. In our series and in Maher's series, this rate is probably underestimated, as specific questions regarding ART have only been asked systematically in the past year.

Although the analysis of the imprinting status at chromosome 15q11-13 in children born after ICSI did not reveal an imprinting defect (Manning et al. 2000), two

recent papers reported three patients with Angelman syndrome (MIM 105830) born after ICSI (Cox et al. 2002; Ørstavik et al. 2002). All three patients exhibited an imprinting defect, which is a rare cause of Angelman syndrome.

As in the previous two reports (DeBaun et al. 2003; Maher et al. 2003), our data suggest that ART may favor imprinting alterations at the centromeric imprinted 11p15 locus and, consequently, the incidence of BWS. These data highlight the need to carefully follow up children born after ART to test for BWS and other diseases related to imprinted regions. Although no specific procedures of ART appear to be associated with a risk of BWS in our series, these data lend support to the importance of precisely recording these different procedures of ART, particularly the stimulation protocol, the biological technique, the stage of maturation of the gametes, the culture media used at each step, and the timing of embryo transfer.

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#### Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for BWS, KCNQ1OT, KCNQ1, IGF2 receptor, H19, IGF2, CDKN1C, and Angelman syndrome)

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### To Trust or Not to Trust an Idiosyncratic Mitochondrial Data Set

*To the Editor:*

In a recent report, Silva et al. (2002) provided partial (8.8 kb) information on the mtDNA coding region (within the region 7148–15946, in the numbering of the Cambridge reference sequence [CRS]; Anderson et al. [1981]) in 40 individuals from Brazil. On the basis of the similarity in nucleotide diversity and age estimates of the four founder haplogroups A, B, C, and D, they claimed to have added new evidence for a single early entry of the founder populations into America. However, a site-by-site audit of the data reveals that their sequences are not of high enough quality to justify such statements. The authors failed to realize that a large number of mutations associated with basal branches of the worldwide mtDNA phylogeny (Finnilä et al. 2001; Maca-Meyer et al. 2001; Torroni et al. 2001; Derbeneva et al. 2002; Herrnstadt et al. 2002; Kivisild et al. 2002) were not correctly scored in their data set.

## Research letters

## Incidence of retinoblastoma in children born after in-vitro fertilisation

Annette C Moll, Saskia M Imhof, Johannes R M Cruysberg, Antoinette Y N Schouten-van Meeteren, Maarten Boers, Flora E van Leeuwen

Every year, 3000 women in the Netherlands are treated with in-vitro fertilisation (IVF), and results from studies suggest that the offspring of these women do not have a heightened risk of cancer. Between November, 2000, and February, 2002, we diagnosed retinoblastoma in five patients born after IVF. To calculate the relative risk of the disease, we assumed that the proportion of children conceived by IVF in the Netherlands is between 1.0 and 1.5%, and that the five patients who we diagnosed with the disease represent all new cases in the Netherlands during that period. Relative risks for retinoblastoma were significantly raised (7.2 [95% CI 2.4–17.0], and 4.9 [1.6–11.3], for 1% and 1.5% rates, respectively). This possible association of an increased risk of retinoblastoma in a population-based study needs to be established.

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See Commentary page 273

Retinoblastoma is a malignant tumour of the retina that occurs in childhood. Since 1945, the incidence of this disease in the Netherlands has been constant at around 1 per 17 000 livebirths.<sup>1</sup> In most cases (60%), the disease is non-hereditary and affects only one eye. However, in the 40% of hereditary cases, both eyes are usually affected.

Every year, 3000 women in the Netherlands undergo in-vitro fertilisation (IVF). Although Klip and colleagues<sup>2</sup> showed that the offspring of women who underwent IVF between 1980 and 1995 did not have an enhanced risk of cancer, the number of cancers (seven in IVF, nine in controls) of any type that was seen was very small. In the Vrije University Medical Centre, where about 95% of all patients with retinoblastoma in the Netherlands are treated, we diagnosed retinoblastoma in five patients born after IVF between November, 2000, and February, 2002. Therefore, we compared the incidence of retinoblastoma in the IVF population with that in the general population, and estimated the relative risk of retinoblastoma in children born after IVF in the Netherlands.

In this analysis, we calculated the ratio of observed to expected numbers of retinoblastoma cases in the study population using data from the Dutch retinoblastoma registry<sup>1</sup> and the Netherlands cancer registry. We derived confidence limits of the ratio obtained by use of the Poisson distribution of the observed numbers.

Incidence of the disease was 2.6 per 100 000 children in the first year of life, 0.9 per 100 000 in those aged between 1 and 4 years, and 0.1 per 100 000 in 5–9 year olds. In the Netherlands, an estimated 1–1.5% of children are conceived after IVF.<sup>3</sup> We calculated that 0.69 retinoblastoma cases would be expected in children conceived after IVF between 1995 and 2001 using numbers of births since 1995 and the 1-year age-specific mortality rates in the Netherlands (obtained by calendar year and sex from Statistics Netherlands), the estimate that 1% of all births are conceived by IVF, and the sex-specific and age-specific retinoblastoma incidence rates. On the assumption that the five patients we saw at our clinic represented all cases in the Netherlands born between Jan 1, 1995, and Dec 31, 2001, we calculated a significantly increased risk ratio (RR) of 7.2 (95% CI 2.4–17.0). If we used the upper estimate that 1.5% of all births are after IVF, then the RR was 4.9 (1.6–11.3). These might be conservative estimates of the true risk since some retinoblastoma cases could have been treated in other hospitals, and the oldest child with retinoblastoma was born in 1997. However, because we based the expected number of cases on all children born from 1995 onwards, we believe our results are conservative.

The table shows characteristics of the five patients with retinoblastoma. None of the patients had a family history of retinoblastoma. Two were one of a twin pair, but the siblings of these two twins have no ocular abnormalities. IVF (supplemented by intracytoplasmic sperm injection in one case) was done in four different IVF centres.

In the three patients with unilateral retinoblastoma, the tumour was enucleated. Of the two patients with bilateral disease, one eye was enucleated; in one, the remaining eye was treated with a radioactive ruthenium plaque, and in the other, an external beam radiation therapy was used. When last seen in October, 2002, all patients were alive and free of disease.

The number of infants born after IVF is increasing steadily. Cancer incidence in this fast growing population of children has been investigated in only very few studies. In their report of ocular disorders in 47 children born after IVF in Israel, Anteby and colleagues<sup>4</sup> describe a case of unilateral retinoblastoma in a child conceived with donor sperm; therefore, close investigation of the association between IVF and retinoblastoma was not possible.

	Age at diagnosis (months)	Eye	Birthweight (g)	Gestational age (weeks)	Number of IVF cycles	Cause of subfertility	DNA 13q14 analysis	Other fertility treatment
Patient 1 (F, 1997)	38	Both	3885	40	8	Unexplained	Exon 8 mutated	6 courses of clomid
Patient 2 (M, 1999)*	15	Left	2005	36	2	Maternal cause	Normal	None
Patient 3 (F, 1998)*	34	Right	2135	41	1	Unexplained	Normal	None
Patient 4 (M, 2001)†	8.5	Both	2330	39	2	Unexplained	Intron 3 mutated‡	8×AI attempts
Patient 5 (F, 1999)	32	Left	4100	41	2	Paternal cause	Normal	1×ICSI at same time

M=male. F=female. AI=artificial insemination. ICSI=intracytoplasmic sperm injection. \*One of a pair of dizygous twins. †One of a pair of twins until co-twin died after spontaneous abortion at 8 weeks' gestation. ‡Variation in a non-coding part of the 13q14 gene, no pathogen mutation found.

#### Characteristics of five patients with retinoblastoma born after IVF in the Netherlands

A large retrospective cohort study from Sweden reported that 5.4% of children born after IVF had developmental problems, but that there was no increased risk of cancer in this group. This finding about cancer risk is lent support by results of a study by Bruinsma and colleagues<sup>5</sup> that did not show a significantly increased incidence of cancer in children born after assisted conception in Australia. In a cohort of 9484 offspring of Dutch women who had ovarian stimulation before IVF, 16 cases of cancer were reported,<sup>2</sup> which suggests that the risk of childhood cancer was not increased compared with the general population and with an internal reference group of subfertile women who were treated without IVF. Although IVF was done during 1980–95, no retinoblastoma cases were reported in children born during this time. Our five patients were all born in the Netherlands after 1995 between 1997 and 2001. That five cases arose during this time, but that none were seen during the earlier period is important, and represents a striking excess in disease frequency. Did something change in the IVF procedure itself (possibly the culture medium), or is our observation only a chance finding? Perhaps the same genetic factors are involved in infertility and retinoblastoma. However, none of the parents of our patients had had retinoblastoma, and we have not identified any published data about infertility problems of unaffected parents of retinoblastoma patients.

Whether treatment with ovulation-inducing drugs increases the risk of childhood cancer is an important matter, especially with the rising numbers of women undergoing treatment for subfertility. Future investigators should consider the number of IVF treatments, other fertility drugs given before IVF, and the possibility that serious disorders in children conceived by IVF are diagnosed earlier than those in other children who do not have such close medical surveillance. Our finding requires further research to confirm the association and to explore a possible causal mechanism.

#### Contributors

A C Moll, F E van Leeuwen, and J R M Cruysberg had the idea for the study. A C Moll and F E van Leeuwen designed the study. A C Moll, S M Imhof, J R M Cruysberg, A Y N Schouten-van Meeteren gathered data, A C Moll and F E van Leeuwen processed data and did statistical analysis. A C Moll, F E van Leeuwen, and M Boers drafted the manuscript. All investigators contributed to the interpretation of data and manuscript revisions.

#### Conflict of interest statement

None declared.

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## Faecal calprotectin concentrations and diagnosis of necrotising enterocolitis

Daniel Carroll, Anthony Corfield, Richard Spicer, Pamela Cairns

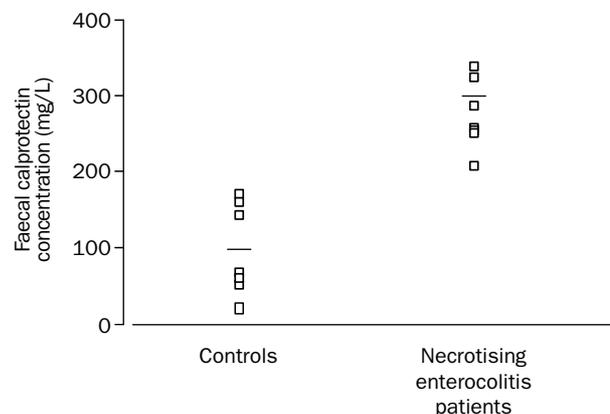
**Calprotectin has been proposed as a useful marker of inflammatory bowel disease in children. We did a pilot study to establish whether it can be used to aid diagnosis of necrotising enterocolitis in preterm infants. Patients with clinical features of necrotising enterocolitis had raised faecal calprotectin concentrations at the time of diagnosis compared with matched controls (288.4 mg/L [SD 49.1] and 98.0 mg/L [60.6], respectively;  $p=0.0006$ ). Faecal calprotectin might be a useful marker of gastrointestinal mucosal inflammation in neonates.**

*Lancet* 2003; **361**: 310–11

Necrotising enterocolitis is a serious disorder of preterm infants, with high mortality and morbidity. Diagnosis is made on the basis of clinical criteria since there are no specific diagnostic tests. Calprotectin is a 36-kDa calcium-binding and zinc-binding protein constituting 60% of soluble cytosol proteins in human neutrophil granulocytes.<sup>1</sup> Faecal calprotectin concentrations are raised in various organic bowel diseases in adults.<sup>1</sup> Calprotectin is resistant to degradation, and has been proposed as a useful laboratory marker of gastrointestinal inflammation in children and adults with active inflammatory bowel disease.<sup>2</sup> Defects in or increased permeability of the mucosal barrier cause migration of large numbers of granulocytes into the intestinal lumen. If mucosal lesions are extensive, as in active inflammatory bowel disease, calprotectin concentrations are generally ten to twenty times the upper reference limit of 30 mg/L in adults.<sup>3</sup>

We identified seven consecutive patients with clinical features consistent with necrotising enterocolitis and who had required at least 7 days' parenteral nutrition, intravenous antibiotics, and enteral starvation; and seven controls matched for age, sex, and gestational age. We obtained written informed consent from the parents of the infants, and obtained ethics approval from United Bristol Healthcare Trust ethics committee.

We obtained stool samples, which were stored at  $-20^{\circ}\text{C}$  before analysis. Stool samples were obtained as soon as possible after the diagnosis of necrotising enterocolitis was made. All patients produced a stool within 24 h of diagnosis. We recorded the date and time at which each stool sample was obtained, patients' gestational age, postnatal age at diagnosis, sex, feeding history, and clinical signs. Samples were thawed and analysed twice for faecal calprotectin concentrations with CALPREST (Eurospital, Trieste, Italy) and the mean concentration calculated. Data analysis was by Mann-Whitney U test with SPSS for Windows (version 10).



**Faecal calprotectin concentrations**

# Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization—embryo transfer or gamete intrafallopian transfer: a meta-analysis

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**Objective:** To perform a systematic review of the literature to determine whether singleton pregnancies resulting from IVF-ET/GIFT are at higher risk for preterm birth (<37 weeks).

**Design:** Literature search and systematic review.

**Setting:** Medical school.

**Intervention(s):** A MEDLINE search (1965–2000) was performed using the terms “premature labor,” “infertility,” “pregnancy complications,” “gonadotropins,” “pregnancy outcome,” “preterm delivery,” and “in vitro fertilization.” Criteria for inclusion were English language, original research article, study patients conceived using IVF-ET (with or without intracytoplasmic sperm injection) or GIFT, pregnancy outcome reported compared with a control group (e.g., naturally conceived singletons at their hospital or a national reference), and prematurity clearly defined. Incomplete articles (e.g., abstracts), reports of other studies, and studies that failed to separate multiple from singleton gestations were excluded.

**Main Outcome Measure(s):** Summary of relative risks of preterm birth.

**Result(s):** Twenty-seven articles met all inclusion/exclusion criteria and were analyzed by meta-analysis. The random-effects summary relative risk of preterm birth in singleton pregnancies resulting from IVF-ET/GIFT was 1.98 (95% confidence interval, 1.77–2.22).

**Conclusion(s):** The risk of preterm birth in singleton pregnancies resulting from IVF-ET/GIFT is twice that of natural conceived pregnancies. (Fertil Steril® 2004;82:1514–20. ©2004 by American Society for Reproductive Medicine.)

**Key Words:** Gonadotropin stimulation, preterm birth, pregnancy outcome, infertility treatment, assisted reproductive technology, in vitro fertilization

Infertility is a growing public health and social problem. According to the latest National Survey of Family Growth (1), in 1995 the number of women with impaired fecundity increased to 10% of the reproductive-age population (6.1 million women), up from 8% in the 1988 survey. More importantly, the number of childless women with impaired fecundity increased dramatically, from 2.2 million in 1988 to 2.8 million in 1995 (a 27% increase). More than 9 million reproductive-age women gave a history of having received infertility services (15% of women aged 15–44 years).

In vitro fertilization—embryo transfer and GIFT have become increasingly used treat-

ments for infertile patients. In 1999, more than 86,000 IVF-ET/GIFT cycles were performed in the United States (2), resulting in 30,285 live births. This represents 0.8% of all live births nationally (0.7% in 1998). In a recent survey by Jain et al. (3), the three states that required complete insurance coverage performed almost three times as many IVF-ET/GIFT cycles as states without infertility legislation (3.35 vs. 1.21 cycles per 1,000 women). This suggests that the potential use of this technology is much larger than current numbers suggest and that a federal law requiring infertility insurance coverage (which has been proposed) would greatly increase the number of children born

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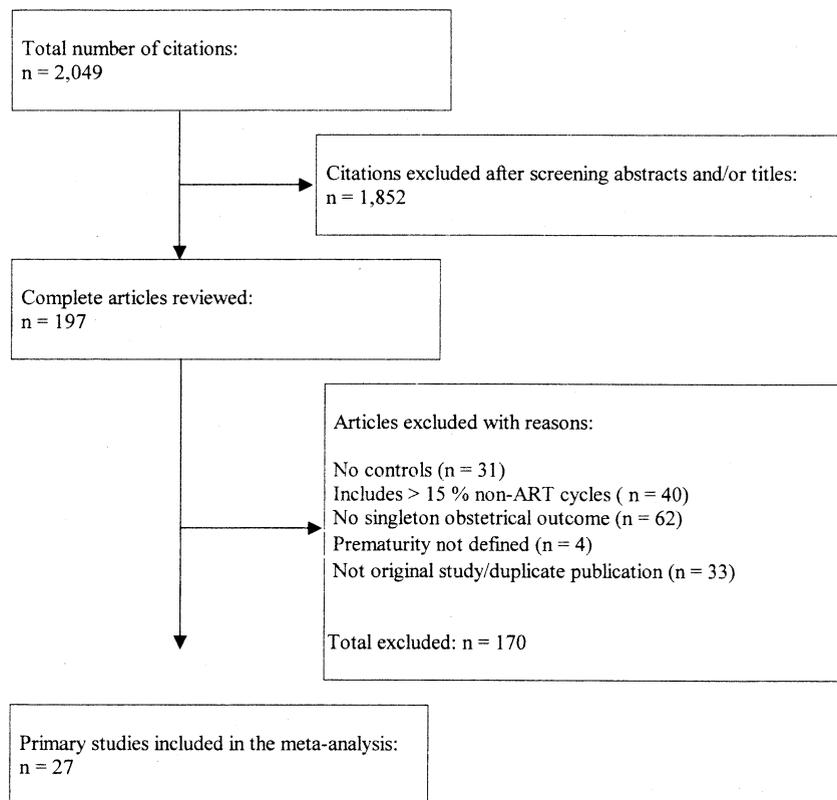
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**FIGURE 1**

Study selection process for systematic review of preterm birth in singleton pregnancies resulting from IVF-ET/GIFT compared with naturally occurring controls. ART = assisted reproductive technology.



McGovern. Preterm birth after IVF-ET/GIFT. *Fertil Steril* 2004.

after these procedures. Almost all IVF-ET/GIFT cycles use gonadotropins to stimulate multiple ovarian follicles.

Infants delivered after IVF-ET/GIFT are known to be more likely to be premature, but most of this risk is thought to be explained by the higher frequency of multiple births. Although a number of small series have found an elevated risk of prematurity in singletons after IVF-ET/GIFT, they have usually had limited statistical power. Therefore, a controversy exists as to whether singleton pregnancies are actually more likely to deliver preterm. The hypothesis of this study is that singleton pregnancies conceived after IVF-ET/GIFT are at increased risk for premature birth. A meta-analysis was performed to answer this question.

## MATERIALS AND METHODS

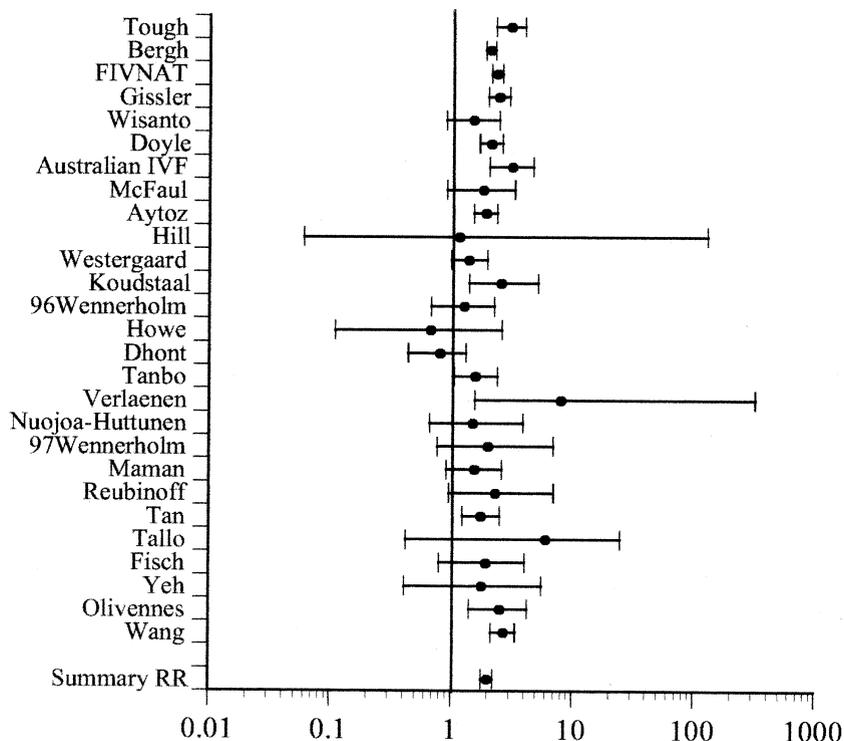
The study followed the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (4) for reporting of meta-analyses of observational studies. The University of Medicine and Dentistry of New Jersey Institutional Review Board indicated that approval was not necessary for

this study. Publications were identified using MEDLINE (1965–2000) for the English language under the terms “pre-mature labor,” “infertility,” “pregnancy complications,” “gonadotropins,” “pregnancy outcome,” “preterm delivery,” and “in vitro fertilization.” Other publications were identified by examining the references of these articles and standard textbooks. Criteria for inclusion were English language publication, original work, publication dates 1965–2000, majority ( $\geq 85\%$ ) of subjects having conceived using IVF-ET or GIFT, prematurity clearly defined, and pregnancy outcome data reporting the incidence of prematurity in comparison with a control group. The control groups accepted were naturally conceived singletons from a hospital database (either age- and/or parity-matched controls or general hospital database) or a national reference. Exclusion criteria were incomplete articles, such as abstracts and reports from other studies, and failure to separate multiple from singleton gestations.

This search identified a total of 2,049 references (Fig. 1), and these abstracts were reviewed. Of these, 1,852 were

**FIGURE 2**

Meta-analysis of the relative risk of preterm birth in singleton pregnancies resulting from IVF-ET/GIFT, including relative risks and confidence intervals for each study. RR = Relative Risks.



McGovern. Preterm birth after IVF-ET/GIFT. Fertil Steril 2004.

clearly not relevant to the research question. The remaining 197 articles were obtained, and two observers blinded to the journal of publication, authors, and results reviewed the isolated methods sections independently. When the methods section review suggested a relevant study, two observers then examined the complete texts independently, and the results were abstracted and compared. This process excluded 170 articles for the following reasons: no controls (n = 31), inclusion of >15% of pregnancies from non-IVF-ET/GIFT treatments (n = 40), no singleton obstetrical outcomes (n = 62), preterm delivery not defined (n = 4), and not an original study (n = 33). A final 27 studies met all criteria and were included in the meta-analysis.

Statistical analysis was performed using SAS (Cary, NC) and Comprehensive Meta-Analysis (Englewood, NJ) software. Relative risks, 95% confidence intervals (CIs), and P values were extracted when present (three studies). Relative risks were computed from gonadotropin-stimulated and control incidence rates cited in the remaining studies. A 95% CI for the study relative risk was estimated using the binomial distribution or asymptotic estimates of the variance of the logarithm of the relative risk, as justified by sample size and

detail of available information. The significance of relative risk >1 was tested in each study by  $\chi^2$  or Fisher's exact test.

Although some studies cited the use of matched designs, unmatched estimates were obtained in the absence of sufficient detail for matched-design computations. When national population rate denominators were not available, a conservatively small value of 100,000 was imputed. Summary relative risks and CIs were first estimated using fixed-effect, general inverse variance-based methods (5). Heterogeneity of relative risks within subgroups was tested by  $\chi^2$  based on variance-based methods. In the presence of significant heterogeneity ( $P < .05$ ), random-effects point estimates and CIs were computed (6) using DerSimonian-Laird estimates.

## RESULTS

Relative risks ranged from 0.67 to 8.00. Combining the relative risks from all 27 studies (Fig. 2) revealed that singleton pregnancies resulting from IVF-ET/GIFT were at a significantly greater risk of delivering prematurely when compared with control groups of spontaneously conceived singletons. The fixed-effects summary relative risk was 2.13

TABLE 1

Summary information from the 27 studies included in the meta-analysis.

Reference	Study group	Controls	Study group (n)	Study group, % preterm birth	Control (n)	Control, % preterm birth	Relative risk
(7)	IVF	Provincial database	203	22.7	116,226	7.4	3.07
(8)	IVF	National database	3,298	11.2	1,505,724	5.4	2.07
(9)	IVF	National database	3,822	9.4	100,000 <sup>a</sup>	4.0	2.35
(10)	IVF	National database	746	11.0	188,381	4.5	2.44
(11)	ICSI only	National database	222	7.6	65,535	5.1	1.49
(12)	IVF	National database	648	13	100,000 <sup>a</sup>	6	2.11
(13)	IVF	National database	108	19.0	100,000 <sup>a</sup>	6.2	3.06
(14)	IVF or GIFT	National database	79	13.0	100,000 <sup>a</sup>	7.0	1.86
(15)	ICSI only	National database	826	9.9	100,000 <sup>a</sup>	5.2	1.90
(16)	IVF or GIFT	Matched age	55	5.5	42	4.8	1.15
(17)	IVF or ICSI	Matched age + parity	1,298	7.3	1,298	5.3	1.38
(18)	IVF	Matched age + parity	307	15.0	307	5.9	2.54
(19)	ICSI only	Matched age + parity	140	9.3	9,753	7.3	1.27
(20)	IVF	Matched age + parity	54	11.1	54	16.6	0.67
(21)	IVF or ICSI	Matched age + parity	311	8.4	622	10.5	0.80
(22)	IVF or GIFT	Matched age + parity	355	14.9	643	9.5	1.57
(23)	IVF	Matched age + parity	140	11.4	140	1.4	8.14
(24)	IVF	Matched age + parity	276	7.6	276	5.1	1.49
(25)	IVF	Matched age + parity	160	11.2	160	5.6	2.00
(26)	IVF	Matched age + parity	169	15.4	646	9.9	1.56
(27)	IVF	Matched age + parity	260	8.8	260	3.9	2.26
(28)	IVF	Matched age + parity	494	14.0	978	8.0	1.75
(29)	IVF	Matched age	62	10.0	62	2.0	5.00
(30)	IVF	Hospital database	64	12.5	3,775	6.5	1.92
(31)	IVF	Hospital database	24	13.0	4,279	7.0	1.86
(32)	IVF	Hospital database	162	11.1	5,096	4.4	2.52
(33)	IVF or GIFT	Hospital database	465	16.7	21,457	6.2	2.69

Note: GIFT = gamete intrafallopian transfer; ICSI = intracytoplasmic sperm injection.

<sup>a</sup> The article did not specify the control n, and a conservative n of 100,000 was assumed for statistical purposes.

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(95% CI, 2.03–2.24). A  $\chi^2$  test for heterogeneity was highly significant ( $P < .0001$ ).

To attempt to explain heterogeneity, articles were classified (Table 1) according to type of control group (the major difference among studies): local hospital-based controls, national or regional database controls, or matched controls (usually age and parity). Four studies with hospital-based controls whose relative risks ranged from 1.78 to 2.71 had a fixed-effects summary relative risk of 2.58 (95% CI, 2.15–3.09). Nine studies with controls from a national or regional database had relative risks ranging from 1.50 to 3.14, with a fixed-effects summary relative risk of 2.23 (95% CI, 2.10–2.36). Fourteen studies with controls matched by age and/or parity had relative risks ranging from 0.67 to 8.00, with a fixed-effects summary relative risk of 1.52 (95% CI, 1.33–1.74). However, within the second and third subgroups (national/regional database or matched controls) there was significant heterogeneity among their relative risks. Finally, a random-effects analysis that included all the studies produced a summary relative risk of 1.98 (95% CI, 1.77–2.22).

Six of the included studies contained additional information about the incidence of extremely preterm birth (<32 weeks). Analysis of these six articles revealed a random-effects summary relative risk of extremely premature birth after IVF-ET/GIFT of 2.49 (95% CI, 0.86–7.21) that was not significantly different from 1. The wide confidence bands reflect the heterogeneity of the results (relative risks ranged from 0.4 to 19.0).

## DISCUSSION

This meta-analysis demonstrates a highly significant two-fold (1.98) relative risk for preterm birth in singleton pregnancies resulting from IVF-ET/GIFT when compared with naturally conceived singletons. The strength of this finding led us to a further analysis of the risk of extreme prematurity (<32 weeks). A trend toward an excess risk for extreme prematurity was found in singleton pregnancies resulting from IVF-ET/GIFT when compared with naturally conceived singletons (relative risk, 2.49). Unfortunately, be-

cause only six studies provided information about delivery before 32 weeks, our analysis was limited and therefore remains inconclusive. Although births before 32 weeks are less common events, even a slight increase in deliveries at this age would have dramatic effects upon healthcare costs and childhood health.

Prematurity is the single largest preventable cause of perinatal morbidity and mortality and continues to increase despite considerable efforts to reverse this trend (34). Of the \$10.2 billion spent in the United States annually for initial hospital care of newborns, 57% is spent on the 9% of infants who deliver before 37 weeks (35). Therefore, a 1% increase in prematurity would increase national healthcare expenditures by more than \$642 million. For initial hospital care, a term infant costs about \$1,197, whereas a preterm infant costs approximately \$16,305 (35).

We believe that publication bias did not influence the results because of [1] the great interest in studying all aspects of pregnancies derived from these new technologies and [2] the interest of physicians performing these procedures to publish data verifying their safety (which would produce significant investigator effort to publish “negative” results). Publication dates of studies included in our analysis ranged from 1985 through 2000. Clearly, over this period, IVF-ET/GIFT went from being investigational to being established. To confirm our hypothesis, we examined and found no correlations among the years of patients studied, the years of publication, the risks of prematurity in either the IVF-ET/GIFT or control groups, and the relative risks of prematurity reported in the article (data not shown). This does not suggest a systematic publication bias as these procedures became more established or a general change in the diagnosis of prematurity over time.

This analysis also does not allow us to determine precisely whether the elevated prematurity risk is related to gonadotropin stimulation or some other aspect of IVF-ET/GIFT. Virtually all IVF-ET/GIFT cycles involve the stimulation of multiple ovarian follicles with injectable gonadotropins, and therefore the resulting pregnancies are associated with multiple corpora lutea (36). Relaxin, a peptide hormone produced by the corpus luteum of pregnancy (37), is a potent stimulator of collagen breakdown. Relaxin stimulates procollagenase (MMP-1) and prostromelysin-1 (MMP-3) production while decreasing tissue inhibitor of metalloproteinase-1 (TIMP-1) production (38). In human pregnancy, collagen breakdown normally plays an important role in the increased elasticity necessary for cervical effacement and dilatation during labor.

The increased relaxin levels observed in gonadotropin-stimulated pregnancies do not fall after the first trimester but persist for the duration of pregnancy (39). These excess levels of relaxin may well lead to premature cervical change and premature delivery. Although other luteal products are also important, relaxin provides a plausible link between excess luteal function and premature cervical change and

delivery. In a study of pregnancies after gonadotropin stimulation (40), it was demonstrated that elevated levels of relaxin were an independent risk factor for prematurity. Because relaxin levels were more closely linked to the number of corpora lutea than to the presence of twins or triplets, all pregnancies resulting from gonadotropin stimulation would be at risk, not just multiple pregnancies.

There could be many differences between spontaneously conceived singleton pregnancies in healthy couples and singleton pregnancies in infertile couples that have been treated with IVF-ET/GIFT. It is possible that the couples themselves have a reproductive pathology, which would result in premature delivery even in treatment-independent pregnancies. An interesting study published after the dates selected for the systematic review (41) addresses this question. These investigators compared the incidence of prematurity in three groups of singleton pregnancies: IVF-ET/GIFT patients, insemination patients (many of whom used “minimal” gonadotropin stimulation), and normal controls. A very large sample size (>1,000 pregnancies in each of the three arms) adds considerable strength to their analysis. They found a significant gradation of prematurity risk, with 12.5%, 8.5%, and 5.5% preterm births, respectively, in the three groups.

Joffe and Li (42) used a large birth database to compare the incidence of prematurity with the time needed to achieve pregnancy (a surrogate indicator of infertility). Their data reveal an incidence of prematurity of 12.6% in “infertile” couples (took longer than 12 months to conceive), vs. 9.3% in couples who conceived within 6 months. Taken together, these studies support the concept that, although infertility itself may slightly increase the prematurity risk above the normal population, gonadotropin stimulation and IVF-ET/GIFT further elevate this risk.

It is possible that some of the excess prematurity in gonadotropin singletons is iatrogenic because of either altered management (early delivery) of a “premium” pregnancy or necessary medical interventions secondary to other obstetrical complications. Both singleton and multiple pregnancies resulting from assisted reproductive technologies have been shown to be at increased risk for a variety of obstetrical complications (22, 26, 28, 43), which can be associated with the need for early delivery.

It has also been suggested that IVF-ET/GIFT pregnancies may have gestational age calculations that are incorrectly estimated to be several days early, leading to a falsely elevated incidence of preterm birth. Although the day of ovulation is known with great certainty, concerns have been expressed that the process of developing embryos in vitro may delay the early stages of implantation. If the increased prematurity of IVF-ET/GIFT infants was a result of mathematical error, then these children would be born at normal or greater-than-normal weights for their miscalculated gestational ages. This is clearly not the case, and multiple studies

document lower birth weights in infants born after IVF-ET/GIFT (22, 23, 44).

It should be emphasized that the reproductive problems of the infertile couple are not resolved at conception. The twofold risks of preterm and extremely preterm birth in singleton pregnancies after IVF-ET/GIFT argue strongly for further study and heightened clinical vigilance during these pregnancies. Obstetricians and patients alike should be aware of this risk so that they can maintain an increased awareness for the signs and symptoms of premature labor.

Our results reveal that the problem of prematurity is not limited to multiple pregnancies but rather exists as a risk for singleton pregnancies resulting from IVF-ET/GIFT. If true, they suggest that the current strategy of making infertility treatments safer by trying to limit the occurrence of multiple pregnancies may be expected to have only partial success. To improve the safety of IVF-ET/GIFT, we must devote more time and effort into understanding the mechanisms of preterm labor so that we may more effectively prevent it.

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# IVF and stillbirth: a prospective follow-up study

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**BACKGROUND:** Previous studies have indicated that the risk of stillbirth is increased in singleton pregnancies achieved after assisted reproduction technology (ART). However, no previous study fully accounted for factors with potential influence on the risk of stillbirth. Further, whether fertility treatment, the possible reproductive pathology of the infertile couples or other characteristics related to being sub-fertile may explain a possible association with stillbirth remains unclear. This study compares the risk of stillbirth in women pregnant after fertility treatment (IVF/ICSI and non-IVF ART) and subfertile women with that in fertile women.

**METHODS:** We used prospectively collected data from the Aarhus Birth Cohort, Denmark and included information about 20 166 singleton pregnancies (1989–2006). Outcome measure was stillbirth.

**RESULTS:** The risk of stillbirth in women who conceived after IVF/ICSI was 16.2 per thousand (‰) and in women who conceived after non-IVF ART 2.3‰. In fertile and subfertile women, the risk of stillbirth was 3.7‰ and 5.4‰, respectively. Compared with fertile women, women who conceived after IVF/ICSI had more than four times the risk of stillbirth [odds ratio (OR): 4.44, 95% confidence interval (CI): 2.38–8.28], and adjustments for maternal age, BMI, education, smoking habits and alcohol and coffee intake during pregnancy had only minor impact on the findings (OR: 4.08; 95% CI: 2.11–7.93). The risk of stillbirth in women who conceived after non-IVF ART and in women who conceived spontaneously with a waiting time to pregnancy of a year or more was not significantly different from the risk in women with a shorter time to pregnancy.

**CONCLUSIONS:** Compared with fertile women, women who conceived by IVF/ICSI had an increased risk of stillbirth that was not explained by confounding. Our results indicate that the increased risk of stillbirth seen after fertility treatment is a result of the fertility treatment or unknown factors pertaining to couples who undergo IVF/ICSI.

**Key words:** assisted reproduction / IVF/ICSI outcome / infertility / stillbirth / epidemiology

## Introduction

Since the first child was born after fertility treatment 30 years ago, the number of assisted pregnancies has increased steadily (Adamson *et al.*, 2006). In European countries, up to 4% of all deliveries now result from fertility treatment (Andersen *et al.*, 2007). Much interest has been put into the efficacy of assisted reproduction technology (ART) (Pandian *et al.*, 2003), but more and more research now focuses on the safety (Ludwig *et al.*, 2006). Safety studies are important not only to be able to provide the couple evidence-based information but also to bring into focus potentially preventable adverse outcomes.

Two register-based studies (Gissler *et al.*, 1995; De Neubourg *et al.*, 2006) and several small case–control studies (Howe *et al.*, 1990; Tanbo *et al.*, 1995; Verlaenen *et al.*, 1995; Reubinoff *et al.*, 1997; Dhont *et al.*, 1999; Nuojua-Huttunen *et al.*, 1999; Westergaard

*et al.*, 1999; Koudstaal *et al.*, 2000a, b; Isaksson *et al.*, 2002) have indicated that the risk of perinatal mortality is increased in singleton pregnancies achieved after ART. However, many previous studies have not had the statistical power to study mortality (Helmerhorst *et al.*, 2004), only a few studies have studied stillbirth (Reubinoff *et al.*, 1997; Koudstaal *et al.*, 2000a, b; Kallen *et al.*, 2005; De Neubourg *et al.*, 2006) and no previous study fully accounted for factors with potential influence on the risk of stillbirth. Thus, whether fertility treatment including hormone stimulation and mechanical procedures, the possible reproductive pathology of the infertile couples or other characteristics related to being subfertile may explain a possible association with stillbirth remains unclear.

The aim of this prospective cohort study was to compare the risk of stillbirth in singleton pregnancies in women pregnant after IVF/ICSI with the risk of stillbirth in women who conceived in less than 1 year. To explore the effect of fertility treatment versus the effect of

infertility, we also included information about women who conceived after non-IVF ART and subfertile women with a time to pregnancy of 1 year or more.

## Study population and methods

All women booking for delivery at the Department of Obstetrics and Gynaecology, Aarhus University Hospital, Denmark, are asked to participate in the Aarhus Birth Cohort. The Aarhus Birth Cohort is a longitudinal cohort of unselected pregnant women that are consecutively recruited in early pregnancy through the antenatal healthcare services (Wisborg *et al.*, 2003). Nearly all women in the area comply with the antenatal care programme. Women who agree to participate (75%), complete two questionnaires before the first routine antenatal care visit at 16 weeks of gestation. The first questionnaire provides information for the hospital records and for study purposes, the second questionnaire only for study purposes. Information from these questionnaires is linked, using the women's unique personal identification number, to information about delivery and the newborn. In the present study, we included information on women booking for delivery from 1 August 1989 to 31 October 2006. The Danish Data Protection Agency, acting as the ethics committee for studies, granted authorization for the implementation of the project.

The first questionnaire provided information on medical and obstetric history, including waiting time to pregnancy and fertility treatment, maternal age, smoking habits before pregnancy and during the first trimester and alcohol intake during pregnancy. The second questionnaire provided information on intake of coffee, marital status, education and any psychological problems. The women were asked whether the pregnancy was planned, and if planned, about waiting time to pregnancy in years and months. We also asked the women to provide information about consultations due to infertility and about infertility treatment. Women who conceived after fertility treatment were categorized into two groups according to treatment (IVF/ICSI and non-IVF ART). The non-IVF ART group included women who conceived after hormone stimulation ( $n = 352$ ) and insemination ( $n = 527$ ). No difference in the risk estimate of the outcome of interest was found between these two groups. Women who conceived spontaneously were categorized into two groups according to waiting time to pregnancy, 0–11 months (fertile women) and 12+ months (subfertile women). Women with unplanned pregnancies were categorized as fertile.

Information about delivery was obtained from birth registration forms filled in by the attending midwife immediately after delivery. Before data entry, all birth registration forms were manually checked and compared with the medical records by a research midwife. Gestational age, measured in completed weeks, was based on early fetal ultrasound measures or detailed information on the woman's last menstrual period. Information about stillbirths was obtained from the birth registration forms and validated with information from the Danish Medical Birth Register through record linkage, using the mother's unique personal identification number. During the study period, the National Board of Health changed the definition of stillbirth. Until April 2004, stillbirth was defined as delivery of a dead child at 28 completed weeks of gestation or later, and after that

time, as delivery of a dead child at 22 completed weeks of gestation or later.

The study population was restricted to primiparous, Danish-speaking women with a singleton pregnancy who filled in the first questionnaire ( $n = 27\,072$ ). Women with chronic illnesses (cardiovascular, pulmonary or kidney diseases, diabetes or other metabolic diseases, or epilepsy) ( $n = 4268$ ) and with missing information on waiting time to pregnancy and infertility treatment ( $n = 2638$ ) were excluded from the study.

## Statistical analyses

The association between fertility treatment and stillbirth is presented as odds ratio (OR) with 95% confidence interval (CI). Potential confounding factors were coded as in Table I and entered into the multivariate logistic regression analyses as a number of dummy variables equal to the number of categories – 1. Missing values were included as a separate category for each variable. Interaction was tested by Mantel–Haenszel analyses. Statistical significance was defined as a two-sided  $P$ -value of  $<0.05$ .

The study was approved by the Regional Ethics Committee, the Danish National Board of Health and the Danish Data Protection Agency.

## Results

In this study of 20 166 primiparous, singleton pregnancies, 16 525 (82%) conceived spontaneously after less than 12 months, 2020 (10%) after more than 1 year of trying, 879 (4%) conceived after non-IVF ART and 742 (4%) conceived after IVF/ICSI. The overall risk of stillbirth was 4.3‰ ( $n = 86$ ).

Characteristics of fertile women, subfertile women and women who conceived after fertility treatment are shown in Table I. Compared with fertile women, women who conceived after IVF/ICSI were older ( $P < 0.01$ ), more often cohabiting ( $P < 0.01$ ), had a higher BMI ( $P = 0.02$ ), and fewer women who conceived after IVF/ICSI drank alcohol during pregnancy ( $P = 0.01$ ). Women who conceived after non-IVF ART were older ( $P < 0.01$ ), had a higher BMI ( $P < 0.01$ ) and a higher intake of coffee during pregnancy ( $P < 0.01$ ). Compared with fertile women, subfertile women were older ( $P < 0.01$ ), more often cohabiting ( $P < 0.01$ ), had a higher BMI ( $P < 0.01$ ) and a shorter education ( $P < 0.01$ ). There were also more smokers among subfertile women ( $P < 0.01$ ) and they had a higher intake of alcohol ( $P < 0.01$ ) and coffee during pregnancy ( $P < 0.01$ ).

The risk of stillbirth was 16.2‰ in women who conceived after IVF/ICSI and 2.3‰ in women who conceived after non-IVF ART. In fertile and subfertile women, the risk was 3.7‰ and 5.4‰, respectively (Table II). After adjustment for maternal age, BMI, education, smoking habits and alcohol and coffee intake during pregnancy, we found a significantly increased risk of stillbirth in women who conceived after IVF/ICSI compared with fertile women (OR: 4.08; 95% CI: 2.11–7.93). The risk of stillbirth in subfertile women and women who conceived after non-IVF ART was not statistically significantly different from the risk in fertile women. Compared with women pregnant after IVF/ICSI, fertile women (OR: 0.25; 95% CI: 0.13–0.48), subfertile (OR: 0.33; 95% CI: 0.14–0.76) and women pregnant

**Table I** Characteristics of primiparous fertile women, subfertile women and women who conceived after non-IVF ART or IVF/ICSI with singleton pregnancies, Aarhus, Denmark, 1989–2006.

	Fertile* (n = 16 525) [n (%)]	Subfertile** (n = 2020) [n (%)]	Non-IVF ART (n = 879) [n (%)]	IVF/ICSI (n = 742) [n (%)]
Maternal age (years)				
15–24	3020 (18)	252 (13)	37 (4)	5 (1)
25–34	12 678 (77)	1583 (78)	664 (76)	498 (67)
35+	827 (5)	185 (9)	178 (20)	239 (32)
Marital status				
Cohabiting	15 113 (92)	1850 (92)	816 (92)	713 (96)
Single	325 (2)	19 (1)	17 (2)	5 (1)
Missing	1087 (7)	151 (8)	46 (5)	24 (3)
Years of education				
7–10	1 120 (7)	188 (9)	51 (6)	44 (6)
11+	13 738 (83)	1571 (78)	736 (84)	646 (87)
Missing	1 667 (10)	261 (13)	92 (11)	52 (7)
Cigarettes/day				
0	13 762 (83)	1531 (76)	737 (84)	633 (85)
1–9	1583 (10)	262 (13)	85 (10)	62 (8)
10+	1 128 (7)	220 (11)	54 (6)	44 (6)
Missing	52 (0.3)	7 (0.3)	3 (0.3)	3 (0.4)
Alcohol intake during pregnancy (drinks/week)				
<1	12 883 (78)	1534 (76)	704 (80)	614 (83)
1–2	2716 (16)	319 (16)	118 (13)	95 (13)
3+	674 (4)	133 (7)	50 (6)	27 (4)
Missing	252 (1)	34 (2)	7 (1)	6 (1)
Coffee (cups/day)				
0–3	12 551 (76)	1365 (68)	624 (71)	585 (79)
4+	2210 (13)	381 (19)	155 (18)	105 (14)
Missing	1764 (11)	274 (14)	100 (11)	52 (7)
Maternal BMI (kg/m <sup>2</sup> ) before pregnancy				
<20	3583 (22)	458 (23)	152 (17)	145 (20)
20–24	9949 (60)	1 105 (55)	498 (57)	429 (58)
25–29	1975 (12)	296 (15)	143 (16)	108 (15)
30+	688 (4)	122 (6)	66 (8)	44 (6)
Missing	330 (2)	39 (2)	20 (2)	16 (2)

\*Fertile: waiting time to pregnancy &lt;12 months.

\*\*Subfertile: waiting time to pregnancy 12+ months.

**Table II** Fertility and risk of stillbirth, Aarhus, Denmark, 1989–2006.

Fertility	Births	Stillbirths (%)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Fertile	16 525	61 (3.7)	Reference	Reference
Subfertile	2020	11 (5.4)	1.48 (0.78–2.81)	1.33 (0.70–2.56)
Non-IVF ART	879	2 (2.3)	0.62 (0.15–2.52)	0.53 (0.13–2.18)
IVF/ICSI	742	12 (16.2)	4.44 (2.38–8.28)	4.08 (2.11–7.93)

%, number of stillbirths per thousand; OR, odds ratio; CI, confidence interval.

\*Adjusted for maternal age, education, marital status, BMI and intrauterine exposure to tobacco smoke, alcohol and coffee.

after non-IVF ART (OR: 0.13; 95% CI: 0.03–0.58) all had a statistically significantly lower risk of stillbirth.

Mean gestational age at delivery was lower in stillborn infants of IVF pregnant women (32 weeks) compared with stillborn infants of women who conceived spontaneously (36 weeks) ( $P < 0.05$ ).

During the study period, the number of assisted pregnancies increased, and in May 2004, the National Board of Health changed the definition of stillbirth. However, compared with fertile women, we found an increased risk of stillbirth in IVF/ICSI conceptions both before (OR: 4.03; 95% CI: 1.90–8.56) and after (OR: 5.55; 95% CI: 1.77–17.36) the change in stillbirth definition (test of homogeneity between strata  $P = 0.65$ ).

## Discussion

In this prospective cohort study, we found that compared with spontaneously conceived singleton pregnancies, singleton IVF/ICSI pregnancies had more than 4-fold increased risk of stillbirth. It has been speculated that the increased risk of adverse outcomes in assisted pregnancies is due to factors related to the underlying infertility of the couples (Romundstad *et al.*, 2008). However, we found that couples with a waiting time to pregnancy of 1 year or more and women who conceived after non-IVF ART had a risk of stillbirth similar to that of fertile couples and statistically significantly lower than women pregnant after IVF/ICSI, which may indicate that the increased risk of stillbirth is not explained by infertility.

## Confounding

In agreement with previous studies (Gissler *et al.*, 1995), we found that women with assisted pregnancies differed from other women in a number of characteristics with potential influence on the outcome of interest. To account for differences in parity and pre-existing disease between women with assisted pregnancies and spontaneously conceived pregnancies, we included only primiparous women with no pre-pregnancy diseases. Furthermore, owing to careful prospective collection of information about a number of factors, we could evaluate variables with potential influence on our results (i.e. maternal age, smoking and socioeconomic factors). None of these factors seemed to explain our results, but residual confounding from crude categorization of variables or confounding from unknown variables cannot be ruled out. IVF and ICSI patients represent a group resistant to low-technology infertility treatment and have a longer infertility period and may accordingly be selected by unknown factors associated with an increased risk of stillbirth.

## Vanishing twins

Several previous studies have found that assisted conceptions are at higher risk of adverse outcomes than are spontaneously conceived pregnancies (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004). However, much of the increased risk is explained by multiple pregnancies in assisted conceptions, and from 1998 to 2005 the average number of embryos transferred decreased from 2.0 to 1.75 in Denmark. In our study, we included only singleton deliveries, but previous studies have shown that 10% of IVF singletons originate from twin gestations because of the transfer of two or more embryos (Pinborg *et al.*, 2005). Compared with singleton conceptions, these

pregnancies carry an increased risk of very preterm delivery and low birthweight (Pinborg *et al.*, 2007). The increased risk of stillbirth in singleton IVF/ICSI pregnancies that we found in our study may therefore, to some extent, be explained by a higher number of twin gestations in early pregnancy. However, the risk of stillbirth in non-IVF ART pregnancies was significantly lower than that in IVF/ICSI pregnancies and comparable with the risk in fertile women. As for preterm delivery (Schieve *et al.*, 2002), the vanishing twins are probably not the sole contributor to the increased risk of stillbirth in IVF singletons.

## Preterm delivery

Compared with women who conceive spontaneously, women with pregnancies after IVF/ICSI treatment have an increased risk of preterm delivery which cannot be explained solely by a higher number of twin pregnancies (Jackson *et al.*, 2004; Halliday, 2007). Preterm delivery is associated with a higher risk of morbidity and mortality (Gardosi *et al.*, 1998) and may be one of the reasons for an increased risk of perinatal mortality in IVF pregnancies. It is also possible that in women pregnant after fertility treatment, there is a shared aetiology leading to either stillbirth or delivery of a live born preterm infant. Compared with stillborn infants of women who conceived spontaneously, we found that mean gestational age at stillbirth was lower for infants of women pregnant after fertility treatment, which might indicate different aetiologies of stillbirth. However, despite the size of the study, we had limited possibilities to fully explore the causes of death, and this needs further investigation in even larger datasets.

During the study period, the National Board of Health changed the definition of stillbirth. However, analysing data according to the two periods defined by different definitions of stillbirth showed an increased risk of stillbirth in IVF/ICSI conceptions both before and after the change in stillbirth definition.

In conclusion, we found that compared with women who conceived spontaneously and women who conceive after non-IVF ART, women who conceived after IVF/ICSI had an increased risk of stillbirth that was not explained by confounding from age, lifestyle habits or socioeconomic factors. Future studies should focus on further exploration of this finding so that the information given to infertile couples seeking treatment can be differentiated to the individual couples.

## Authors' roles

The authors qualify for authorship by having contributed substantially to this work, as specified by criteria (a), (b) and (c) of the *Uniform Requirements for Manuscripts Submitted to Biomedical Journals*, and they are able to accept public responsibility for it. They have reviewed the final version of the manuscript and approve it for publication.

## Ethics

Ethical approval not required for this study. The Aarhus Birth Cohort is approved by the Danish Data Protection Agency.

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Review

# Long-term and transgenerational effects of *in vitro* culture on mouse embryos

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## Abstract

The mouse is a convenient model to analyze the impact of *in vitro* culture (IVC) on the long-term health and physiology of the offspring, and the possible inheritance of these altered phenotypes. The preimplantation period of mammalian development has been identified as an early ‘developmental window’ during which environmental conditions may influence the pattern of future growth and physiology. Suboptimal culture media can cause severe alterations in mRNA expression in the embryo, which are associated with embryo quality reduction. In addition, the embryonic epigenetic reprogramming may also be severely affected by IVC, modifying epigenetic marks particularly in imprinted genes and epigenetically sensitive alleles. These altered epigenetic marks can persist after birth, resulting in adult health problems such as obesity, increased anxiety and memory deficits. Furthermore, some epigenetic modifications have been found to be transmitted to the offspring (epigenetic transgenerational inheritance), thereby providing a suitable model to assess risks of cross-generational effects of perturbing early embryo development. This review will highlight how preimplantation environment changes can not only affect developmental processes taking place at that time, but can also have an impact further, affecting offspring health and physiology; and how they may be transmitted to the next generation. We will also analyze the emerging role of epigenetics as a mechanistic link between the early environment and the later phenotype of the developing organism.

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**Keywords:** *In vitro* culture; Epigenetic; Placental development; Long-term effect; Transgenerational

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## 1. Introduction

The first week of development represents the interval called preimplantation or pre-attachment development (depending on the species), which is a uniquely mammalian phenomenon and encompasses the free-living period of mammalian development during which the early conceptus traverses the oviduct and gains access to the uterine environment. During the short mammalian preimplantation embryo development, a highly controlled genetic developmental program occurs, as well as major epigenetic reprogramming events (demethylating non-imprinted DNA regions and other multiple copy DNA regions, histone modifications, X-activation, etc.) that determine the future of the embryo. Immediately after fertilization, the oocyte and sperm-derived chromatin undergo extensive remodeling. During preimplantation development, the embryonic genome becomes demethylated. In most mammalian species analyzed, male pronuclei become demethylated by an active mechanism, which requires the action of specific enzymes (demethylases), while the methylation marks of the female gamete are passively diluted by the successive rounds of chromatin replication and segregation throughout embryo division [1]. During this period, imprinted genes participate in the establishment of normal embryonic development, and simultaneously, new methylation marks are established de novo (<http://www.mgu.har.mrc.ac.uk/research/imprinting/>) [2]. At the blastocyst stage, the embryonic genome is mostly remethylated again. De novo methylation is lineage specific, such that the inner cell mass (ICM, from which all adult tissues arise) is highly methylated and the trophectoderm (which gives rise to most extra-embryonic tissues, including the placenta) remains methylated at the same level as at morula stage [3]. DNA methylation is also responsible for the transcriptional control of some retrotransposon elements (RTEs) that play a key role in providing common regulation to a group of functions during the development of preimplantation embryos [4].

Although the only optimal microenvironment for embryo development is the oviduct [5], early-cleavage embryos are able to cope with environmental stress and can grow in a wide range of culture conditions. Con-

trary to the view that early embryo is the most fragile stage of life, mammalian preimplantation embryos exhibit remarkable plasticity and will attempt to develop to blastocysts adapting to suboptimal culture conditions, although this adaptation may impair further stages of development. Environmental perturbations, including suboptimal conditions of *in vitro* embryo culture, can affect preimplantation development, leading to a developmental arrest prior to the blastocyst stage, or decreased quality of the resulting blastocysts. Thus, embryo viability in the short term (pregnancy failure) or in the long term (viability of the offspring born after embryo transfer) may be compromised.

The culture of early embryos outside of the female tract (IVC) is an essential technique common to *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI), and most assisted reproductive technology (ART) procedures in humans, farm animals and laboratory animals. Moreover, the IVC of embryos is a requirement for many scientific, medical, and commercial interventions including the generation of transgenic animals, gene targeting, cloning from embryonic and adult cells, and embryonic stem cell derivation. Since embryo culture was developed in the early 1960s, it has been an intensive effort to improve the *in vitro* embryo culture environments; however, for many of the mammalian species, culture conditions have not been fully optimized. In many *in vitro* culture systems result in a high development to blastocysts, but the embryo development to term may be affected [6]. In this sense, the term embryo quality usually refers to the generation of viable offspring after embryo transfer. However some phenotypic consequences in the offspring may result from the application of ART including IVC, that may not be able to supply the right signaling cues, and can lead to the misregulation of genes and aberrant epigenetic modifications [7]. In the last 10 y it has been reported that cultured embryos display significant changes in patterns of gene expression and imprinting [8], and these changes affect the embryo implantation ability, the fetal development, the neonatal health and the susceptibility to suffer diseases in adult life [9].

The use of IVC techniques has revealed that the plasticity in the developmental program may exceed the adaptive capacity of the embryo and has fostered im-

portant research directions aimed at alleviating culture-induced changes in embryonic programming. A better knowledge of the mechanisms that produce these developmental effects would help to improve the embryo culture systems. The purposes of this review are: (i) to provide an overview of the reported effects of *in vitro* culture of mouse embryos on the health and physiology of the offspring, (ii) to describe the transmission of the epigenetic defects produced by IVC, and (iii) to describe possible mechanisms that mediate the effect of *in vitro* culture in both long-term and transgenerational effect.

## 2. Influence of culture on gene expression and imprinting of preimplantation embryo

The literature is replete with studies in a variety of mammalian species, which have documented significant changes in mRNA expression levels in cultured versus *in vivo* derived embryos [10–14]. To address the specific effect on mRNA transcription of blastocysts produced by IVC, we performed a detailed analysis of global gene expression in mouse embryos using an Affymetrix (Santa Clara, CA, USA) oligonucleotide array (MOE430 2.0 chip) containing more than 45,000 transcripts and variants, together with a T7-based linear double amplification method. Embryos collected at the zygote stage (day 0.5) were cultured *in vitro* 96 h (in KSOM medium either supplemented or not with FCS) or were collected directly from the uterus at the blastocyst stage (day 3.5) [15]. Embryos cultured *in vitro* in KSOM and in KSOM+FCS displayed a reduced number of cells in the inner cell mass at the blastocyst stage compared with *in vivo* derived embryos; however, only culture in KSOM+FCS leads to a reduction in the number of trophoblast cells. IVC in KSOM affected the quality of the blastocysts but did not have any long-term consequence in the adult animal [6]. However, culture in presence of FCS reduced by half the capacity of the resulting blastocysts to implant into the uterus following embryo transfer when compared to culture without FCS [9], and lead to abnormal foetal growth and development [9,16]. Microarray analysis identified a panel of genes that are differentially expressed in IVC mouse embryos in KSOM, in the presence or absence of FCS, which may explain the blastocysts adaptation/damage and the phenotype of the adult animals derived. The presence of FCS affected the expression of 198 genes. Metabolism, proliferation, apoptosis and morphogenetic pathways were the most common processes affected by IVC in KSOM, how-

ever, the presence of FCS during IVC preferentially affected genes associated with certain molecular and biological functions related to epigenetic mechanisms indicating that culture-induced alterations in transcription at the blastocyst stage are linked to epigenetic alterations. These results provide a foundation for understanding the molecular origin at the time of preimplantation development of the long-term consequences of IVC in mice. In agreement with the quiet embryo hypothesis [17] a higher number of genes are up-regulated in IVC conditions, indicating that the genome is more actively transcribed *in vitro*, suggesting a decrease of repressive epigenetic marks. These results are in agreement with Giritharan et al [18] who, using the same Microarray chip, reported that IVC in Whitten's medium produced misregulation of 4660 transcripts, the majority of which were up-regulated by IVC (3693 up-regulated). Also, gene chip technology has shown that blastocysts cultured in Whitten's medium had aberrant expression of 114 genes compared with *in vivo* developed embryos, and blastocysts cultured in KSOM displayed only 29 genes misexpressed [13]. Moreover, a comparison of IVC using KSOM in 20% oxygen versus *in vivo* [19] had reported differences in 456 genes between groups.

In addition to the effect of IVC on gene expression, the epigenetic reprogramming of the embryo may also be severely affected by IVC, particularly in imprinted genes [9,16,20]. Imprinted genes are particularly implicated in the regulation of fetal growth, placental function, brain development and post-natal behavior. It has been determined that the formulation of the medium used for embryo culture has a profound effect on the methylation patterns in the resultant two-cell embryos [21], indicating that, in addition to imprinted genes, other epigenetic alterations may intensely alter the gene expression. For example, it has been reported that IVC leads to biallelic expression of the imprinted gene H19 at the blastocysts stage, which persists in extra-embryonic tissues after implantation [22]. A recent study analyzed the effect of five commercial embryo culture systems (KSOMaa, Global, Human Tubal Fluid, Preimplantation 1/Multiblast, and G1v5PLUS/G2v5PLUS) and observed that embryo culture in all commercial media systems resulted in imprinted methylation loss compared to *in vivo*-derived embryos [23].

In addition to imprinted genes, genes expressed in a tissue-specific manner and regions of non-coding repetitive DNA, both of which are frequently epigenetically modified, are likely to be influenced by environmental factors during development [24]. Another group of el-

ements that can be altered by IVC are the retrotransposable elements and related epialleles. Retrotransposable elements are a group of DNA sequences that in some cases may be able to retain parental epigenetic marks, from fertilization through embryonic development to adulthood [25]. Transposable and retrotransposable elements comprise approximately 30–45% of the mammalian genome and are remnants of ancestral viral infections that became incorporated into the DNA of the germline, playing a major role in genomes evolution. Most transposons and retrotransposons have accumulated a sufficient number of mutations to render them functionless. However, many possess functionally competent promoters, which tend to be silenced by extensive methylation during embryogenesis. Interestingly, a comprehensive analysis of transposable elements (TE) expression in mammalian oocytes and embryos reveals that some retrotransposons make an unexpectedly high contribution to the maternal mRNA pool, which persists in cleavage stage embryos. Furthermore, TEs act as alternative promoters and first exons for a subset of host genes, regulating their expression in full-grown oocytes and cleavage stage embryos [26]. These authors indicated that differential TE expression triggers sequential reprogramming of the embryonic genome during the oocyte to embryo transition and in preimplantation embryos. The Agouti viable yellow (Avy) allele and the Axin 1 fused (Axin1Fu) allele in mice are two examples whereby a repetitive element (a retrotransposon) situated within a gene (in these cases controlling coat color and kinky tail respectively) can be regulated by DNA methylation, although the precise molecular mechanism remains controversial [27]. IVC has been used to experimentally alter the methylation status of these loci providing a visual read-out of methylation change via the coat color or the kinky tail of the mouse [28–30]. *In vitro* mouse embryo manipulations have been shown to reduce methylation at the Avy and Axin1Fu loci, shifting coat colors or kinky tail phenotypes in the offspring. Other elements similar to Avy and Axin1Fu are susceptible to be altered by IVC.

There are many aspect related with IVC that can affect the quality of the embryos and that should be mentioned. It has been reported that the preimplantation mouse embryo is highly sensitive to a small decrease in intracellular pH [31] and that the presence of ammonium in culture medium has a detrimental effect on embryo physiology and biochemistry [32] and also ammonium induces aberrant blastocyst differentiation, metabolism, pH regulation, gene expression and sub-

sequently alters fetal development in the mouse [33]. It has been also reported that reductions in oxygen availability can influence both embryonic gene expression and subsequent fetal development [34]. In addition to IVC, the hormonal stimulation of the ovary is a common practice in ART, and, it has been suggested, an adverse effect of hormonal stimulation on gene imprinting [8,21]. Accumulative effect of superovulation, artificial fertilization (ICSI and IVF) and IVC could increase the genetic and epigenetic alterations of the early embryos.

### 3. IVC affects placental and fetal development

The subacute nature of some of these aberrant embryo modifications induced by IVC hampers its detection in the short term, and blastocyst production, a hallmark for the efficiency of IVC systems, often remains unaltered. Some progress has been made in uncovering genes influencing embryo development, although much research is still required to precisely characterize the association between gene expression patterns and embryo, foetal, and adult phenotype. Interestingly, the alterations in genomic imprinting caused by IVC are easier to be restored in tissues derived from ICM than in those resulting from the trophoctoderm, suggesting that mechanisms that safeguard imprinting might be more robust in the embryo than in the placenta [22].

The alterations produced in the early embryo by the IVC could result in abnormal fetal and/or placental development [35]. Changes in the intrauterine availability of nutrients, oxygen, and hormones program tissue development and lead to abnormalities in adult cardiovascular and metabolic function [36]. IVC can affect differentially ICM or trophoctoderm. Some culture media can be suboptimal for the trophoctoderm and then produce situations of placental insufficiency, fetal undernutrition, undergrowth, and impaired development, linking trophoctoderm anomalies with the development of disease in adult life. In a recent study it has been analyzed the transcriptomic effects of *in vitro* embryo manipulations on the expression profile of placental genes at 10.5 d post-fertilization [37]. The authors found that 6% of transcripts were altered at the two-fold threshold in placentas of IVC derived embryos, and that the X-chromosome harbors 11% of altered genes. Interestingly, expression of X and imprinted genes was greatly modulated probably to adapt to adverse conditions, indicating that some genes from the X chromosome display a similar susceptibility to

epigenetic changes following IVC than imprinted genes. A recent study indicated also the importance of mitochondrial function in regulating pre- and post-implantation development [38]. After inhibiting mitochondrial function with amino-oxyacetate, they observed a reduced blastocyst development without a reduction in implantation rates. However, it significantly reduced placental growth but not fetal growth. These results indicated that the IVC modify the placental expression profile long after embryo manipulation, probably to adapt to adverse conditions, and that placental tissues are more sensitive to preimplantation epigenetic disturbance than embryonic tissues [39,40]. These placental alterations have the potential to influence long-term patterns of gene expression that might be associated with increased risk of adult diseases.

#### 4. Long-term effect of IVC in mice

There is evidence from many laboratories that the periconceptual period is a window of enhanced sensitivity to environmental conditions both *in vivo* and *in vitro*. Culture media can have serious consequences for the embryo and results in long term effects (LTE) on postnatal development and behavior [9,41] (Fig. 1). It has been found that suboptimal IVC produces long term effects not only in mice, but also in farm animals. The large offspring syndrome (LOS), which has been widely described in sheep and cattle, is associated with IVC and results from alterations in the genetic imprinting established during preimplantation development [42]. In mice there are several studies [9,41,43] reporting long-term consequences of IVC, such as obesity, increased anxiety, and memory deficits. We have observed that IVC in presence of serum reduces embryo viability and is responsible for alterations in genetic imprinting during preimplantation development [9]. Suboptimal IVC also caused organomegaly of liver and heart, suggesting that a careful post-mortem histological examination of apparently normal individuals may reveal severely compromised welfare [9]. Similar developmental and behavioral alterations in adult mice derived from *in vitro* produced embryos not exposed to serum were reported by others [41], suggesting that IVC environments in general, not only those supplemented with serum, are able to induce aberrant phenotypes. Taken together, it seems that the well-documented developmental alterations seen in mouse, sheep and cattle after IVC are probably applicable to most eutherian mammals, including humans. Progeny resulting from IVC procedures might have subtle genetic and

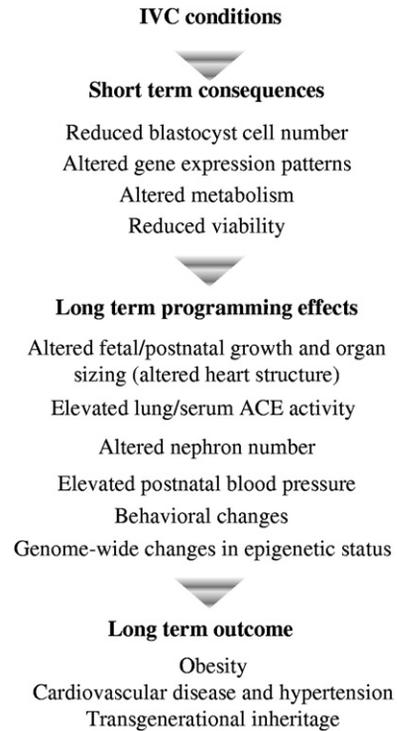


Fig. 1. Long-term programming of postnatal growth and physiology can be induced irreversibly during the preimplantation period of development by adverse preconceptional environment *in vitro* (for example suboptimal *in vitro* culture).

epigenetic defects that are below the threshold, threaten viability, which are only detected at long-term [44].

It has been reported that IVC of mouse preimplantation embryos has also been associated with elevation of offspring systolic blood pressure (SBP) following either prolonged (two-cell to blastocyst stage) or brief (2 h prior to uterine transfer) IVC [45]. It has been also reported that culture and transfer of mouse preimplantation embryos resulted in altered postnatal growth and organ sizing in the offspring which persisted into a second generation, indicating epigenetic modifications [39]. In a recent review the relationship between preimplantation embryo environment and postnatal cardiovascular disease risk has been described, and biochemical, molecular, genetic, and physiological pathways have been implicated in this association [46]. The authors described various factors including endothelial function, vascular responsiveness, the renin-angiotensin system, kidney structure, and early postnatal growth dynamics as potential contributors (Fig. 2). However, although there is a significant increase in risk associated with IVC, no detrimental effect on longevity has been reported in mice [47].

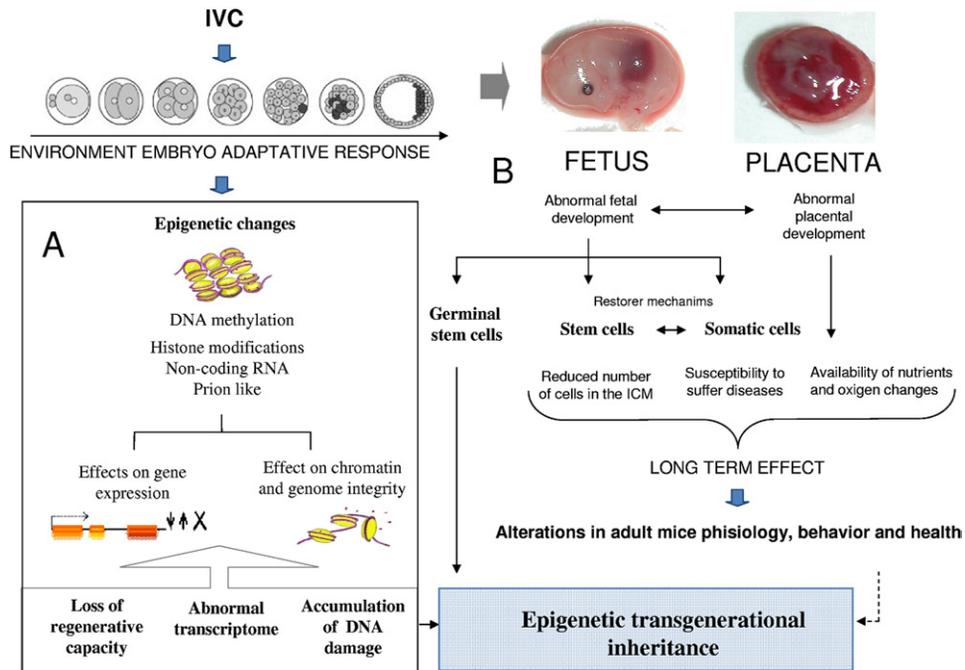


Fig. 2. The IVC can produce epigenetic alterations during periconceptual period of embryo development (A) that should be later mediate through diverse foetal organ (somatic or stem cells) and through placenta (B). IVC may produce epigenetic modifications (cytosine methylation, histone modifications), and crosstalk among chromatin marks and regulators result in changes in chromatin conformation that modulate different outcomes, such as transcriptional activation or repression, chromatin condensation or DNA repair. These will affect the phenotype of the individual and also if germ cells are affected may influence the next generation offspring.

It is important to remark that the transcriptional level of a large number of genes differs between male and female preimplantation embryos [26,28,48]. Due to the differences in sex chromosome dosage, mammals are sexually dimorphic before gonadal differentiation, and epigenetic differences between sexes occur during preimplantation [49], which provides an explanation for the gender specific short- and long-term effects produced by IVC [50–52].

## 5. Transgenerational effects of IVC

A transgenerational effect occurs when the alterations in the epigenetic marks caused by IVC persist into subsequent generations. There is evidence that epigenetic information can be inherited across generations in mammals, despite extensive reprogramming both in the gametes and in the early developing embryo. It has been reported that culture and transfer of mouse preimplantation embryos resulted in altered postnatal growth and organ sizing in the offspring which persisted into a second generation, suggesting heritable epigenetic modifications [39]. There are some specific types of transposons, for instance intracisternal-A par-

ticles (IAPs, a long-terminal repeat retrotransposon), that are resistant to postfertilization demethylation. The differential methylation of these transposons can regulate the transcription of neighboring genes. As we have indicated above, several alleles in mice have been shown to exhibit transgenerational epigenetic inheritance mediated by an IAP element, for example the *Avy* and the *Axin1Fu* alleles. It has been reported that IVC can affect the postnatal expression of these defined epigenetically sensitive allele that exhibit transgenerational inheritance [28]. Interestingly, the IAP element present in the *Axin1Fu* is demethylated during preimplantation and its histone modifications marks allow the transgenerational inheritance of the modifications induced by IVC in the *Axin1Fu* epiallele, indicating that histone modifications may serve as heritable marks in the absence of DNA methylation. We do not know how widely the mechanisms involved in the regulation of *Axin1Fu* allele are used throughout the rest of the genome. However, given the conservation during evolution of epigenetic mechanisms between mice and humans, it is likely that similar mechanisms of metastable epialleles will act in humans. Indeed, 30% of all transcript identify by cap-analysis of gene expression

(CAGE) tags (method to measure expression levels by counting large amounts of sequenced capped 5' ends of transcripts, essential for identification of novel promoters) derived from human embryonic tissues have been associated with repetitive elements (16% retrotransposons) [53].

In addition to the epigenetic marks of DNA, the germ cells transmit various cytoplasmic RNAs (which include mRNAs, siRNAs, piRNAs, and miRNAs) that are essential for post-fertilization development. There is now accumulating evidence that the RNAs (particularly non-coding RNAs) of germ cells may carry functional epigenetic information that can be inherited transgenerationally through the germline. One example of this RNA-mediated inheritance comes from studies of paramutation in which the interaction between two alleles of a single locus results in heritable variation [54].

An alternative method of transgenerational inheritance of aberrant phenotypes could be that de novo mutations in the DNA sequence of preimplantation embryos may be induced by exposures to suboptimal culture media and conditions, and that these genetic effects account for the changes observed in offspring. In the field of developmental toxicology, there is evidence for an association between exposure to various drugs/toxins and an increased occurrence of mutations, including numerical and structural chromosomal abnormalities, point mutations, copy number variant (CNV) changes, and duplications/deletions of microsatellites [55]. However, there are no studies to determine whether IVC produce de novo mutations, and if these mutations account for some of the induced phenotypic variation observed in offspring.

## 6. Conclusions

Developmental programming is an important physiological process that allows a single genotype to produce different phenotypes. Early embryonic development is of special interest because this period is critical for establishing and maintaining epigenetic marks, and because the adaptive responses of the developing early embryo (during preimplantation and during early organ development) to anticipated future adverse environments, may have maladaptive consequences if the environment is mismatched to the predicted. Under suboptimal IVC there is an adaptive response by the early embryo to suboptimal nutrition that may predispose it to certain adult diseases. Under suboptimal conditions, the preimplantation embryo activates physiological

mechanisms of developmental plasticity to stabilize conceptus growth and extra embryonic lineage and enhance postnatal fitness, that may lead to adult excess growth and cardiovascular, behavioural, and fertility alterations. The understanding of the mechanisms that cause these maladaptive developmental responses is necessary to predict and prevent pathologies. We hypothesize that such adaptations are due to epigenetic changes occurring during sensitive periods in early development (preimplantation and peri-implantation). The use of suboptimal *in vitro* culture of preimplantation mouse embryos will serve to determine the epigenetic marks susceptible to nutritional and environmental conditions and their possible transmission to the next generation. We ought to identify epigenetic labile genes in the mouse and human embryo genomes that allow us to identify the best IVC to produce competent and healthy embryos, to ensure that animal and human assisted reproductive technologies are utilized in the most efficient and safest possible manner. In addition, because epigenetic marks are reversible, we will need to determine when, how, and whether to use preventive treatments, such as specific diets, drugs or lifestyle changes to reduce the transmission of maladaptive acquired phenotypes.

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## LOW AND VERY LOW BIRTH WEIGHT IN INFANTS CONCEIVED WITH USE OF ASSISTED REPRODUCTIVE TECHNOLOGY

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**ABSTRACT**

**Background** The increased risk of low birth weight associated with the use of assisted reproductive technology has been attributed largely to the higher rate of multiple gestations associated with such technology. It is uncertain, however, whether singleton infants conceived with the use of assisted reproductive technology may also have a higher risk of low birth weight than those who are conceived spontaneously.

**Methods** We used population-based data to compare the rates of low birth weight ( $\leq 2500$  g) and very low birth weight ( $< 1500$  g) among infants conceived with assisted reproductive technology with the rates in the general population.

**Results** We studied 42,463 infants who were born in 1996 and 1997 and conceived with assisted reproductive technology and used as a comparison group 3,389,098 infants born in the United States in 1997. Among singleton infants born at 37 weeks of gestation or later, those conceived with assisted reproductive technology had a risk of low birth weight that was 2.6 times that in the general population (95 percent confidence interval, 2.4 to 2.7). The use of assisted reproductive technology was associated with an increased rate of multiple gestations; however, its use was not associated with a further increase in the risk of low birth weight in multiple births. Among twins, the ratio of the rate of low birth weight after the use of assisted reproductive technology to the rate in the general population was 1.0 (95 percent confidence interval, 1.0 to 1.1). Infants conceived with assisted reproductive technology accounted for 0.6 percent of all infants born to mothers who were 20 years of age or older in 1997, but for 3.5 percent of low-birth-weight and 4.3 percent of very-low-birth-weight infants.

**Conclusions** The use of assisted reproductive technology accounts for a disproportionate number of low-birth-weight and very-low-birth-weight infants in the United States, in part because of absolute increases in multiple gestations and in part because of higher rates of low birth weight among singleton infants conceived with this technology. (N Engl J Med 2002;346:731-7.)

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INFANTS who have low birth weight, either because of early delivery or because of fetal growth restriction, are at increased risk for short- and long-term disabilities and death.<sup>1,2</sup> The use of assisted reproductive technology is an important contributor to the rate of low birth weight in the United States because it is associated with a higher rate of multiple birth,<sup>3,4</sup> which, in turn, is associated with low birth weight.<sup>5</sup> By 1997, the use of assisted reproductive technology accounted for more than 40 percent of triplets born in the United States.<sup>4</sup> In addition, studies have suggested that there is a higher rate of low birth weight among singleton infants conceived with assisted reproductive technology than among naturally conceived singleton infants<sup>6-8</sup> or among all infants in the general population.<sup>9-13</sup> However, these studies had methodologic limitations. In particular, they did not address the issue of whether infants born as singletons were conceived as part of a multiple gestation that was later reduced either medically or spontaneously to a singleton pregnancy.

In addition, it remains unclear whether the risk of low birth weight among singleton infants conceived with assisted reproductive technology is a direct effect of the procedure involving such technology<sup>14,15</sup> or reflects some other factor related to the underlying infertility of the couples who conceive using these procedures.<sup>16-18</sup> Studies have been limited by small sample sizes and lack of data regarding such potentially confounding variables as the factors causing infertility and their severity.

We used population-based data from records of procedures performed with assisted reproductive technology in the United States to compare the risk of low birth weight among infants conceived with assisted reproductive technology with that found in the general population. The large sample and detailed data on the procedures and resulting pregnancies provided an opportunity to analyze outcomes according to several important factors, including the number of infants born, the number of fetuses early in the pregnancy, cause of infertility, and factors involved in treatment.

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## METHODS

### Study Population

Clinics and medical practices in the United States are required to report data on every procedure involving assisted reproductive technology to the Centers for Disease Control and Prevention (CDC).<sup>19</sup> Each year, the Society for Assisted Reproductive Technology collects data on such procedures performed in clinics in the United States and provides these data to the CDC. Procedures involving assisted reproductive technology are defined as procedures for the treatment of infertility in which both oocytes and sperm are handled outside the body; these include in vitro fertilization with transcervical embryo transfer, gamete and zygote intrafallopian transfer (in which gametes or zygotes are transferred into the fallopian tube rather than the uterus), frozen-embryo transfer, and donor-embryo transfer. Data abstracted from patients' records and submitted to the CDC include each patient's demographic characteristics and medical history, as well as clinical information on the procedures performed and resultant pregnancies and births. In 1996, 300 clinics reported more than 60,000 procedures; in 1997, 335 clinics reported more than 70,000 procedures. Five to 7 percent of clinics that were in operation during these years did not report data, despite the federal requirement; because most of these were known to be small practices, we estimate that the data reported represent more than 95 percent of all procedures performed with the use of assisted reproductive technology.

We included in the present analysis infants conceived through procedures performed in 1996 and 1997 in which the mother was between 20 and 60 years of age. Of 136,972 procedures, 23 percent (31,767) resulted in the delivery of one or more liveborn infants. Because some of these were multiple-birth deliveries, the total number of infants was 45,886. Although a delivery could include both liveborn and stillborn infants, we excluded from our analysis the 182 stillborn infants. A total of 3241 infants with missing data on birth weight were also excluded. Our final sample included 42,463 infants conceived with assisted reproductive technology.

### Internal Comparisons

We classified infants according to the number at birth (singleton, twin, triplet, or quadruplet or higher-order birth). Although only liveborn infants were included in this study, the assignment of the number born was based on the total number of liveborn and stillborn infants delivered. Within each birth-number group we examined the risk of low and very low birth weight. Birth weight was recorded as a categorical variable in 500-g strata. We defined low birth weight as 2500 g or less and very low birth weight as less than 1500 g.

We assessed variations in risk according to maternal and treatment-related factors, using stratification and multivariable logistic regression. The factors we evaluated included the number of fetal hearts observed on early ultrasonography (i.e., the number of fetuses in the pregnancy), maternal age, parity, primary cause of infertility, previous procedures involving assisted reproductive technology, and the type of procedure that resulted in the current conception. Procedures were classified according to whether the embryos had been fertilized during the current procedure (i.e., were fresh) or had been previously fertilized and frozen until the current procedure and whether the source of the oocytes was the mother herself (nondonor) or another woman serving as an oocyte or embryo donor. In addition, procedures in which a woman other than the mother served as a gestational carrier or surrogate were classified separately. Thus, the procedure was categorized as involving fresh embryos and nondonor oocytes, frozen embryos and nondonor oocytes, fresh embryos and donor oocytes, frozen embryos and donor oocytes, or a gestational carrier. We considered separately whether intracytoplasmic sperm injection (a procedure in which a single sperm is injected directly into the oocyte) was

used in procedures involving fresh embryos and nondonor oocytes and those involving fresh embryos and donor oocytes.

### External Comparison

We compared the observed numbers of low-birth-weight and very-low-birth-weight infants conceived with assisted reproductive technology with expected numbers. Expected numbers were calculated with the use of the public-use computer file containing the 1997 birth-certificate data for the United States (on 3,389,098 infants born to women who were 20 years of age or older)<sup>20</sup> and were adjusted to match the age and parity distributions for women who conceived with assisted reproductive technology. We computed standardized ratios for low birth weight and very low birth weight by dividing the observed numbers by the expected numbers and calculated 95 percent confidence intervals for each estimate.<sup>21</sup>

To rule out the possibility that the reduction of gestations that had initially involved multiple fetuses might explain a higher rate of low birth weight in singletons, we performed secondary analyses that included only those births in which the number of fetal hearts noted on ultrasonography did not exceed the number of infants who were born. (Data were not available to permit the differentiation of spontaneous reductions from medically induced reductions.) To separate the effects of treatment from underlying characteristics of the patients or embryos, we performed several additional analyses in this subsample. In one analysis, we restricted the sample to infants conceived with the use of donor oocytes among couples without a diagnosis of male-factor infertility, since these infants were considered most likely to have been conceived with healthy gametes. In a second analysis, we restricted the sample to infants born to couples with a diagnosis of male-factor infertility, since women in this subgroup were considered unlikely to have uterine or other infertility-related disease. And in a third analysis, we restricted the sample to infants who had been carried by a gestational surrogate, since these surrogates were presumably healthy women.

Analyses were conducted separately for singletons and twins. In addition, we subdivided low-birth-weight infants into term and preterm infants. Preterm delivery was defined as delivery at less than 37 completed weeks of gestation. We calculated gestational age as the interval from the date of oocyte retrieval and fertilization to the date of birth. For procedures performed with the use of frozen embryos and for other procedures for which the date of oocyte retrieval was missing, the gestational age was calculated as the interval from the date of embryo transfer to the date of birth. To make the estimates comparable with those in the general population, we computed the estimated postmenstrual age (the age according to the last menstrual period) as the gestational age in days plus 14. Term low birth weight was defined as a weight of 2500 g or less with delivery at term; preterm low birth weight was defined as a weight of 2500 g or less with preterm delivery.

To assess the contribution of the use of assisted reproductive technology to low birth weight in the United States, we examined 20,369 infants from our study population who were born in 1997; some had been conceived with assisted reproductive technology in 1996 and some in 1997. For singleton infants, twins, and infants from higher-order multiple births, we divided the number of low-birth-weight and very-low-birth-weight infants conceived with assisted reproductive technology by the total number of low-birth-weight and very-low-birth-weight infants born to women 20 years of age or older in the United States in 1997.

This study was approved by the institutional review board of the CDC; in accordance with federal regulations, the requirement to obtain informed consent was waived for this retrospective analysis.

## RESULTS

The study population was similar to the total population of women treated with assisted reproductive

technology in terms of the characteristics of the women and the infertility treatment they received (Table 1). However, some factors associated with higher success rates for assisted reproductive technology — an age of less than 35 years, previous deliveries, no previous procedures involving assisted reproductive technology, and the use of fresh embryos (particularly fresh donor embryos) — were slightly more common among the study population.

A total of 43 percent of the infants in the study population were singletons, 43 percent were twins, 12 percent were triplets, and 1 percent were quadruplets or higher-order multiples. The percentage of infants with low birth weight varied from 13.2 percent among singletons to almost 100 percent among quadruplets or higher-order multiples. The percentage of infants with low birth weight varied with maternal characteristics and treatment-related factors (Table 2). Singletons were more likely to have low birth weight if there had been more than one fetal heart on early ultrasonography, and twins were more likely to have low birth weight if there had been more than two fetal hearts.

The rate of low birth weight was also higher among singletons and twins born to nulliparous women and women who had no previous procedures involving assisted reproductive technology (Table 2). Low birth weight was less common in infants conceived by couples with male-factor infertility, conceived with intracytoplasmic sperm injection, or carried by a gestational surrogate. Among triplets, 90 percent or more of the infants had low birth weight, regardless of maternal characteristics or treatment-related factors. The percentages of quadruplets and higher-order multiples with low birth weight are not shown in Table 2 but were nearly 100 percent in all groups. The results for singletons and twins were materially unchanged after multivariable adjustment for the maternal characteristics and treatment factors listed in Table 2.

The rate of very low birth weight ranged from 2.6 percent for singletons to 66.9 percent for quadruplets or higher-order multiples. The rate of very low birth weight also varied with maternal and treatment-related factors, but to a lesser degree than the rate of low birth weight did (data not shown).

As compared with all singleton infants born in the United States to women 20 years of age or older in 1997, singletons conceived with assisted reproductive technology in 1996 or 1997 were at increased risk for low and very low birth weight (Table 3). When the analysis was restricted to infants who were carried by a gestational surrogate the risk was no longer significantly increased, but there were relatively few infants in this group.

We stratified low-birth-weight infants according to whether they were born at term or were preterm

**TABLE 1. MATERNAL CHARACTERISTICS AND CHARACTERISTICS OF ASSISTED REPRODUCTIVE TECHNOLOGY USED.\***

CHARACTERISTIC	PERCENTAGE OF ALL PROCEDURES INVOLVING ART (N=136,972)	PERCENTAGE OF PROCEDURES THAT RESULTED IN A LIVE BIRTH (N=31,767)	PERCENTAGE OF INFANTS INCLUDED IN FINAL SAMPLE (N=42,463)
Age of mother			
20–29 yr	10.5	13.1	14.0
30–34 yr	31.2	37.2	38.4
35–39 yr	35.8	34.3	33.4
40–44 yr	19.2	12.3	11.2
≥45 yr	3.3	3.2	3.1
Parity			
0	76.9	75.6	75.3
1	17.0	18.2	18.5
≥2	6.2	6.1	6.2
Primary cause of infertility			
Female factor	69.4	68.0	68.1
Male factor	23.0	24.2	24.1
Idiopathic	7.7	7.8	7.8
Previous procedures involving ART			
0	49.8	55.2	55.8
1	24.7	23.1	22.7
2	12.1	10.8	10.7
≥3	13.4	11.0	10.8
Type of procedure†			
Fresh embryo, nondonor oocyte	76.3	76.9	78.0
Frozen embryo, nondonor oocyte	14.2	9.9	8.9
Fresh embryo, donor oocyte	6.6	10.3	10.5
Frozen embryo, donor oocyte	2.0	1.7	1.6
Gestational carrier	0.9	1.2	1.1
Use of intracytoplasmic sperm injection‡			
No	61.3	62.1	62.9
Yes	38.7	37.9	37.1

\*Data on age, parity, primary cause of infertility, and type of procedure were missing for less than 1 percent of infants; data on previous procedures were missing for 3 percent of infants; and data on the use or nonuse of intracytoplasmic sperm injection were missing for 6 percent of infants. The final sample included all live-born infants with data on birth weight. ART denotes assisted reproductive technology.

†Procedures were classified according to whether the embryos had been fertilized during the current procedure (i.e., were fresh) or had been previously fertilized and frozen until the current procedure and whether the source of the oocytes was the mother herself (nondonor) or another woman serving as an oocyte or embryo donor; procedures in which a woman other than the mother served as a gestational carrier were classified separately.

‡Data are for procedures involving fresh embryos and nondonor oocytes and those involving fresh embryos and donor oocytes only.

(Table 4). Singleton infants conceived with assisted reproductive technology had a risk of term low birth weight that was more than twice that of singleton infants in the general population, and they had a smaller but still significant increase in the risk of preterm low birth weight. The risk of term low birth weight

**TABLE 2. PERCENTAGE OF INFANTS WITH LOW BIRTH WEIGHT ( $\leq 2500$  g) AMONG SINGLETONS, TWINS, AND TRIPLETS CONCEIVED WITH ASSISTED REPRODUCTIVE TECHNOLOGY IN 1996 AND 1997.\***

VARIABLE	SINGLETONS	TWINS	TRIPLETS
	(N=18,408)	(N=18,399)	(N=5127)
	% with low birth weight		
Total	13.2	55.2	92.4
No. of fetal hearts on early ultrasonography			
1	12.6		
2	17.6	53.2	
3	25.4	61.1	92.4
4	50.0	70.7	94.4
5	—	70.3	—
$\geq 6$	—	89.7	—
Age of mother			
20–29 yr	12.4	61.7	92.4
30–34 yr	13.4	55.3	92.7
35–39 yr	13.1	53.6	91.8
40–44 yr	13.5	51.3	91.0
$\geq 45$ yr	12.3	53.5	98.1
Parity			
0	13.7	57.3	93.1
1	11.3	48.4	90.0
$\geq 2$	12.4	49.8	90.6
Primary cause of infertility			
Female factor	13.6	56.1	92.4
Male factor	12.2	52.7	93.0
Idiopathic	12.5	54.7	90.4
Previous procedures involving ART			
0	14.3	56.6	93.3
1	11.5	54.0	91.5
2	12.7	53.3	93.2
$\geq 3$	11.9	51.1	89.6
Type of procedure†			
Fresh embryo, nondonor oocyte	13.6	56.0	92.1
Frozen embryo, nondonor oocyte	10.5	49.5	92.1
Fresh embryo, donor oocyte	14.0	53.6	94.5
Frozen embryo, donor oocyte	11.8	57.1	97.4
Gestational carrier	8.7	50.0	90.0
Use of intracytoplasmic sperm injection‡			
No	14.3	56.8	92.4
Yes	12.7	54.0	93.0

\*Data on age, parity, primary cause of infertility, and type of procedure were missing for less than 1 percent of infants; data on previous procedures were missing for 3 percent of infants; and data on the use of intracytoplasmic sperm injection were missing for 6 percent of infants. The percentage of infants with low birth weight is not provided if there were fewer than 20 infants in the category. Global P values were calculated by the chi-square test and were adjusted for correlations between infants within each birth-number group. Global  $P < 0.05$  for all variables except the age of the mother among singletons and all variables among twins. ART denotes assisted reproductive technology.

†Procedures were classified according to whether the embryos had been fertilized during the current procedure (i.e., were fresh) or had been previously fertilized and frozen until the current procedure and whether the source of the oocytes was the mother herself (nondonor) or another woman serving as an oocyte or embryo donor; procedures in which a woman other than the mother served as a gestational carrier were classified separately.

‡Data are for procedures involving fresh embryos and nondonor oocytes and those involving fresh embryos and donor oocytes only.

remained elevated in analyses restricted to subgroups of the study population conceived with presumably healthy gametes or carried by a presumably healthy woman. The risk of preterm low birth weight was no longer increased in analyses restricted to study infants who had been carried by presumably healthy women.

Singletons conceived with assisted reproductive technology and delivered at term tended to be born slightly earlier than singletons in the general population (mean gestational age, 39.1 vs. 39.5 weeks). We therefore further adjusted our analyses for the week of gestation at delivery (37 to 41 or more) in addition to maternal age and parity. This adjustment did not substantially change our findings (adjusted term-low-birth-weight ratio, 2.4; 95 percent confidence interval, 2.3 to 2.6).

Among twins conceived with assisted reproductive technology, the risks of both term and preterm low birth weight were similar to those in the general population of twins. The ratio of the rate of low birth weight at term among twins conceived with assisted reproductive technology to the rate among all twins born at term was 1.0 (95 percent confidence interval, 1.0 to 1.1).

The 20,369 infants conceived with assisted reproductive technology and born in 1997 represented 0.6 percent of the 3,389,098 infants born to women 20 years of age or older in the United States in that year. However, we estimate that the use of assisted reproductive technology accounted for 3.5 percent of the infants with low birth weight and 4.3 percent of the infants with very low birth weight born to women in this age group. The excesses were due in large part to the increased number of infants from multiple births who were conceived with assisted reproductive technology. However, the increased rates of low birth weight among singletons conceived with assisted reproductive technology also played a small part (0.6 percent of low-birth-weight singletons were conceived with assisted reproductive technology, as compared with the 0.2 percent that would have been expected).

## DISCUSSION

Singleton infants conceived with assisted reproductive technology were at increased risk for low birth weight at term relative to singletons in the general population of the United States. This risk was not explained by known differences between the two populations in the distribution of maternal age, maternal parity, or gestational age at delivery. In addition, there was an increased risk even in analyses in which the sample was restricted to infants from pregnancies that had not originated as multiple gestations, infants conceived with gametes from apparently fer-

**TABLE 3.** OBSERVED AND EXPECTED CASES OF LOW BIRTH WEIGHT AND VERY LOW BIRTH WEIGHT AMONG SINGLETON INFANTS CONCEIVED WITH ASSISTED REPRODUCTIVE TECHNOLOGY IN 1996 AND 1997.\*

VARIABLE	TOTAL NO.	NO. OF CASES OBSERVED	NO. OF CASES EXPECTED†	STANDARDIZED RISK RATIO (95% CI)
<b>Low birth weight</b>				
All infants	18,398	2423	1339.4	1.8 (1.7–1.9)
Pregnancies with one fetal heart	16,730	2104	1197.1	1.8 (1.7–1.8)
Use of donor oocytes, no diagnosis of male-factor infertility	1,397	190	119.3	1.6 (1.4–1.8)
Diagnosis of male-factor infertility	2,759	329	195.9	1.7 (1.5–1.9)
Use of gestational carrier	180	16	13.3	1.2 (0.6–1.8)
<b>Very low birth weight</b>				
All infants	18,398	480	263.4	1.8 (1.7–2.0)
Pregnancies with one fetal heart	16,730	408	239.2	1.7 (1.5–1.9)
Use of donor oocytes, no diagnosis of male-factor infertility	1,397	49	23.5	2.1 (1.5–2.7)
Diagnosis of male-factor infertility	2,759	78	38.5	2.0 (1.6–2.5)
Use of gestational carrier	180	0	2.6	—

\*Ten infants with missing data on parity were not included in these analyses. CI denotes confidence interval.

†The number of expected cases was calculated by applying the rates of low birth weight from the 1997 U.S. birth-certificate data to the population of infants conceived with assisted reproductive technology. The values were adjusted to account for differences in the distributions of age (in the following categories: 20 to 29 years, 30 to 34 years, 35 to 39 years, 40 to 44 years, and ≥45 years) and parity (0, 1, or ≥2) between the two populations.

tile persons, and infants from pregnancies carried by women who were unlikely to have an underlying uterine or other infertility-related disease. Thus, this study suggests that the increased risk of low birth weight in singleton infants born at term who were conceived with assisted reproductive technology may be directly related to such treatments for infertility.

Singletons who were conceived with assisted reproductive technology also had a moderately elevated rate of preterm low birth weight. However, increased risks were not observed among all subgroups; in particular, the risk was not increased among infants delivered by a gestational carrier rather than the mother. These subgroup analyses involved greatly reduced samples and must therefore be interpreted cautiously. However, a possible explanation is that the risk of preterm low birth weight associated with assisted reproductive technology may be related to some underlying condition in the women who undergo procedures involving such technology rather than to the procedures themselves.

The mechanisms underlying the association between the use of assisted reproductive technology and low birth weight among infants born at term remain unclear and warrant further research. The use of human menopausal gonadotropin as part of procedures

involving assisted reproductive technology has been associated with increases in insulin-like growth factor-binding protein 1; this protein has been linked to intrauterine growth restriction.<sup>22</sup> During pregnancies initiated with assisted reproductive technology, altered levels of other endometrial proteins and increased rates of structural abnormalities of the placenta have also been found.<sup>23,24</sup> These factors may also contribute to growth restriction. A less direct mechanism is also possible. The use of assisted reproductive technology has been linked to such maternal complications as pregnancy-induced hypertension.<sup>25-27</sup>

Studies also suggest that women who have conceived with assisted reproductive technology are more likely to undergo elective cesarean section, resulting in deliveries that occur earlier than those following spontaneous pregnancies.<sup>6,7,25-27</sup> We did not have data on complications of pregnancy or type of delivery, but we did find that singletons conceived with assisted reproductive technology and born at term were delivered slightly earlier than term singletons in the general population. Adjustment for the week of gestation at delivery did not substantially reduce the risk ratio for low birth weight at term. We observed an excess risk of low birth weight among the singletons conceived with assisted reproductive technolo-

**TABLE 4.** OBSERVED AND EXPECTED CASES OF LOW BIRTH WEIGHT AMONG TERM AND PRETERM SINGLETON INFANTS CONCEIVED WITH ASSISTED REPRODUCTIVE TECHNOLOGY IN 1996 AND 1997.\*

VARIABLE	TOTAL NO.	NO. OF CASES OBSERVED	NO. OF CASES EXPECTED†	STANDARDIZED RISK RATIO (95% CI)
<b>Term low birth weight</b>				
All infants	18,182	1180	455.2	2.6 (2.4–2.7)
Pregnancies with one fetal heart	16,530	1059	413.1	2.6 (2.4–2.8)
Use of donor oocytes, no diagnosis of male-factor infertility	1,390	80	42.4	1.9 (1.5–2.3)
Diagnosis of male-factor infertility	2,730	190	66.5	2.9 (2.5–3.3)
Use of gestational carrier	180	8	4.7	1.7 (0.5–2.9)
<b>Preterm low birth weight</b>				
All infants	18,182	1206	859.6	1.4 (1.3–1.5)
Pregnancies with one fetal heart	16,530	1011	780.3	1.3 (1.2–1.4)
Use of donor oocytes, no diagnosis of male-factor infertility	1,390	110	75.7	1.5 (1.2–1.7)
Diagnosis of male-factor infertility	2,730	131	126.1	1.0 (0.9–1.2)
Use of gestational carrier	180	8	8.5	0.9 (0.3–1.6)

\*Term infants were defined as those born at or after 37 weeks of gestation, and preterm infants were defined as those born at less than 37 weeks of gestation. Ten infants with missing data on parity and 216 infants (1 percent) with missing data required to calculate gestational age were not included in these analyses; of the infants missing gestational-age data, 37 had low birth weight and 179 had normal birth weight. CI denotes confidence interval.

†The number of expected cases was calculated by applying the rates of low birth weight from the 1997 U.S. birth-certificate data to the population of infants conceived with assisted reproductive technology. Values were adjusted to account for differences in the distributions of age (in the following categories: 20 to 29 years, 30 to 34 years, 35 to 39 years, 40 to 44 years, and  $\geq 45$  years) and parity (0, 1, or  $\geq 2$ ) between the two populations.

gy who were born at every week of gestation between 37 and 41 weeks.

Twins conceived with assisted reproductive technology and born at term were not at higher risk of low birth weight than twins in the general population. It is possible that the additional risk associated with the use of assisted reproductive technology is negligible in twin pregnancies, which are already at high risk. Twins conceived with the use of medications for ovarian stimulation but without assisted reproductive technology may also be at increased risk for low birth weight and may have accounted for a sizable proportion of twins in the general population. We do not have data on the use of these medications among the mothers of the general birth cohort.

We did not compare the birth weights of triplets and higher-order multiples in our study population with those in the general population. More than 40 percent of the triplets and higher-order multiples in the general population were conceived with assisted reproductive technology, and the risk of low birth weight was greater than 90 percent among such infants in both groups.

We estimate that more than 3 percent of the low-birth-weight infants and more than 4 percent of the very-low-birth-weight infants born in 1997 were conceived with assisted reproductive technology — six times the proportions that would be expected on the basis of the frequency of these procedures. These higher-than-expected proportions are largely explained by the increased rate of multiple births. Although the use of assisted reproductive technology did not appear to increase the already high risk of low birth weight among infants from multiple gestations, the increased risk of low birth weight among singletons conceived with assisted reproductive technology and delivered at term indicates that infants from both singleton and multiple births must be considered in assessing the effect of assisted reproductive technology on the rate of low birth weight in the United States.

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## MAIN RESEARCH ARTICLE

# Maternal and child outcome after in vitro fertilization – a review of 25 years of population-based data from Sweden

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## Key words

In vitro fertilization, pregnancy, neonatal outcome, long-term morbidity, multiple births

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## Abstract

**Objective.** To summarize data on deliveries after in vitro fertilization (IVF) performed in Sweden up to 2006. **Design.** Cohort study of women and children, conceived after IVF, with comparisons of deliveries after IVF before and after 1 April 2001. **Setting.** Study based on Swedish health registers. **Population.** Births registered in the Swedish Medical Birth Register with information on IVF from all IVF clinics in Sweden. **Methods.** Results from the second study period are summarized, and outcomes between the two periods are compared. Long-term follow-up is based on data from both periods. **Main outcome measures.** Maternal and perinatal outcomes, long-term sequels. **Results.** Some maternal pregnancy complications decreased in rate, notably pre-eclampsia and premature rupture of membranes. The rate of multiple births and preterm births decreased dramatically, with a better neonatal outcome, including reduced neonatal mortality. No difference in outcome existed between IVF and intracytoplasmic sperm injection or between the use of fresh and cryopreserved embryos, but children born after blastocyst transfer had a slightly higher risk for preterm birth and congenital malformations than children born after cleavage stage transfer. An increased risk for cerebral palsy, possibly for attention deficit and hyperactivity disorder, for impaired visual acuity and for childhood cancer was noted, but these outcomes were rare also after IVF. An increased risk for asthma was demonstrated. No effect on maternal cancer risk was seen. **Conclusion.** A marked decrease in multiple births was the main reason for better pregnancy and neonatal outcome and may also have a beneficial effect on long-term results, notably cerebral palsy.

**Abbreviations** CI, confidence interval; ICD, international classification of diseases; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; OR, odds ratio; RR, risk ratio; SGA, small for gestational age

## Introduction

Even though most pregnancies after in vitro fertilization (IVF) have a normal outcome, an increased risk for a number of obstetric and neonatal complications exists, such as preterm delivery and congenital malformations. These appear to depend only slightly on the IVF technique used, but seem mainly to relate to the subfertility status of the parents and notably the mother. One exception is the high rate

of multiple births, which was due to the transfer back of two or more embryos. During the past 25 years, some important changes in IVF technology have occurred. In 1992, intracytoplasmic sperm injection (ICSI) was introduced, which opened up the treatment of male infertility. Other new techniques were freezing of embryos followed by transfer of thawed embryos, increase of culture time with transfer of blastocyst stages, and a trend towards single embryo transfer. During the period, changes in the selection of couples

for IVF may also have occurred because of the increasing access to IVF facilities, which permits treatment of less severe subfertility cases.

In order to evaluate possible changes with time of IVF, there should ideally be access to national data comprising both a large number of IVF pregnancies and an adequate amount of background material for comparison. The presence of national health registers in Sweden makes it possible to perform such analyses and to compare changes over time in delivery outcome and also to conduct long-term follow-up of women who delivered after IVF and their children. In 2005, a series of articles was published on outcome after IVF during the years 1982–2001 (1). A new set of data was collected for IVF up to and including 2006, and articles on specific problems have been published or are in press (2–10). Here we summarize results from the second study period, compare outcomes between the two periods and describe long-term follow-up, based on data from both periods.

## Material and methods

Methodological issues in studies of delivery outcome after IVF were extensively discussed by Nygren *et al.* (1), to which the reader is referred. Pregnancies after IVF which ended with a delivery were reported from all IVF clinics in Sweden, and further information was obtained by linkage with national health registers as specified below. In principle, data collection was made in the same way during the study periods, but more detailed IVF data were obtained for the second period (Table 1). It was then possible to analyze some new features, such as the effect of blastocyst transfer and vanishing twins.

The first study was based on embryo transfers from 1982 to 31 March 2001 and therefore deliveries up to and including 2001. The second study was based on embryo transfers from 1 April 2001 to the end of 2006 and therefore including some deliveries in 2007. The first study analyzed data from 13 261 women who had IVF and gave birth to 16 280 infants. The second study analyzed data from 14 126 women who gave

birth to 15 570 infants. The numbers were thus similar for the two periods.

Delivery outcome was studied using the Swedish Medical Birth Register (11), which contains medical data on the pregnancy, delivery and pediatric examination of the newborn. Information on indication for IVF was not available. Supplemental information on congenital malformations was obtained from the Birth Defects Register (previously Register of Congenital Malformations) and the Patient Register on hospitalized children (12). Long-term follow-up was carried out using the Swedish Cancer Register (13), the Patient Register (14) and the Prescribed Drug Register (15). Small for gestational age (SGA) as a measure of intrauterine growth retardation was based on sex- and parity-specific standard graphs derived from the Medical Birth Register (16).

Analyses were performed using the Mantel–Haenszel technique. Risk estimates were in most cases made as odds ratios (ORs) with 95% confidence intervals (95% CI) according to Miettinen's method. Two ORs were compared based on the Mantel–Haenszel variances. When the expected number of outcome was below 10, risk ratios as observed over expected numbers were used instead, with 95% CI from Poisson distributions.

A special study was made of the risk for asthma in children born after IVF compared with other children. Asthma was identified by five or more prescriptions for anti-asthmatic drugs after the age of two years, based on data in the Swedish Prescribed Drug Register (15) from 1 July 2005 up to and including April 2010. Adjustment was made for the following maternal characteristics: year of delivery, maternal age, parity, smoking in early pregnancy and body mass index. Children whose mothers were born abroad or with either parent of non-Swedish citizenship were excluded.

The study was performed within the responsibilities of the National Board of Health and Welfare and therefore no ethical approval from outside ethical committees was required.

## Results

The percentage of ICSI among all IVF increased considerably over time and is now around 50%. The use of cryopreserved embryos also increased to about 10%, and the proportion of single embryo transfers is at present about 70%.

The median age for women having their first child after IVF decreased from 34 to 32 years, while among all women who gave birth it increased from 25 to 28–29 years. The length of the reported involuntary childlessness decreased slightly. Maternal smoking decreased markedly in both groups.

### Pregnancy complications

In our previous study (17), we verified that a number of maternal complications during pregnancy occurred at a higher rate than expected after IVF in comparison with the general

**Table 1.** Available information on IVF pregnancies, obtained from the IVF clinics.

Variable	Years of embryo transfer	
	1982–2001	2002–2006
Maternal identification	Yes	Yes
Date of embryo transfer	Year, month	Date
IVF clinic	Yes	Yes
IVF method	Yes	Yes
Blastocyst transfer	No	Yes
Number of transferred embryos	No	Yes
Number of sacs at early ultrasound	No	Yes
Date of early ultrasound	No	Yes

**Table 2.** Some pregnancy complications after IVF.

Pregnancy complication	First period		Second period	
	Odds ratio	95% CI	Odds ratio	95% CI
Pre-eclampsia	1.63	1.53–1.74	1.31	1.22–1.40
Placental abruption	2.17	1.74–2.72	1.82	1.53–2.17
Placenta previa	3.65	3.15–4.23	4.18	3.64–4.81
Premature rupture of membranes	2.54	2.34–2.76	1.63	1.49–1.79
Cesarean section	2.04	1.97–2.12	1.43	1.37–1.48

Note: Odds ratio with 95% confidence intervals (95% CI) was estimated after adjustment for year of birth, maternal age, parity and smoking in early pregnancy. Estimates for the first (17) and second study periods are compared.

population, namely pre-eclampsia, placental abruption, placenta previa and premature rupture of membranes. There was also an increased rate of cesarean sections. For some of these outcomes, a decline with time was found (2). Table 2 compares the ORs for each condition between the two periods; for all conditions except placenta previa, the OR was lower during the second than during the first period. For placental abruption, the decline may be random ( $z=1.21$ ,  $p=0.19$ ).

There was a sharp decline in multiple births after IVF during the years 2002–2006 (2). In 2002, the rate had gone down to 21%, from a maximum of over 30% in 1991. It continued to decrease, and reached 6% (95% CI 5.1–6.9%) in 2006. During these years, there were only 11 triplet deliveries after IVF.

The decline in the rate of multiple births was a result of the increasing rate of single embryo transfer. Among embryo transfers made in 2002, single embryo transfer represented 25%, in 2003 54%, in 2004 70%, in 2005 74% and in 2006–2007 75%. In only six instances, three embryos had been transferred (2002–2003).

Among 195 pregnancies, two sacs were identified by ultrasound in early pregnancy but only one infant was born; the phenomenon called 'vanishing twin'. They represent 16.5% of the 1 183 pregnancies where two sacs were identified. An unpublished analysis of these cases showed no deviations from other singletons with respect to preterm birth, low birthweight, intrauterine growth retardation or congenital malformations.

Among all twins born in 2001–2007 after IVF, there were 728 pairs with the same sex of both twins and 692 pairs with different sex of the twins. An estimate based on Weinberg's differential rule (18) suggests that 36 twin pairs (95% CI 25–50) were monozygotic; the expected number from the population was 61, adjusted for year of birth and maternal age, risk ratio = 0.60, 95% CI 0.42–0.82. There were thus significantly fewer than expected monozygotic twin pairs, which contrasted with the excess noted in the previous analysis of the years 1982–2001; an adjusted OR of 3.0 (95% CI 1.4–3.5) based on an estimated number of monozygotic twins of 148.

The decline in twinning will directly affect rates of preterm birth, low birthweight and intrauterine growth retardation (SGA), which all declined sharply (2). After embryo transfer during 2002–2006, the average percentage of preterm births (<37 weeks) was 14.5% against the population average of 6.2%. It declined from 23.2% in the 2002 cohort to 10.9% in the 2006 cohort. The average percentage of low-birthweight infants (<2500g) was 11.7, declining from 18.7 to 8.4%, and the population average was 4.3%. The average percentage of SGA infants was 5.1%, declining from 7.3% to a minimum (in 2005) of 3.7%. The population average was 2.3%.

In one study, we compared the outcome among unlike-sexed and therefore dizygotic twins after IVF with that of such twins in the population (3), using the total IVF material from 1982 to 2007. We found evidence for a worse neonatal outcome in the former group, with a significantly higher rate of very preterm births (<32 weeks) and of neonatal jaundice.

### Neonatal characteristics

Among singleton births, changes in rates of preterm birth, low birthweight or SGA were less marked than among all IVF infants. The crude rates had gone down during the whole observation period (2), but there was no further certain decline during 2002–2007. The average preterm rate after IVF was 7.5%, against the population rate of 5.1%; the average low-birthweight rate was 5.3%, against the population rate of 3.1%; and the average SGA rate was 3.3%, against the population rate of 2.1%. For all outcomes, the risks were thus about 50% increased compared with the population. This was to a large extent due to maternal characteristics. If adjustment was made for year of birth, maternal age, parity, smoking, body mass index and number of previous miscarriages, the odds ratio for preterm birth was 1.32 (95% CI 1.22–1.47), for low birthweight 1.34 (95% CI 1.23–1.47), and for SGA 1.16 (95% CI 1.03–1.27). If adjustment was also made for known period of unwanted childlessness as a proxy for subfertility, all odds ratios were close to 1.0; preterm birth 1.01 (95% CI 0.90–1.12), low birthweight 0.98 (95% CI 0.86–1.11), and SGA 0.89 (95% CI 0.77–1.03).

**Table 3.** Odds ratios for neonatal conditions in all infants and in singletons, comparing infants born after IVF with other infants, for the time period 2001–2006.

Neonatal condition	All infants		Singletons	
	Odds ratio	95% CI	Odds ratio	95% CI
Apgar <7 at five minutes	1.27	1.31–1.44	1.04	0.89–1.22
Cerebral bleeding	1.66	1.19–2.33	1.11	0.70–1.77
Neonatal convulsions	1.08	0.74–1.58	1.04	0.68–1.57
Respiratory diagnoses	1.63	1.52–1.77	1.19	1.08–1.31
Mechanical ventilation	2.68	2.03–3.54	1.64	1.10–2.45
Continuous positive airway pressure	2.20	1.90–2.53	1.37	1.11–1.68
Sepsis/pneumonia	1.20	1.01–1.43	1.08	0.89–1.32

Note: Odds ratios were adjusted for year of birth, maternal age, parity, smoking in early pregnancy, body mass index and number of previous miscarriages.

Over the total observation time, a decline could be observed in the rate of cerebral bleeding, convulsions, respiratory diagnoses, use of continuous positive airway pressure or mechanical ventilation and of sepsis/pneumonia (2). To a large extent, this was due to the declining rate of multiple births, and changes among singletons were hardly noticeable.

For infants born after IVF during the second study period, the odds ratios for the various neonatal conditions are shown in Table 3. For all variables except neonatal convulsions, an increased odds ratio was seen when all infants were studied. When the analysis was restricted to singletons, all ORs decreased and only those related to respiratory diagnoses, mechanical ventilation and continuous positive airway pressure remained significant.

There was a weak decline in stillbirth rates during the observation period (2). For the second study period, the OR for stillbirth (after adjustment for year of birth, maternal age, parity, smoking, body mass index and previous miscarriages) was 0.94 (95% CI 0.73–1.20), similar to the adjusted OR for the first period of 1.06 (95% CI 0.85–1.33).

The total risk for early neonatal death declined markedly during the total observation period (2), while the risk for singletons remained relatively unchanged. For the second period, the adjusted OR was 2.22 (95% CI 1.89–3.10) for all infants and 1.79 (95% CI 1.17–2.75) for singletons.

A small risk increase for any congenital malformation was seen after IVF. For the second period, the OR was 1.23 (95% CI 1.14–1.32), and for the first period 1.42 (95% CI 1.32–1.52), but follow-up times differed. There was only a relatively weak decline in the congenital malformation rate during the observation period (2). Some malformations showed a specifically high risk in the first period. For some of them, a risk increase of the same magnitude remained during the second period: any or major cardiovascular defect and limb reduction defects. For others, a risk increase remained, but the risk estimate was significantly lower for the second period than for the first: neural tube defects, cardiac septum defects and esophageal atresia. For a third group of malformations,

a risk was seen during the first but not during the second period: orofacial clefts, small bowel atresia, anal atresia and hypospadias (7).

During the second period, the use of International Classification of Diseases, 10th Edition (ICD-10) made it possible to study cases coded as syndromes (ICD Q87) and compare their occurrence after IVF with that in the population. The risk for a syndrome defined in this way was doubled, but based on only 14 cases. In the total material, seven children were identified with syndromes which at least sometimes are due to imprinting errors: Prader–Willi ( $n=4$ ), Silver–Russell ( $n=2$ ) and Beckwith–Wiedemann ( $n=1$ ) (7).

#### Long-term follow-up

In a previous study (19), we showed that children born after IVF required more hospital care than other children, at least up to the age of eight years. Excess use was found for conditions associated with central nervous system damage, including cerebral palsy, congenital malformations, infections, asthma and accidents. In order to study long-term effects on some specific conditions, we conducted new studies on the following five different outcomes: cerebral palsy (4), attention deficit hyperactivity disorder (5), poor visual acuity (10), asthma and cancer (6). Table 4 summarizes data from the three published studies and adds data on asthma.

A follow-up study of the occurrence of maternal cancer after IVF treatment and delivery compared with other women who had given birth showed an increased risk for most cancer types before the IVF, but a lower risk for breast and cervix cancer (mainly cancer in situ) and a normal rate of most other cancers after IVF (9). The only exception was an increased risk for ovarian cancer, but this was seen already before IVF was performed.

#### Differences in outcome according to IVF method used

Very few differences were found between outcomes after different types of IVF techniques and notably between standard

**Table 4.** Odds ratios at long-term follow-up of children conceived after IVF.

Outcome	Reference	Number	Odds ratios	95% CI
Cerebral palsy	4	138	1.96	1.66–2.31
Attention deficit hyperactivity disorder	5	212	1.18	1.03–1.36
Poor visual acuity	10	25	1.55	1.04–2.32
Asthma	Present study	1388	1.28	1.21–1.35
Cancer	6	53	1.42	1.09–1.87

IVF and ICSI. We could not verify the postulated difference in congenital malformation rates after cryopreserved ICSI compared with fresh ICSI or cryopreserved standard IVF (7). In contrast, we found some evidence for a higher malformation rate and possibly also a higher rate of preterm birth after blastocyst transfer than after early cleavage transfer (8).

## Discussion

It is important to monitor effects of medical interventions. This is usually done with some kind of epidemiological approach. In most instances, no specific effect is expected, but a number of short-term and long-term outcomes are studied in order to identify deviations which could be the result of the intervention. Related to IVF, most concern has been directed to the offspring, but maternal complications and long-term effects of the hormonal treatments, usually given before IVF, have also caused concern. As many outcomes are studied, the problem with multiple testing is important. Findings in one epidemiological study are seldom conclusive, even in the presence of statistical significance, but have to be repeated in separate studies or by continued observation.

Our studies of the outcome after IVF comprise data for 25 years (embryo transfers made from 1982 to 2006). We have divided the material into two periods of different lengths but with approximately the same number of cases. This gives an opportunity for comparisons and studies of change over time. There can be many explanations for a difference in risk for a specific outcome between the periods. One is that the risk has genuinely changed, but one should consider the possibility that the estimated ORs could be randomly high or low. Straightforward statistical tests may not help because of the multiple testing situation. One can use pregnancy complications as an example. A number of pregnancy complications were studied during the first period, and some were statistically significantly increased among women who have had IVF. They may genuinely have been increased or they may have randomly high as a consequence of multiple testing. When these outcomes were studied again during the second period, and a lower OR was found, this may have been due to a decline in the risk or due to a more adequate estimate of a true and unchanged lower risk. Most of these outcomes have shown a decreasing occurrence in the total population, but by using ORs, the IVF pregnancies were compared with

non-IVF pregnancies and therefore such background trends were eliminated.

The majority of outcomes studied have shown a similar pattern, with a decline in risk, such as pre-eclampsia, premature rupture of membranes, cesarean section, preterm birth, low birthweight, SGA, early neonatal death and some congenital malformations. Some have remained with an unchanged risk increase, such as placenta previa and some congenital malformations.

There was, however, a clear-cut and easily understandable decrease in the multiple birth rate as a consequence of the agreement to reduce the number of transferred embryos. A lower rate of multiple births could easily explain the large decline in many neonatal outcomes. The idea that a reduction of the number of multiple births would be beneficial has been challenged (20,21). One argument has been that IVF twins had a better outcome than ordinary twins. This statement was, to a large extent, based on comparisons between IVF twins (who are mainly dizygotic) with other twins (a large proportion being monozygotic). We compared unlike-sex twin pairs (who are dizygotic) after IVF with such twins conceived spontaneously and found evidence that the former had more neonatal problems than the latter (3). The strong decline of the twinning rate while the neonatal outcome became markedly better supports the opinion that single embryo transfer is beneficial.

Among singleton infants, relatively small changes occurred, and these changes were more difficult to evaluate. If genuine, they could be the result of a change in case mix, for instance women with less pronounced subfertility (decreasing period of unwanted childlessness) problems were included during the second period compared with the first period (2). Indications for IVF were not registered in the present study. Mounting evidence supports the idea that a major cause of the problems lies in parental and notably maternal subfertility, which was illustrated in the present study of preterm birth, low birthweight and SGA, where the risks disappeared after adjustment for years of unwanted childlessness as a proxy for subfertility. Other possibilities were advances in IVF methodology or obstetric treatment of women after IVF. During the observation period, major changes in IVF technology had taken place, most notably by the introduction and spreading of ICSI techniques. Our analyses so far had not identified any major differences between standard

IVF and ICSI with respect to delivery outcome, so that was probably not an explanation for the noted effects. The only possible effect of a technical modification was the indicated increased risks seen after blastocyst transfer (9), but these findings are preliminary and motivate further studies.

The decline in preterm birth rates will affect neonatal morbidity, which is strongly linked to prematurity. Among the neonatal diagnoses studied during the second period, only respiratory problems were increased among singletons. Other neonatal morbidity appeared to be linked to twinning, and a further reduction of multiple births will most likely diminish the total risk further. The risk for early neonatal death remains elevated also among singletons.

Some specific outcomes are worth further comments. Our data for the whole study period and for the two periods separately indicated no signs of an increased risk for intrauterine death in pregnancies after IVF. This is in contrast to the recent study from Denmark (22), where a marked increase in stillbirth risk was claimed. We cannot explain the discrepancy between the two studies, but it seems unlikely that the IVF technique by itself increases the risk for stillbirth.

Monozygotic twinning has repeatedly been described to occur at an increased rate after IVF (23–27), and this was found in data from the first study period (28). In the data from the second study period, no indications of an increased monozygotic twinning rate were found. There has been some discussion on the cause of monozygosity after IVF, and zona manipulation has been suggested (24), but in other studies this could not be verified (such as 25,27). The decline in monozygosity rate in our material is unexplained. It is of some interest that it has occurred in parallel with the decline in the risk for certain congenital malformations which are thought to be linked to the origin of monozygotic twinning, such as spina bifida, esophageal, small gut and anal atresia (29). In a recent study (30), a specific increase after IVF was described in a group of malformations called ‘blastogenesis birth defects’, which contains most of the above-mentioned conditions and includes monozygotic twins. Whatever the cause of monozygosity after IVF may be, it may have declined in strength at the same time as the rate of dizygotic twinning declined.

Many of the neonatal morbidities found after IVF increased the risk for early neonatal death, but also for late sequelae. One would therefore expect long-term effects on the development of children born after IVF. This was also seen. An increased risk of cerebral palsy exists, but was strongly linked to multiple births. In our analysis (4), we found some evidence that the risk for cerebral palsy declined when the rate of multiple births went down, but it is still too early to draw firm conclusions. A moderate increase in the risk of attention deficit hyperactivity disorder was noted (5), but this condition is difficult to study epidemiologically, and an important component in its etiology is genetic. Twinning seemed not

to increase the attention deficit hyperactivity disorder risk and, if the risk is present at all, it may remain basically unchanged. Poor visual acuity was also demonstrated, mainly a result of cerebral damage (10). A much more common effect was that on asthma, where many perinatal factors have been shown to act as risk factors, including cesarean section. Finally, a weak increase in the cancer risk among children after IVF was identified (6). Possible intermediary factors could be preterm birth and neonatal asphyxia. All outcomes except for asthma are of low prevalence, and a moderate risk increase still represents only a low absolute risk for a child born after IVF.

A concern has repeatedly been expressed that the hormone treatments associated with IVF could increase the risk for maternal cancer. We found no evidence for such an effect (9). It is true that the risk for ovarian cancer was higher after IVF than among the control women, but even before the IVF, an increased risk for ovarian cancer was seen. This indicates an association between ovarian pathology, leading both to infertility and the need for IVF, and to an increased risk for ovarian cancer. The hormonal treatments taking place at IVF did not seem to increase cancer risk, at least not in premenopausal women. The lower risks for breast and cervical cancers are difficult to explain, but may be a result of more intensive screening and therefore identification and treatment of pre-cancerous conditions, or to less use of oral contraceptives.

## Conclusions

The 25 year survey of delivery outcome after IVF in Sweden verified the presence of a number of deviations, but also that most of them have declined in rate, mainly as a result of the lower rate of multiple births after the agreement to reduce the number of eggs transferred. Other factors may have contributed, such as changes in patient mix or IVF methodology. It is yet unclear whether there will also be a positive effect on long-term sequelae. We found little evidence for a difference in outcome between standard IVF and ICSI or between fresh and cryopreserved embryos used for transfer.

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# Natural versus induced twinning and pregnancy outcome: a Dutch nationwide survey of primiparous dizygotic twin deliveries

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**Objective:** To compare obstetrical outcome of twin pregnancies after assisted reproduction with that of natural twin pregnancies.

**Design:** Retrospective national database study.

**Setting:** Academic Medical Centre.

**Patient(s):** One thousand ninety-three primiparous mothers registered in the Dutch National Birth Registry who gave birth to a dizygotic (DZ) twin (male/female) in 1994, 1995, and 1996. We compared 613 natural twin pregnancies and 480 twin pregnancies born after assisted reproduction.

**Main Outcome Measure(s):** Gestational length, mode of delivery, mode of presentation of the children, birth weight, APGAR score, congenital anomalies, perinatal mortality rate, highest recorded maternal diastolic blood pressure, and maternal postpartum complications.

**Result(s):** Rates of perinatal mortality and very premature parturition (<29 weeks) were lower in natural twin mothers. Overall, induced DZ twins were born 3.5 days earlier with a lower birth weight and APGAR score compared with controls. Rates of congenital anomalies and incidence of cesarean section were not different. The highest recorded diastolic blood pressure was lower in induced twinning with a 30% lower incidence of diastolic blood pressure >90 mm Hg.

**Conclusion(s):** Obstetric outcome for induced DZ twin pregnancy is less optimal than in natural DZ twin pregnancy. Twinning in assisted reproduction is known for its contribution to the high rate of premature deliveries, but in addition being a subfertile patient undergoing treatment makes an intrinsic contribution to adverse events as well. (*Fertil Steril*® 2001;75:731–6. ©2001 by American Society for Reproductive Medicine.)

**Key Words:** Twinning, IVF, pregnancy, blood pressure, perinatal mortality, preterm delivery, low birth weight, ovulation induction

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Following the worldwide introduction of in vitro fertilization (IVF), there was immediate concern about pregnancy complications and adverse effects on the fetus. Over the past several years, a number of studies were conducted addressing this issue, and in general it can be said that subfertile patients show an increased rate of preterm delivery, have children with lower birth weight, and experience a higher rate of perinatal mortality even after adjusting for age, parity, and multiplicity (1–11).

Negative effects on obstetrical outcome in case of artificially induced pregnancy are pre-

dominantly related to multiplicity. Most reported studies in this field combine singleton and multiple pregnancies. Furthermore, it seems that the subfertile status itself, and not the hormonal or semisurgical treatments of infertility, is a negative determinant (3, 12, 13). To further substantiate these findings, it would be advantageous to selectively compare twin pregnancies and thus avoid the abundant influence of twinning itself.

More adverse outcomes should be seen in cases of induced twinning compared with natural twinning if they are intrinsic to subfertile status and/or subfertility-related procedures.

TABLE 1

Maternal age at time of delivery.

	Natural twin pregnancy (n = 613)	Twin pregnancy after ovulation induction (n = 176)	Twin pregnancy after IVF (n = 304)	All induced twin pregnancy: IVF + ovulation induction (n = 480)
Mean ( $\pm$ SD) age, y	29.2 $\pm$ 4.1	29.3 $\pm$ 4.1	32.3 $\mp$ 3.9(S)	31.2 $\pm$ 4.2

Note: S = significant different from natural twins and twins after ovulation induction ( $P < .00001$ ).

IVF = in vitro fertilization.

Lambalk. Natural vs. induced twin pregnancy. *Fertil Steril* 2001.

Those studies comparing pregnancy outcome in natural versus induced multiple pregnancies report various results. Some describe an increase of perinatal mortality (2), higher rates of prematurity (2, 5), and lower birth weight (2, 14), while others conclude that there are no differences (4, 8, 15, 16). Lower risks of adverse outcome in induced twinning have even been described (17).

A number of factors may have contributed to these contradictory findings. Apart from considerable variation in methodology, most studies were single centered and no consistent corrections were made for maternal age, parity, and, most importantly, type of twin (monozygotic or dizygotic).

Most twins born after artificial reproductive assistance are dizygotic (DZ). Therefore, comparisons for obstetric outcome should only be made with natural DZ twin pregnancies. Usually, when this was taken into consideration, numbers of pregnancies became too low to provide good statistical power.

Twinning is a very important feature of artificial reproductive assistance. Therefore, we need to firmly establish whether a twin pregnancy in the subfertile patient has additional obstetrical risks besides those established for the singleton pregnancy (3).

We therefore conducted a nationwide comparison of obstetric outcome in all 1,093 opposite sex natural or induced twin pregnancies born from primiparous women in the Netherlands in the period between 1994 and 1996.

## MATERIAL AND METHODS

### Database

Data were obtained from the Dutch Nationwide Obstetric Registration over the years 1994–1996. This registry covers more than 98% of all hospital deliveries in the Netherlands that occurred after more than 15 weeks of gestation. Although home delivery is very common, all twins are delivered in the hospital. Therefore, the data on twin pregnancies and deliveries in this registry concern all twin deliveries in the Netherlands during the study period. For the purpose of this study, we extracted information only from male/female

twin pregnancies to ensure dizygosity. Furthermore, we only used data of primi-parae.

From the registry we extracted information on whether the twin pregnancy had occurred naturally, after IVF, or after other forms of ovulation induction. The total number of pregnancies available for the analysis was 1,093, of which 613 were natural twin pregnancies and 480 were reported to have occurred after ovulation induction (either for IVF,  $n = 304$ , or otherwise,  $n = 176$ ). Furthermore, we extracted data on maternal age at time of delivery; highest measured diastolic blood pressure registered anywhere during the pregnancy; duration of pregnancy (weeks); mode of initiation of delivery; mode of presentation and mode of delivery per child; birth weight, sex, and APGAR score and following order of the individual children; perinatal mortality; and presence of all reported congenital anomalies and presence of maternal postpartum complications (infection, hemorrhage, tromboembolism, or brain injury).

The study was approved by the Ethical Board of the Dutch Nationwide Obstetric Registration.

### Statistical Analysis

The data were analyzed with the BMDP statistical package (BMDP, Cork, Ireland). Means of two groups were compared with Student's  $t$ -test, and means of more than two groups with one-way analysis of variance. Proportions in two or more groups were compared with  $\chi^2$  analysis. The results are presented as odds ratios with 95% confidence intervals (CIs). As maternal age differed significantly between the assisted reproduction technique (ART) and the natural DZ twinning group, all odds ratios were adjusted for maternal age using logistic regression analysis. Continuous data were adjusted for differences in maternal age with analysis of covariance.

## RESULTS

### Maternal Parameters

The mean age of the women who had a twin after IVF treatment was significantly higher than that of women who had received other hormonal treatment and those who had natural twins (Table 1).

TABLE 2

Number of mothers with a highest diastolic blood pressure > 90 mm Hg registered at any time during pregnancy.

	No. of mothers (%)	Odds <sup>a</sup> ratio	95% CI
Natural twin pregnancy	221 (36.6)		
Twin pregnancy after ovulation induction	64 (36.8)	1.04 (NS)	0.73–1.47
Twin pregnancy after IVF	87 (28.8)	0.68 (S)	0.49–0.95

Note: <sup>a</sup> Adjusted for age.

S = significant; NS = nonsignificant.

IVF = in vitro fertilization.

Lambalk. Natural vs. induced twin pregnancy. *Fertil Steril* 2001.

The mean of the highest diastolic blood pressure recorded anywhere during pregnancy was  $84.8 \pm 12.3$  mm Hg in the natural twins and slightly lower in the induced twins ( $83.2 \pm 12.3$  mm Hg;  $P < .05$ ).

In particular, in the IVF-induced twin group there was a significant 30% lower number of twin pregnancies with the highest diastolic blood pressure >90 mm Hg (Table 2).

There was no maternal mortality in any of the studied pregnancies, and there were no significant differences in incidence of maternal obstetric complications (data not shown).

### Pregnancy and Delivery Parameters

The duration of the pregnancy in the natural twin was 0.5 weeks longer than in the induced twins ( $P < .02$ ), and there was a significant increase in the incidence of very premature or immature parturition in the latter group (<29 weeks). However, there was no difference in the number of pregnancies that went for less than 26 weeks of gestation (Table 3).

The incidence of secondary cesarean section was slightly higher in the case of an induced twin pregnancy (odds ratio 1.4; 95% CI [1.02–1.93]), but statistical significance disappeared after correction for maternal age (Table 4). There

were no differences with respect to rates of primary cesarean section, induction of labor, operative delivery, and mode of cephalic presentation of the first and the second child (Table 4).

### Neonatal Parameters

Birth weight and APGAR score were slightly but significantly higher in the natural twins. Overall perinatal mortality was 1.5 times higher in the induced twins. When only deliveries at >29 weeks were considered, no significance in perinatal mortality was seen (Table 5).

A statistically significant increased risk of congenital anomalies of 1.67 [1.0–2.9] was seen for the induced twin pregnancy, which disappeared after correction of maternal age.

## DISCUSSION

We show that obstetric outcome for primiparous mothers of induced DZ twins is slightly worse than in primiparous mothers with a natural DZ twin pregnancy. Overall, the differences are modest. However, our data corroborate, in particular, the worrisome finding of an increase in perinatal mortality rate as described by Moise et al. (2). That study indicates that in assisted reproduction, multiple pregnancies, aside from their intrinsic contribution to adverse obstetrical outcome, also maintain the additional risks of adverse outcomes as observed in the singletons, which are related to some still unknown particular negative feature of the sub-fertile patient.

In our study, the very premature parturition contributed strongly to the observed differences in neonatal outcome and in particular to the higher mortality rate. This is in line with similar observations in singletons (1–11). We found no difference in mortality rate when only pregnancies beyond 29 weeks of gestation were taken into consideration. Obstetric intervention is often put forward as one important factor contributing to higher rates of premature birth in “precious” ART pregnancies. We found only slightly higher rates of

TABLE 3

Duration of pregnancy.

	Natural twin pregnancy (n = 613)	Induced twin pregnancy (n = 480)	Odds <sup>a</sup> ratio	95% CI	P
Mean duration $\pm$ SD (weeks)	$35.4 \pm 3.8$	$34.9 \pm 4.0$	—	—	<.02 (S)
No. of twin pregnancies with duration of <26 weeks	21 (3%)	22 (5%)	1.36 (NS)	0.73–2.47	
No. of twin pregnancies with duration of <29 weeks	29 (5%)	31 (6%)	1.37 (S)	1.0–1.94	

Note: <sup>a</sup> Adjusted for age. S = significant; NS = not significant.

Lambalk. Natural vs. induced twin pregnancy. *Fertil Steril* 2001.

TABLE 4

Parameters for mode of presentation of the children and mode of delivery.

	No. of natural twin pregnancies, % (n = 613)	No. of induced twin pregnancies, % (n = 480)	Odds <sup>a</sup> ratio	95% CI
Cephalic presentation, first child	458 (75)	370 (77)	1.17 (NS)	0.87–1.56
Cephalic presentation, second child	398 (64)	290 (60)	0.82 (NS)	0.62–1.05
Induction of labor	204 (33)	152 (32)	1.1 (NS)	0.83–1.39
Operative vaginal delivery	125 (20)	74 (15)	0.88 (NS)	0.67–1.25
Primary cesarean section	94 (15)	83 (17)	1.06 (NS)	0.75–1.49
Secondary cesarean section	98 (16)	98 (20)	1.18 (NS)	0.85–1.64

Note: <sup>a</sup> Adjusted for age.

S = significant; NS = not significant.

Lambalk. Natural vs. induced twin pregnancy. *Fertil Steril* 2001.

secondary cesarean sections, which, according to our analysis, is likely to be related to the higher maternal age. Moreover, there were no differences in rates of induction of parturition between the two groups, which further substantiates that obstetrical interference is not involved.

So far, there does not seem to be a satisfactory explanation for the consequent observation of impaired obstetrical outcome of pregnancies after ART. Hormonal and technical manipulation of ovaries, follicles, oocytes, sperm, and embryos may exert some influence, but the condition of subfertility per se has been shown to be crucial. Obstetric outcome of subfertile patients who become pregnant naturally while waiting for treatment is impaired for controls the same as it is for ART-treated patients (12). Suggested explanations for the association between subfertility and impaired obstetric outcome are cigarette smoking, pelvic infections, prenatal exposure to diethylstilboestrol, occupational exposure to solvents and pesticides, folate deficiency, and stress (18).

It is plausible that in the subfertile patients some comorbidity is present such as thyroid disease or autoimmune or

antiphospholipid diseases, which lead to preterm delivery and a higher rate of perinatal mortality. Unfortunately, the information in the database regarding such pathology was limited and did not allow further evaluation.

An entirely alternative explanation is a genetically determined link between optimal obstetric performance and fertility, particularly in twinning. In other words, induced twin mothers do not perform worse but natural twin mothers may actually perform better. In the human, perinatal and maternal morbidity and mortality are higher in twin pregnancies compared with singleton pregnancies (19). According to Darwinian principles of survival of the fittest, this disadvantage of twinning should contribute to a decrease in the proportion of women carrying the hereditary trait of DZ twinning. However, heredity remains one of the main factors contributing to DZ twinning (20). The reason why hereditary DZ twinning is still very frequent in humans despite the assumed reproductive costs of maternity is that overall reproductive success is equal or even better than in singletons (21).

If we assume that a substantial number of natural twin mothers have genetic etiology for their twinning and that this

TABLE 5

Neonatal outcome.

	Natural twins (n = 1226)	Induced twins (n = 960)	Odds <sup>a</sup> ratio	95% CI	P
Mean ( $\pm$ SD) birth weight, g	2,319 $\pm$ 663	2,250 $\pm$ 686			<.02 (S)
Mean ( $\pm$ SD) APGAR score	8.8 $\pm$ 2.0	8.6 $\pm$ 2.3			<.05 (S)
No. of perinatal deaths	58 (5%)	63 (7%)	1.47 (S)	1.01–2.15	
No. of perinatal deaths with birth at >29 weeks	18 of 1,158 twins (2%)	16 of 884 twins (2%)	0.97 (NS)	0.48–1.95	
Birth defects	24 (2%)	31 (3%)	1.54 (NS)	0.88–2.69	

Note: <sup>a</sup> Adjusted for age.

S = significant; NS = not significant.

Lambalk. Natural vs. induced twin pregnancy. *Fertil Steril* 2001.

is not the case among the induced twin mothers, then our findings would support the hypothesis that along with a hereditary trait of twinning, which causes higher gonadotropin serum levels, multiple follicle growth, and multiple ovulation (22–25), some other coinherited factors may favor obstetric outcome.

The ideal study to test this hypothesis would have been an age-controlled comparison of the obstetrical outcome in natural primiparous DZ twin mothers with and without a family history of DZ twinning. Unfortunately, this information was not collected in the database we used and we therefore could not carry out this evaluation.

There were some other limitations in the use of this database that also need to be addressed. No reliable information is available on certain factors that may be confounding, such as type of infertility present, socioeconomic status, ethnic and geographical distribution, and type of prenatal care (either second or third referral hospital). Absence of this information is mainly the result of privacy protective measures in the data handling process of the registry.

Remarkably, we found that mothers of induced twins had significantly lower blood diastolic pressure, and in the IVF group a significantly lower number had highest diastolic blood pressure over 90 mm Hg. This implies that hypertensive disorders such as preeclampsia apparently are less frequent in the induced twin pregnancy. In fact, similar findings were reported on singletons in a large study on obstetric outcome of IVF pregnancies: the French in Vitro National Study (1). This study described 5% preeclampsia in IVF singleton pregnancies compared with 8% in the general French population. These observations could mean that some protection comes along with artificially induced pregnancies against one of the greatest threats in pregnancy, namely, hypertensive disorders. In our study, differences in parity and maternal age could not have been an influence because it only concerned primiparous women and age was actually highest in the mothers with induced twins. Explaining this unexpected finding is challenging. Over the past several years, a number of studies have reported that immunological adaptation between couples mediated via semen compounds is strongly related to a lower chance of having hypertensive disorders in pregnancy (26, 27). We would like to put forward the possibility that in pregnancies of subfertile couples a longer sexual relationship effectuated this immunological adaptation to a greater extent than in couples experiencing their first natural pregnancy.

Finally, we found a nonsignificant, slightly higher rate of congenital malformations in the induced twins, which seems to be related to the higher maternal age in this group. In a recent study from Sweden it appeared that malformations occurred in 5.4% of singletons born after IVF with a risk ratio of 1.25 [1.07–1.46], while there was no increased risk in multiple pregnancy babies (1.08 [0.93–1.25]) (3). It also appeared from this study that risks of malformations of the

central nervous system were largely confined to the multiple pregnancies, which is in agreement with findings in earlier population-based studies (28). This is possibly related to the lesser likelihood of termination of a multiple pregnancy with one child with a congenital anomaly (29).

These data and ours point to the relatively low additional risk from ART per se with respect to occurrence of congenital malformations in multiple pregnancy (1).

In summary, twin pregnancies after assisted reproduction have a higher rate of perinatal mortality and lower birth weight as result of a higher rate of premature parturition. Twinning in assisted reproduction evidently contributes to the high rate of premature deliveries, but aside from that, additional risks, intrinsic to assisted reproduction, remain to the same extent as with singleton pregnancies. We conclude that still more effort needs to be put into designing new assisted reproduction protocols that prevent twinning in females that normally only would bear singletons. Single embryo transfer seems an obvious approach. There are now a number of reports that show very high ongoing pregnancies with this procedure (30–31).

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# NEONATAL OUTCOME OF INFANTS BORN AFTER *IN VITRO* FERTILIZATION AT NATIONAL TAIWAN UNIVERSITY HOSPITAL

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**Background and Purpose:** This study compared the neonatal outcome between infants born after *in vitro* fertilization (IVF) and after natural conception at National Taiwan University Hospital.

**Methods:** All medical records of women who underwent IVF and gave birth at our hospital from January 1995 to December 1996 were reviewed. The charts of their offspring were also reviewed. We compared the neonatal outcome of infants born after IVF with that of infants born after natural conception. Neonatal outcome was evaluated based on preterm birth, very low birth weight (VLBW), perinatal morbidity, and neonatal mortality.

**Results:** A total of 75 women underwent IVF and gave birth to a total of 100 live newborns and two fetuses with intrauterine death during the 2-year study period. Among these newborns, the prevalence of preterm birth was 28%, of perinatal morbidity was 17%, and of neonatal mortality was 3%, which were significantly higher than those among the 7,736 neonates born after natural conception. However, the rate of VLBW was similar between the two groups. The rate of preterm birth for twin pregnancies was higher than that for singleton pregnancies in both groups.

**Conclusion:** This study showed that infants born after IVF had a higher risk of preterm birth and higher perinatal morbidity and neonatal mortality.

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**Key words:**  
*in vitro* fertilization  
preterm birth  
very low birth weight

Since the first successful birth after *in vitro* fertilization and embryo transfer (IVF/ET) reported in 1978 [1], the technique has been used widely and a great number of successful pregnancies have been achieved [2]. This technique brings hope for infertile couples who would like to have their own child. However, the obstetric, perinatal, and neonatal outcomes of IVF pregnancies are always the major concern for both physicians and parents. Previous studies of IVF pregnancies found discrepant outcomes [3-6]. IVF pregnancies may lead to higher frequencies of preterm birth, low birth weight, small size for gestational age, and perinatal mortality compared with pregnancies from natural conception [4, 5]. The increase in preterm birth may be due to the high rate of multiple pregnancies with IVF [4]. However, later studies revealed that the obstetric and perinatal outcome of IVF

pregnancies seemed not dissimilar from those of natural pregnancies [6].

IVF was introduced in Taiwan in 1985 [7]. Its use has increased since then, and has produced many newborns each year in Taiwan. While both physicians and parents are eager to learn about the prognosis of these newborns, reports about neonatal outcome of IVF pregnancies are still lacking in Taiwan. This study compared the neonatal outcome between infants conceived by IVF and by natural conception.

## Patients and Methods

All medical records of women who underwent IVF and gave birth at National Taiwan University Hospital

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(NTUH) from January 1995 through December 1996 were reviewed. The charts of their offspring were also reviewed for data on gestational age, birth weight, perinatal morbidity, and neonatal mortality. Preterm birth was defined as birth before 37 weeks' gestational age, and very low birth weight (VLBW) was defined as birth weight of less than 1,500 g. Perinatal morbidity was defined as admission to the neonatal intensive care unit (NICU) immediately after birth except for those infants admitted purely due to VLBW.

If more than two fetuses were noted in IVF pregnancies, fetal reduction to not more than two fetuses was performed after informed consent was obtained from parents.

The total number of infants who were born as a result of natural conception during the 2-year period was also recorded. The incidence of preterm birth, VLBW, perinatal morbidity, and neonatal mortality were compared between the IVF and natural conception groups. Chi-square test was used to evaluate the difference between these two groups and a *p* value of less than 0.05 was defined as significant.

## Results

Seventy-five women underwent IVF and delivered a child at NTUH during the 2-year period, including 100 live babies and two fetuses with intrauterine death. Among them, 50 infants were born to 25 mothers (33%) with twin pregnancies. Due to the policy of fetal reduction for pregnancies with more than two fetuses, there were no triplets or higher-order births in our IVF group. Eleven mothers (14.7%) underwent the fetal reduction procedure in the second trimester. During the study period, 7,736 infants were born from natural conception.

Neonatal outcome was compared between IVF and natural conceptions (Table 1). Nineteen women

(25.3%) in the IVF group delivered prematurely. The prevalence of preterm birth in the IVF group (28%) was higher than that in the natural conception group (4%). In the IVF group, preterm birth was more prevalent in twin than in singleton pregnancies (*p* = 0.05). However, the prevalences of VLBW, morbidity, and mortality were similar between singleton and twin pregnancies in the IVF group (Table 2). The rate of VLBW in the IVF group was similar to that in the natural conception group. More infants in the IVF group were admitted to the NICU immediately after birth than in the natural conception group. After exclusion of those infants admitted to the NICU purely due to VLBW, infants in the IVF group were still admitted to the NICU more than those in the natural conception group (*p* < 0.01). The causes of admission to the NICU immediately after birth included respiratory distress (7 cases), VLBW (6), severe anemia (1), ileus (1), imperforate anus (1), and severe asphyxia (1). The seven infants with respiratory distress recovered within 2 days although no definite etiology of the condition was identified.

Two infants with congenital anomaly were born in the IVF group (2%), including one with Down syndrome and one with imperforate anus.

The mortality rate in the IVF group was significantly higher than that in the natural conception group. Two infants in the IVF group died of extremely low birth weight and one infant died of ileus with acute renal failure. However, among those infants admitted to the NICU, the mortality rates were similar for those conceived by IVF and natural conception (*p* = 0.74).

## Discussion

The prevalence of preterm birth in this series of IVF pregnancies (28%) was similar to those in previous reports (25–30%) [4, 8]. Our study found a higher

**Table 1.** Comparison of neonatal outcome between infants in the *in vitro* fertilization (IVF) and natural conception groups

	IVF, n (%) (n = 100)	Natural conception, n (%) (n = 7,736)	<i>P</i>
Preterm birth	28 (28.0)	309 (4.0)	< 0.01
VLBW	6 (6.0)	325 (4.2)	0.70
Perinatal morbidity			
VLBW included	17 (17.0)	451 (5.8)	< 0.01
VLBW excluded*	11 (11.7)	126 (1.7)	< 0.01
Neonatal mortality			
Total	3 (3.0)	49 (0.6)	< 0.01
NICU only†	3 (17.7)	49 (10.9)	0.74

\*Excluding infants admitted to neonatal intensive care unit (NICU) purely due to very low birth weight (VLBW); †calculation according to the population admitted to NICU.

**Table 2.** Comparison of the neonatal outcome between twin and singleton pregnancies in the *in vitro* fertilization (IVF) group

	Twin n (%)	Singleton n (%)	<i>p</i>
Preterm birth	9 (36)	10 (20)	0.05
VLBW	3 (6)	3 (6)	1
Morbidity	8 (16)	9 (18)	0.70
Mortality	2 (4)	1 (2)	0.80

VLBW = very low birth weight.

prevalence of preterm birth but no difference in the incidence of VLBW, morbidity, or mortality in twin pregnancies compared to singleton pregnancies in the IVF group, which is compatible with the findings of Wennerholm et al that the high rate of preterm birth may be due to multiple pregnancies [6]. However, in addition to multiple pregnancies, the underlying cause of infertility and higher maternal age may also play important roles in preterm birth [9]. The rate of twin pregnancies in our IVF group was similar to previous reports [2, 10]. Owing to the policy of fetal reduction in our hospital, the rate of VLBW infants in the IVF group was similar to that in the natural conception group, and there were no triplets or higher-order births. These results are different from a previous study [4], and suggest that fetal reduction to two fetuses or less may promote mothers to bear slightly larger babies despite a higher incidence of preterm birth [11].

Because the NICU mortality rate was similar between the two groups, the severity of illness requiring admission to the NICU might have been comparable between the two groups. Perinatal morbidity was higher in IVF infants than in natural conception infants, even after exclusion of those infants admitted to the NICU purely due to VLBW, which is compatible with a previous report [6]. Respiratory distress was the major cause of the need for neonatal intensive care in our series. Further population study is needed to determine the reason for this finding.

Similar to the findings of Wennerholm et al [4], the neonatal mortality rate in this study was higher in the IVF group than in the natural conception group. The most common cause of death was extremely low birth weight (2/3). This finding indicates that prevention of extreme preterm birth is the most important factor to decrease the rate of neonatal death in infants born after IVF pregnancies.

In conclusion, infants born after IVF pregnancies were at increased risk of preterm birth, perinatal morbidity, and neonatal mortality in our study. Multiple pregnancies made an important contribution to preterm birth. Fetal reduction to two fetuses or less may decrease the rate of VLBW infants. Determination of the most effective methods to prevent preterm birth and to decrease morbidity and mortality will require further study.

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# Outcomes From Assisted Reproductive Technology

Bradley J. Van Voorhis, MD

The use of assisted reproductive technology (ART) for treating the infertile couple is increasing in the United States. The purpose of this paper is to review the short-term outcomes after ART. Pregnancy rates after ART have shown nearly continuous improvement in the years since its inception. A number of factors affect the pregnancy rate, with the most important being a woman's age. Certain clinical diagnoses are associated with a poorer outcome from ART, including the presence of hydrosalpinges, uterine leiomyomata that distort the endometrial cavity, and decreased ovarian reserve. Multiple gestations are the major complication after ART. New laboratory techniques, including extended embryo culture, may allow the transfer of fewer embryos to maintain pregnancy rates while reducing the risk of multiple gestations. Although much of the morbidity in children born after ART is the result of multiples, recent analysis suggests that even singletons are at higher risk for perinatal morbidity, including preterm delivery and small for gestational age infants. In vitro fertilization may be associated with a slight increased risk for birth defects. The major short-term complication of ART in women is the development of ovarian hyperstimulation syndrome. This syndrome is difficult to predict, but new treatments are being developed that may limit its frequency. Because of its high pregnancy rate, couples are moving to ART more quickly in the management of their infertility. All outcomes of ART, including pregnancy rates and adverse complications, need to be compared with standard non-ART therapy when deciding the appropriate course of treatment for a given couple.

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It would be difficult to name a field of medicine that has undergone more rapid and profound advancement over the past 50 years than the field of infertility treatment. The use of human gonadotropins and antiestrogens for induction of ovulation was first reported in the late 1950s,<sup>1,2</sup> paving the way for the first medical treatment of anovulatory infertility. Perhaps the most remarkable feat in our field, the birth of the first child from in vitro fertilization (IVF), occurred just over 25 years ago.<sup>3</sup> Although originally developed for women with tubal factor infertility, IVF

is now used for all causes of infertility, and use has grown to the point that nearly 1% of babies born in the United States are now conceived by IVF.

Because of intense research efforts, pregnancy rates with assisted reproductive technology (ART) have shown continuous improvement. However, in recent years, outcomes other than live birth rates have become an important focus of investigation. Problems including multiple gestations, ovarian hyperstimulation, and the health of children born from these techniques have gained new prominence as researchers are now evaluating the health consequences of these procedures. The focus of this review is to evaluate the short-term outcomes from ART, including expected pregnancy rates and complications. Clinical factors that may affect the outcomes of ART will be discussed. Finally, the appropriate implementation of ART procedures within the treatment strategy of infertile couples will be examined.

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## OUTCOMES OF ART: PREGNANCY RATES

### Treatment-Independent Pregnancy Rates

Although commonly referred to as infertile, many couples seeking treatments are actually subfertile and capable of conception without treatment. Often, the difference between undergoing infertility treatment or not is conceiving sooner rather than later.<sup>4</sup> Therefore, the background fertility rate in subfertile couples is important for counseling and for weighing the risks and benefits of a proposed treatment. The treatment-independent pregnancy rate varies depending upon the population studied. In a primary care population of infertile patients, a treatment-independent cumulative pregnancy rate of 27.4% after 12 months was found.<sup>5</sup> In a population of infertile patients referred to specialists for non-ART infertility evaluation and treatments, a 12.3% cumulative pregnancy rate after 12 months was reported.<sup>6</sup> Finally, in couples referred for IVF, a 12-month cumulative pregnancy rate of 2–6% was determined.<sup>7</sup>

Factors that have been found to negatively affect the treatment-independent pregnancy rate include the presence of tubal disease, anovulation, male factor infertility, and endometriosis.<sup>6</sup> Factors associated with a higher spontaneous pregnancy rate include history of a previous pregnancy, a duration of infertility of less than 24 months, and female age less than 30 years.<sup>5</sup> Thus, it can be seen that the expectations for a given couple will vary depending on the “severity” of the infertility problem. Couples in which the woman is relatively younger or who have a shorter duration of infertility will have a significantly better chance of spontaneous conception than couples with a long history of infertility, previous infertility treatment cycles, and certain infertility diagnoses mentioned above. After a complete infertility evaluation, couples, who often believe there is no hope for spontaneous conception, should be advised regarding their prognosis for pregnancy without treatment (often in the range of 1–3% per month for unexplained infertility).

### Definitions and Pregnancy Rates

Assisted reproductive technology includes all fertility treatments in which both eggs and sperm are handled in vitro. Assisted reproductive technology procedures typically involve stimulating the growth of multiple ovarian follicles, surgically removing eggs from a woman’s ovaries, and then combining them with sperm in the laboratory. With gamete intrafallopian transfer (GIFT), a laparoscopy is performed, and sperm and unfertilized eggs are placed into the fallopian tube immediately after egg retrieval. With all

other ART procedures, eggs are fertilized in vitro, either by culturing eggs with sperm or by injecting a single sperm into the egg by a process known as intracytoplasmic sperm injection (ICSI). Zygote intrafallopian transfer (ZIFT) is the laparoscopic transfer of fertilized eggs (zygotes) to the fallopian tube. The large majority of ART cycles are IVF cycles, performed by culturing embryos for variable numbers of days before transferring the embryos through the cervix to the uterus. Assisted reproductive technology includes the use of donor eggs and cryopreserved embryos.

Although previously very intuitive to clinicians treating infertile couples, the fact that IVF is more effective than awaiting spontaneous conception is now evidence-based.<sup>8</sup> In a Canadian prospective multicenter trial, couples were randomized to immediate IVF or 3 months without treatment. Study entrance criterion ensured that only couples with a relatively good prognosis (female age < 40 years, open fallopian tubes, motile sperm in the ejaculate) were included. Of 71 couples randomized to expectant management, only 1 couple conceived a twin gestation in the 3 months of waiting. In contrast, of the 68 couples randomized to IVF, 29 couples conceived an ongoing pregnancy resulting in a live birth. Thus, this trial demonstrated a 21-fold increase in the live birth rate after one IVF cycle, compared with 3 months of nontreatment.

In 1992, Congress passed the Fertility Clinical Success Rate and Certification Act requiring all clinics performing ART in the United States to annually report success rates to the Centers for Disease Control and Prevention (CDC). Data accuracy is verified by medical directors at individual clinics and validated by committee members performing site visits. This reporting system serves as a rich source of outcomes from ART in the United States. Although the outcome data are not corrected for all possible confounding variables, because of the large size and comprehensive nature of the report, important insights into outcomes of ART can be gleaned from the composite statistics.

The most recent data available on the CDC Web site (<http://www.cdc.gov/reproductivehealth/ART02/index.htm>) is from the year 2002, the delay being necessary to collect the most important outcome of ART, the live birth rate. In that year, 391 clinics out of a total of 428 ART clinics reported data on 115,392 cycles. Clinics that did not report either were not functioning in 2002 or simply failed to report as required.

By analyzing annual reports, several trends can be seen in the practice of ART in the United States.



Most importantly, pregnancy rates have improved over time (Fig. 1). Pregnancy rates from IVF have steadily increased in the United States virtually every single year since these rates have been compiled, with a current (2002 data) live birth rate of 28.3% per cycle.<sup>9</sup> The live birth rate per retrieval was 32.6%, and the live birth rate per transfer was 34.8%. Rates are higher in the latter categories because of the smaller denominator as couples with canceled cycles or failed fertilization and no embryo transfer are eliminated.

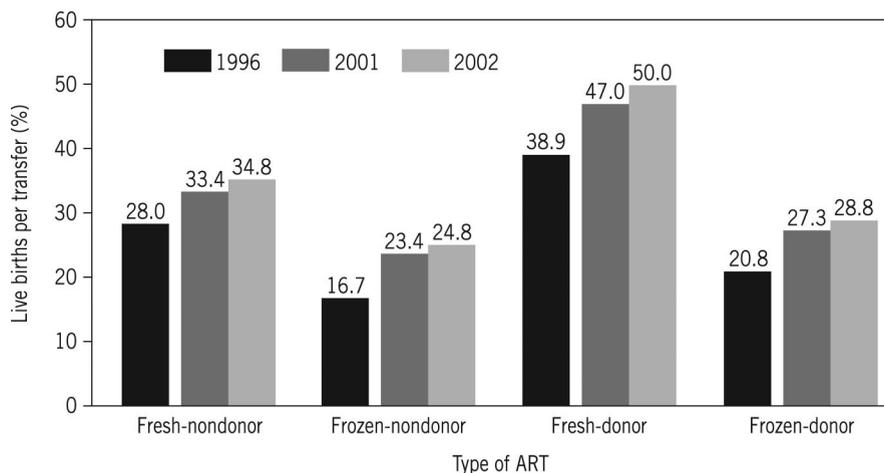
Pregnancy rates with IVF now equal or exceed those of both GIFT and ZIFT. As a result, GIFT and ZIFT, procedures which once represented about 25% of all ART cycles, currently represent less than 1% of ART cycles. This is a major advancement in the treatment of infertile women because the cost and morbidity of a laparoscopic procedure is avoided.

Pregnancy rates are also improving for the transfer of cryopreserved embryos (Fig. 1). Cryopreserved embryos result in a lower pregnancy rate than fresh embryo transfers (live birth rate of 24.8% versus 34.8% per transfer). However, frozen embryo transfer cycles are very cost-effective because ovarian stimulation and retrieval are not necessary before embryo transfer.<sup>10</sup> In addition, when couples are finished with fertility treatments, excess cryopreserved embryos can be donated to other infertile couples, a practice which is also quite cost-effective for the recipient couple.<sup>11</sup>

Another major advancement in ART is ICSI,

which was developed for the treatment of severe male factor infertility sperm.<sup>12</sup> These men, previously at very high risk for failed fertilization using routine insemination, are now selected to have ICSI, resulting in similar fertilization rates as for men without sperm abnormalities. It has since been discovered that ICSI can be used with ejaculated sperm, sperm from the epididymis, and even sperm recovered from testicular tissue. Intracytoplasmic sperm injection is being used with increasing frequency in ART cycles. In 2002, 53% of ART cycles used ICSI for fertilization of eggs. Furthermore, pregnancy rates with ICSI are now very similar to (although slightly below) rates achieved with IVF and standard insemination (CDC Web site).

Intracytoplasmic sperm injection is being used more routinely in some clinics in an attempt to reduce the rate of failed fertilization of oocytes. This complication of IVF is rare, difficult to predict based on seminal fluid parameters, and very disappointing to couples after the effort of ovarian stimulation and egg retrieval. A recent meta-analysis of studies evaluating the use of ICSI in nonmale factor infertility cases concluded that there was no advantage to using ICSI rather than IVF and standard insemination.<sup>13</sup> In fact, the fertilization rates were slightly higher with standard insemination in nonmale factor cases. Because ICSI is more expensive, most centers are restricting the use of ICSI for the indication of male factor infertility.



**Fig. 1.** Trends in live birth rates per embryo transfer. Results are shown for in vitro fertilization cycle using fresh and frozen embryos in both nondonor cycles and donor egg cycles. ART, assisted reproductive technology. Modified from Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2002 Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Reports. Atlanta (GA): Centers for Disease Control and Prevention; 2004. Available at <http://www.cdc.gov/reproductivehealth/ART/index.htm>. Retrieved November 2, 2005.

Van Voorhis. *Outcomes From ART. Obstet Gynecol* 2006.



## PROGNOSTIC FACTORS FOR PREGNANCY AFTER ART

### Demographic Factors

A woman's age is the most important factor affecting the chances for live birth after ART (Fig. 2). This point needs to be emphasized with older patients who may have inflated perceptions about the chances for pregnancy. Misperceptions may arise from media reports of celebrity pregnancies that fail to distinguish between donor and nondonor oocyte sources. From 2002 CDC data, live birth rates per cycle range from just over 40% in women aged 27 years, down to 6% at age 43, and only 2% in women who are over 43 years of age. A large increase in miscarriage rate with aging (reaching nearly 45% at age 43) contributes to the low live birth rate after IVF in relatively older women. Primary care physicians should consider prompt referral of older women (> 36 years of age) with infertility.

Data from donor oocyte cycles demonstrate that the reduced fertility associated with aging is linked primarily to aging of the ovary and oocyte rather than aging of the uterus and endometrium. Donor oocyte cycles result in high pregnancy rates among recipient women that are independent of the age of the recipient (Fig. 2).

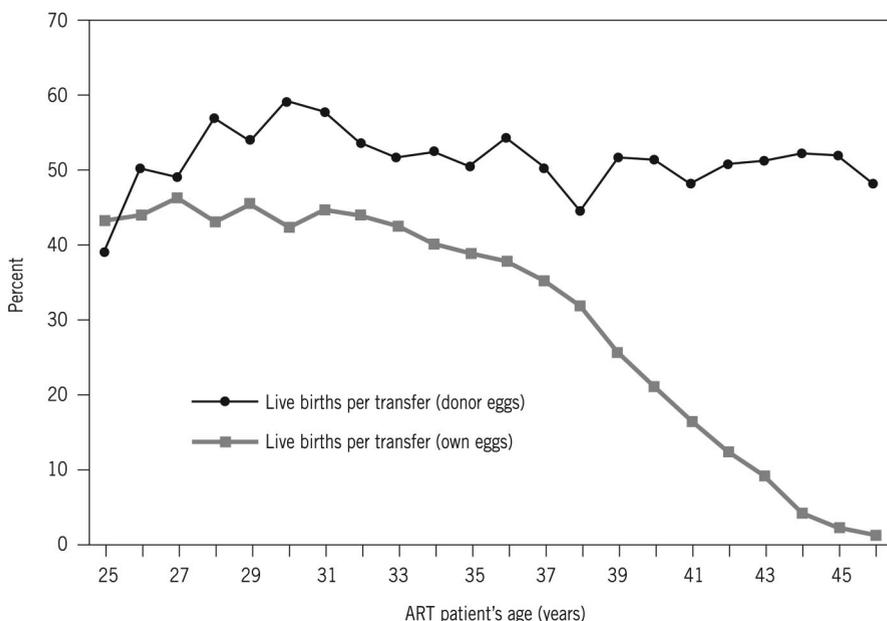
Based on 2002 CDC data, parous women have consistently higher pregnancy rates with ART than nulliparous women. Women who have been pregnant, but miscarried, have the same pregnancy rate as age-matched women who have never been pregnant. Among women aged 40 or younger, those who have

had no previous IVF cycles have a slightly higher pregnancy rate than those women who have had 1 or more previous IVF cycles that have not resulted in pregnancy.

### Infertility Diagnosis

Based on CDC data, several infertility diagnoses appear to be associated with a worsened prognosis for pregnancy after ART. Compared with the average live birth rate of 28.3%, a distinctly lower live birth rate of 13.9% is seen with the diagnosis of reduced ovarian reserve. Women with multiple diagnoses had a live birth rate of 23.4%, and couples with both male and female diagnoses had a live birth rate of 26.4%. Uterine factor infertility, defined as a structural or functional disorder of the uterus, was associated with a reduced pregnancy rate of 22.9%. In contrast, very little difference in pregnancy rates is seen when comparing couples with the diagnosis of tubal factor infertility, ovulation dysfunction, endometriosis, male factor infertility, or unexplained infertility. Couples in all of these diagnostic groups had similar live birth rates per cycle of 30–35%. It is important to remember that, with these national statistics, the extent to which the infertility workup was completed is not known and definitions may vary between clinics. In addition these pregnancy rates per diagnostic group are not corrected for other potentially important confounding variables, including the age of the woman.

The effect of endometriosis on ART outcomes is difficult to determine from national data due to selec-



**Fig. 2.** The effect of female age on assisted reproductive technology (ART) pregnancy rate in couples using a woman's own eggs or using donor eggs. Modified from Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2002 Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Reports. Atlanta (GA): Centers for Disease Control and Prevention; 2004. Available at <http://www.cdc.gov/reproductivehealth/ART/index.htm>. Retrieved November 2, 2005.

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tion bias because not all cases have been ascertained in the diagnostic evaluation of infertile couples. A meta-analysis of studies evaluating endometriosis and IVF outcomes has concluded that patients with endometriosis have a significantly lower pregnancy rate (odds ratio 0.56, 95% confidence interval 0.44–0.70) than women with tubal factor infertility. Women with more severe disease had lower pregnancy rates than women with mild disease.<sup>14</sup> It should be noted that women with endometriosis still had reasonably high pregnancy rates with ART, and this finding does not imply that treatment of endometriosis will result in higher pregnancy rates with ART.

### Hydrosalpinges and Pregnancy After ART

In vitro fertilization was initially developed as a treatment of tubal factor infertility, although it is now used as treatment for virtually all causes of infertility. Ironically, several retrospective series noted that women with tubal disease appeared to have poorer results from IVF than women with other types of infertility. In 1994, the first study found a worsened prognosis for women with a hydrosalpinx.<sup>15</sup> Since then, numerous studies have confirmed that women with hydrosalpinges have approximately a 50% reduction in both clinical pregnancy and delivery rates compared with age-matched women without hydrosalpinges but being treated by ART.<sup>16</sup> Some have also noted an association between the presence of a hydrosalpinx and an increased miscarriage rate after ART.

Hydrosalpinges vary in the amount of fluid they contain and how they are diagnosed. Large hydrosalpinges that are easily visible by ultrasonography may differ in prognostic significance from hydrosalpinges not present by ultrasonography but only demonstrated after filling of the tubes with fluid either at hysterosalpingography (HSG) or laparoscopy. Several retrospective studies have found that ultrasonography-visible hydrosalpinges carry the worst prognosis, indicating that the size of the hydrosalpinx and volume of fluid contained are important factors.<sup>15,17,18</sup>

The mechanism of the impaired pregnancy rates with hydrosalpinges and IVF is unknown. Theories have focused on the toxic effect of this fluid on embryo development, the effect of the fluid on endometrial receptivity and implantation, and the simple mechanical wash out of embryos by hydrosalpinx fluid. Each of these theories is supported by in vitro data, but the true cause of reduced pregnancy rates is unknown.

Retrospective data suggested that women who had a hydrosalpinx removed by salpingectomy had

an improved pregnancy rate after ART treatment. As a result, a randomized, multicenter, controlled trial was performed in several Scandinavian countries.<sup>19</sup> A total of 204 patients with uni- or bilateral hydrosalpinges were randomized to either laparoscopic salpingectomy or no intervention before an IVF cycle. Because of slow recruitment, the trial was stopped before the targeted sample size was reached. In total, the women treated by salpingectomy before IVF had a 30% delivery rate compared with an 18% delivery rate in women with no treatment, a difference that did not reach statistical significance. However, statistically improved delivery rates were noted in the subset of women who had ultrasonography-visible hydrosalpinges, particularly if they were bilateral. A recent meta-analysis combining the Scandinavian study with 2 other prospective randomized trials concluded that surgical treatment for hydrosalpinges significantly increased the odds of a live birth after IVF.<sup>20</sup> In addition, a later analysis of the cumulative results experienced by the women enrolled in the Scandinavian trial found a significantly higher pregnancy rate with ART after salpingectomy.<sup>21</sup> Again, the improvement was most notable in patients with hydrosalpinges visible by ultrasonography.

Salpingectomy is not without some morbidity. In addition to the usual morbidities associated with operative laparoscopy, concern has been raised that dissection of the fallopian tube from the ovary may compromise blood flow to the ovary and thus impair ovarian responsiveness in the subsequent IVF cycle.<sup>16</sup> Therefore, experienced laparoscopists should perform this surgery, and if extensive dissection is necessary to completely remove the tube, then consideration should be given to simply proximally obstructing the fallopian tube. Given the proposed mechanisms of impaired IVF pregnancy rates, theoretically, this should be successful and is supported by retrospective data showing equivalent outcomes after proximal obstruction compared with salpingectomy.<sup>22</sup>

Suggested alternatives to salpingectomy have included aspiration of the hydrosalpinx at the time of oocyte retrieval or prolonged treatment with antibiotics before ART.<sup>17,23,24</sup> Retrospective series have reached different conclusions regarding the effectiveness of these practices, and they require further study. Consideration can always be given to performing operative salpingostomy, which would have the advantage of possibly permitting pregnancies without ART. However, with large hydrosalpinges, the pregnancy rates following tubal surgery are disappointingly low and the risk of ectopic pregnancy is quite



high. In addition, hydrosalpinges often reform after distal salpingostomy.

The data now seem to clearly suggest that the presence of a hydrosalpinx, particularly when visible with ultrasonography, is associated with worsened outcome from IVF. Furthermore, evidence is mounting that salpingectomy is associated with improved outcomes in these patients with their IVF cycle. In our clinical practice, we perform ultrasound examinations of all women who are suspected of having tubal disease, either by clinical factors or based on hysterosalpingogram. If a hydrosalpinx is present on ultrasonogram, we always determine whether or not ART is an option for the couple before performing a laparoscopic procedure. If ART is a possibility, it has been our practice to perform laparoscopic salpingectomy for its beneficial effects on subsequent ART cycles. Only if ART is not possible for the couple, either for economic or ethical reasons, will salpingostomy and lysis of adhesions be performed in an attempt to improve the fertility of the woman.

### Leiomyomata and ART Outcomes

The effect of uterine leiomyomata on outcomes with ART likely depends on the size and location of the tumor. Current expert opinion is that uterine leiomyomata that are intracavitary or distort the endometrium have an adverse effect on live birth rates after ART. However, this opinion is based on only 2 retrospective series<sup>25,26</sup> that reported a 50–70% reduction in pregnancy rates in women with leiomyomata in this location. Most studies do not include women with intracavitary leiomyomata because they have typically been removed empirically before any ART attempts. The effect of intramural leiomyomata with no apparent distortion of the endometrial cavity is more controversial, with some studies reporting impaired pregnancy<sup>27,28</sup> or embryo implantation rates,<sup>29</sup> whereas others find no effect.<sup>30</sup> The differences in these studies may be related to how the location of the leiomyomata and how “cavity distortion” was determined. The techniques of hysteroscopy and saline infusion sonography may more sensitively detect leiomyomata impinging on the endometrial cavity than a hysterosalpingogram or standard transvaginal ultrasonography.<sup>30</sup> Based on the available evidence, if intramural or subserosal leiomyomata that do not impinge on the endometrial cavity have an effect on pregnancy rates after ART, the effect is likely to be small. Certainly there is no evidence that myomectomy improves ART outcomes. We currently recommend removal of leiomyomata that impinge on the endometrial cavity before ART although evidence

from a randomized trial is lacking. Clinical judgment that takes into consideration patient symptomatology and reproductive history is necessary in deciding whether or not to perform surgery for intramural leiomyomata not impinging on the endometrium.<sup>30</sup>

### Smoking and ART Outcomes

Cigarette smoking is associated with reduced ovarian function because it contributes to an earlier age of menopause.<sup>31</sup> Meta-analyses of studies evaluating the effects of smoking on IVF outcomes have concluded that smoking reduces the pregnancy rate by approximately 50%.<sup>31</sup> The mechanism of this effect is not clear, but constituents of cigarette smoke can be detected in follicular fluid and thus could affect the health of oocytes or embryos. We found that cigarette smoking was associated with a prolonged and dose-dependent reduction in numbers of oocytes and embryos obtained in an ART cycle.<sup>32</sup> Smoking also appears to have a more transient adverse effect on fertility because current, but not past smokers, had a markedly reduced pregnancy rate after ART in our study. For many reasons, women undergoing ART cycles should be encouraged to stop smoking for as long as possible before a cycle although the time frame for the reversal of the adverse effect of smoking on ART outcomes is not clear.

### Decreased Ovarian Reserve and ART Outcomes

In general, women have a decline in ovarian function and fertility with aging. However, age alone is not always a reliable predictor of ovarian function, so investigators have searched for means of testing ovarian reserve. Decreased ovarian reserve is defined by poor ovarian follicular response to gonadotropin stimulation during ART, probably secondary to a reduced number and quality of ovarian follicles available for stimulation. This results in a much higher rate of cycle cancellation, fewer eggs retrieved, fewer embryos, and a lower pregnancy rate with ART.<sup>33</sup>

A number of hormones, including follicle-stimulating hormone (FSH), inhibin, and müllerian inhibiting substance, have been assessed for their ability to predict decreased ovarian reserve. The most widely used test for estimating ovarian reserve is a basal FSH value.<sup>33,34</sup> Follicle-stimulating hormone values have been shown to be elevated early in the menstrual cycle of women with reduced ovarian reserve. The basal FSH is typically drawn on cycle day 2, 3, or 4, and values above 15 mIU/mL (in many laboratories) suggest a decreased ovarian reserve and a significantly reduced probability of pregnancy after ART. If



the FSH is above 20 mIU/mL, the chances for pregnancy are virtually zero.

Another test for ovarian reserve is the clomiphene citrate challenge test.<sup>35</sup> This test requires a basal FSH value on cycle day 3, followed by the administration of 100 mg of clomiphene citrate on cycle days 5–9. A second FSH value is then obtained on cycle day 10. If either FSH value is elevated, reduced ovarian reserve is diagnosed. This test is thought to be more sensitive because it has a provocative component that may unmask reduced ovarian reserve not detected by the basal FSH screening alone.

A recent meta-analysis of studies using either the basal FSH measurement or the clomiphene citrate challenge test concluded that both tests were similar in their ability to predict a clinical pregnancy after ART and therefore recommended that a basal FSH be preferred over the clomiphene citrate challenge test because it is simpler and less expensive.<sup>36</sup> With either test, the sensitivity was very low, but specificity was very high for clinical pregnancy after ART. Thus, a normal result is not useful because of its poor sensitivity; the woman may still have reduced ovarian reserve or may not conceive after ART for some other reason. It has been suggested that ovarian reserve testing reflects oocyte numbers but not quality, and thus older women (with reduced egg quality) still have a low chance for pregnancy with ART even after the finding of a normal FSH value.<sup>37</sup> An abnormal result for FSH is highly predictive of a poor outcome from ART.<sup>36</sup> Some ART centers use an FSH cutoff for admitting patients into their program for ART cycles. It should be remembered that an elevated FSH value does not necessarily mean that a woman will be unable to conceive a pregnancy naturally. There are many examples of patients with elevated FSH values that were subsequently able to conceive without treatment. The ovarian reserve tests are better used as a predictor of who is not likely to benefit from ART.

There are some problems with using the serum FSH levels for ovarian reserve testing. The first is that different studies use different cutoffs for a normal value. There is considerable difference among laboratories measuring FSH because of the use of different assays and reference standards for the assay.<sup>38</sup> As a result, physicians must be aware of the assay and references used in their laboratory to find relevant values for their clinical situation. The poor sensitivity of a basal FSH can be improved by also measuring the estradiol (E2) level, with values above 80 pg/mL being abnormal.<sup>39</sup> The improved sensitivity is seen because some “normal” basal FSH values will be

falsely normal because, in some patients, E2 is abnormally elevated in the early follicular phase and is actively suppressing FSH. Sensitivity of the test can also be improved by repeating basal FSH values in several cycles, with any one elevated value signaling reduced ovarian reserve.<sup>40,41</sup>

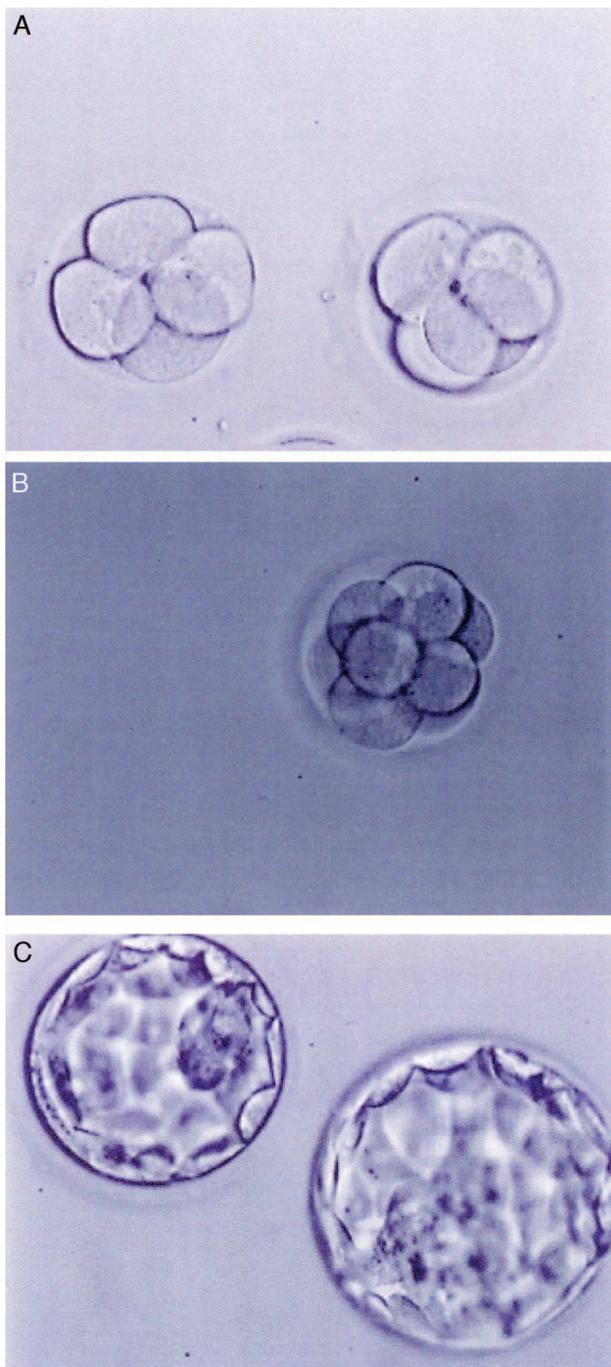
Reduced ovarian reserve can also be diagnosed through ultrasound observation of the ovaries. We first reported that the volume of the ovaries was a predictor of ovarian reserve because women with small ovarian volumes had an increased rate of cycle cancellation, achieved a lower E2 level after stimulation, had fewer oocytes retrieved, and had a lower pregnancy rate.<sup>42,43</sup> Others have confirmed these initial observations. More recently, investigators have found that low numbers of ovarian antral follicles (< 10 total follicles with a diameter between 2 and 10 mm) indicates reduced ovarian reserve and diminished chance for pregnancy after ART.<sup>44,45</sup>

### Laboratory Techniques and ART Outcomes

New laboratory techniques have been introduced for the purpose of improving pregnancy rates from ART. One innovation is prolonged culture of embryos before transfer to the uterus.<sup>46</sup> Embryo transfer was traditionally performed 2 days after oocyte retrieval when embryos are at the 2- to 4-cell stage. The day 2 transfer of embryos may have some advantages because earlier placement in the uterus limits the amount of time spent in the in vitro environment. On the other hand, more prolonged culture (often for 3–5 days) allows embryologists to observe embryos for growth and morphology and select presumably “healthier” embryos for transfer to the uterus. Recent changes in culturing techniques have allowed for the culture of embryos for 5–6 days when they have developed to the blastocyst stage. (Fig. 3) The disadvantage of prolonged culture is that average rates of blastocyst formation have ranged from 28% to nearly 50% in various series.<sup>47</sup> Thus, more embryos need to be cultured to produce a suitable number of blastocysts for transfer, and some women may not have a good-quality blastocyst to transfer. Many programs restrict blastocyst embryo transfer to patients with a good prognosis, as determined by ovarian response to ovulation induction or a high number of embryos obtained in a given cycle.

A systematic review of trials comparing day 2 with day 3 embryo transfer found no significantly different live birth rates.<sup>48</sup> Likewise, an analysis of trials comparing day 2 and day 3 embryo transfers with blastocyst transfers found no significantly different live birth rates.<sup>45</sup> However, these conclusions are





**Fig. 3.** Human embryos cultured in vitro. **A)** Embryos after 2 days of culture at the 4-cell stage of development. **B)** An 8-cell embryo on culture day 3. **C)** Blastocyst embryos on culture day 5. All photographs were taken at the same magnification.

Van Voorhis. *Outcomes From ART. Obstet Gynecol* 2006.

based on a small number of prospective randomized trials. Because blastocyst transfer is a relatively new practice, there are great variations in the culture

media used in various trials, and this may make a large difference in ultimate implantation and pregnancy rates. Further studies are needed to determine the optimal strategy for embryo culture and transfer. Blastocysts appear to have a higher implantation rate than embryos transferred at cleavage stages (day 2 or day 3), allowing for transfer of fewer embryos to achieve the same pregnancy rate. Although the pregnancy rate may not differ, the major advantage of prolonged culture may ultimately be a reduction in multiple gestations after ART.

## ADVERSE OUTCOMES FROM ART

### Multiple Gestations

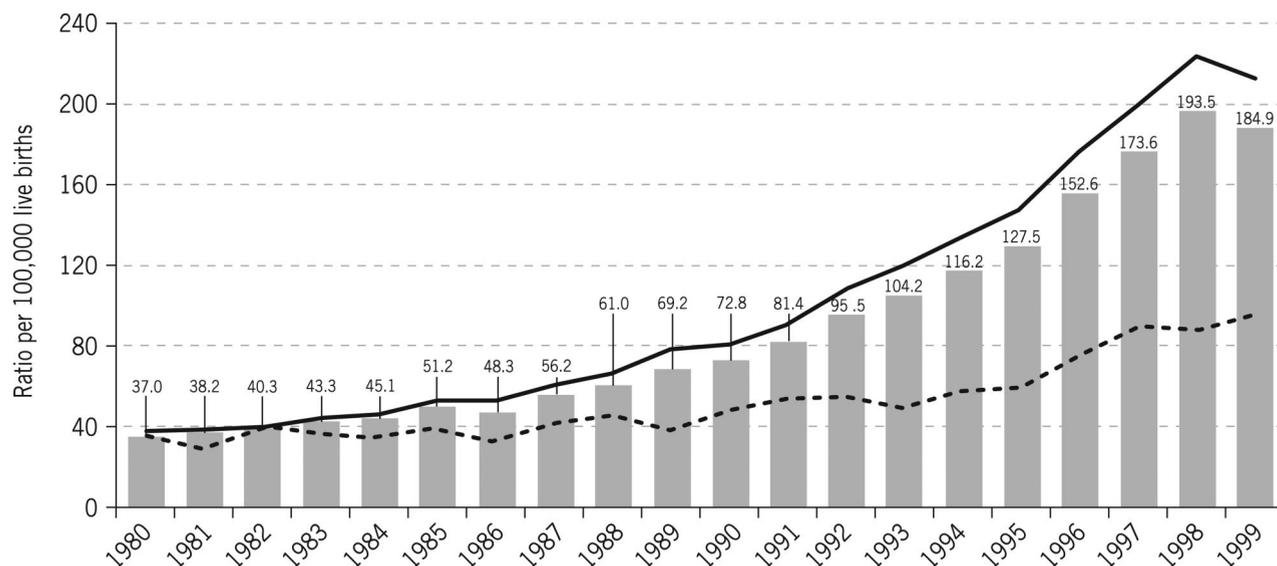
In the United States, rates of multiple births are increasing at a rapid rate (Fig. 4). The greatest increases in multiple birth rates have been in women of advanced maternal age and in white women.<sup>49</sup> Two potentially linked causes are a societal trend toward delayed childbearing and increasing use of ART. Older women are at increased risk for multiple gestations in naturally conceived pregnancies. They are also at increased risk of being infertile and of using ART and donor eggs, placing them at higher risk of multiple gestations.

Multiple births are now the biggest challenge facing infertility specialists in the United States. In the year 2000, of the 35,000 infants that were born after ART procedures, 44% were twins and 9% were triplets or higher-order multiple gestations.<sup>50</sup> With ART, twinning rates are 22 times higher than what is seen in the general population, and triplets and higher order multiples are 50 times the natural rate of 0.18%.<sup>50</sup> Nationally, it has been estimated that more than 40% of the triplet and higher-order births in 1997 were the result of ART and another 40% due to use of ovulation-inducing drugs.<sup>51</sup>

Multiple gestations conceived naturally or through ART are associated with significant morbidity. Although multifetal births account for only 3% of all live births nationally, they account for 17% of all preterm births (< 37 weeks of gestation), 23% of early preterm births (< 32 weeks of gestation), and 26% of very low birth weight infants (< 1,500 g).<sup>52,53</sup>

There is evidence of adverse long-term health consequences for infants of multiple gestations born after ART. Although most commonly associated with higher-order multiple gestations, this is true for twins as well. Children born after IVF have been found to have an increased risk of cerebral palsy<sup>54</sup> and a higher hospitalization rate, mainly due to the high twinning rate associated with IVF. A recent Danish national





**Fig. 4.** High-order (triplets and greater) multiple-birth ratios among infants of all races (shaded bars), white infants (solid line), and black infants (broken line) in the United States from 1980 to 1999. Modified from Russell RB, Petrini JR, Damus K, Mattison DR, Schwarz RH. The changing epidemiology of multiple births in the United States. *Obstet Gynecol* 2003;101:129–35, with permission from Lippincott Williams & Wilkins.

Van Voorhis. *Outcomes From ART. Obstet Gynecol* 2006.

study found that, compared with IVF singletons, more IVF twins were admitted to a neonatal intensive care unit (NICU), had a surgical intervention, and had special needs and poor speech development. More mothers of IVF twins rated their infants' general health as being poor than did IVF singleton mothers. Furthermore, analysis showed that parents of twins experienced more marital stress than did parents of singletons.<sup>55</sup>

Multiple gestations also increase the maternal risks of hypertension, postpartum bleeding, premature labor with prolonged bedrest, and cesarean deliveries. Although rare, maternal mortality is increased in multiple gestations.

Multiple gestations are costly to the health care system. The estimated charges associated with a singleton delivery are approximately \$10,000, whereas with a twin delivery the charges are closer to \$40,000.<sup>56,57</sup> Excess hospital costs for multiple gestations born from IVF cycles have been estimated at \$640 million in the United States in the year 2000.

There are several reasons for the increased multiple birth rate after ART. Historically, low embryo implantation rates led clinicians to place multiple embryos in the uterus in an effort to improve the efficacy of a given IVF procedure. Although the large preponderance of twins after IVF are dizygotic as a result of transferring multiple embryos, monozygotic twinning has also been shown to be increased. We

found the incidence of monozygotic twinning of 3.2% after IVF, compared with a background rate of 0.4% in the general population.<sup>58</sup> Others have confirmed the increased rate of monozygotic twinning after ART, with particular risk factors being manipulation of the zona pellucida with the use of assisted hatching or extended culture and blastocyst embryo transfer.

In addition to the desire for high pregnancy rates among IVF clinicians, patient input may be partially responsible for the high multiple gestation rate after ART. We have found that over 20% of infertile patients surveyed actually desired multiples (predominantly twins) over a singleton gestation as an outcome from treatment. Our study found that younger women who were nulliparous, had a lower family income, and had a longer duration of infertility were more likely to desire a twin gestation over a singleton. Importantly, a lack of knowledge of the health consequences and risks of twins gestations was also associated with the desire for multiple gestations. Perhaps by educating our patients about the risks, the desire for multiple gestations might be lessened.<sup>59</sup>

Physicians performing ART are aware of the problem, and improvements are being made. Advances in embryo culture techniques, as well as embryo selection (the practice of culturing multiple embryos and then selecting the most morphologically favorable embryos for transfer), have led to improvements in embryo implantation rates. In response to



these improvements and to changes in the Society for Assisted Reproductive Technology (SART) guidelines for numbers of embryos to transfer, IVF clinicians have reduced the number of embryos transferred over time. This has likely contributed to a recent reduction in triplet and higher-multiple gestation rate in the United States after ART.<sup>60</sup> Increased use of fetal reduction procedures could also contribute to the reduction. However, as yet, twinning rates remain high and have not dropped.

One obvious solution to the problem of multiple gestations, and particularly twins, after ART is to transfer a single embryo in an IVF cycle. Although it is intuitive that putting in more embryos will result in a higher live birth rate, this is not necessarily the case according to CDC data. In women who are under 35 years of age and who have excess cryopreserved embryos, pregnancy rates are not greatly affected by numbers of embryos transferred, with a pregnancy rate of 47.4% after single-embryo transfer compared with 51.8% if 2 embryos were transferred. Transfer of additional embryos did not result in a higher pregnancy rate but simply increased the high-order multiple gestation rate. Therefore, among patients with a good prognosis (characterized by young age and having extra embryos for cryopreservation), single-embryo transfer results in a high pregnancy rate and virtually eliminates the risk of twins.

A recent large multicenter randomized prospective trial to test this practice was conducted in several Scandinavian countries.<sup>61</sup> This trial compared the strategy of transferring 2 fresh embryos with the strategy of transferring a single fresh embryo (followed by the transfer of a single cryopreserved frozen and thawed embryo if needed). Entrance criteria required that women be under the age of 36 and undergoing their first or second IVF cycle. In addition, they had to have at least 2 embryos of good morphologic quality for this study. Approximately one third of patients presenting to these clinics had these favorable prognostic factors and qualified for the trial. Transferring 2 embryos resulted in a higher pregnancy rate than transfer of a single embryo (43.4% versus 29.6%). However, after the women who received a single-embryo transfer and did not conceive subsequently had a single cryopreserved embryo replaced, the cumulative pregnancy rate was 38.8%, a number similar to the double-embryo transfer group. This study did not demonstrate equivalence of the 2 treatment protocols but suggested that any reduction in live birth rate by transferring one embryo was unlikely to exceed 11.6%. Importantly, twinning was markedly reduced in the single-embryo

transfer arm. These findings emphasize the importance of a high-quality embryo cryopreservation program as a means of allowing fewer embryos to be transferred, with the promise that future frozen cycles may be successful. They further suggest that single-embryo transfer should be strongly considered in couples with a good prognosis for pregnancy after IVF.

In this study, a large majority of the embryos were transferred after 2 days in culture, at which time the average embryo is typically at a 4-cell stage. Some have advocated that culturing embryos for 5 days to the blastocyst stage may allow for selection of embryos that are more likely to implant, thus improving the pregnancy rate after single-embryo transfer. One pilot study has already demonstrated a very high pregnancy rate after single-blastocyst embryo transfer.<sup>62</sup>

Unfortunately, in many states, infertility is not covered by insurance companies, resulting in out-of-pocket payments for ART services. This economic limitation to multiple treatment cycles may lead patients to take more risks in a given ART cycle in the hope that, by placing more embryos, the pregnancy rate will be improved. Indeed, a recent study found an association between state-mandated insurance coverage for IVF services and lower average numbers of embryos transferred per IVF cycle. This, in turn, resulted in a decrease in the percentage of pregnancies, with 3 or more fetuses in these states.<sup>63</sup> Single-embryo transfer is more likely to be embraced by couples that have insurance coverage for infertility. This strategy may be attractive to insurers because the practice of single-embryo transfer has been shown to be cost-effective compared with double-embryo transfer due to the much reduced twinning rate with single-embryo transfer.<sup>64,65</sup>

### Perinatal Outcomes for Singletons Conceived After ART

Recently, data has accumulated suggesting that even singleton pregnancies conceived by IVF are at higher risk for adverse outcomes. A meta-analysis of studies evaluating perinatal outcomes for singletons conceived after IVF included 15 cohort studies from around the world.<sup>66</sup> All studies included in this analysis compared IVF-conceived singletons with spontaneously conceived singletons and controlled for maternal age and parity because these are 2 factors that are known to affect perinatal outcome. Singletons conceived by IVF were at approximately a 2-fold increase risk for perinatal mortality, low birth weight, very low birth weight, preterm delivery, and small for gestational age infants (Table 1). Increased risks for gestational diabetes, placenta previa, preeclampsia,



**Table 1. Perinatal Outcomes in Singletons After In Vitro Fertilization and Natural Conception: Results of a Meta-Analysis of Clinical Trials**

Outcome	Approximate Absolute Risk (%)		Odds Ratio (95% CI)
	IVF	Spontaneous	
Perinatal mortality	2	0.7	2.9 (1.61–2.98)
Preterm delivery	11.5	5.3	1.95 (1.73–2.20)
Birth weight < 2,500 g	9.5	3.8	1.77 (1.40–2.22)
Birth weight < 1,500 g	2.5	1.0	2.70 (2.31–3.14)
Small for gestational age	14.6	8.9	1.60 (1.25–2.04)

Adapted from Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004;103;551-63, with permission from Lippincott Williams & Wilkins. IVF, in vitro fertilization; CI, confidence interval.

and stillbirth were also found among IVF-conceived singletons although these were secondary outcomes for this analysis. The odds ratios for these complications ranged from 1.55 for preeclampsia to 2.87 to placenta previa.

The cause for these adverse perinatal outcomes among IVF-conceived singletons is unknown. One possibility is that some aspect of the IVF treatment (eg, ovarian stimulation or embryo culture) may increase the risk for subsequent adverse pregnancy outcomes. Alternatively, there may be an underlying disorder in the infertile couple that contributes both to the infertility and the adverse perinatal outcomes. It has been noted that IVF-conceived singletons are at a significantly higher risk of having induction of labor and both emergent and elective cesarean deliveries.<sup>66</sup> Thus, some of the adverse outcomes, including low birth weight, very low birth weight, and preterm delivery, may be attributable, in part, to iatrogenic intervention. Regardless of the cause, current evidence suggests that pregnancies conceived after IVF are at higher risk for adverse outcomes, and thus, patients should be advised of this risk before undergoing infertility treatment.

### Birth Defects After ART

Several studies have been conducted to determine whether or not ART is associated with an increased risk of birth defects.<sup>67</sup> The results from these studies are mixed, although a majority of published studies report a slight increase in the rate of birth defects after IVF. In the largest study reported from the United States, we compared children conceived by IVF at the University of Iowa and a matched cohort of naturally conceived children.<sup>68</sup> Birth defects were prospectively diagnosed and recorded in a statewide birth defects registry. We found that 6.2% of IVF-conceived children had major birth defects, compared with a rate of 4.4% in naturally conceived children. There was a

statistically significant difference in major birth defect rate after IVF, with an adjusted odds ratio for a major birth defect of 1.30 (95% confidence interval 1.00–1.67). Specific birth defects that were increased in the IVF population included cardiovascular and musculoskeletal defects as well as certain known birth defect syndromes.

Our study supports the findings of some, but not all, previous studies evaluating birth defects after IVF as compared with either matched cohort or national registry rates after correction for important variables. The studies listed in Table 2 are the largest studies of birth defect rates that control for maternal age and plurality.<sup>68–74</sup> This is important because birth defects are increased in multiple-gestation pregnancies. In addition, all of these studies detected and reported birth defects in a standardized fashion. However, even these studies cannot be directly compared because birth defects were detected in children for varying lengths of time after birth, and different classification systems for birth defects were used. Our interpretation of current evidence is that IVF may be associated with an increase in birth defects, but the effect is small. Findings to date are not likely to dissuade many couples from pursuing infertility treatments.

Recent attention has been directed toward epigenetic errors that might be inherent in the infertile couple or induced as an adverse effect of ART itself. Differential DNA methylation leading to expression of only 1 of 2 parental alleles is a mechanism of gene regulation known as genomic imprinting. Defects in imprinting may cause either over- or underexpression of certain genes, leading to birth defects or cancer. Several syndromes caused by imprinting defects, including Beckwith-Wiedemann syndrome and Angelman syndrome, have been reported to be more prevalent in children born after IVF as reviewed in Niemitz and Feinberg.<sup>75</sup> Some have proposed that



**Table 2. Association of Birth Defects and In Vitro Fertilization**

Study	Country	Years	Infants Studied	IVF Defects n/N (%)	Controls Defects n/N (%)	Adjusted OR	Specific Defects Increased
Matched cohort studies							
Dhont <sup>69</sup>	Belgium	1992–1997	Singleton Twin	84/3,048 (2.8) 86/2,482 (3.5)	62/3,048 (2) 73/2,482 (2.9)	Nonsignificant Nonsignificant	None None
Westergaard <sup>70</sup>	Denmark	1994–1995	All	107/2,245 (4.8)	103/2,245 (4.6)	Not stated	None Cardiovascular, musculoskeletal, chromosomal, urogenital
Population-based studies							
Hansen <sup>71</sup>	Australia	1993–1997	Singleton All	50/527 (9.5) 75/837 (9.0)	164/3,906 (4.2) 168/4,000 (4.2)	2.2 (1.5–3.2) 2.0 (1.5–2.9)	Cardiovascular, musculoskeletal, chromosomal, urogenital
Koivurova <sup>72</sup>	Finland	1990–1995	All	20/309 (6.6)	24/569 (4.4)	Not stated	Cardiovascular
Olson <sup>68</sup>	United States	1989–2002	Singleton All	38/645 (5.9) 90/1,462 (6.2)	171/4,590 (3.7) 369/8,422 (4.4)	1.44 (0.98–2.12) 1.3 (1.0–1.67)	Cardiovascular, musculoskeletal, syndromal
Population-based studies							
Ericson <sup>73</sup>	Sweden	1982–1991	All	516/9,111 (5.6)	Not stated	0.89 (0.74–1.06)	Omphalocele, neural tube defects, anal atresia
Anthony <sup>74</sup>	Netherlands	1995–1996	All	137/4,224 (3.2)	8,526/314,605 (2.7)	1.03 (0.86–1.23)	Cardiovascular, musculoskeletal

IVF, in vitro fertilization; OR, odds ratio.



prolonged exposure to embryo culture media used in IVF may predispose to imprinting defects in the human embryo as has been demonstrated in bovine embryos cultured in vitro under certain conditions. It is important to realize that the number of infants affected by known imprinting disorders after ART is extremely small. However, this does not exclude the possibility of more subtle subclinical effects of imprinting defects in children after ART. Further studies regarding the effect of ART on genetic imprinting are needed.

### **Birth Defects After Intracytoplasmic Sperm Injection**

Because some couples who would otherwise be incapable of reproduction are having children after ICSI, a valid concern has been the health of the infants conceived by this new process.<sup>76</sup> This is especially pertinent because male infertility is being increasingly found to be secondary to genetic disorders. In infertile males, chromosomal aberrations increase as sperm counts decrease. Men with azoospermia are at increased risk of having numerical sex chromosome aberrations. Moreover, in men with severely disordered spermatogenesis (usually characterized by sperm concentrations of < 5 million sperm/mL), Y chromosome microdeletions are found at an increased rate. Finally, congenital bilateral absence of the vas deferens leading to azoospermia is linked to mutations in the cystic fibrosis transmembrane conductance regulator gene. Because reproduction can occur using ICSI in all these cases, careful genetic evaluation and counseling is required.

Male children conceived after ICSI carry the same Y chromosome microdeletions as their fathers.<sup>77,78</sup> In addition, a higher rate of karyotypic abnormalities have been described in pregnancies conceived after ICSI than in pregnancies conceived by IVF with standard insemination.<sup>79</sup> Furthermore, the incidence of karyotypic abnormalities after ICSI can be correlated with the number of sperm in the ejaculate, showing the link between the severity of male factor infertility and subsequent chromosomal aberrations in offspring.<sup>80</sup> Of course, the children born from ICSI are too young to yet know the effect of these genetic abnormalities on their subsequent reproduction. However, this further underscores the importance of adequate pretreatment screening and genetic counseling of males before ICSI.

Several studies have demonstrated a higher birth defect rate among children born after ICSI compared with natural conception.<sup>71,81,82</sup> In the largest study evaluating this question, the major malformation rate

after ICSI was 8.7%, compared with 6.1% in the population-based control cohort.<sup>81</sup> After adjusting for risk factors including multiple birth and maternal age, the odds ratio of a birth defect was 1.24 (95% confidence interval 1.02–1.50). This study described a trend toward increased malformation rate in the male genitalia after ICSI. This trend supports other reports of an increased rate of hypospadias among children born after ICSI.<sup>82</sup> However, others have failed to find an increased risk for male genital defects after ICSI.<sup>68,71</sup> Although children born after ICSI may have a slightly higher risk of birth defects than naturally conceived controls, most studies have concluded they are not at significantly higher risk than children conceived by IVF with standard insemination techniques.<sup>83</sup>

The cause of the increased birth defect rate after ART cycles, with or without ICSI, is unclear. It remains possible that the reported increase is secondary to an ascertainment bias due to more careful scrutiny of ART-conceived babies, either prenatally by ultrasonography or postnatally by physical exams. Alternatively, it may result either from problems inherent in the infertile population or from a poorly understood effect of the ART treatment process. Thus far, the effect, if real, on birth defects appears to be small but deserving of further study.

### **Ovarian Hyperstimulation Syndrome**

Ovarian hyperstimulation syndrome is an iatrogenic complication of ovarian stimulation for ART presenting in the luteal phase (early-onset ovarian hyperstimulation syndrome) or in early pregnancy (late-onset ovarian hyperstimulation syndrome). Ovarian hyperstimulation syndrome can occur in a mild form, consisting of increased ovarian size accompanied by abdominal discomfort. Severe ovarian hyperstimulation syndrome is characterized by significant abdominal distention and pain, often accompanied by nausea and vomiting. The abdominal distension is secondary to enlarged ovaries and a protein-rich ascites accumulating in the peritoneal cavity but also occasionally in the pleural and paracardiac space. Severe ovarian hyperstimulation syndrome patients have significant intravascular volume depletion and hemoconcentration and are at risk for potentially fatal thromboembolisms.<sup>84</sup> Ovarian hyperstimulation syndrome is a self-limiting process that gradually resolves over several weeks although patients may need to be supported during this time by intravenous fluid hydration, pain control, and paracenteses to control ascites and reduce distension and shortness of breath. The cause of ovarian hyperstimulation syndrome is



unknown, although most recent evidence supports a role for vascular endothelial growth factor production from granulosa cells leading to increased vascular permeability and development of the ascites.

Mild forms of ovarian hyperstimulation syndrome are quite common and occur in approximately 25% of IVF patients. More severe forms are present in approximately 0.1–2% of ART patients.<sup>84</sup> Risk factors for the development of ovarian hyperstimulation syndrome include younger age, polycystic ovarian syndrome, high serum E2 concentrations, high follicle numbers, and a history of ovarian hyperstimulation syndrome. Despite knowing these risk factors, it remains very difficult to predict exactly who will develop ovarian hyperstimulation syndrome.

With gonadotropin stimulation cycles for ART, ovarian hyperstimulation syndrome seldom occurs unless human chorionic gonadotropin (hCG) is administered. Human chorionic gonadotropin is given to mimic the midcycle luteinizing hormone (LH) surge necessary to stimulate oocyte maturation. Because hCG has a longer half-life and a higher receptor affinity than LH, the hCG effect lasts significantly longer. Early-onset ovarian hyperstimulation syndrome occurs in response to the hCG injection given before egg retrieval in an ART cycle. Strategies to lower the incidence of the syndrome include canceling the cycle and not giving hCG when high-risk factors are present and the use of “coasting,” whereby women are not given additional gonadotropins for several days, allowing estrogen levels to drop before hCG administration. Alternatives to hCG injection, including use of recombinant LH and use of gonadotropin-releasing hormone agonists to stimulate a native LH surge, are being studied as newer means of preventing early-onset ovarian hyperstimulation syndrome.<sup>85</sup>

Late-onset ovarian hyperstimulation syndrome, occurring in early pregnancy, is often a more prolonged and serious form of ovarian hyperstimulation syndrome. In addition to the risk factors previously mentioned, the risk for late-onset ovarian hyperstimulation syndrome is increased by higher serum concen-

trations of hCG in early pregnancy. Thus, one way of reducing the later onset of ovarian hyperstimulation syndrome is to avoid multiple gestations in ART cycles. Another strategy is to cryopreserve all embryos that result from an ART cycle and transfer them when the ovaries have recovered from gonadotropin stimulation. Retrospective series have shown this to be successful with high pregnancy rates and avoidance of late-onset ovarian hyperstimulation syndrome.<sup>85</sup>

## WHEN TO USE ART IN THE TREATMENT OF INFERTILITY

There are some causes of infertility that clearly need to be treated initially with ART. Examples include ovarian failure requiring donor oocytes or embryos and males with azoospermia requiring sperm retrieval procedures and ICSI. On the other hand, simple cases of anovulation due to polycystic ovarian syndrome are often easily treated by a variety of ovulation induction techniques, and to move directly to ART would be inappropriate. For many couples, the decision as to when to perform ART is not so clear and should be the result of careful consideration of the outcomes of all infertility treatment options. Couples are being increasingly drawn to ART because of the high per-cycle pregnancy rate that is achieved compared with average pregnancy rates achieved with other therapies (Table 3).<sup>87–89</sup> The frequent visits and intensive monitoring associated with ART are less important than the potential frustration that may occur with failed treatment cycles using other options.

One way to evaluate various treatment options is to perform a cost-effectiveness analysis. Economic evaluation of practice has been defined as “the comparative analysis of alternative courses of action in terms of both their costs and consequences.”<sup>90</sup> A number of economic analyses have been performed with regard to infertility treatments, but there are some caveats that must be considered when evaluating these studies. Cost-effectiveness is best determined after a prospective randomized trial. Although there are an increasing number of these studies in the

**Table 3. Outcomes of Common Non-ART Infertility Treatments**

Treatment	Live Birth Rate/Cycle (%)	Multiple Birth Rate (%)	OHSS Risk (%)	References
IUI alone	4.2–6	1	None	86, 87
Clomiphene Citrate-IUI	7–8.3	10	Rare	87, 88
hMG alone	5.7–7.7	23–28	0.1–5	86, 88
hMG-IUI	8.1–13	23–28	0.1–5	86, 89

ART, assisted reproductive technology; OHSS, ovarian hyperstimulation syndrome; IUI, intrauterine insemination; hMG, human menopausal gonadotropin.



infertility field, many of the previous economic analyses are based on retrospective data. In addition, the effectiveness of various treatments is a moving target. As already noted, IVF pregnancy rates have improved from year to year, which may lead to changes in conclusions regarding the cost-effectiveness of IVF compared with other infertility treatments. Finally, findings of cost-effectiveness studies are best applied to large populations and not necessarily to the individual who may have different priorities when making highly personal treatment decisions.

### Cost-Effectiveness of ART

There is no question that IVF is expensive. The initial estimate of the cost/delivery of IVF ranged somewhere between \$44,000 and \$212,000 per delivery.<sup>91</sup> However, this was based on IVF pregnancy rates that were very low by today's standards. Based on data in 1992, we found the cost/delivery for IVF to be \$37,000. In addition, when factoring in later cryopreserved embryo transfer, the total cost/delivery was reduced to \$30,000. As mentioned previously, the cost-effectiveness of IVF is likely to continue to improve as pregnancy rates improve.<sup>92</sup>

In all cost-effectiveness studies published thus far, relatively "low tech" treatments like intrauterine insemination (IUI) or ovulation induction combined with IUI are more cost-effective than ART for the majority of infertile couples. In a retrospective cohort study, we found that IUI, use of clomiphene citrate combined with IUI, and use of gonadotropin injections plus IUI, all had approximately one third the cost per delivery compared with IVF.<sup>93</sup> A number of other studies have reached a similar conclusion.<sup>94-96</sup>

It often becomes difficult to know how many cycles should be attempted before moving on to other therapies. With IUI in particular, several large series have demonstrated that most pregnancies are obtained in the first few cycles of insemination. This may be secondary to a selection bias because more favorable candidates get pregnant more quickly.<sup>97,98</sup> However, from a practical standpoint, couples are often anxious to try something different after 3-4 failed cycles. We generally schedule a consultation at this point to counsel couples about an appropriate next step. Of paramount importance is consideration of the woman's age, and generally a more "aggressive" approach with earlier treatment with ART is recommended in older women.

From a cost-effectiveness standpoint, ART may be the favored initial treatment for couples with certain causes of infertility. For women with tubal disease, all published studies agreed that IVF is at

least as cost-effective as tubal surgery. In a retrospective study, we found the cost per delivery to be twice as high for tubal surgery by laparotomy as for IVF.<sup>93</sup> There is no question that outcomes from tubal surgery will depend greatly on the degree of tubal damage. However, even experienced surgeons have reported live birth rates of only 20-30% after surgery for distal tubal occlusion, and these rates are generally achieved only after 2-3 years of follow-up compared with higher pregnancy rates achieved in a single month of IVF treatment.

In certain cases of male factor infertility, ART using ICSI may be more cost-effective than attempts at conception using IUI. Defining what constitutes male factor infertility based on seminal fluid analysis parameters can be difficult because of the great overlap in these parameters when comparing fertile with infertile males.<sup>99</sup> However, we have found that men with an average total motile sperm count (calculated by multiplying the ejaculate volume by the concentration by the motility) of less than 10,000,000 have an extremely low pregnancy rate with intrauterine insemination.<sup>100</sup> Others have noted a similar threshold effect of low motile sperm counts, either in the ejaculate or in the sample used for insemination.<sup>101,102</sup> We found that treatment with ART was more cost-effective than IUI in couples with this type of severe male factor infertility.

### SUMMARY

Assisted reproductive technology is one of the great success stories in the field of obstetrics and gynecology. Pregnancy rates continue to improve because of new laboratory techniques and recognition of clinical factors that impact outcomes. Although generally safe, adverse outcomes in the short term have been described both in the women undergoing ART and in infants born from these procedures. Continued research is required to understand the causes of these adverse consequences. Of great importance is the need to make ART safer by reducing the incidence of multiple gestations. Further research regarding the long-term implications of these new treatments on infertile women and the children born therefrom is also required.

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# Perinatal Outcome Among Singleton Infants Conceived Through Assisted Reproductive Technology in the United States

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**OBJECTIVE:** To examine perinatal outcome among singleton infants conceived with assisted reproductive technology (ART) in the United States.

**METHODS:** Subjects were 62,551 infants born after ART treatments performed in 1996–2000. Secular trends in low birth weight (LBW), very low birth weight (VLBW), preterm delivery, preterm LBW, and term LBW were examined. Detailed analyses were performed for 6,377 infants conceived in 2000. Observed numbers were compared with expected using a reference population from the 2000 U.S. natality file. Adjusted risk ratios were calculated.

**RESULTS:** The proportion of ART singletons born LBW, VLBW, and term LBW decreased from 1996 to 2000. The proportion delivered preterm and preterm LBW remained stable. After adjustment for maternal age, parity, and race/ethnicity, singleton infants born after ART in 2000 had elevated risks for all outcomes in comparison with the general population of U.S. singletons: LBW standardized risk ratio 1.62 (95% confidence interval 1.49, 1.75), VLBW 1.79 (1.45, 2.12), preterm delivery 1.41 (1.32, 1.51), preterm LBW 1.74 (1.57, 1.90), and term LBW 1.39 (1.19, 1.59). Risk ratios for each outcome remained elevated after restriction to pregnancies with only 1 fetal heart or any of 7 other categories: parental infertility diagnosis of male factor, infertility diagnosis of tubal factor, conception using in vitro fertilization without intracytoplasmic sperm injection or assisted hatching, conception with intracytoplasmic sperm injection, conception in a treatment with extra embryos available, embryo culture for 3 days, and embryo culture for 5 days.

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*The data used for this study were collected with the assisted reproductive technology reporting system of the Society for Assisted Reproductive Technology. Since 1995, data from this system have been used by the Centers for Disease Control and Prevention (CDC) to calculate pregnancy success rates for assisted reproductive technology clinics operating in the United States. This system is jointly supported by Society for Assisted Reproductive Technology, the American Society for Reproductive Medicine (ASRM), and CDC. We thank the Society for Assisted Reproductive Technology and ASRM, without whose contributions this work would not have been possible.*

**CONCLUSION:** Singletons born after ART remain at increased risk for adverse perinatal outcomes; however, risk for term LBW declined from 1996 to 2000, whereas preterm LBW was stable. (Obstet Gynecol 2004;103:1144–53. © 2004 by The American College of Obstetricians and Gynecologists.)

**LEVEL OF EVIDENCE:** III

In the United States and worldwide, assisted reproductive technologies (ARTs) are increasingly used to overcome infertility.<sup>1</sup> In 2000, close to 100,000 procedures were performed in the United States, resulting in more than 35,000 infants, nearly 1% of the U.S. birth cohort.<sup>1</sup> Although ART is thus providing benefits to thousands of couples, adverse outcomes associated with these procedures have been reported. In addition to the known risk for multiple gestation and multiple birth resulting from high-order embryo transfer, recent studies also suggest that singleton infants may be at increased risk for adverse outcome compared with naturally conceived singleton infants. A series of studies reported that singletons conceived through ART are at increased risk for low birth weight (LBW), very low birth weight (VLBW), preterm delivery, and fetal growth restriction.<sup>2–13</sup>

We recently reported increased risks for LBW and VLBW among singletons recorded in the U.S. population-based registry of ART procedures maintained by the Centers for Disease Control and Prevention (CDC). Our analysis included more than 18,000 infants conceived with ART in 1996 and 1997.<sup>2</sup> We found an increased risk for LBW and VLBW among ART singletons compared with the general U.S. population of singletons. The risks remained elevated after restricting analyses to subgroups conceived with presumably healthy gametes or carried by a presumably healthy woman, suggesting that the increased risks were, at least in part, due to a treatment effect.

Recent evidence from Finland suggests that the risk for LBW and preterm delivery among ART infants may



have declined in that country.<sup>3</sup> For singleton infants, adjusted odds ratios for the association between ART and LBW declined from 2.4 in 1991–1993 to 1.7 in 1998–1999. The authors suggested that this finding may have been related to improved antenatal care for women who conceived with ART.

Both ART treatment and patient care after treatment may have changed in the United States since our initial study of this issue. To determine whether the risk of LBW or preterm delivery among ART infants has declined in the United States, we have expanded on our earlier work. We examined secular trends from 1996 to 2000 for LBW, VLBW, preterm delivery, preterm LBW, and term LBW among singleton infants conceived with ART in the United States. We further examined the risks for each of these outcomes in greater detail among a subset of infants conceived in the most recent year, 2000.

## MATERIALS AND METHODS

Clinics and medical practices in the United States are mandated to report data for every ART procedure to the CDC (Fertility Clinic Success Rate and Certification Act of 1992 [FCSRCA], Public Law No. 102–493, October 24, 1992). *Assisted reproductive technology* is defined as infertility treatments in which both oocytes and sperm are handled outside the body. These include in vitro fertilization–transcervical embryo transfer (IVF-ET), gamete and zygote intrafallopian transfer (GIFT and ZIFT), frozen embryo transfer, and donor embryo transfer. Data abstracted from patient records and submitted to the CDC include patient demographics, medical history, and clinical information about the ART procedure and resultant pregnancies and births. Five to seven percent of clinics do not report data each year. Most of these are known to be small practices; thus, we estimate the data reported represent more than 95% of all ART procedures performed. For the present study, we selected ART procedures that were performed during the period 1996–2000 and resulted in a singleton live-birth delivery. The total study population included 62,551 infants, with a range of 9,078–16,422 per year.

We defined *LBW* and *VLBW* as 2,500 g or less and less than 1,500 g, respectively. We defined *preterm delivery* as gestational age less than 37 completed weeks. We calculated gestational age as date of birth minus date of oocyte retrieval (and fertilization). If date of retrieval was missing, and for all frozen embryo procedures, gestational age was calculated as date of birth minus date of embryo transfer. For comparability with the general population, we adjusted for date of theoretical last menstrual period (LMP) by adding 14 days to our gestational age estimate.

*Preterm LBW* was defined as gestational age less than 37 weeks and birth weight of 2,500 g or less. *Term LBW* was defined as gestational age 37 weeks or greater and birth weight of 2,500 g or less.

Data used to calculate gestational age were collected in a comparable manner in all years of the study. Data collection for birth weight underwent revision between the 1998 and 1999 data collection periods. From 1996 to 1998, birth weight was collected as a categorical variable in 500-g increments up to 2,500 g and an additional category of greater than 2,500 g. In 1999 and 2000, birth weight was collected as a continuous variable, with options for entering grams, pounds, and ounces, or ounces only. For this analysis, all birth weights collected in 1999 and 2000 were converted to grams. We considered the possibility that changing the variable from categorical to continuous might have impacted its validity over time. We examined data from a separate study underway in which birth certificate data were obtained for ART infants born to Massachusetts resident mothers in 1997–2000. Preliminary analyses suggest high (> 90%) concordance rates between singleton birth weights reported in the ART registry and those reported on the birth certificate in both early (1997–1998) and late (1999–2000) time periods (unpublished data).

We examined secular trends for LBW, VLBW, preterm, preterm LBW, and term LBW among all ART singleton infants reported to the registry for 1996 to 2000 and among subsets based on the type of ART procedure. Procedure type was classified as 1) transferred embryos created from the patient's oocytes fertilized during the current procedure (fresh, nondonor), 2) transferred embryos created using oocytes from a woman serving as an egg donor fertilized during the current procedure (fresh, donor), and 3) transferred embryos previously fertilized (using either patient or donor oocytes) and frozen until the current procedure (thawed). Within each group we calculated the relative change in each outcome from 1996 to 2000 and the  $\chi^2$  test for trend.

More detailed analyses of the risks for each outcome among a subset of the infants born after an ART treatment in 2000 were performed. These analyses included stratification on maternal and ART treatment characteristics, and comparison of observed numbers of adverse outcomes among ART singletons with expected numbers based on the general U.S. population of singleton births, with adjustment for potential confounding factors. For these analyses, our original study population was 16,422 infants from 380 clinics. We restricted the sample to ensure complete data for key outcome and adjustment factors and homogeneity in ART type. Because maternal race/ethnicity was an important adjustment factor but was incompletely reported in some clin-



ics, we first selected infants from clinics with less than 20% missing data for this variable (N = 9,145 infants from 294 clinics). We further limited the sample to infants conceived using fresh, nondonor embryos, IVF-ET (ie, procedures with transfer to the fallopian tubes were excluded), and gestation by the intended mother (ie, procedures using a gestational surrogate were excluded). This further restriction to obtain a more homogeneous subset of ART births reduced our sample to 6,709. Finally, we excluded infants with maternal age less than 20 or more than 44 years (n = 12), missing or out-of-range birth weight data (n = 23), missing or out-of-range gestational age data (n = 101), missing maternal parity (n = 4), and missing maternal race (n = 192). Our final ART study population was 6,377 infants.

For each of the 5 outcomes of interest, we assessed variations in risk by maternal, treatment, and pregnancy factors. Factors evaluated included maternal age, parity, race/ethnicity, parental infertility diagnosis, use of intracytoplasmic sperm injection (ICSI), use of assisted hatching, number of days in embryo culture, supernumerary embryos cryopreserved for future use (a marker for high embryo quality independent of embryos transferred), and number of fetal hearts observed on early ultrasound (ie, pregnancy plurality). Intracytoplasmic sperm injection, which is used often in male-factor infertility, involves injecting a single sperm directly into the oocyte. Assisted hatching includes various treatments in which chemicals, lasers, or mechanical means are used to create an opening in the zona pellucida of the embryo so that the implantation potential might be increased.

We compared the observed numbers for each outcome among infants in our final ART study population with expected numbers. Expected numbers were calculated using a referent population from the 2000 U.S. natality public use computer file.<sup>14</sup> From this file we selected singleton births with a maternal age between 20 and 44 years, maternal race/ethnicity of non-Hispanic white, non-Hispanic black, Hispanic, or Asian, and no missing data for parity. Maternal age, race/ethnicity, and parity were our primary adjustment factors. The restrictions to specific groups of age and race were made because of small samples in other categories for either the natality file (age above 44) or the ART study population (age below 20 and other race/ethnicity groups, such as Native American). To provide comparability in gestational age estimates between the referent population and the ART population, we also limited our selection of births from the natality file to those with maternal initiation of prenatal care in the first trimester and a gestational age estimate based on LMP. From this referent group of births, we computed rates for each of the 5 outcomes within strata based on maternal age, race/

ethnicity, and parity (48 strata in all). These rates were applied to the distribution of the ART study population on these same factors to compute expected numbers. We computed standardized risk ratios for each outcome and calculated 95% confidence intervals for each estimate.

Slight differences between the natality and ART registry files in data definitions, collection, and categorization for the 3 adjustment factors should be noted. Maternal age was based on age at delivery in the natality file and age at conception (ART treatment) in the ART registry. Parity was based on previous live births in the natality file and previous births (both live and stillbirths) in the ART registry. Maternal race and Hispanic origin were separately collected on birth certificates and then combined as a single variable. In the ART registry, race/ethnicity was collected as a single categorical variable. We do not believe these differences in collection and classification had an appreciable effect on the calculation of expected numbers.

We also calculated risk ratios for selected subsets of our ART study population. Because it is plausible that ART singletons were more likely than singletons in the general population to have been from pregnancies that originated as multiple gestations with subsequent reduction of one or more fetuses, we separately examined the subset of ART singletons with only one fetal heart documented in early pregnancy. We also examined subsets based on the 2 most common parental infertility diagnoses, tubal factor and male factor. For both of these subsets, we selected infants with only a single diagnosis reported. The tubal factor group was considered the most likely ART subset to have been conceived with healthy gametes (ie, no reports of ovarian abnormalities, diminished ovarian reserve, or male factor infertility). The male factor group was considered the most likely subset to have been gestated by a woman without an underlying infertility abnormality. Finally, we examined several subsets based on specific treatment characteristics to disentangle some of the heterogeneity in ART treatment. Accordingly, we separately evaluated infants conceived with IVF only, ie, without the more invasive ICSI and assisted hatching procedures. We also separately evaluated infants conceived in ICSI procedures (a group of special interest because of the rapid growth of this treatment), procedures with supernumerary embryos cryopreserved (an indication that the embryos transferred were deemed of good quality), procedures with embryo culture for 3 days (the current standard of care for embryo culture), and procedures with embryo culture for 5 days (extended culture to the blastocyst stage).

This study was approved by the Institutional Review Board at CDC.



**Table 1.** Adverse Perinatal Outcomes Among Liveborn Singleton Infants Conceived With Assisted Reproductive Technology, United States, 1996–2000

	1996	1997	1998	1999	2000	Relative change, 1996–2000 (%)	P for trend
Number of singleton infants							
Total	9,078	10,658	12,580	13,813	16,422	80.9	
Patient oocytes, freshly fertilized embryos	6,943	8,119	9,578	10,511	12,435	79.1	
Donor oocytes, freshly fertilized embryos	899	1,019	1,250	1,459	1,805	100.8	
Thawed embryos	1,236	1,520	1,752	1,843	2,182	76.5	
Percentage LBW							
Total	13.3	13.1	11.8	9.6	9.2	-31.3	< .001
Patient oocytes, freshly fertilized embryos	13.7	13.5	12.0	9.9	9.1	-33.8	< .001
Donor oocytes, freshly fertilized embryos	15.2	13.0	13.1	10.0	10.6	-30.3	< .001
Thawed embryos	9.7	11.2	9.8	7.6	8.4	-13.5	.004
Percentage VLBW							
Total	2.2	3.0	1.9	1.8	1.9	-12.1	< .001
Patient oocytes, freshly fertilized embryos	2.2	3.1	2.1	1.8	1.9	-15.5	< .001
Donor oocytes, freshly fertilized embryos	3.1	3.8	2.0	1.9	2.3	-26.5	.023
Thawed embryos	1.3	1.8	1.0	2.0	1.8	41.7	NS
Percentage preterm delivery							
Total	13.8	13.4	14.3	13.0	14.1	2.7	NS
Patient oocytes, freshly fertilized embryos	13.0	12.7	13.1	12.2	13.1	0.6	NS
Donor oocytes, freshly fertilized embryos	18.5	17.3	18.2	15.2	16.2	-12.5	NS
Thawed embryos	14.5	14.5	18.1	15.6	18.3	26.6	.002
Percentage preterm LBW							
Total	6.6	6.6	6.8	6.3	6.7	1.1	NS
Patient oocytes, freshly fertilized embryos	6.7	6.5	6.7	6.2	6.4	-4.1	NS
Donor oocytes, freshly fertilized embryos	8.8	8.1	8.9	7.5	8.2	-6.3	NS
Thawed embryos	4.4	6.2	6.1	5.8	6.8	52.9	.040
Percentage term LBW							
Total	6.6	6.2	4.9	3.3	2.4	-64.0	< .001
Patient oocytes, freshly fertilized embryos	6.9	6.7	5.2	3.7	2.5	-63.3	< .001
Donor oocytes, freshly fertilized embryos	6.3	5.0	4.1	2.6	2.4	-61.9	< .001
Thawed embryos	5.1	4.6	3.6	1.6	1.5	-71.4	< .001

LBW = low birth weight; VLBW = very low birth weight; and NS = not significant.

Samples for calculation of percentages of outcomes were reduced from totals because of missing values for birth weight and gestational age. Percentage of observations missing data for birth weight ranged from 8% in 1996 to 1% in 2000; percentage of observations missing data for gestational age was 1–2% in all years.

## RESULTS

The number of singleton infants reported to the ART registry increased each year from 1996 to 2000; the total increase was 81% (Table 1). Increases were observed among all procedure types. The rise in ART singletons paralleled increases in total ART procedures performed and associated live-birth deliveries during the same time period (data not shown). The proportion of singletons born LBW and term LBW declined for all ART types (Table 1). Overall, LBW declined 31%, from 13.3% to 9.2%, and term LBW declined 64%, from 6.6% to 2.4%. The percent VLBW declined 12% overall, although one ART type (thawed embryos) had a nonsignificant 42% increase. There was little change in the proportion of singletons born preterm (13.8% versus 14.1% in 1996 and 2000, respectively) or both preterm and LBW (6.6% versus 6.7%), except for the subset conceived with thawed embryos. For this group, the trends were similar to that observed with VLBW.

Singleton infants in our final ART study population selected for in-depth analysis ( $n = 6,377$ ) were comparable with the total population of singleton infants reported from fresh, nondonor ART procedures in 2000 on maternal and treatment variables (Table 2). For the vast majority of infants in both groups, maternal age was 30 to 39 years, there were no prior births, and maternal race was non-Hispanic white. The most common single infertility conditions were tubal factor and male factor; additionally multiple diagnoses were commonly reported. For over half of the infants, ICSI had been used for fertilization, and approximately 40% were conceived from treatments involving assisted hatching of transferred embryos. For about 70%, embryos were cultured for 3 days before transfer. Supernumerary embryos were available and cryopreserved in about one third of the procedures from which these infants were conceived. For over 97% (data not shown), 2 or more embryos had been transferred; however, only 9% had more than 1



**Table 2.** Percentage Distribution of Study Population by Maternal Characteristics and Treatment Factors

Factor	All singletons from ART in 2000 using freshly fertilized embryos from patient oocytes (N = 12,435)	Singletons included in final study population (N = 6,377)
Maternal age (y)		
< 30	14.9	16.7
30–34	39.7	39.4
35–39	36.4	35.7
40–44	8.9	8.1
≥ 45	0.1	0.0
Parity		
0	73.6	73.4
1	19.8	19.0
≥ 2	6.6	7.6
Maternal race/ethnicity		
Non-Hispanic white	86.3	86.2
Non-Hispanic black	3.5	3.6
Hispanic	5.8	6.1
Asian	4.5	4.2
Missing*	n = 4,629	n = 0
Parental infertility diagnosis <sup>†</sup>		
Tubal factor	16.3	17.4
Ovulation disorder	5.6	6.0
Diminished ovarian reserve	2.3	2.2
Endometriosis	8.4	8.9
Uterine factor	1.2	0.9
Male factor	21.6	20.4
Other factor	5.7	4.4
Unknown/idiopathic	11.5	9.6
Multiple female factors	11.2	12.6
Female and male factors	16.2	17.8
Use of ICSI	52.9	53.1
Use of assisted hatching	39.0	40.6
Days in embryo culture		
1	0.5	0.1
2	4.7	4.9
3	71.2	70.0
4	2.5	2.9
5	18.6	19.4
6	2.4	2.8
Supernumerary embryos cryopreserved	35.9	35.4
Fetal hearts in early pregnancy		
1	91.1	90.8
2	7.8	8.1
3+	1.0	1.1

ART = assisted reproductive technology; ICSI = intracytoplasmic sperm injection.

\* Other than race/ethnicity, percentage of missing values for all variables was either 0% or < 3%.

<sup>†</sup> Other infertility diagnoses include serious illness, cancer chemotherapy, and immunologic factors.

fetal heart in early pregnancy. The risks for the perinatal outcomes of interest were also comparable between the total population of singletons conceived in fresh, nondonor ART in 2000 (Table 1) and our final study population (Table 3).

In comparison with the singletons in the referent population, ART singletons had increased risks for all 5 perinatal outcomes (Table 3). We observed some variation in risk across ART infant subgroups based on maternal and treatment characteristics; however, risks in specific subgroups were nonetheless increased in comparison with the referent population. In general, there was little variation in risk of any outcome according to maternal age. Lower risks were observed with a parity of 1 and a maternal race/ethnicity of non-Hispanic white. The variation by parity and race is in keeping with known variation in the general U.S. population and was, thus, also observed in the referent population (data not shown).

Among ART infants significant variations in risks of preterm delivery and preterm LBW were also observed according to parental infertility diagnosis; the lowest risks were observed in the male-factor subgroup (Table 3). In keeping with these results, infants from ICSI procedures had decreased risks for all outcomes but term LBW. Infants from procedures with presumed high-quality embryos (ie, supernumerary embryos were cryopreserved) had an increased risk for LBW. Infants from pregnancies with more than 1 fetal heart had substantially higher risks for all outcomes, although the difference did not reach statistical significance for VLBW.

After adjustment for maternal age, parity, and race/ethnicity, infants from the ART study population had significantly elevated risks for all outcomes—LBW, VLBW, preterm delivery, preterm LBW, and term LBW (Table 4). Risk ratios for each of these 5 outcomes remained elevated when the analysis was restricted to infants with only 1 fetal heart in early pregnancy, parental infertility diagnosis of male factor, parental infertility diagnosis of tubal factor, conception using IVF with no ICSI or assisted hatching, conception with ICSI, conception in a procedure with supernumerary embryos cryopreserved, embryo culture for 3 days, and embryo culture for 5 days. However, in 5 (of 45) subsets with smaller sample sizes, confidence intervals overlapped 1.0.

## DISCUSSION

We previously reported that singletons conceived with ART in 1996 and 1997 had a greater than 2-fold increase in term LBW compared with the expected rate adjusted for age and parity.<sup>2</sup> The present study demonstrates that, by 2000, the absolute risk for term LBW among



**Table 3.** Risks for Adverse Perinatal Outcomes Among Singleton Infants in Referent and Final ART Study Population by Maternal and ART Treatment Factors

	LBW (%)	VLBW (%)	Preterm delivery (%)	Preterm LBW (%)	Term LBW (%)
Referent population	5.1	0.9	9.0	3.2	1.9
Final ART study population	9.4	1.7	13.1	6.6	2.8
Maternal age (y)					
< 30	10.0	1.5	14.8	6.8	3.2
30–34	9.0	1.8	12.4	6.2	2.8
35–39	9.3	1.6	12.5	6.5	2.8
40–44	10.2	2.5	15.4	8.3	1.9
Parity					
0	10.1*	1.8	13.4	7.1*	3.0
1	6.2	1.2	11.3	4.1	2.1
≥ 2	10.5	2.3	14.4	7.6	2.9
Maternal race/ethnicity					
Non-Hispanic white	8.6*	1.4*	12.7*	6.1*	2.5 <sup>†</sup>
Non-Hispanic black	19.7	7.9	21.9	15.4	4.4
Hispanic	12.4	2.8	13.7	7.5	4.9
Asian	12.0	... <sup>‡</sup>	12.8	8.3	3.8
Parental infertility diagnosis <sup>§</sup>					
Tubal factor	10.8	2.3	14.4 <sup>†</sup>	8.1 <sup>†</sup>	2.7
Ovulation disorder	8.9	... <sup>‡</sup>	14.4	6.0	2.9
Diminished ovarian reserve	8.7	... <sup>‡</sup>	15.2	... <sup>‡</sup>	... <sup>‡</sup>
Endometriosis	8.4	... <sup>‡</sup>	11.8	6.0	2.5
Uterine factor	... <sup>‡</sup>	... <sup>‡</sup>	19.3	... <sup>‡</sup>	... <sup>‡</sup>
Male factor	8.0	1.4	10.6	4.8	3.2
Other factor	10.0	... <sup>‡</sup>	15.4	8.6	... <sup>‡</sup>
Unknown/Idiopathic	8.1	... <sup>‡</sup>	12.0	5.4	2.6
Multiple female factors	10.6	2.3	15.3	8.0	2.6
Female and male factors	9.7	1.7	12.7	6.9	2.8
Use of ICSI					
No	10.3 <sup>†</sup>	2.1 <sup>†</sup>	14.9*	7.8*	2.5
Yes	8.6	1.4	11.5	5.6	3.0
Use of assisted hatching					
No	9.7	1.6	13.1	6.8	2.9
Yes	9.0	1.9	13.1	6.3	2.7
Days in embryo culture					
2	9.1	... <sup>‡</sup>	12.3	6.2	... <sup>‡</sup>
3	9.0	1.7	12.7	6.3	2.7
4	13.5	... <sup>‡</sup>	15.7	8.1	5.4
5	10.4	2.1	14.0	7.6	2.8
6	9.2	... <sup>‡</sup>	16.1	6.3	... <sup>‡</sup>
Supernumerary embryos cryopreserved					
No	8.7 <sup>†</sup>	1.6	12.6	6.2	2.6
Yes	10.4	2.0	14.0	7.3	3.2
Fetal hearts in early pregnancy					
1	8.4*	1.5	12.4*	5.9*	2.6*
2	16.7	2.7	18.9	12.7	4.1
3+	30.6	... <sup>‡</sup>	27.3	22.2	... <sup>‡</sup>

ART = assisted reproductive technology; LBW = low birth weight; VLBW = very low birth weight; and ICSI = intracytoplasmic sperm injection  
 \*  $P < .01$ .

<sup>†</sup> Chi-squared  $P < .05$  for variability in risk by maternal or ART treatment factors.

<sup>‡</sup> Risk for outcome not provided if number of observed cases in a given subgroup  $< 10$ .

<sup>§</sup> Other infertility diagnoses include serious illness, cancer chemotherapy, and immunologic factors.

ART singletons had declined by 64%. Moreover, although the risk for term LBW was still significantly increased above expected rates, the magnitude of the increase was less than that previously reported. In 2000, term LBW was moderately increased (40%) among

ART singletons compared with the expected rate adjusted for age, parity, and race/ethnicity.

The reasons for the decline in term LBW are unclear. A similar trend was observed for all ART types. Moreover, detailed analyses of the 2000 data do not reveal any subset



**Table 4.** Observed and Expected Outcomes Among Liveborn Singleton Infants Conceived With ART, 2000

Outcome	Total N	Observed cases	Expected cases*	Standardized risk ratio (95% confidence interval)†
<b>LBW</b>				
Total	6,377	598	369.46	1.62 (1.49, 1.75)
Limit to 1 fetal heart	5,764	488	333.08	1.47 (1.34, 1.60)
Limit to male factor	1,301	104	73.46	1.42 (1.14, 1.69)
Limit to tubal factor	1,109	120	63.00	1.90 (1.56, 2.25)
Limit to no ICSI, no assisted hatching	2,030	212	115.20	1.84 (1.59, 2.09)
Limit to ICSI	3,387	291	196.54	1.48 (1.31, 1.65)
Limit to supernumerary embryos cryopreserved	2,255	236	126.04	1.87 (1.63, 2.11)
Limit to embryo culture for 3 days	4,432	398	259.20	1.54 (1.38, 1.69)
Limit to embryo culture for 5 days	1,227	127	68.77	1.85 (1.53, 2.17)
<b>VLBW</b>				
Total	6,377	110	61.60	1.79 (1.45, 2.12)
Limit to 1 fetal heart	5,764	88	55.47	1.58 (1.26, 1.92)
Limit to male factor	1,301	18	12.01	1.50 (0.81, 2.19)
Limit to tubal factor	1,109	25	11.02	2.27 (1.38, 3.16)
Limit to no ICSI, no assisted hatching	2,030	39	19.31	2.02 (1.39, 2.65)
Limit to ICSI	3,387	48	32.60	1.47 (1.06, 1.89)
Limit to supernumerary embryos cryopreserved	2,255	46	21.05	2.19 (1.55, 2.82)
Limit to embryo culture for 3 days	4,432	74	43.18	1.71 (1.32, 2.10)
Limit to embryo culture for 5 days	1,227	26	11.44	2.27 (1.40, 3.15)
<b>Preterm delivery</b>				
Total	6,377	834	590.94	1.41 (1.32, 1.51)
Limit to 1 fetal heart	5,764	715	533.15	1.34 (1.24, 1.44)
Limit to male factor	1,301	138	117.87	1.17 (0.98, 1.37)
Limit to tubal factor	1,109	160	103.72	1.54 (1.30, 1.78)
Limit to no ICSI, no assisted hatching	2,030	291	186.32	1.56 (1.38, 1.74)
Limit to ICSI	3,387	388	312.74	1.24 (1.12, 1.36)
Limit to supernumerary embryos cryopreserved	2,255	315	204.15	1.54 (1.37, 1.71)
Limit to embryo culture for 3 days	4,432	561	413.30	1.36 (1.25, 1.47)
Limit to embryo culture for 5 days	1,227	172	111.36	1.54 (1.31, 1.78)
<b>Preterm LBW</b>				
Total	6,377	421	242.53	1.74 (1.57, 1.90)
Limit to 1 fetal heart	5,764	340	218.58	1.56 (1.39, 1.72)
Limit to male factor	1,301	62	48.10	1.29 (0.97, 1.61)
Limit to tubal factor	1,109	90	41.24	2.18 (1.73, 2.63)
Limit to no ICSI, no assisted hatching	2,030	158	75.51	2.09 (1.77, 2.42)
Limit to ICSI	3,387	188	128.95	1.46 (1.25, 1.67)
Limit to supernumerary embryos cryopreserved	2,255	164	82.44	1.99 (1.68, 2.29)
Limit to embryo culture for 3 days	4,432	279	170.29	1.64 (1.45, 1.83)
Limit to embryo culture for 5 days	1,227	93	45.15	2.06 (1.64, 2.48)
<b>Term LBW</b>				
Total	6,377	177	127.32	1.39 (1.19, 1.59)
Limit to 1 fetal heart	5,764	148	114.87	1.29 (1.08, 1.50)
Limit to male factor	1,301	42	25.40	1.65 (1.15, 2.15)
Limit to tubal factor	1,109	30	21.81	1.38 (0.88, 1.87)
Limit to no ICSI, no assisted hatching	2,030	54	39.79	1.36 (1.00, 1.72)
Limit to ICSI	3,387	103	67.74	1.52 (1.23, 1.81)
Limit to supernumerary embryos cryopreserved	2,255	72	43.57	1.65 (1.27, 2.03)
Limit to embryo culture for 3 days	4,432	119	89.26	1.33 (1.09, 1.57)
Limit to embryo culture for 5 days	1,227	34	23.66	1.44 (0.95, 1.92)

ART = assisted reproductive technology; LBW = low birth weight; ICSI = intracytoplasmic sperm injection; and VLBW = very low birth weight.

\* Expected numbers based on rates among singleton infants included in the 2000 U.S. natality file with maternal age 20–44 years, maternal race non-Hispanic white, non-Hispanic black, Hispanic, or Asian, complete data for parity, maternal initiation of prenatal care in first trimester, and gestational age estimate based on last menstrual period.

† Risk ratios adjusted for maternal age, parity, and race/ethnicity.



based on patient or treatment characteristics that approach the high rate of term LBW (6.6%) observed in 1996. Thus, the available data do not illuminate a particular change in either the patient population undergoing ART or the specific aspects of this population's treatment that are responsible for the change in risk. However, the ART registry captures only limited patient and treatment variables. For example, the ART registry does not include data on some maternal characteristics that are strongly associated with fetal growth, such as maternal body mass index and weight gain.<sup>15</sup> Additionally, several changes in ART treatment have occurred over the time period of interest that we were not able to evaluate. One example is the increased focus on techniques such as ultrasound-guided embryo transfer. Several randomized studies have demonstrated that ultrasound-guided transfer results in increased implantation and pregnancy rates.<sup>16</sup> It is conceivable that more optimal embryo placement might lead to a more optimal site for implantation and placentation, which could lead to improvements in fetal growth. Previous studies have demonstrated that placentas from ART pregnancies had increased pathologic features compared with naturally conceived pregnancies.<sup>17,18</sup> Finally, the trend might in part reflect a change in obstetric practice, such as closer monitoring and intervention before term for pregnancies with an indication of fetal growth restriction. A recent Canadian study suggests this may be related to the increase in average birth weight among term infants in that country.<sup>19</sup>

Although term LBW declined, the risk for preterm LBW was stable and remained increased in comparison with expected rates. The greater risks for LBW and preterm delivery among singletons conceived with ART have been hypothesized to be related to the underlying infertility among women using ART.<sup>20</sup> Our results of slightly reduced risks for the subset of infants born to couples with male factor as the only infertility diagnosis suggest that part of the association between ART and preterm delivery might be explained by maternal infertility. However, risks in the male-factor subset were still elevated in comparison with the general population of singletons born to nonteenaged mothers in the United States, which served as our referent population. Thus, it appears that part, but not all, of the elevated preterm risk is explained by the underlying infertility, and part remains unexplained and may be related to a treatment effect. As in other analyses, the risk ratios in the male-factor subset were most pronounced for the most restrictive preterm outcomes, preterm LBW and VLBW, rather than for the more general category of preterm delivery (which included many normal birth weight [50%] infants).

We were unable in this analysis to identify a specific treatment factor that may underlie the preterm risk.

Infants conceived with freshly fertilized embryos, thawed embryos, maternal oocytes, donor oocytes, ICSI, IVF without ICSI, 3 days in embryo culture, and 5 days in embryo culture were all at increased risk for preterm delivery, preterm LBW, and VLBW. It is possible that the risk is related to some aspect of treatment common to all or most ART procedures. ART pregnancies may be different from naturally conceived pregnancies in several ways. Ovarian hyperstimulation and administration of hCG and/or progesterone for luteal support may result in nonphysiological levels of estrogen, progesterone, and relaxin, which in turn may have effects on endometrial and cervical tissues and placentation and/or may impair embryo-endometrial synchronization.<sup>21-26</sup> The *in vitro* environment in which embryos develop can affect various embryo parameters<sup>27</sup> and might also impact subsequent *in vivo* development of the embryo and fetus.

It is feasible that part of the preterm risk in ART singletons is related to maternal exposures such as cigarette smoking, maternal stress, subclinical pelvic infection, deficiencies in micronutrients such as folate, or environmental exposures such as pesticides. To explain the excess preterm risk associated with ART, such a factor would need to be increased, not only among women with a diagnosis of infertility, but also among women who sought ART because their partners had male-factor infertility. This is plausible with many of the above factors; however, the data are not collected in the ART registry.

Finally, the possibility exists that increased monitoring and intervention among ART pregnancies may lead to a higher rate of medically indicated preterm deliveries and that these births are accounting for the preterm excess. We cannot evaluate this hypothesis directly because the ART registry does not capture detail on type of preterm birth, induction or cesarean delivery. However, as noted above, the increase in preterm risk among ART singletons is not solely attributable to moderately preterm births but is actually greatest among very preterm infants.

This study had many strengths, most notably the large sample that allowed us to adjust for important potential confounders and to examine specific infertility and treatment subsets. This study also must be interpreted in the context of certain limitations. We lacked data needed to explore specific mechanisms for the effects observed, including data on maternal progesterone, estrogen and relaxin levels, embryo culture constituents, embryo quality grade, maternal exposures associated with preterm delivery, and type of preterm delivery. Because the ART registry data set is based on ART procedures performed each year and patients undergoing more than one procedure cannot be linked, our evaluation of secular trends may not



be based on completely independent samples of births across years.

This study provides evidence that, from 1996 to 2000, there was little change in the risk for preterm LBW among singletons born after assisted reproductive technology; however, term LBW declined. Nonetheless, ART singletons had greater than expected risks for all adverse perinatal outcomes evaluated, including term LBW. Further study of the mechanisms for these effects may help to clarify the exact roles of the underlying infertility, other maternal-fetal exposures, obstetric practice, and direct effects stemming from the ART treatment, which are responsible for this risk.

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ences versus expectations.<sup>8-10</sup> As self assessments continue to play a central role in the measurement of health outcomes, including vignettes in national surveys and clinical research can improve the use of self reports by confronting important problems of interpersonal comparability.

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## Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies

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### Abstract

**Objective** To compare the perinatal outcome of singleton and twin pregnancies between natural and assisted conceptions.

**Design** Systematic review of controlled studies published 1985-2002.

**Studies reviewed** 25 studies were included of which 17 had matched and 8 had non-matched controls.

**Main outcome measures** Very preterm birth, preterm birth, very low birth weight, low birth weight, small for gestational age, caesarean section, admission to neonatal intensive care unit, and perinatal mortality.

**Results** For singletons, studies with matched controls indicated a relative risk of 3.27 (95% confidence interval 2.03 to 5.28) for very preterm (<32 weeks) and 2.04 (1.80 to 2.32) for preterm (<37 weeks) birth in pregnancies after assisted conception. Relative risks were 3.00 (2.07 to 4.36) for very low birth weight (<1500 g), 1.70 (1.50 to 1.92) for low birth weight (<2500 g), 1.40 (1.15 to 1.71) for small for gestational age, 1.54 (1.44 to 1.66) for caesarean section, 1.27 (1.16 to 1.40) for admission to a neonatal intensive care unit, and 1.68 (1.11 to 2.55) for perinatal mortality. Results of the non-matched studies were similar. In matched studies of twin gestations, relative risks were 0.95 (0.78 to 1.15) for very preterm birth, 1.07 (1.02 to 1.13) for preterm birth, 0.89 (0.74 to 1.07) for very low birth weight, 1.03 (0.99 to 1.08) for low birth weight, 1.27 (0.97 to 1.65) for small for gestational age, 1.21 (1.11 to 1.32) for caesarean

section, 1.05 (1.01 to 1.09) for admission to a neonatal intensive care unit, and 0.58 (0.44 to 0.77) for perinatal mortality. The non-matched studies mostly showed similar trends.

**Conclusions** Singleton pregnancies from assisted reproduction have a significantly worse perinatal outcome than non-assisted singleton pregnancies, but this is less so for twin pregnancies. In twin pregnancies, perinatal mortality is about 40% lower after assisted compared with natural conception.

### Introduction

There is a widespread belief that pregnancy outcome is substantially worse after assisted than after natural conception.<sup>1-3</sup> The difference, however, relates predominantly to the higher frequency of multiple pregnancies.<sup>3</sup> The first indication that assisted singleton pregnancies may also have poorer outcomes appeared in 1985,<sup>2</sup> but it was not clear how much related to assisted reproduction or to confounders, such as maternal age and parity. Several matched cohort studies have since confirmed these findings.<sup>1-4-8</sup>

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**P+** Nine tables of detailed results and a list of excluded studies can be found on [bmj.com](http://bmj.com)

**ELPS** This is the abridged version of an article that was posted on [bmj.com](http://bmj.com) on 23 January 2004: <http://bmj.com/cgi/doi/10.1136/bmj.37957.560278.EE>

**Table 1** Summary of risk of various outcomes in singleton and twin pregnancies after assisted conception compared with those conceived naturally. Figures are relative risk (95% confidence intervals)

Outcome	Singleton births		Twin births	
	Matched studies	Non-matched studies	Matched studies	Non-matched studies
Gestational age (weeks):				
<32	3.27 (2.03 to 5.28)	Not tested	0.95 (0.78 to 1.15)	1.20 (0.82 to 1.78)
32-36	2.05 (1.71 to 2.47)	Not tested	1.07 (1.00 to 1.14)	0.88 (0.66 to 1.17)
>37	2.04 (1.80 to 2.32)	1.94 (1.31 to 2.88)	1.07 (1.02 to 1.13)	0.99 (0.80 to 1.23)
Birth weight (g):				
<1500	3.00 (2.07 to 4.36)	1.57 (0.21 to 11.7)	0.89 (0.74 to 1.07)	1.46 (1.01 to 2.11)
1500-2499	1.54 (1.30 to 1.82)	3.28 (2.04 to 5.27)	1.02 (0.97 to 1.08)	1.05 (0.96 to 1.15)
>2500	1.70 (1.50 to 1.92)	2.58 (1.80 to 3.68)	1.03 (0.99 to 1.08)	1.12 (1.06 to 1.19)
Small for gestational age	1.40 (1.15 to 1.71)	1.46 (0.98 to 2.15)	1.27 (0.97 to 1.65)	0.93 (0.73 to 1.18)
Caesarean section	1.54 (1.44 to 1.66)	2.33 (1.95 to 2.79)	1.21 (1.11 to 1.32)	1.17 (1.06 to 1.29)
Neonatal intensive care unit	1.27 (1.16 to 1.40)	1.38 (0.67 to 2.86)	1.05 (1.01 to 1.09)	1.26 (1.16 to 1.36)
Perinatal mortality	1.68 (1.11 to 2.55)	3.77 (1.15 to 12.4)	0.58 (0.44 to 0.77)	0.84 (0.53 to 1.32)

Some studies found an opposite trend,<sup>9, 10</sup> while most reported differences that were compatible with chance. Moreover, for twin pregnancies the general consensus, with few exceptions,<sup>11-13</sup> seems to be that assisted twin pregnancies have outcomes that are either similar to or slightly better than those conceived naturally.<sup>1, 9, 14-17</sup>

We identified all published studies on birth outcomes after assisted conception that distinguished singleton from multiple pregnancies and that incorporated an appropriate control group from the same population. We examined whether there are genuine differences in outcome between assisted and natural

conceptions and whether they apply to both singleton and twin pregnancies.

## Methods

We searched Medline, Embase, LILACS, and POP-LINE for 1985-2002. This search was supplemented with the references of the articles, review articles, and theses.

We selected reports with categorical data on any of the following outcomes: gestational age and weight at birth, caesarean section, perinatal death, and admission to neonatal intensive care. Studies without a control group of natural conceptions or that did not distinguish singleton from multiple pregnancies were excluded.

International definitions were followed for preterm (<37 weeks), very preterm (<32 weeks), low birth weight (<2500 g), very low birth weight (<1500 g), small for gestational age (birth weight <10th centile for gestation), and perinatal mortality (stillbirths and deaths in first week  $\geq 500$  g per 1000 total births  $\geq 500$  g). We used Review Manager (Update Software, Oxford) to calculate relative risks.

## Results

Included studies are listed and described in tables A and B on www.bmj.com. Seventeen (14 matched and three non-matched) dealt with singleton pregnancies and 17 (10 matched and seven non-matched) with twin pregnancies.

Table 1 summarises relative risks of the outcomes in singleton and twin pregnancies after assisted and natural conception. Analyses from individual studies for perinatal mortality are given in table 2. Analyses from individual studies for all other outcomes are given in web tables (see www.bmj.com). The website also lists the excluded studies with reasons for exclusion.

### Preterm birth

Very preterm singletons (<32 weeks) were reported in only three studies with a prevalence of 1.3-2.1% in assisted conceptions and 0.3-2.9% in natural conceptions, a relative risk of 3.27 (95% confidence interval 2.03 to 5.28) (see the full version of this paper on bmj.com).<sup>1, 9, 19</sup> Mildly preterm singletons (32-36 weeks) accounted for 6.5-9.2% and 3.8-7.6%, respectively, (2.05, 1.71 to 2.47) (see web table C).<sup>1, 9, 19</sup> Preterm singletons (<37 weeks) accounted for 5.8-15% and 1.4-10.5%, respectively (see bmj.com). The relative risk in both the 12 matched<sup>1, 4-10, 12, 19, 21, 22</sup> and two non-matched<sup>23, 25</sup> studies showed a doubling of the risk of preterm birth after assisted conception.

Very preterm twins were reported in three matched studies<sup>1, 9, 19</sup> (see bmj.com) and two non-matched<sup>17, 26</sup> studies. After we excluded one study that reported live infants only,<sup>19</sup> the frequency range was 7.0-10.5% in assisted conceptions and 4.9-10.7% in natural conceptions and was not statistically different (see web table D). Mildly preterm twins accounted for 41.7-45.2% of cases and 33.0-40.5% of controls in the matched studies (1.07, 1.00 to 1.14).<sup>1, 9, 19</sup> Preterm twins differed widely in frequency from 18.8-60.0% and 20.0-52.4%, respectively. The relative risk was 1.07 (1.02 to 1.13) in the nine matched studies<sup>1, 4, 9-13, 19, 22</sup> (see bmj.com) and

**Table 2** Perinatal mortality in singleton and twin pregnancies after assisted conception compared with natural conception

Study	No (%) assisted	No (%) natural	Relative risk (95% CI)
<b>Singletons</b>			
Matched singleton studies:			
Dhont et al <sup>1*</sup>	41/3048 (13.5)	18/3048 (5.9)	2.28 (1.31 to 3.96)*
Dhont et al <sup>9</sup>	2/311 (6.4)	10/622 (16.1)	0.40 (0.09 to 1.81)
Isaksson et al <sup>10</sup>	1/69 (14.5)	5/345 (14.5)	1.00 (0.12 to 8.43)
Koudstaal et al <sup>8</sup>	3/307 (9.8)	1/307 (3.3)	3.00 (0.31 to 28.7)
Nuojua et al <sup>21</sup>	1/92 (10.9)	2/276 (7.2)	1.50 (0.14 to 16.4)
Reubinoff et al <sup>7</sup>	2/260 (7.7)	1/260 (3.8)	2.00 (0.18 to 21.9)
Tanbo et al <sup>5</sup>	4/355 (11.3)	6/643 (9.3)	1.21 (0.34 to 4.25)
Verlaenen et al <sup>6</sup>	3/140 (21.4)	2/140 (14.3)	1.50 (0.25 to 8.84)
Total	57/4582 (12.4)	45/5641 (8.0)	1.68 (1.11 to 2.55)
Non-matched singleton studies:			
Olivennes et al <sup>25†</sup>	3/162 (18.5)	25/5096 (4.9)	3.77 (1.15 to 12.4)
<b>Twins</b>			
Matched twin studies:			
Dhont et al <sup>1*</sup>	61/2482 (24.6)	82/2482 (33.0)	0.74 (0.54 to 1.03)*
Dhont et al <sup>9</sup>	0/230	6/230 (26.1)	0.08 (0.00 to 1.36)
Fitzsimmons et al <sup>16</sup>	4/112 (35.7)	48/216 (222.2)	0.16 (0.06 to 0.43)
Isaksson et al <sup>10</sup>	0/40	6/200 (30.0)	0.38 (0.02 to 6.56)
Koudstaal et al <sup>13</sup>	3/192 (15.6)	1/192 (5.2)	3.00 (0.31 to 28.6)
Tallo et al <sup>12</sup>	4/72 (55.6)	4/72 (55.6)	1.00 (0.26 to 3.85)
Total	72/3128 (23.0)	147/3392 (43.3)	0.58 (0.44 to 0.77)
Non-matched twin studies:			
Agustsson et al <sup>14</sup>	2/138 (14.5)	16/906 (17.6)	0.82 (0.19 to 3.53)
Lambalk et al <sup>28</sup>	18/1158 (15.5)	16/884 (18.1)	0.86 (0.44 to 1.67)
Olivennes et al <sup>17</sup>	10/144 (69.4)	28/328 (85.4)	0.81 (0.41 to 1.63)
Total	30/1440 (20.8)	60/2118 (28.3)	0.84 (0.53 to 1.32)

\*Early neonatal deaths in this paper are erroneously labelled as early fetal deaths, but they are included in perinatal deaths.

†Two cases lost to follow up.

0.99 (0.80 to 1.23) in the two non-matched studies (see web table D).<sup>17 23</sup>

#### Birth weight

Singletons <1500 g were reported for six matched studies<sup>1 5 6 9 10 19</sup> and one non-matched study.<sup>25</sup> Frequencies in the matched studies were 1.5-3.9% for assisted conceptions and 0.3-2.7% for natural conceptions with a relative risk of 3.00 (2.07 to 4.36) (see web table E). Singletons <2500 g were more common among cases than among controls in both matched ( $n=12$ )<sup>1 4-10 12 19 21 22</sup> and non-matched ( $n=2$ )<sup>23 25</sup> studies. Percentages of low birth weight were 2.9-15.7% in cases, 0-11.5% in matched controls, and 3.6-4.8% in non-matched controls (see web table E).

Twins <1500 g accounted for 5.0-25.0% of cases and 3.8-10.4% of controls (omitting one study reporting live infants only).<sup>19</sup> The relative risk was 0.89 (0.74 to 1.07) for the five matched<sup>1 9-11 19</sup> and 1.46 (1.01 to 2.11) for the two non-matched studies (see web table F).<sup>17 27</sup> Twins <2500 g accounted for 37.5-70.6% and 50.0-98.6% of cases versus 38.1-58.8% and 52.5-94.5% of controls, with relative risks of 1.03 (0.99 to 1.08) and 1.12 (1.06 to 1.19), respectively, in the eight matched studies<sup>1 4 9-13 19 22</sup> and the four non-matched studies.<sup>17 23 26 27</sup>

#### Small for gestational age

The 12 studies that reported on infants who were small for gestational age applied various reference charts. The frequency in singleton cases and controls was 1.6-16.3% versus 1.6-13.1% with a relative risk of 1.40 and 1.46, respectively, for the six matched<sup>4 6-8 12 20</sup> and two non-matched studies (see web table G).<sup>23 25</sup> The four matched<sup>1 11-13</sup> and three non-matched<sup>17 23 26</sup> twin studies showed no significant difference between assisted and natural conceptions.

#### Caesarean section

Rates of caesarean section were significantly higher after assisted than after natural conception (see web table H). The effect was more marked for singleton than for twin pregnancies in both matched<sup>1 4-8 10 11 13 18 20 21</sup> and non-matched studies.<sup>14 17 24-27</sup>

#### NICU admissions

Admissions to neonatal intensive care were more common after assisted conception in both matched and non-matched studies, and the difference was larger for singletons<sup>1 5-7 9 10 19 21 23</sup> than for twins (see web table I).<sup>1 9-11 14 19 23 26 27</sup>

#### Perinatal mortality

Perinatal mortality differed widely among studies (table 2). In singleton pregnancies it was significantly higher after assisted than after natural conception in both matched and non-matched studies. All of the difference in the matched studies was accounted for by the study of Dhont et al in 1999, which contributed 67% of the cases.<sup>1</sup> Without this study mortality was 10.4 per 1000 for both cases and controls.

Matched twin studies were also dominated by the same study, which contributed 78% of the cases,<sup>1</sup> and by another with an extraordinarily high mortality among controls.<sup>16</sup> However, most twin studies showed a lower mortality after assisted than after natural conception, with a relative risk of 0.58 (0.44 to 0.77) for matched and 0.84 (0.53 to 1.32) for non-matched studies (table 2).

## Discussion

### Bias and confounding

Though assisted conception has had many successes, it seems that resulting singleton pregnancies have a worse outcome at birth compared with naturally conceived singleton pregnancies. Women with assisted pregnancies differ from other women in many characteristics that influence outcome, including age, parity, and socioeconomic status,<sup>1 2 12</sup> while subfertility itself also contributes to the difference.<sup>29</sup> We therefore subdivided studies into those with matched and those with non-matched, population specific controls and placed greater emphasis on the former. These virtually all matched for prominent confounders, such as age and parity, but they varied widely in controlling for other known confounders, such as socioeconomic status, smoking, and pre-existing disease. Although none controlled for all factors that might be important, they are likely to estimate true differences between assisted and natural conceptions better than the population based studies.

Nevertheless, our study uncovered major limitations of the matched cohort approach to differences in perinatal outcome between assisted and natural conceptions. Our summary results are largely dominated by a matched cohort study from Flanders, which contributed 54% of the cases in the singleton studies and 68% in the twin studies.<sup>1</sup> Its authors used three different control groups of singletons to match for various combinations of characteristics.<sup>1</sup> This led to disparate comparison groups, with perinatal mortality, for example, being 5.2 per 1000 in controls matched for maternal age and infant sex and 12.1 per 1000 in those matched also for parity and gestational age. The validity of matching for gestational age is questionable because gestational age is clearly influenced by assisted conception and affects other outcomes, such as birth weight and mortality. We therefore included only the controls matched for maternal age, infant sex, and parity. In another study, controls, but not cases, included several twin to twin transfusions in babies referred for special care.<sup>16</sup> Similar degrees of arbitrariness may have applied to other matched cohort studies without being apparent from the data.

### Risk factors

Despite these limitations it is clear that the rate of preterm birth in singleton pregnancies after assisted reproduction is twice that seen with natural conceptions. This means that assisted reproduction is as much of a predictor for preterm birth as history of preterm birth.<sup>30</sup> The effect was larger for very preterm than for mildly preterm births and contributed to higher rates of (very) low birth weight, admission to intensive care, and perinatal death. There were also 40% more infants who were small for gestational age after assisted conception.

Differences between assisted and natural conceptions were all much smaller in twin pregnancies than in singleton pregnancies, often with confidence intervals that included unity. An added risk, such as assisted conception, may have a marked impact on a low risk singleton pregnancy, but only a small effect on the heavily weighted balance of twin pregnancy. Assisted twin pregnancies may actually start off with a relative

### What is already known on this topic

There is a widespread belief that pregnancy outcome is worse after assisted than after natural conception

The worse outcome as been attributed to the higher frequency of multiple pregnancies and by confounding

### What this study adds

Compared with non-assisted singleton pregnancies, singleton pregnancies from assisted conception have a significantly worse perinatal outcome

This is less so for twin pregnancies

In twin pregnancies, perinatal mortality is about 40% lower after assisted compared with natural conception

Results of the non-matched studies were similar

advantage over singleton pregnancies. As these studies were conducted when 85% of cycles of in vitro fertilisation entailed transfer of several embryos,<sup>3 31</sup> most births must have originated from the transfer of more than one embryo. Development of two rather than one may reflect an implantation advantage that accounts for the smaller difference in outcome between assisted and natural conceptions in twins than in singletons.

### Conclusions and recommendations

Whatever the explanation, singletons from assisted conception are significantly disadvantaged compared with other singletons, but this is substantially less so for twins. Women undergoing assisted reproduction should be informed of the increased risks in singleton pregnancies. With a twin pregnancy they may be relatively advantaged compared with other twin gestations, but this is poor consolation for the much greater risks of twin pregnancy overall. Virtually all perinatal and infant morbidity occurs more frequently in twins than in singletons.<sup>3</sup>

Twenty five years after the birth of the first baby conceived by in vitro fertilisation, our data draw attention to three challenges. Firstly, emphasis needs to shift, more than it has already,<sup>3 31</sup> from achieving pregnancy to achieving a successful outcome. Secondly, it may be timely to consider any multiple pregnancy after assisted conception as a failure of that technology to achieve what it ought to achieve. Thirdly, there is a need to narrow the gap in perinatal outcome between assisted and other singleton pregnancies. This may also enhance understanding of how gestational age, fetal growth, and birth weight interact with each other.

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# Post-neonatal hospitalization and health care costs among IVF children: a 7-year follow-up study

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**BACKGROUND:** The objective of this study was to evaluate whether the post-neonatal hospitalization and resulting health care costs are increased among *in vitro* fertilization (IVF) children up to 7 years of age. **METHODS:** We conducted a population-based cohort study with linkage to a national hospital discharge register including 303 IVF children, born from 1990 to 1995, and 567 control children (1:2) randomly chosen from the Finnish Medical Birth Register and matched for sex, year of birth, area of residence, parity, maternal age and socioeconomic status. The cost calculations were stratified for singleton ( $n = 152$  vs.  $n = 285$ ) and twin ( $n = 103$  vs.  $n = 103$ ) status. Main outcome measures were hospitalizations and societal health care costs. **RESULTS:** The full-sample and singleton analyses showed that IVF children were significantly more frequently admitted to hospital (mean 1.76 vs. 1.07,  $P < 0.0001$ ; 1.61 vs. 1.07,  $P = 0.0004$ , respectively) and spent significantly more days in the hospital (mean 4.31 vs. 2.61,  $P < 0.0001$ ; 3.47 vs. 2.56,  $P = 0.0014$ , respectively) than control children. No differences were detected between IVF and control twins. The costs of post-neonatal hospital care per child were 2.6-fold for IVF singletons, but 0.7-fold for IVF twins when compared with controls. Cost estimation showed 2.6-fold costs for total IVF population in comparison to general population based controls. **CONCLUSIONS:** The incidence of multiple births increases the utilization of post-neonatal health care services and costs among IVF children in comparison to naturally conceived children. Increased hospitalization and costs were also seen among IVF singletons.

**Keywords:** IVF; health care costs; post-neonatal hospitalization

## Introduction

*In vitro* fertilization (IVF) has proved to be an effective treatment for infertility and has become widely used around the globe. Although offering relief to many couples suffering from involuntary childlessness, concern has risen over the long-term outcome of children born after this treatment. At this point, it has been shown that IVF predisposes children to adverse events such as preterm birth and low birth weight, these factors are strongly related to the increased incidence of multiple births after IVF (Koivurova *et al.*, 2002). However, IVF singletons are also prone to similar neonatal problems (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; McGovern *et al.*, 2004) suggesting that factors related to infertility itself (Basso and Baird, 2003; Basso and Olsen, 2005) and IVF technology in the form of 'vanishing embryo syndrome' (Pinborg *et al.*, 2005) may play a role on the outcome.

As a consequence of the poorer neonatal outcome, IVF children have been shown to need hospital care more often during

the neonatal period than children born after natural conception (Koivurova *et al.*, 2002; Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004). So far, to our knowledge, only five studies have been published concerning post-natal hospitalization after IVF (Leslie *et al.*, 1998; Ericson *et al.*, 2002; Pinborg *et al.*, 2004; Bonduelle *et al.*, 2005; Källén *et al.*, 2005). A large register study showed that the utilization of hospital care was at an increased level up to 6 years of age after IVF, although the hospital care utilization was most prominent during the neonatal period (Ericson *et al.*, 2002). The same phenomenon was also seen in another publication that additionally showed an excess in discharge diagnoses indicating brain damage after IVF (Källén *et al.*, 2005). Bonduelle *et al.* (2005) showed that IVF and ICSI (intracytoplasmic sperm injection) children were more likely to be admitted to hospital up to the age of 5 years than children conceived naturally. Danish IVF/ICSI twins did not over-utilize hospital care resources in comparison to control twins followed until 2–7 years of age, but the

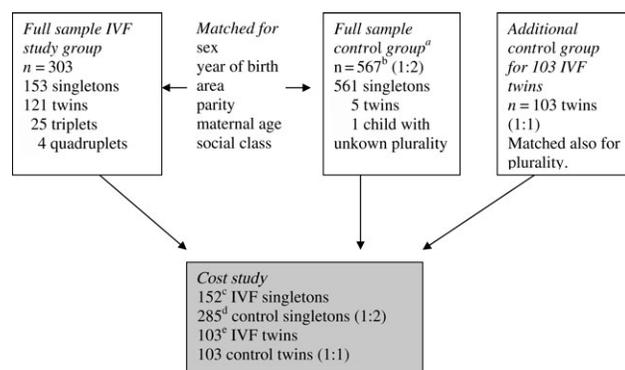
over-use in comparison to IVF/ICSI singletons was clear pointing out the strong effect of multiple birth on the post-natal child outcome (Pinborg *et al.*, 2004). A Finnish study following children up to the age of four years showed that the odds ratio for having a hospital visit was increased (OR 1.32) and the mean length of hospitalization was higher (3.8 vs. 2.6 days) among IVF singletons ( $n = 4559$ ) in comparison to control children ( $n = 190\ 398$ ) (Klemetti *et al.*, 2006). On the contrary, a small Australian study showed no post-natal over-utilization of health care resources after IVF (Leslie *et al.*, 1998).

To fill in the gap in the present literature, we established a follow-up study, based on our earlier cohort study that followed children from birth until 3 years of age (Koivurova *et al.*, 2002; 2003). The present study used a linkage to the Finnish Hospital Discharge Register (FHDR) on a cohort of IVF and control children to test the hypothesis that IVF children over-utilize hospital care resources post-neonatally up to the age of 7 years in comparison to naturally conceived children. Furthermore, we calculated the resulting health care costs to study whether the post-neonatal health care costs continue to be higher for the IVF children, as they were during the neonatal period (Koivurova *et al.*, 2004).

## Materials and Methods

### Study design

This study is a continuation to our previous population-based 3-year follow-up study on a cohort of IVF children born from 1990 to 1995 (Koivurova *et al.*, 2003). The study design, study population and drop-out figures are presented in Fig. 1. The information on IVF children were derived from the register of the IVF outpatient clinic at the University Hospital of Oulu and from the Infertility Clinic of the Family Federation of Finland in Oulu, where all IVF treatments in Northern Finland (provinces of Oulu and Lapland) are performed. The pre-study sample size calculations were based on clinical developmental outcomes and are presented in our previous publications (Hadders-Algra and Touwen, 1990; Koivurova *et al.*, 2002; Koivurova *et al.*, 2003).



**Figure 1:** Study design and the study population. Shaded presentation for the cost study. Full-sample IVF group represents the total cohort of IVF children studied. Singleton control group was derived from the full-sample control group and for IVF twins an additional twin control group was randomly chosen. <sup>a</sup>Children from multiple pregnancies in the same proportion as in a general population. <sup>b</sup>Thirty-nine IVF children missing second control. <sup>c</sup>One singleton control missing in plurality matching. <sup>d</sup>Nineteen IVF children missing second control. <sup>e</sup>Eighteen twin controls missing in plurality matching

The analyses regarding post-neonatal hospitalization were performed for the full-sample group ( $n = 303$ , 153 singletons, 121 twins, 25 triplets and 4 quadruplets) where the control group ( $n = 567$ , (1:2)) represents the general population in the proportion of multiple births, as well as for groups stratified according to singleton and twin status. The cost analyses were performed by plurality: the singleton analysis consisted of 152 IVF singletons and their 285 controls (1:2, derived from the full-sample control group) and the twin analysis consisted of 103 IVF twins and their additional control cohort of 103 (1:1) naturally conceived twins. In the present analyses, these children were group matched for sex, year of birth, area of residence, parity, maternal age and socioeconomic status defined by the occupation of the mother. The IVF children were born after conventional fresh ova IVF pregnancies mostly after transfer of two or three embryos. Control children were identified from the Finnish Medical Birth Register that covers all births in the country since 1987 (Gissler *et al.*, 1995). In practise, all children were of the same ethnic origin, since up to 99% of population in the study area was of Finnish genetic origin in 1990.

### Data collection and analysis

The data regarding post-neonatal hospitalization including diagnoses and the length of the hospital stay were collected by a linkage to the Finnish Hospital Discharge Register where all hospitalizations in Finland have been recorded since 1967. FHDR covers all general and mental hospitals and beds in local health care centres in the public sector as well as private hospitals nationwide. Additionally, day surgery at public and private hospitals is included. We obtained full data until the end of 2002 when all children were at least 7 years old. Hospital treatments during the neonatal period (0–27 days from birth) were excluded from this analysis as they have been presented previously (Koivurova *et al.*, 2002; 2004).

We calculated the mean numbers of admissions to hospitals and days in hospital for the IVF and control groups. The mean unit prices (year 2001) of hospital treatment for different diagnoses (Diagnoses-Related Groups, DRGs) were collected from the data of National Research and Development Centre for Welfare and Health (STAKES) (Hujanen, 2003). To calculate the health care costs per child, all diagnoses appearing during the 7-year follow-up were reviewed and categorized into corresponding DRGs. DRGs have been used internationally since 1990s and they classify patients into separate groups with a specific diagnosis and treatment. This classification helps to estimate the costs of the treatment in each group (Hujanen, 2003). The costs were calculated for each DRG, but only a fraction of DRGs are shown in Tables 1 and 2. The cost calculations were performed by using the DRG-based unit prices to calculate the cost of one day, and then multiplying it by the mean number of days in the hospital for the corresponding diagnosis to get the costs for the actual length of the hospitalization in question. This was further multiplied by the proportion of children with the diagnosis in question in IVF and control groups to get the cost for each DRG per child (Koivurova *et al.*, 2004). The unit prices were inflated to correspond to the prices during 2004 using the consumer price index compiled by Statistics Finland. Wilcoxon Two-Sample Test was used for group matched data in the statistical analysis (two-sided *t*-test approximation applied) and Bonferroni correction was used for multiple testing regarding the median of post-natal hospital days per age period among hospitalized children.

## Results

In the full-sample analysis, the mean number of admissions to the hospital was significantly higher for IVF children than for control children during the 7-year follow-up (1.76 vs. 1.07,

**Table 1:** Post-neonatal health care costs of the main DRGs per child appearing among the IVF and control singletons up to 7 years of age

DRG	Price <sup>a</sup> (€)/days	IVF singletons ( <i>n</i> = 152)			Control singletons ( <i>n</i> = 285)		
		<i>n</i> (%) <sup>b</sup>	Mean number of days per child	Cost (€)/per child	<i>n</i> (%) <sup>b</sup>	Mean number of days per child	Cost (€) per child
Brain damage	1311.4/2.29	1 (0.66)	0.013	0.05	3 (1.05)	0.133	0.80
CNS disorder	839.1/2.24	4 (2.63)	0.076	0.75	1 (0.35)	0.025	0.03
CNS seizures and headache	1867.6/2.15	7 (4.61)	0.579	23.19	6 (2.11)	0.051	0.93
CNS infection	7089.4/11.01	–	–	–	1 (0.35)	0.165	0.37
Psychiatric/psychological disorder	3888.0/4.43	3 (1.97)	0.03	0.52	10 (3.51)	0.153	4.71
Otitis media/upper respiratory infection	966.2/1.89	68 (44.74)	0.434	99.26	85 (29.82)	0.305	46.50
Asthma/obstructive bronchitis	1495.5/2.96	6 (3.95)	0.089	1.78	3 (1.05)	0.018	0.10
Oesophagitis/gastroenteritis	1095.6/2.08	30 (19.74)	0.477	49.6	26 (9.1)	0.295	14.14
Juvenile rheumatoid arthritis	1044.8/2.39	7 (4.61)	0.826	16.65	10 (3.51)	0.2	3.07
Prematurity	6613.2/13.39	1 (0.66)	0.007	0.02	2 (0.7)	0.256	0.89

<sup>a</sup>Corresponds to a unit price calculated for a certain length of hospitalization for a certain diagnosis. Prices have been inflated to correspond to the year 2004.

<sup>b</sup>Number and percentage of children treated for a certain (DRG). A child is counted only once for a certain (DRG), but can be counted for many different DRGs.

– Indicates no hospitalizations for the DRG in question.

€, cost of hospitalization per child in the IVF or control group in a specific DRG.

$P < 0.0001$ ). The mean number of days in the hospital was also significantly higher for IVF children (4.31 vs. 2.61,  $P < 0.0001$ ). In the full-sample group, 61% of IVF children were hospitalized during the follow-up in comparison to 46% of control children. The descriptive presentation of the median of hospital days per age period among hospitalized children showed that the utilization of hospital care appeared to be higher for IVF children during infancy and in the fifth year of life when compared with controls (Fig. 2), but after Bonferroni correction, no significant results remained.

Singleton analysis showed similar results: iVF singletons were significantly more frequently admitted to the hospital during the follow-up (1.61 vs. 1.07,  $P = 0.0004$ ) and were admitted for significantly longer periods (3.47 vs. 2.56,  $P = 0.0014$ ) than control singletons. Of the IVF singletons, 61% were hospitalized by the age of 7 years in comparison to 43% of control singletons.

The twin analyses showed no significant differences between the groups with regard to the number of admissions (1.76 vs.

1.43,  $P = 0.5304$ ) and mean number of hospital days (3.85 vs. 5.50,  $P = 0.7658$ ). The percentage of hospitalized twins by age 7 was also similar in both groups (43% vs. 44%).

The DRG-based diagnoses showed that risks of having diagnoses related to diseases affecting the central nervous system (CNS) (7.9% of IVF singletons and 13.6% of IVF twins having one or many CNS related diagnoses vs. 3.9% of control singletons and 1.9% of control twins) were doubled among IVF singletons, and further multiplied among IVF twins, in comparison to control children (Tables 1 and 2). No cerebral palsy (CP) diagnoses occurred among IVF singletons, whereas one occurred among control singletons. Among IVF twins, 7 out of 11 visits in the DRG regarding brain damage were due to CP, whereas among control twins both visits were due to CP.

Tables 1 and 2 show the unit prices for the main DRGs, number of days the unit price accounts for, number (%) of children treated at hospital per DRG, mean number of days spent at hospital per child in each DRG and cost per child in each DRG.

**Table 2:** Post-neonatal health care costs of the main DRGs per child appearing among the IVF and control twins up to 7 years of age

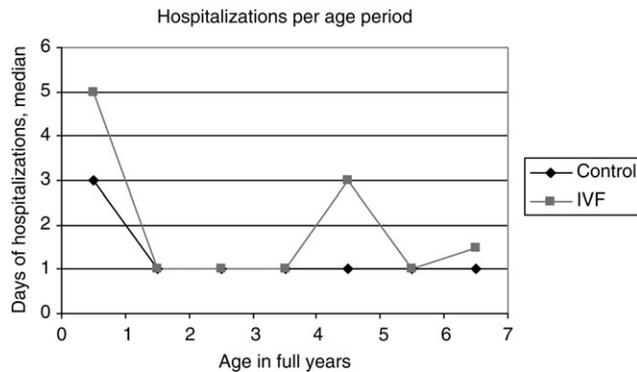
DRG	Price <sup>a</sup> (€)/days	IVF twins ( <i>n</i> = 103)			Control twins ( <i>n</i> = 103)		
		<i>n</i> (%) <sup>b</sup>	Mean number of days per child	Cost (€)/per child	<i>n</i> (%) <sup>b</sup>	Mean number of days per child	Cost (€) per child
Brain damage	1311.4/2.29	11 (10.68)	0.335	20.49	2 (1.94)	0.039	0.43
CNS disorder	839.1/2.24	2 (1.94)	0.024	0.17	–	–	–
CNS seizures and headache	1867.6/2.15	1 (0.97)	0.010	0.08	–	–	–
Psychiatric/psychological disorder	3888.0/4.43	3 (2.91)	0.194	5.03	–	–	–
Otitis media/upper respiratory infection	966.2/1.89	41 (39.81)	0.519	105.6	40 (38.83)	0.471	93.50
Asthma/obstructive bronchitis	1495.5/2.96	4 (3.88)	0.117	2.29	8 (7.77)	0.388	15.23
Oesophagitis/gastroenteritis	1095.6/2.08	15 (14.56)	0.413	31.67	21 (20.39)	0.515	55.31
Juvenile rheumatoid arthritis	1044.8/2.39	4 (3.38)	0.170	2.88	1 (0.97)	0.005	0.02
Prematurity	6613.2/13.39	10 (9.71)	0.844	40.48	10 (9.71)	2.544	122.00
Neonatal problems	2937.9/5.00	1 (0.97)	0.010	0.06	3 (2.91)	0.369	6.31

<sup>a</sup>Corresponds to a unit price calculated for a certain length of hospitalization for a certain diagnosis. Prices have been inflated to correspond to the year 2004.

<sup>b</sup>Number and percentage of children treated for a certain (DRG). A child is counted only once for a certain (DRG), but can be counted for many different DRGs.

– Indicates no hospitalizations for the DRG in question.

€, cost of hospitalization per child in the IVF or control group in a specific DRG.



**Figure 2:** Median of post-natal hospital days per age period during the 7-year follow-up of hospitalized IVF and control children (full-sample analysis) *P*-values from Wilcoxon Two-Sample Test (two-sided *t*-approximation): first year ( $P = 0.061$ ), second year ( $P = 0.630$ ), third year ( $P = 0.900$ ), fourth year ( $P = 0.330$ ), fifth year ( $P = 0.024$ ), sixth year ( $P = 0.370$ ), seventh year ( $P = 0.110$ )

An example of the health care cost calculations (IVF singletons with brain damage in Table 1) divide the unit price (€) 1311.4 by 2.29 (to obtain the cost of one day treatment), multiply this by 0.013 (to obtain the cost of 0.013 days treatment) and multiply this result by 0.0066 (proportion of IVF singletons, 1/152, with brain damage) to obtain the cost of brain damage hospitalization per child in the IVF group (€0.05).

The total costs of post-neonatal hospital care constituting of all DRG based discharge diagnoses per IVF singleton were 2.6-fold (€205.8 per child) compared with that per control singleton (€79.6 per child). For twins the situation was reversed: the post-neonatal health care costs were 1.3-fold for a control twin (€302.4 per child) compared with that for an IVF twin (€224.1 per child). The costs between IVF singletons and IVF twins were almost equal, but for a control twin the costs were nearly four-fold in comparison to a control singleton. The most common illnesses requiring hospital care among IVF and control children were middle ear infections, upper respiratory tract infections and gastroenteritis. The most costly illnesses among IVF and control singletons were those mentioned above, but for IVF children also the care of seizures affecting the CNS (convulsions, headache) and juvenile rheumatoid arthritis resulted in significant costs. For twins, additional significant costs resulted from the care of preterm birth related conditions, and among IVF twins a significant proportion of costs were due to diagnoses indicating brain damage.

Total post-neonatal health care costs of the full-sample IVF group ( $n = 303$ , multiple birth rate 49.5%) were ~€65 000. In a similar size population with a natural multiple birth rate around 1.0%, the costs would be ~€25 000, indicating a 2.6-fold post-neonatal health care costs for the IVF population.

## Discussion

In the present population-based cohort study on carefully matched IVF and control children of women with similar age and parity, we found that in the full-sample and singleton analyses IVF children were significantly more often admitted to hospital and spent significantly more days there during the

7-year follow-up than their naturally conceived controls. Consequently, the post-neonatal costs of hospital care of IVF singletons were also markedly higher than those for control singletons. Among twins, no differences were found reflecting the more complex nature of twin comparisons with the inter-mediating effect of the alteration in the zygosity and chorionicity rate after IVF as well as decreased power in this respect. To our knowledge, this is the first study that compares the hospital care costs based on DRG-classification between the groups.

Knowledge on long-term outcome and utilization of hospital care of children born after IVF is limited at the moment. So far, it has been shown that IVF children need more hospital care services than other children during the first month of their lives due to events related to preterm birth and low birth weight which have a higher incidence among IVF children, mostly as a result of the increased proportion of multiple births. It is of importance to find out whether the hospital care resources are also over-utilized by IVF children after the neonatal period as this reflects the general health of the IVF offspring as well as allowing an estimation of the societal expenditure followed by assisted reproduction.

In our previous publication based on manual data collection from hospital records, we showed that the cumulative morbidity up to 3 years of age of IVF singletons was higher than that for control singletons pointing out a higher incidence of respiratory diseases, especially obstructive bronchitis, and diarrhoea after IVF (Koivurova *et al.*, 2003). Those results are in line with the present study with a different data source showing that IVF children were hospitalized more often and for longer time periods during the 7-year follow-up. Our results are in accordance with recent previous literature that has shown an increased hospitalization of IVF children up to 5–6 years of age in larger series of study subjects (Ericson *et al.*, 2002; Bonduelle *et al.*, 2005; Källén *et al.*, 2005). The Swedish register studies included all IVF children regardless of plurality in the study, showing the natural effect of multiple birth and relating adverse outcomes on the results (Ericson *et al.*, 2002; Källén *et al.*, 2005). Bonduelle *et al.* (2005) studied only singletons with similar results, indicating that the long-term health even of IVF singletons is poorer than that of other children.

Previous studies have raised concern over the neurological health of IVF children with an excess of CP and other diagnoses indicating brain damage (Ericson *et al.*, 2002; Strömberg *et al.*, 2002; Källén *et al.*, 2005; Hvidtjørn *et al.*, 2006). This finding is to a large extent due to the increased incidence of multiple and preterm births after IVF. Our present study population has not the power to confirm a statistically significantly increased risk for such a rare event as CP among IVF children; however, 7.9% of IVF singletons and 13.6% of IVF twins were hospitalized with disorders affecting the CNS in comparison to 3.9 and 1.9% of control children, respectively. It is notable also that seven CP diagnoses were detected among IVF twins. Furthermore, diseases with immunological backgrounds such as asthma, rheumatoid arthritis and infectious diseases were overrepresented among IVF singletons suggesting that there might be some kind of disturbance in the immunological response after IVF, but this observation needs to be evaluated in further studies.

In general, the main diagnoses leading to hospitalization in IVF and control groups were common middle ear, upper respiratory tract and gastrointestinal infections reflecting the benign nature of the health problems during the follow-up period. Naturally, conditions related to preterm birth caused additional and significant hospitalizations among twin groups. In both twin groups, the rate of preterm birth related conditions was 10%, but among control twins the hospitalizations were markedly longer. In this small sample of twins, this finding is probably due to chance. The overall level of hospitalization was similar between the twin groups in this study indicating that multiple birth and relating factors such as zygosity and chorionicity are stronger determinants of child outcome among twins than IVF technique or infertility. Due to our sample size, we cannot detect more severe and rare conditions that might occur in the IVF offspring as a result of the manipulative nature of IVF technique. As another deficiency of this study, we are not aware of the possible deaths in the IVF and control groups after 3 years of age (hospital records have been manually checked up to 3 years of age), because FHDR only records deaths in hospitals and no information on all deaths is gathered to FHDR. It is probable that some deaths have occurred among both groups during the end of the study period, but we believe that no major bias is caused by this lack of information because children's deaths are likely to happen at hospitals or confirmed at hospitals, although some, such as accidental deaths, may have happened outside the hospitals. In addition, in a larger Finnish register-based study, IVF singletons and control singletons had similar mortality rates during a 2-year follow-up period (Klemetti *et al.*, 2006).

The costs of post-neonatal hospital care among IVF singletons in this study were greater than two-fold in comparison to singletons born after natural conception. In our previous publication on neonatal health care costs after IVF, we showed that neonatal health care costs of IVF singletons until the age of 28 days were 1.5-fold compared with control singletons (Koivurova *et al.*, 2004). Therefore, it seems that societal health care costs after IVF rise up to the age of 7 years in proportion to that for natural conception as far as singletons are concerned. For twins the situation was reversed: for control twins the post-neonatal costs were higher, than for IVF twins, as were the neonatal costs in our previous publication (Koivurova *et al.*, 2004). The costs of post-neonatal care between IVF singletons and IVF twins were, due to relatively high-IVF singleton costs, almost equal, whereas the costs of control twins were nearly four-fold higher than that for control singletons. Nevertheless, it can be concluded that multiple birth increases the health care costs post-neonatally even without the potential effects of IVF technology and infertility. No previous studies exist on the long-term health care costs after IVF, so further studies with larger study populations are needed to confirm these findings.

As our full-sample analysis shows, multiple birth and related factors probably play the major role in determining the post-neonatal outcome of the IVF offspring. Still, the post-neonatal outcome of IVF singletons measured with the level of hospitalization seems more adverse than that of control singletons, indicating that IVF technology and parental factors relating

to infertility may also be of importance. Previously it has been shown that infertility as such is a risk factor for adverse perinatal outcome reflecting the differences between fertile and infertile populations as well as the effect of underlying causes of infertility such as Chlamydia infections, prenatal diethylstilbestrol exposure, solvent exposure and psychological stress (Basso and Baird, 2003). Furthermore, the length of involuntary infertility has been shown to be an independent risk factor for child hospitalization (Ericson *et al.*, 2002) even for term children (Källén *et al.*, 2005). Parents of IVF children may seek medical help more easily than other parents, and that may lead to more loose indications of hospital treatments. Unfortunately, we were not able to control for the length of infertility in this study. We believe that the diagnoses set at the hospitals are accurate and non-biased, since the physicians at the hospitals at that time were not necessarily aware of the IVF status of the child. Furthermore, the inpatient hospital care in Finland is almost entirely based on public funding with no private hospitals for children. Only minor procedures such as tympanotomies and adenotomies are partly performed in the private sector as day surgery. The admission policy is homogenous across the country, thus diminishing bias.

In conclusion, the present study shows that there is an increase in the use of post-neonatal hospital care among IVF children, in accordance with previous larger studies. Furthermore, it shows that resulting post-neonatal health care costs are higher among IVF children. Societal costs after IVF also consist of the even higher costs resulting from the IVF procedure itself, as well as from prenatal and neonatal care. The elevated incidence of multiple births after IVF markedly increases the post-neonatal health care costs among IVF offspring in comparison to the costs of naturally conceived children. Considering long-term child outcome as well as the finances, elective single embryo transfer is recommended.

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## Article

# Preimplantation genetic diagnosis for gender selection in the USA



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## Abstract

Preimplantation genetic diagnosis (PGD) for gender selection for non-medical reasons has been considered an unethical procedure by several authors and agencies in the Western society on the basis that it could disrupt the sex ratio, that it discriminates against women and that it leads to disposal of normal embryos of the non-desired gender. In this study, the analysis of a large series of PGD procedures for gender selection from a wide geographical area in the USA shows that, in general, there is no deviation in preference towards any specific gender except for a preference of males in some ethnic populations of Chinese, Indian and Middle Eastern origin that represent a small percentage of the US population. In cases where only normal embryos of the non-desired gender are available, 45.5% of the couples elect to cancel the transfer, while 54.5% of them are open to have embryos transferred of the non-desired gender, this fact being strongly linked to cultural and ethnic background of the parents. In addition this study adds some evidence to the proposition that, in couples with previous children of a given gender, there is no biological predisposition towards producing embryos of that same gender. Based on these facts, it seems that objections to gender selection formulated by ethics committees and scientific societies are not well founded.

**Keywords:** ethics committees, FISH, gender selection, preimplantation genetic diagnosis

## Introduction

Preimplantation genetic diagnosis (PGD) analysis is being used to improve the outcome of assisted reproduction treatment (Gianaroli *et al.*, 1999; Munné *et al.*, 1999, 2003), for couples with idiopathic recurrent pregnancy loss (Munné *et al.*, 2005; Garrisi *et al.*, 2008), carriers of structural chromosome abnormalities (Otani *et al.*, 2006; Escudero *et al.*, 2008) and gene defects (Harper *et al.*, 2002; Fiorentino *et al.*, 2003).

Because aneuploidy of sex chromosomes in human embryos can lead to offspring with Turner syndrome, Klinefelter syndrome and other abnormalities compatible with post-natal viability, probes for chromosomes X and Y have been included in most PGD protocols using fluorescence in-situ hybridization (FISH), with occasional exceptions for indications of structural chromosome abnormalities. Currently, most X-linked genetic

defects are diagnosed by PGD using molecular methods that allow specific identification of the mutation (Amor and Cameron, 2008). However, in the recent past, karyotype-based gender determination was used to prevent X-linked disorders like haemophilia. For those syndromes with no clear genetic association and an increased male incidence like autism, FISH is still used for gender determination.

While sex selection of embryos for medical indications is well accepted (Ethics Committee of the American Society for Reproductive Medicine, 2001), controversy arises regarding sex selection for family balancing or gender preference purposes, which many people believe to be unethical. Two main reasons are cited: (i) the risk of biasing sex ratios in the population at large and/or gender discrimination; and (ii) the risk that chromosomally normal embryos are being

discarded. Considering gender bias, many believe gender selection is discriminatory and sexist: they argue that it would lead to a severe distortion of the sex ratio based on the assumption that a large proportion of couples would select male offspring (Robertson, 2002; Human Fertilisation and Embryology Authority, 2003; FIGO Committee for the Ethical Aspects of Human Reproduction and Women's Health, 2006; Committee on Ethics, American College of Obstetricians and Gynecologists [ACOG], 2007).

However, several studies have demonstrated that, although in some countries like China and India the sex ratio can be distorted in favour of males, in Western societies there is no evidence of such effect and that gender preferences are usually the result of a desire to have a family with children of both genders (family balancing) (Dahl *et al.*, 2003, 2006a,b; Heyd, 2003; Jain *et al.*, 2005; Fejes *et al.*, 2006). All but one of these studies have been carried out through opinion surveys, questionnaires or analysis of newborn data. The one exception is based on results from a series of 92 PGD assays for gender selection in the New York area (Gleicher and Barad, 2007). Overall, the data suggest a strong sex selection towards males remains confined in that area to some minority ethnic groups of Chinese, Middle Eastern/Muslim and Indian origin and that no bias or a slight preference for females is observed among couples of Western origin.

The second controversial issue regarding gender determination is the disposal of chromosomally normal embryos because they are the unwanted gender. As a result of this concern, pre-fertilization techniques like sperm sorting are favoured over post-fertilization techniques like PGD (Robertson, 2002; Committee on Ethics, ACOG, 2007). No studies have been performed to determine the proportion of couples seeking PGD for gender determination who are willing (non-absolute preference) or not willing (absolute preference) to transfer embryos from the unwanted gender when these are the only ones available.

The policy of the study centre's laboratory has been to offer PGD for all indications but not specifically for gender determination. However, because FISH procedures usually involve the analysis of X and Y chromosomes and regulating agencies (i.e. New York State Department of Health) request the disclosure of all genetic information obtained, it is known that the test is being used by some doctors for gender determination as well as aneuploidy detection. Before deciding whether to modify policy or not, this study involved requesting further information from those IVF centres sending us PGD samples so that any bias in gender prediction could be revealed.

A second purpose of this study was to evaluate the popular belief that families with all same-gender children are predisposed to produce either more girls or more boys than the population at large. Previous studies of very large birth cohorts have not supported this belief (Maconochie and Roman, 1997). But no published reports have looked directly for gender bias at the stage of fertilization and embryo production within the two subpopulations of couples who have children all of one gender or the other. This study presents the confirmed data of 276 PGD cycles involving gender selection from 53 different IVF centres throughout the USA.

## Materials and methods

### Sample population

The couples included in this study were selected from PGD cycles performed in the referring facilities from January 2007 to August 2008. Centres referring these cycles were asked to provide information on the first (advanced maternal age, recurrent pregnancy loss, etc.) and second (gender selection) indications for PGD and, if gender selection was mentioned, race and desired gender was recorded.

Those confirmed to have requested gender determination were classified according to reason for the request, including X-linked diseases, family balance, gender bias or unknown, and classified by ethnicity into Chinese, Indian, Middle Eastern and Western (Caucasian, Hispanic and African-American).

Statistical comparisons between groups were made using chi-squared and Fisher's exact test, with a level of significance of  $P < 0.05$  (GraphPad InStat 3).

### Biopsy, fixation and FISH

Embryos were biopsied on day 3 of development by removing a single blastomere, followed by nuclear fixation using the slightly modified Carnoy method (Velilla *et al.*, 2002). The fixed cells were sent to Reprogenetics laboratories either at Livingston, NJ or South San Francisco, CA for FISH analysis, and results were provided on days 3–5 of embryo development.

PGD was performed by FISH as part of the analysis of five (X, Y, 13, 18, 21), 9 (X, Y, 13, 15, 16, 17, 18, 21, 22) or 12 chromosomes (X, Y, 8, 13, 14, 15, 16, 17, 18, 20, 21, 22) as previously reported (Munné *et al.*, 1998a; Colls *et al.*, 2007). In cycles analysing nine and 12 chromosomes, chromosomes X and Y were analysed by using probes [CEP X, DXZ1 within Xp11.1–q11.1] and [CEP Y, DYZ1 Yq12]. For cases where five chromosomes were analysed, the FISH analysis was performed by using the MultiVysion PGT panel (Abbott, Downers Grove, IL, USA), which includes the same probe for chromosome X used in the nine- or 12-chromosome test and the probe CEP Y, DYZ3 within Yp11.1–q11.1 for chromosome Y.

The no result rescue (NRR) approach was applied after the regular FISH panels in cases where doubtful results for one or more of the analysed chromosomes were obtained (Colls *et al.*, 2007). NRR for chromosomes X and Y was performed by using one of these probes: [Telomeric Xp22.3/Yp11.3, DXYS129], [Telomeric Xq28/Yq12, EST Cdy 16c07] or [LSI Xq12, Androgen Receptor] (Abbott). FISH signals were scored applying criteria previously described (Munné *et al.*, 1998b).

## Results

A total of 3339 PGD cycles using a five-, nine- or 12-chromosome test were reviewed. Of these, 381 (11.4%) were ascertained to be for gender selection from 53 different US-based IVF centres

among more than 150 centres referring case material to us. Of them, 276 (72.4%) stated the preferred gender, while in the remaining 105 (27.6%) there was no disclosure so they could not be used here.

Of the 276 cases included in the study, 145 (52.5%) were referred from IVF centres located in the western half of the USA versus 131 (47.5%) referred from the eastern half of the country. The reasons provided were as follows: 21 (7.6%) were selecting females due to X-linked diseases, nine (3.3%) were selecting females to potentially reduce the chances of autism, 36 (13%) were selecting females or males due to family balance, 97 (35.2%) were selecting females or males due to primary gender selection and 113 (40.9%) were selecting females or males without disclosure of the reason.

After excluding the 30 cases where selection of female embryos was requested for therapeutic reasons, 119/246 (48.4%) of the non-therapeutic requests were for female embryos while 127/246 (51.6%) were selecting for male embryos. However, when gender preference was analysed, taking into account the reason for gender selection and ethnicity, a significant bias towards male selection was seen in couples of Chinese, Indian and Arab/Muslim origin compared with patients of Western origin. Results are summarized in **Table 1**.

Information regarding the gender of previous offspring was obtained in 30 cases requesting gender selection for family balance, showing that eight cases (26.7%) requested family balance after having one child of the opposite requested gender, 15 cases (50.0%) after having two children, six (20.0%) after having three and one (3.3%) after having four.

A total of 1647 embryos were analysed from the 246 cases of gender selection for non-therapeutic reasons. The gender outcome of these embryos showed that there was no difference in the embryo sex ratio of couples wanting either males or females. Limiting the analysis to only Western couples, who are less likely to have a bias towards males and more likely to want family balance, the results showed no difference in the sex ratios regardless of the desired gender. Likewise, no difference in the sex ratio was observed for normal embryos or for embryos abnormal for other chromosomes. Results are summarized in **Table 2**.

In 33 cases of gender selection for non-therapeutic reasons, none of the normal embryos obtained were of the desired gender. Of these, 18/33 (54.5%) elected to have a transfer of embryos of the initially undesired gender while 15 (45.5%) decided to cancel the transfer. Regarding ethnicity, in the first group, 2/18 (11.1%) were of Indian, Chinese or Middle Eastern origin versus 6/15 (40.0%) in the second group.

**Table 3** compares a series of 6977 PGD cycles for aneuploidy testing (PGD-A), with known number of embryos replaced, with the cycles of PGD for gender selection (PGD-G) identified in this study. Although IVF centres referring PGD cycles do not always specify the indication for the test, it is obvious from this table that the vast majority of cycles in which five or less chromosomes are tested are for the indication of gender selection.

It is interesting to observe that, in both groups of PGD cycles, the same number of embryos was transferred (1.5) (**Table 3**), although the number of chromosomally normal

**Table 1.** Gender preference by reason and ethnicity.

<i>Reason</i>	<i>Ethnicity</i>	<i>Cases</i>	<i>Male<sup>a</sup></i>	<i>Female<sup>a</sup></i>	<i>Significance</i>
Family balance	Middle East	1	1 (100)	0 (0)	
	Chinese	2	1 (50.0)	1(50.0)	
	Indian	10	10 (100)	0 (0)	
	Total	13	12 (92.3)	1 (7.7)	<i>P</i> < 0.05
	Western	23	12 (52.2)	11 (47.8)	NS
Primary selection	Middle East	8	6 (75.0)	2 (25.0)	
	Chinese	6	4 (66.6)	2 (33.3)	
	Indian	12	9 (75.0)	3 (25.0)	
	Total	26	19 (73.1)	7 (26.9)	NS
	Western	71	21 (29.6)	50 (70.4)	<i>P</i> = 0.0252
Unknown reason	Middle East	8	8 (100)	0 (0)	
	Chinese	7	7 (100)	0 (0)	
	Indian	8	7 (87.5)	1 (12.5)	
	Total	23	22 (95.7)	1 (4.3)	<i>P</i> = 0.0017
	Western	90	44 (48.9)	46 (51.1)	NS
Total	Middle East	17	15 (88.2)	2 (11.8)	<i>P</i> = 0.0255
	Chinese	15	12 (80.0)	3 (20.0)	NS
	Indian	30	26 (86.7)	4 (13.3)	<i>P</i> = 0.0048
	Total	62	53 (85.5)	9 (14.5)	<i>P</i> < 0.0001
	Western	184	74 (40.2)	110 (59.8)	NS

NS = not statistically significant.  
<sup>a</sup>Values are number (percentage).

**Table 2.** Gender outcome of analysed embryos from different groups of patients.

<i>Group</i>	<i>Total male<sup>a</sup></i>	<i>Total female</i>	<i>Normal male</i>	<i>Normal female</i>
Total patients	784 (47.6)	863 (52.4)	507 (49.6)	515 (50.4)
Select male	418 (49.2)	432 (50.8)	278 (50.9)	268 (49.1)
Select female	366 (45.9)	431 (54.1)	229 (48.1)	247 (51.9)
Total Western patients	581 (47.1)	653 (52.9)	361 (48.7)	381 (51.3)
Select male	251 (49.4)	257 (50.6)	158 (50.6)	154 (49.4)
Select female	330 (45.5)	396 (54.5)	203 (47.2)	227 (52.8)
Total Family balancing	89 (50.6)	87 (49.4)	60 (52.6)	54 (47.4)
Select male	58 (49.6)	59 (50.4)	36 (52.2)	33 (47.8)
Select female	31 (52.5)	28 (47.5)	24 (53.3)	21 (46.7)

<sup>a</sup>Values are number (percentage).**Table 3.** Comparison of preimplantation genetic diagnosis (PGD) cycles for aneuploidy and gender selection.

	<i>PGD for aneuploidy</i>	<i>PGD for gender selection</i>
Age <35 years		
No. of cycles	1867	85
Mean age	31.1	31.1
Embryos tested <sup>a</sup>	19,548 (10.5)	610 (7.2)
Normal embryos <sup>a</sup>	6497 (3.5)	423 (5.0)
Embryos transferred <sup>a</sup>	3456 (1.9)	119 (1.4)
Mean no. of normal non-transferred	1.6	3.6
Cycles with five chromosomes tested <sup>b</sup>	150 (8.0)	61 (71.8)
Age 35–39 years		
No. of cycles	2321	75
Mean age	37.2	36.9
Embryos tested <sup>a</sup>	20,314 (8.8)	467 (6.2)
Normal embryos <sup>a</sup>	5452 (2.3)	245 (3.3)
Embryos transferred <sup>a</sup>	3783 (1.6)	113 (1.5)
Mean no. of normal non-transferred embryos	0.7	1.8
Cycles with five chromosomes tested <sup>b</sup>	108 (4.7)	41 (54.7)
Age >39 years		
No. of cycles	2789	86
Mean age	43.5	44.1
Embryos tested <sup>a</sup>	20,947 (7.5)	570 (6.6)
Normal embryos <sup>a</sup>	3589 (1.3)	354 (4.1)
Embryos transferred <sup>a</sup>	2941 (1.1)	139 (1.6)
Mean no. of normal non-transferred embryos	0.2	2.5
Cycles with five chromosomes tested <sup>b</sup>	102 (3.7)	40 (46.5)
All ages		
No. of cycles	6977	246
Mean age	38.1	37.4
Embryos tested <sup>a</sup>	60,809 (8.7)	1647 (6.7)
Normal embryos <sup>a</sup>	15,538 (2.2)	1022 (4.2)
Embryos transferred <sup>a</sup>	10,180 (1.5)	371 (1.5)
Mean no. of normal non-transferred embryos	0.8	2.6
Cycles with five chromosomes tested <sup>b</sup>	360 (5.2)	142 (57.7)
Total cycles with 9–12 chromosomes tested		
No. of cycles	6617	104
Mean age	38	38.5
Embryos tested <sup>a</sup>	58,167 (8.8)	703 (6.8)
Normal embryos <sup>a</sup>	14,477 (2.2)	306 (2.9)
Embryos transferred <sup>a</sup>	9600 (1.4)	138 (1.3)
Mean no. of normal non-transferred embryos	0.8	1.6

<sup>a</sup>Values are total number (number per cycle).<sup>b</sup>Values are total number (percentage). The rest of cycles had 9–12 chromosomes tested.

and not transferred was higher in the PGD-G (2.6 embryos) than in the PGD-A group (0.8 embryos). Because the average number of embryos tested was actually lower in the PGD-G group (6.7) than in the PGD-A group (8.7), the difference in non-transferred normal embryos is due to the facts that more chromosome abnormalities are detected with 9–12 probes tested than with five probes and that only 5.2% of PGD-A tested for five chromosomes compared with 57.7% of PGD-G (Table 3). Indeed, on average, 25% (2.2/8.7) embryos were classified as normal by PGD-A compared with 63% (4.2/6.7) by PGD-G. Assuming a similar rate of chromosome abnormalities in the PGD-G group, 1.7 embryos (25% of 6.7), would be normal, barely enough to replace 1.5 of them on average. This also means that, by only testing five chromosomes in 58% of cycles, those cycles are having fewer than 1.5 normal embryos replaced, and that potentially normal embryos are left behind for transfer.

Indeed, when only PGD-G cycles with 9–12 chromosomes tested are taken into account (Table 3), the average number of normal embryos not replaced in the PGD-G group decreased from 2.6 to 1.6, much closer to the 0.8 embryos in PGD-A.

## Discussion

In contrast to poll studies, which survey opinions regarding gender selection in the general population, this study was designed to evaluate the choices actually made by couples who have decided to gender-select through the use of PGD. Overall, the results obtained in this study are in agreement with previous findings (Gleicher and Barad, 2007) that suggest that, in an ethnically diverse Western society like the USA, sex ratio cannot be disrupted by sex selection. Indeed, the data showed no significant differences regarding gender preference. In the group of patients of Western origin, there is actually a slight but not significant preference for females. This finding invalidates the unsubstantiated ethical claim, suggested by some, that sex selection is always a sexist procedure favouring males (United Nations, 1995; Hanson *et al.*, 2002; Shenfield, 2005; Committee on Ethics, ACOG, 2007).

However, a significant deviation towards preference for males was observed in patients of Chinese, Indian and Middle Eastern/Muslim ethnicity. Similar results were obtained by Gleicher and Barad (2007) in the analysis of a series of PGD cases for sex selection in the New York area, which showed a strong preference for males in the same ethnic subpopulations but not a significant difference when the overall population is taken into account, although a slight deviation towards males is described. This bias towards males in Gleicher and Barad (2007) and perhaps towards females in this study may be a reflection of the ethnic composition of the group of patients included in the study and thus a reflection of the geographical origin of the samples. In the Gleicher and Barad study, the percentage of Chinese, Indian and Muslim patients is higher and, therefore, the overall results show a slight deviation towards male. However, because the patients included in this study come from 53 different IVF centres throughout the USA, the ethnic composition of the patients included in this study can be considered a more accurate representation of the population composition of the whole country and, therefore, the present results are a more accurate representation of the lack of effect of sex selection on the sex ratio.

Dahl *et al.* (2006b) says that, for a severe disruption in the sex ratio of a population, there must be a strong preference for a specific gender and at the same time there must be a high demand of assisted reproductive technology with PGD for sex selection. The authors can agree with the former but simple observation shows the latter need not be present. In China, where the population control laws of the late 1970s require not more than one child per family, a strong preference for males has led to abortion and infanticide of female newborns and created an excess of males. Chinese society has long fiercely discriminated against females, just as is often the case in Hindu and Muslim societies. The results of male bias mean many males will not find wives and – in theory – that females at last have a choice of suitors. Few women in this situation will accept forcibly arranged marriages or discriminatory mistreatment when multiple choices of husband are available. There are many examples in the natural world, particularly among herbivores (Fisher, 1930; Maynard Smith, 1980) and in human history (Trivers, 1985; Sureau, 1999), that echo this situation. It is the authors' opinion, therefore, that in the longer term an excess of males in a society will have two obvious effects: (i) discriminatory behaviour against females will diminish and eventually disappear; and (ii) any continued activities for direct sex selection will change to return the sex ratio to equilibrium. Thus an unbalanced sex ratio can only be self-correcting in the longer term.

However, in the ethnically diverse USA, there is no overall preference for any particular gender when PGD for sex selection is requested, with the exception of some ethnic populations, which represent an extremely small percentage of the US population (Chinese 0.9%, Indian 0.6%) (United States Census Bureau, 2000). Neither does the USA have a high demand for assisted reproductive technology with PGD for sex selection, since only a small fraction of the total population requests PGD and, of those, only 11.4% also request gender selection. In this study, only 10.9% of the cases referred for sex selection were for a medical reason, usually for female embryos because of X-linked diseases or to decrease the chance of autism, which primarily affects males (Yeargin-Allsopp *et al.*, 2003; Chakrabarti and Fombonne, 2005).

There is also the popular belief that some couples with multiple children of the same gender must have been predisposed in this direction. To assess the validity of this belief, a 1997 study of all 549,048 births in Scotland over a 14-year period looked at the gender of fourth and fifth newborns from families in which all previous children were of one gender or the other (Maconochie and Roman, 1997). If gender predisposition is a real phenomenon, the gender of the later-born children should be skewed towards the gender of their older siblings. But the data failed to support this hypothesis.

This study extended these findings by considering the gender ratio of embryos produced by couples who were proactive in their desire to gender-balance an unbalanced family. If there was a predisposition to conceive embryos of one sex, it would be expected to show up within these particular patient groups. But no difference was found in the sex ratio of their embryos, either as a total or by taking into account only the embryos diagnosed as normal, which suggests that such predisposition does not exist or, that if there is any biological selection against one gender, it did not occur at this stage of development.

However, as 76.6% of couples in the family balance group requested gender selection after having only one or two previous children, they may not be realistically considered to have a predisposition to produce embryos of a given gender and a much larger population of couples with three or more babies of the same sex would need to be screened.

Based on large-scale cross-cultural statistics of newborns, it can be seen that there is a slight but uniform skewing of sex ratio worldwide in favour of males with an average of 1.05 (Central Intelligence Agency, 2004). Different biological factors have been proposed to explain this shift towards males, such as different survival rates between male and female embryos during early embryo development (Crawford *et al.*, 1987; Boklage, 2005), nutritional factors (Jimenez *et al.*, 2003; Rosenfeld and Roberts, 2004; Ménézo, 2006), evolutionary degeneration of the Y chromosome and differential fertilization potential of X-bearing and Y-bearing spermatozoa (Cheng *et al.*, 2007). However, the present results demonstrate that if there is any factor leading to a bias towards male at the newborn stage, it does not affect the early stages of embryo development or, if so, assisted reproductive technologies neutralize that effect. It certainly does not support a better fertilization potential of Y-bearing spermatozoa (Cheng *et al.*, 2007) at least for babies conceived through assisted reproduction treatment.

The fate of normal embryos that are not transferred is a difficult moral aspect of this procedure for some people. This study showed that 54.5% of couples undergoing gender selection PGD for non-therapeutic reasons elected to have any-sex embryos available transferred when there were no embryos of the desired gender; meaning that 45.5% chose to discard. Taken into account that only 13.4% of the PGD-G failed to produce normal embryos of the desired gender, 6.1% of all cycles had no transfer due to lack of normal embryos of the preferred gender. In those cases where no embryos were transferred, there was a significant deviation towards Indian, Chinese and Middle Eastern origin (40.0%) versus the 11.1% found in the group that elected to have embryos transferred even when not the desired gender, which is a natural reflection of those cultural backgrounds.

One can argue that, in addition to this 6.1% of cycles with no transfer, many other normal embryos are not replaced because of gender in cycles with transfer. However **Table 3** shows that the same average number of embryos is transferred (1.5 embryos) in PGD-A and PGD-G cycles. Extrapolating the number of abnormalities seen in PGD-A (75%) to PGD-G, the number of normal embryos not transferred would be actually less than in PGD-A. In PGD-A, the residual normal embryos not transferred are usually those of poor morphology, since on average <2% of cycles with PGD-A produce frozen embryos. Apparently, PGD-G does not increase significantly the number of non-transferred embryos, but this is misleading. For those PGD-G cycles in which only five chromosomes were tested, less detection of chromosome abnormalities means that less normal embryos were transferred in total. Thus, to have a less controversial PGD-G programme, one should test for as many chromosome abnormalities as possible, even more so now it is known that embryo biopsy produces a slight-to-significant detrimental impact on implantation, depending on the embryologist and cells biopsied (Cohen *et al.*, 2007; Munné *et al.*, 2007; Goossens *et al.*, 2008); if the biopsy is to be done,

it should confer the maximum selection and thus maximum advantage to that cycle. Testing more chromosomes in PGD-G would reduce the number of normal embryos left non-transferred, because couples may decide to replace those with the other gender if none of the desired gender are found and this possibility will increase with more chromosomes tested. In addition, by testing more chromosomes after the same biopsy, more normal embryos in general will be transferred and, thus, the pregnancy outcome should increase. This should be further analysed with a large dataset.

To conclude, this study demonstrates that sex selection by PGD in an ethnically diverse Western society, like the USA, does not have any significant effect on population sex ratio, does not discriminate against female embryos and seldom results (6%) in the non-transfer of any normal embryos because such embryos were not of the desired gender, provided that the PGD-G test includes analysis of as many chromosomes as possible. Because these are the main concerns that ethics committees and scientific societies from Western countries raise in support of their opposition to gender selection by PGD, it seems clear that Western objections to gender selection are not well founded. Furthermore, the alternative to PGD or sperm selection for gender in some Asian countries is infanticide, which is universally repugnant. Thus, for those couples who desire gender selection, earlier methods are clearly preferable. It is believed that it is incumbent upon committees and scientific societies that have formulated policy statements on gender selection to start anew with the actual facts in the pursuit of rational policymaking that protects private interests when those interests bear no negative consequences for society at large.

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# Preterm birth and low birth weight after assisted reproductive technology–related pregnancy in Australia between 1996 and 2000

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**Objective:** To describe patterns of preterm birth and low birth weight (LBW) for infants born after assisted reproductive technology (ART) and determine whether these were associated with maternal or treatment characteristics.

**Design:** Retrospective cohort study of national population data of infants conceived through ART.

**Setting:** Australian birth records from 1996 to 2000.

**Patient(s):** Eighteen thousand, four hundred twenty-nine liveborn and stillborn infants conceived through ART.

**Intervention(s):** In vitro fertilization, intracytoplasmic sperm injection, and gamete intrafallopian transfer.

**Main Outcome Measure(s):** Preterm birth and LBW.

**Result(s):** Preterm birth and LBW were more common among singletons and twins conceived with IVF and born to nulliparous mothers. Preterm birth and LBW were, respectively, 1.3 times and 1.5 times more likely to occur among singletons conceived by transfer of fresh embryos, compared with transfer of frozen embryos. Preterm birth and LBW was more common among couples who had female-factor infertility compared with male-factor infertility.

**Conclusion(s):** The transfer of fresh embryos and female-factor infertility were independently associated with preterm birth and LBW for both singletons and twins after ART. (Fertil Steril® 2005;83:1650–8. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** ART, singletons, multiples, preterm birth, LBW

The number of births after the use of assisted reproductive technology (ART) has increased steadily around the world, especially in developed countries (1). In Australia, the number of live births conceived through ART has risen from 2,009 (0.8% of total live births) in 1991 to 4,750 (1.9% of total live births) in 2001 (2).

The benefits of ART are evident in that almost 1 in 50 births were associated with ART procedures in Australia in 2001. However, the international literature shows that ART has been associated with an increased number of adverse perinatal outcomes, most notably multiple births, preterm birth, low birth weight (LBW), very low birth weight (VLBW), perinatal death, and increased risk for short and long term disabilities (3–9). Schieve et al. (6) found that ART singletons were 2.6 times more likely to be term-LBW compared with the general population. Furthermore, Ericson and Källén (8) concluded that ART had a threefold excess

risk for some specific birth defects. Other studies suggest that the high incidence of multiple births after the use of ART is responsible for the majority of adverse perinatal outcomes, including preterm delivery, LBW, and VLBW (3, 4, 7, 10–15). The role of maternal characteristics and ART procedures in adverse perinatal outcomes remains unclear.

During the period 1991 to 2001, the proportion of preterm births in Australia increased from 6.8% to 7.8%, whereas the proportion of liveborn ART infants rose from 0.8% to 1.9%, suggesting that ART may have contributed to the increase. We used data from the Assisted Conception Data Collection (ACDC) to assess the occurrence of preterm birth and LBW for liveborn singletons and twins conceived through ART and to determine whether there was any association with maternal characteristics or ART procedures.

## MATERIALS AND METHODS

### Data Collection

A retrospective cohort study was conducted of infants born between 1996 and 2000 who were conceived through ART procedures performed in Australia. The study used data from two national collections. The ACDC provided data for the study population, and the Australian National Perinatal Data Collection (NPDC) provided data for a proxy comparison to the study population. Both data collections are maintained at

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the Australian Institute of Health and Welfare's National Perinatal Statistics Unit (NPSU).

The ACDC consists of unit records of clinical pregnancy data and summary of ART procedure data reported by ART clinics to the NPSU. It includes details of treatment procedures, the number of cycles commenced, the number of cycles progressing to the stage of oocyte retrieval, the number and type of embryos transferred (ET), clinical pregnancy, and viable pregnancy. Clinics also provide data on maternal age, parity, cause of infertility, date of ET, gestation, birth status (livebirth or stillbirth), sex, plurality, and birth weight.

The NPDC is a national Australian aggregation of state and territory data collections containing cross-sectional data of pregnancy outcomes. It includes maternal age, parity, gestational age, and birth status, sex, plurality, and birth weight. The 1999 NPDC was used to establish a baseline of pregnancy outcomes in this study.

## Definitions

The following definitions are used in this article.

*Gestational age:* Difference between the date of ET and the date of end of pregnancy plus 2 weeks.

*Viable pregnancy:* Pregnancy of at least 20 weeks' gestation and/or 400-g birth weight.

*Live birth:* Infant with signs of life after pregnancy of at least 20 weeks' gestation and/or 400-g birth weight.

*Still birth:* Birth resulting from a viable pregnancy in which the fetus does not exhibit any sign of life when completely removed from the birth canal.

*Term birth:* Liveborn or stillborn infant of gestation of at least 37 weeks but less than 42 weeks.

*Preterm birth:* Liveborn or stillborn infant of gestation of at least 20 weeks but less than 37 weeks.

*Very preterm birth:* Liveborn or stillborn infant of gestation of at least 20 weeks but less than 32 weeks.

*Low birth weight:* Liveborn or stillborn infant weighing <2,500 g.

*Very low birth weight:* Liveborn or stillborn infant weighing <1,500 g.

*IVF:* Fertilization of an egg by a sperm in vitro, that is, in the laboratory.

*Intracytoplasmic sperm injection (ICSI):* One sperm is injected through the zona pellucida, across the perivitelline space, through the vitelline membrane (the egg cell's membrane), and into the substance (or cytoplasm) of the egg.

*Gamete intrafallopian transfer (GIFT):* Unfertilized eggs plus sperm (i.e., gametes) are transferred to the fallopian tube.

## Study Population

The ACDC study population included infants conceived through ART procedures and born in Australia during the period 1996 to 2000. A total of 15,074 pregnancies were reported, resulting in 18,429 liveborn and stillborn infants. Of the total pregnancies, 15,035 (99.7%) were reported as viable, and 14,629 (97.1%) resulted in the delivery of one or more liveborn infant. We excluded 286 stillborn infants, 269 infants whose birth status was not stated, and 148 infants whose gestational age and/or birth weight was not stated. This provided a final data set of 17,726 infants.

For the year 1999, the NPDC was used to compare the outcomes of all Australian births with the outcomes of the ART births as recorded in the ACDC. The ACDC and NPDC are not linked databases, and the births making up the ACDC also are included in the NPDC; therefore the two data collections are not mutually exclusive. The ACDC singletons account for approximately 1% of the total singletons in the NPDC. Notwithstanding this limitation, a comparison of all Australian birth outcomes with ACDC was undertaken as described in the data analysis. A total of 253,352 confinements were reported for women aged 20 years and older, resulting in 257,394 live and stillborn infants in 1999 from NPDC. These data were used to examine the hypothesis that use of ART was associated with preterm birth and LBW. Ethics approval for the study was granted by the Human Research Ethics Advisory Panel of the University of New South Wales, Australia.

## Data Analysis

To examine whether a higher rate of preterm birth and LBW would be expected in ART births compared with all births in Australia, we undertook an univariate analysis to calculate the expected number of preterm birth and LBW for ART-associated singletons who were born in 1999 using overall national birth-cohort rates of preterm birth and LBW for all Australian singletons in the 1999 NPDC. Furthermore, confidence intervals (CIs) of the rates of preterm birth and LBW among ART singletons were calculated and compared with the rates of preterm birth and LBW among all Australian-born singletons, keeping in mind that the NPDC contains the total population of all births in Australia.

We compared the potential risk factors for preterm birth and LBW by using stratification and univariate and multivariate stepwise logistic regression. The ART population sample was stratified by plurality (singleton, twin, and high-order multiple births). Assignment of plurality was based on the total number of liveborn and stillborn infants who were delivered, with only liveborn infants included in the final data set. Within each study group, we examined the factors associated with preterm birth, very preterm birth, LBW, and VLBW. We assessed variations in risk according to maternal, infant, and treatment-related factors, including maternal age and parity, primary cause of infertility, the number and type (fresh or frozen) of embryos transferred, and type of procedure (IVF, ICSI, and GIFT). Odds ratios (ORs), adjusted odds ratios (AORs), and CIs were cal-

**TABLE 1**

**Observed and expected cases of preterm birth, very preterm birth, LBW, and VLBW among liveborn ART singletons in Australia in 1999.**

Parameter	Total no.	Observed no.	Standard rate <sup>a</sup>	Expected no.
Preterm birth	2,555	343	5.7	145.6
Very preterm birth	2,555	59	0.9	23.0
LBW	2,555	249	4.6	117.5
VLBW	2,555	52	0.7	17.9

<sup>a</sup> Rate of preterm birth, very preterm birth, LBW, and VLBW for all Australian live born singletons in 1999.

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culated to estimate the likelihood of preterm birth and LBW in each study group. The level of significance was set at .01, and 99% CIs were used to minimize the risk of chance findings.

All data were analyzed by Statistical Package for the Social Sciences, version 11.5 for Windows (SPSS, Chicago, IL) (16).

## RESULTS

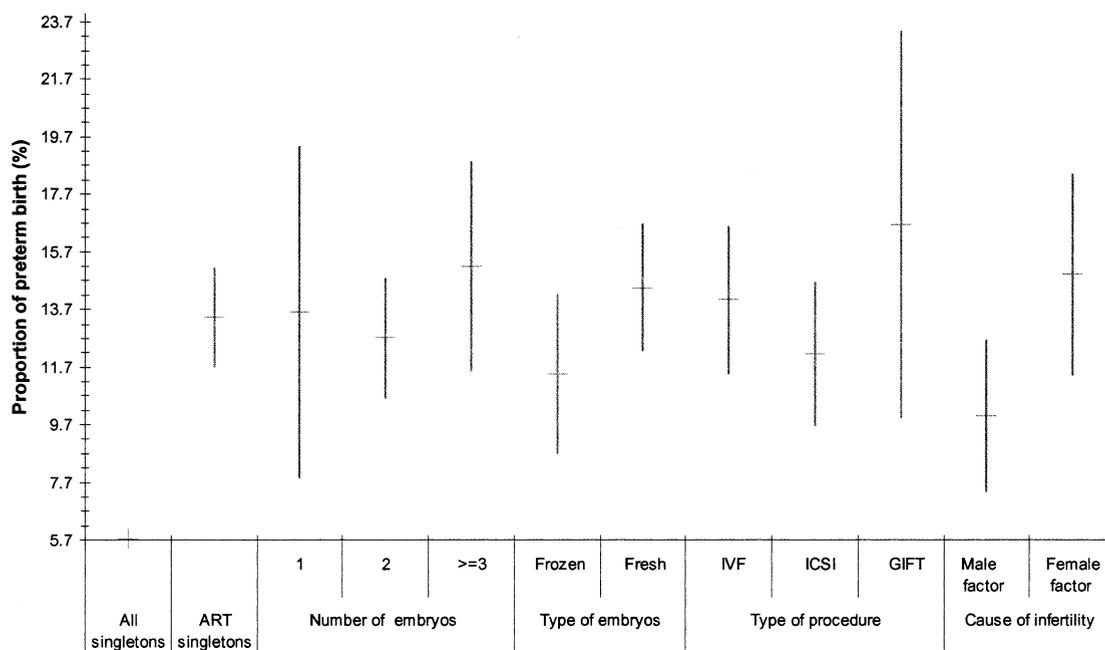
### Comparison of ART Singleton Births With All Australian Singleton Births, 1999

Table 1 details the expected number of preterm birth and LBW for ART-associated singletons born in 1999. The ob-

served number of preterm births was 2.4 times higher than the expected number when applying national birth cohort rates. Likewise, the observed number of LBW infants was 2.1 times higher than expected. Figures 1 and 2 show the proportion of preterm birth and LBW, respectively, for liveborn ART-associated and for all Australian singletons for the year 1999. The 99% CIs of the proportion of preterm and LBW for ART liveborn singletons are outside the proportion of preterm birth and LBW for all Australian liveborn singletons (5.7% and 4.7%, respectively). Singletons conceived from the transfer of only one embryo had a higher proportion of preterm birth than did all Australian singletons, suggest-

**FIGURE 1**

Proportion of preterm birth and 99% CI for assisted reproductive technology (ART) compared with all Australian liveborn singletons Australia, 1999.

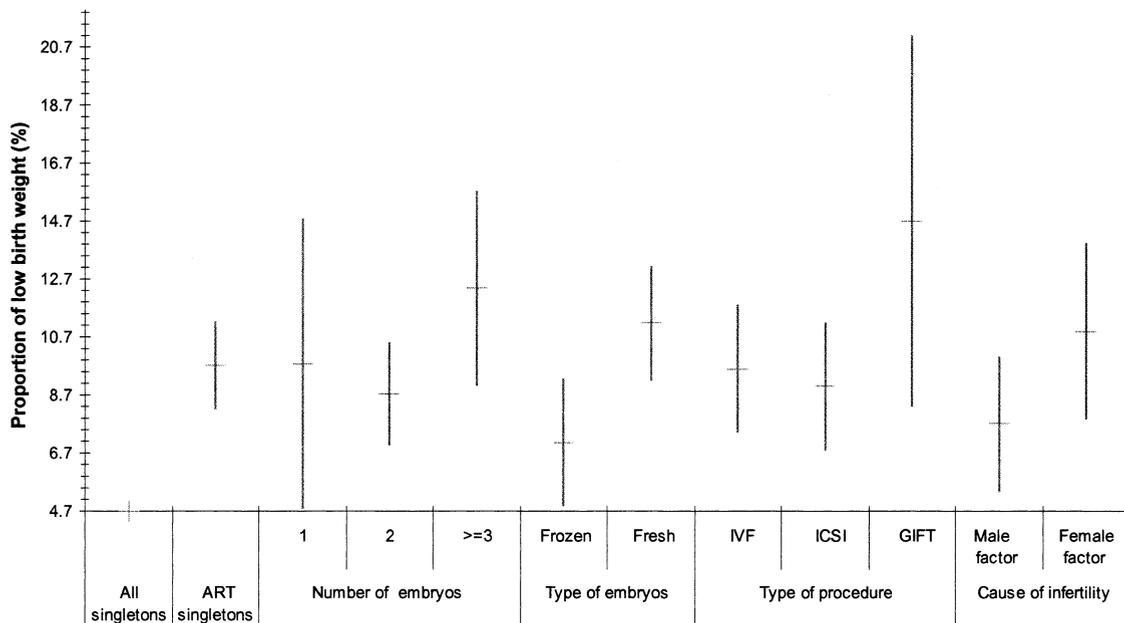


Note: X axis at 5.7% represents the proportion of preterm birth in all Australian liveborn singletons

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**FIGURE 2**

Proportion of low birth weight (LBW) and 99% CI for assisted reproductive technology (ART) compared with all Australian liveborn singletons Australia, 1999.



Note: X axis at 4.7% represents the proportion of low birth weight in all Australian liveborn singletons

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ing that singletons conceived with ART were at increased risk for preterm birth and LBW.

### Overall Maternal Characteristics, ART Procedures, and Birth Outcomes

From 1996 to 2000, a total of 17,726 live infants were born after 15,035 ART cycles. Women with live births after ART had a mean age of 34.0 years at delivery (range 20–55 years). The average gestational age was 37.4 weeks. The median and mode of embryos transferred was 2 (range, 1–8) during the study period.

The proportion of live births due to ICSI increased from 30.4% in 1996 to 47.3% in 2000. Maternal characteristics and ART procedures of the study population are detailed in Table 2. The majority of women in this study were aged 30 to 39 years at delivery and were nulliparous. The majority of liveborn infants were conceived with fresh embryos. Almost half of the liveborn infants were delivered by Caesarean section, with mean gestational age of 36.1 weeks.

Multiple births accounted for 34.8% of all ART births in the study population. Of the 17,726 ART infants, 65.2% were singletons, 31.1% were twins, and 3.7% were higher order multiples. Among multiple births, only 0.7% of live births resulted from the transfer of one embryo, 60.1% resulted from the transfer of two embryos, and 36.2%, from the transfer of three embryos or more.

The proportion of very preterm birth ranged from 2.4% for singletons, 11.5% for twins to 44.3% for higher order multiples. The proportion of VLBW infants was 2.1% among singletons, 10.0% in twins, and 39.1% in higher order multiples. The occurrence of very preterm birth and VLBW varied with maternal characteristics and ART procedures. Very preterm birth and VLBW were less common among infants born to older mothers or to couples with male-factor infertility. They were also less common in infants resulting from procedures in which ICSI or frozen embryos had been used.

### Preterm Birth and LBW Among ART Singletons

A higher proportion of preterm birth was observed among singletons born to older and multiparous mothers. Low birth weight was more common for singletons born to women aged 40 years or older and was less common in multiparous women. The higher number of embryos transferred was related to higher proportions of preterm birth and LBW among singletons. Preterm birth and LBW were less prevalent for singletons conceived by using ICSI, transfer of frozen embryos, and male-factor infertility (Table 3).

Modeling of the singleton data permitted assessment of those factors that contribute independently to preterm birth and LBW. We found that preterm birth was more likely to

TABLE 2

Selected maternal characteristics and ART procedures by number of pregnancies and infants reported in Australia from 1996 to 2000.

Parameter	Pregnancies				Infants	
	Viable pregnancies (n = 15,035)		Pregnancies with a live birth (n = 14,629)		Infants in final sample (n = 17,726)	
	Count	%	Count	%	Count	%
Maternal age (y)						
20–29	2,345	15.6	2,273	15.5	2,820	15.9
30–34	5,904	39.3	5,755	39.4	7,117	40.2
35–39	5,226	34.8	5,084	34.8	6,069	34.3
40–44	1,426	9.5	1,389	9.5	1,564	8.8
≥45	128	0.9	122	0.8	147	0.8
Parity						
≥2	3,038	20.2	2,958	20.2	3,521	19.9
1	4,279	28.5	4,163	28.5	5,023	28.4
0	7,703	51.3	7,499	51.3	9,172	51.8
Cause of infertility						
Obstructive fallopian disorder	1,512	10.1	1,463	10.0	1,755	9.9
Male factors	4,738	31.5	4,606	31.5	5,527	31.2
Endometriosis	998	6.6	975	6.7	1,213	6.8
Other female factors	1,394	9.3	1,353	9.2	1,648	9.3
Other specified infertility	4,253	28.3	4,145	28.3	5,034	28.4
Unexplained infertility	2,140	14.2	2,078	14.3	2,549	14.4
Type of embryo						
Frozen	4,619	30.7	4,506	30.8	5,120	28.9
Fresh	10,413	69.3	10,120	69.2	12,604	71.1
Mode of delivery						
Vaginal	8,517	56.8	8,231	56.4	9,184	51.9
Caesarean section	6,483	43.2	6,369	43.6	8,512	48.1
Type of procedure						
IVF	7,101	47.2	6,897	47.1	8,299	46.8
ICSI	6,118	40.7	5,963	40.8	7,174	40.5
GIFT	1,813	12.1	1,766	12.1	2,251	12.7

Note: Data on maternal age, parity, type of procedure, and type of embryo were missing <0.2% of infants. Data on mode of delivery were missing for 0.2% of infants.

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result from the use of fresh embryos compared with frozen embryos (AOR, 1.3; 99% CI, 1.1–1.6; Table 4). Likewise, preterm birth was more likely to be an outcome when couples had female-factor infertility compared with male-factor infertility (AOR, 1.4; 99% CI, 1.2–1.8).

In the final model (Table 4), the transfer of fresh embryos was 1.6 times more likely to result in LBW singletons compared with the transfer of frozen embryos (99% CI, 1.3–2.0). Singletons born to couples with female-factor infertility were more likely to have LBW compared with singletons born to couples with male-factor infertility (AOR, 1.4; 99% CI 1.1–1.7).

### Preterm Birth and LBW Among ART Twins

A higher proportion of LBW was observed among twins born to younger mothers. Low birth weight was more prevalent among twins born to nulliparous women and women aged ≤29 years or ≥45 years. The higher number of embryos transferred resulted in a higher rate of preterm birth and LBW among twins. Preterm birth and LBW were less common among twins conceived through ICSI or frozen-embryo transfer and among twins born to couples with male-factor infertility (Table 3).

Twins with preterm birth and LBW were a more likely outcome for women who were nulliparous or who had only

TABLE 3

Proportion of preterm birth and LBW by maternal characteristics and ART procedures (Australia, 1996–2000).

Parameter	Singletons (n = 11,556)		Twins (n = 5,513)		Higher order multiples (n = 657)	
	Preterm	LBW	Preterm	LBW	Preterm	LBW
Total	12.5	9.6	60.9	53.9	99.2	94.7
Maternal age (y)						
20–29	12.7	10.5	65.5	57.7	97.8	96.6
30–34	11.1	8.6	61.2	53.7	99.1	95.7
35–39	13.5	9.8	57.8	50.9	100.0	93.2
40–44	13.7	10.6	61.5	52.5	100.0	89.3
≥45	15.5	12.4	59.1	61.4	100.0	83.3
Parity						
≥2	14.6	9.7	59.9	49.1	100.0	96.4
1	12.8	9.2	60.7	51.0	100.0	91.1
0	11.5	9.7	61.3	56.3	98.7	95.8
Cause of infertility						
Obstructive fallopian disorder	15.1	10.3	61.9	52.4	100.0	97.2
Male factors	10.2	8.2	57.6	51.4	97.1	93.6
Endometriosis	11.0	9.4	61.0	54.8	100.0	97.3
Other females factors	14.9	12.2	65.6	60.0	100.0	98.4
Other specified infertility	13.9	10.3	64.4	54.9	100.0	93.8
Unexplained infertility	12.2	9.1	57.4	51.4	100.0	91.7
No. of embryos transferred						
1	13.3	8.6	46.3	43.9	—	—
2	11.7	9.0	60.9	52.1	100.0	93.4
3	14.1	11.2	60.4	56.3	99.0	94.9
≥4	14.2	11.6	70.6	56.6	100.0	95.4
Type of embryo						
Fresh	13.1	10.8	61.3	55.2	99.1	95.0
Frozen	11.4	7.2	59.5	47.3	100.0	92.2
Type of procedure						
IVF	13.6	9.8	63.4	54.2	98.8	94.6
ICSI	11.4	9.1	59.8	51.9	99.1	93.5
GIFT	11.8	10.4	55.5	56.1	100.0	96.2

Note: Data on maternal age, parity, type of procedure, and type of embryo were missing for <0.2% of infants.

Wang. Preterm birth and low birth weight after ART. *Fertil Steril* 2005.

one previous pregnancy, compared with the case of women with two or more previous pregnancies (Table 5). Moreover, twins with preterm birth and LBW were more likely to be born to couples with female-factor infertility (AOR, 1.3; 99% CI, 1.1–1.6 and AOR, 1.3; 99% CI, 1.1–1.5, respectively), compared with couples with male-factor infertility. The use of fresh embryos was associated with an increase in LBW among twins compared with the use of frozen embryos (AOR, 1.3; 99% CI, 1.1–1.6).

## DISCUSSION

Our study found that liveborn ART singleton infants were more likely to be preterm and to have LBW compared with

the national Australian birth cohort. Almost a third of ART infants were delivered preterm, and a quarter were LBW, indicating that ART contributes disproportionately to preterm birth and LBW among the general population. Not surprisingly, for ART infants, twins and higher order multiples were more likely to be preterm birth and LBW than singletons, whereas for both singletons and twins, the likelihood of preterm birth and LBW varied according to mother's parity, cause of infertility, and the type of ART procedures received.

The majority of mothers in our study were aged 30 to 39 years and were nulliparous. Nulliparity was independently

**TABLE 4**

**Singletons born after ART with preterm birth and LBW by maternal and ART factors (Australia, 1996–2000).**

Parameter	n	%	OR (99% CI) in univariate model	AOR (99% CI) in final multivariate model
<b>Preterm birth</b>				
Type of embryo				
Frozen	437	11.4	1.0	1.0
Fresh	1,008	13.1	1.2 (1.0–1.4) <sup>a</sup>	1.3 (1.1–1.6) <sup>a</sup>
Cause of infertility				
Male factors	372	10.2	1.0	1.0
Female factors	420	14.0	1.4 (1.2–1.8) <sup>a</sup>	1.4 (1.2–1.8) <sup>a</sup>
<b>LBW</b>				
Type of embryo				
Frozen	277	7.2	1.0	1.0
Fresh	829	10.8	1.6 (1.3–1.9) <sup>a</sup>	1.6 (1.3–2.0) <sup>a</sup>
Cause of infertility				
Male factors	298	8.2	1.0	1.00
Female factors	322	10.7	1.4 (1.1–1.7) <sup>a</sup>	1.4 (1.1–1.7) <sup>a</sup>

Note: Factors put in multivariate model were maternal age and parity, cause of infertility (male or female), number of embryos transferred, type of embryos, and type of procedure. Collinearity relationship among factors in multivariate model was examined in linear regression.

<sup>a</sup> *P* < .01.

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associated with preterm birth and LBW for ART twins but not for singletons. There is general agreement that pregnancy outcomes are more favorable for younger, multiparous women (17–19) and that pregnancies in older primigravida are associated with a high risk of preterm birth and LBW (20–22). Thus, the poorer perinatal outcomes found in this study could be associated with an increased maternal age and decreased parity of ART mothers, and not necessarily related to any factors that were intrinsic to ART procedures. Our study was consistent with others in the literature in demonstrating how maternal age and parity interacts with and confounds the relationship among infertility, ART procedures, and poor perinatal outcomes such as preterm birth and LBW (7, 11, 23).

Our data indicate that female-factor infertility increased the likelihood of preterm birth and LBW for ART singletons and twins. This is closely related to the finding that the IVF procedure, as compared with ICSI, resulted in a higher proportion of preterm birth and LBW. Female-factor infertility is more highly correlated with treatment by IVF than ICSI, and as our final model confirms, it is the cause of infertility and not the type of ART procedure. As other studies have found, our model suggests that the higher occurrence of preterm birth and LBW was most likely associated with the physiological characteristics of women with female-factor causes of infertility, such as tubal disease, endometriosis, or hormonal disorders (24–26). One possible

explanation is that the pathophysiology of retarded fetal growth involves defective uteroplacental interaction, which may be of uterine origin. For instance, reduced uterine vasculature is implicated and also has been suggested as a possible cause of infertility (27). It has been suggested by others that IVF pregnancies incur a higher risk for hypertension and bleeding (15, 28), which can in turn lead to reduced birth weight and gestation. In women with normal fertility once male factors are overcome, their uterine environment is healthy, and so more normal growth can be expected.

In our study, the likelihood of preterm birth and LBW also varied according to the type of embryos used in the ART procedure. Preterm birth and LBW were less likely to occur among ART singletons and among twins who were conceived by using frozen embryos, compared with those conceived by using fresh embryos. This finding has been demonstrated in other studies (29, 30) in which it was found that the cryopreservation process did not adversely influence fetal development or increase perinatal risk and, in fact, that cryopreservation appeared to have a protective effect. The better outcome of frozen embryos found in our study is likely related to the embryo selection process in which those embryos chosen for freezing are of better quality. Another possible selection bias is that couples who had excess embryos available for cryopreservation were more likely to have a better response to ovulation induction therapy and have good-quality oocytes to begin with.

TABLE 5

Twins born after ART with preterm birth and LBW by maternal and ART factors (Australia, 1996–2000).

Parameter	No.	%	OR (99% CI) in univariate model	AOR (99% CI) in final multivariate model
Preterm birth				
Parity				
≥2	616	59.9	1.0	1.0
1	936	60.7	1.0 (0.8–1.3)	1.3 (1.0–1.7)
0	1,801	61.3	1.1 (0.9–1.3)	1.4 (1.1–1.8) <sup>a</sup>
Cause of infertility				
Male factors	980	57.6	1.0	1.0
Female factors	889	63.0	1.4 (1.1–1.5) <sup>a</sup>	1.3 (1.1–1.6) <sup>a</sup>
LBW				
Parity				
≥2	505	49.1	1.0	1.0
1	787	51.0	1.1 (0.9–1.3)	1.3 (1.1–1.7) <sup>a</sup>
0	1,654	56.3	1.3 (1.1–1.6) <sup>a</sup>	1.6 (1.3–2.0) <sup>a</sup>
Type of embryo				
Frozen	565	47.3	1.0	1.0
Fresh	2,385	55.2	1.4 (1.2–1.6) <sup>a</sup>	1.3 (1.1–1.6) <sup>a</sup>
Cause of infertility				
Male factors	873	51.4	1.0	1.0
Female factors	788	55.8	1.2 (1.0–1.4)	1.3 (1.1–1.5) <sup>a</sup>

Note: Factors put in multivariate model were maternal age and parity, cause of infertility (male or female), number of embryos transferred, type of embryos, and type of procedure.

Collinearity relationship among factors in multivariate model was examined in linear regression.

<sup>a</sup>  $P < .01$ .

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Our study confirmed the high rate of Caesarean section after the use of ART seen in other studies (11, 31, 32). In Australia in 2000, 25.4% of all births involved Caesarean section, compared to 47.1% in the ART births (1). Daniel et al. (32) found that ART is associated with maternal complications such as pregnancy-induced hypertension, which may partially explain the higher proportion of Caesareans among our study population. However, the high rate of Caesarean section in our study population also could be related to older maternal age, lower parity, increased incidence of multiple pregnancies (33), and the increased concern and anxiety that often are experienced by ART mothers (29).

The comparison between ART singletons and all Australian singletons suggests that ART infants have a higher risk for preterm birth and LBW. This increased risk persists even when comparing ART singletons conceived with the transfer of only one embryo with singletons in the general population. However, our study was unable to further explore the relationship between ART and poor perinatal outcome because of the unavailability of matched data identifying ART infants within the Australian birth cohort. Schieve et al. (6) faced similar data-related problems in their study. They approached this by applying the rates of LBW in the 1997

population data (from the U.S. birth certificate data) to the population of ART infants to derive the expected number of ART LBW infants. They then calculated standardized risk ratio (SRR)(adjusted for maternal age and parity) by dividing the observed number by the expected number to conclude that singletons conceived through ART had a risk of LBW that was 2.6 times that in general population.

We also calculated the expected number of preterm and LBW infants according to the rate of these in Australian singletons. Yet, even though ART singletons accounted for only 1% of all Australian-born singletons, we hesitated to use SRR to estimate the risk of preterm birth and LBW for ART infants because ART contributed a disproportionate part to both preterm birth and LBW. Instead, we chose to calculate a proxy indicator in the form of 99% CIs for ART singletons and concluded that because our CIs did not cross the rate of all Australian singletons, ART infants were at increased risk of preterm birth and LBW compared with all Australian infants. A more methodologically sound approach to comparing perinatal outcomes between ART and their naturally conceived counterparts would be to undertake a linked study that identifies infants conceived through ART in the national Australian birth cohort. This would then provide

two mutually exclusive populations in which the true risk for preterm birth and LBW could be properly assessed.

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# Risk of borderline and invasive ovarian tumours after ovarian stimulation for *in vitro* fertilization in a large Dutch cohort

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**BACKGROUND:** Long-term effects of ovarian stimulation for IVF on the risk of ovarian malignancies are unknown.

**METHODS:** We identified a nationwide historic cohort of 19 146 women who received IVF treatment in the Netherlands between 1983 and 1995, and a comparison group of 6006 subfertile women not treated with IVF. In 1997–1999, data on reproductive risk factors were obtained from 65% of women and data on subfertility (treatment) were obtained from the medical records. The incidence of ovarian malignancies (including borderline ovarian tumours) through 2007 was assessed through linkage with disease registries. The risk of ovarian malignancies in the IVF group was compared with risks in the general population and the subfertile comparison group.

**RESULTS:** After a median follow-up of 14.7 years, the risk of borderline ovarian tumours was increased in the IVF group compared with the general population [standardized incidence ratio (SIR) = 1.76; 95% confidence interval (CI) = 1.16–2.56]. The overall SIR for invasive ovarian cancer was not significantly elevated, but increased with longer follow-up after first IVF ( $P = 0.02$ ); the SIR was 3.54 (95% CI = 1.62–6.72) after 15 years. The risks of borderline ovarian tumours and of all ovarian malignancies combined in the IVF group were significantly increased compared with risks in the subfertile comparison group (hazard ratios = 4.23; 95% CI = 1.25–14.33 and 2.14; 95% CI = 1.07–4.25, respectively, adjusted for age, parity and subfertility cause).

**CONCLUSIONS:** Ovarian stimulation for IVF may increase the risk of ovarian malignancies, especially borderline ovarian tumours. More large cohort studies are needed to confirm these findings and to examine the effect of IVF treatment characteristics.

**Key words:** ovarian stimulation / ovarian malignancies / fertility drugs / infertility / *in vitro* fertilization

## Introduction

Currently, 1.2–2.3% of children born in the Western world are conceived by assisted reproductive technologies (Kremer *et al.*, 2008; Wright *et al.*, 2008). In the Netherlands, it has been estimated that the number of treatment cycles increased by 40% from 1996 till 2005 (Kremer *et al.*, 2008). Fertility drugs (FDs) used in IVF treatment temporarily raise serum levels of exogenous gonadotrophins and gonadal hormones, and consequently increase the chances of multiple folliculogenesis and ovulations. The long-term effects of ovarian stimulation are unknown. In view of the assumed role of 'incessant ovulation' (Fathalla, 1972) and increased gonadotrophin levels in ovarian cancer pathogenesis (Cramer and Welch, 1983; Risch, 1998; Vlahos *et al.*, 2010) concerns have been raised that ovarian stimulation and multiple ovarian punctures as used in IVF may increase the risk of ovarian malignancies (Fishel and Jackson, 1989). Invasive ovarian cancer accounts for 6% of female cancer deaths in the USA (Jemal *et al.*, 2008).

Over the past decades, several studies reported a significant increase of ovarian cancer risk after FD use (Whittemore *et al.*, 1992; Rossing *et al.*, 1994; Brinton *et al.*, 2005; Sanner *et al.*, 2009; Källén *et al.*, 2011), but others did not observe such an elevated risk (Franceschi *et al.*, 1994; Bristow and Karlan, 1996; Mosgaard *et al.*, 1997; Modan *et al.*, 1998; Venn *et al.*, 1999; Parazzini *et al.*, 2001; Dor *et al.*, 2002; Doyle *et al.*, 2002; Ness *et al.*, 2002; Rossing *et al.*, 2004; Dos Santos Silva *et al.*, 2009; Jensen *et al.*, 2009), or reported non-significant risk increases for subgroups (Shushan *et al.*, 1996; Ness *et al.*, 2002; Brinton *et al.*, 2004). Some studies noted an elevated risk of borderline ovarian tumours following the use of FDs (Harris *et al.*, 1992; Rossing *et al.*, 1994; Shushan *et al.*, 1996; Parazzini *et al.*, 1998; Ness *et al.*, 2002; Sanner *et al.*, 2009). Borderline ovarian tumours are low-grade ovarian malignancies with far less aggressive behaviour than invasive ovarian cancer (Bell, 2005; Hart, 2005).

Short follow-up, low statistical power and lack of control for important confounders, such as cause of subfertility and parity, have limited the conclusions from previous studies. We report here on a large nationwide cohort study in the Netherlands (the OMEGA study) that was designed to examine long-term risk of ovarian malignancies (both invasive ovarian cancer and borderline ovarian tumours) after ovarian stimulation for IVF. A unique feature of our study is that data on reproductive factors were obtained from the participating women, whereas detailed information on subfertility cause and treatment was abstracted from the medical files.

## Patients and Methods

### Study population

In 1995–1996, we identified a nationwide historical cohort of 19 861 subfertile women who received at least one IVF cycle with ovarian stimulation between 1983 and 1995 in 1 of the 12 IVF hospitals with legal permission to provide IVF treatment in the Netherlands. Since the registration of IVF treatment was obligatory by law, all IVF clinics in the Netherlands could provide a minimal data set with names, birth dates and addresses of eligible women. The institutional ethics committees of all IVF clinics approved the study procedures, which have been described previously (Klip *et al.*, 2001; Klip, 2002; de Boer *et al.*, 2003).

To obtain a large enough comparison group of subfertile women not treated with IVF, we identified women who were diagnosed with fertility problems shortly before IVF became a routine procedure for subfertile patients. The non-IVF comparison group consisted of 6604 women whose subfertility was diagnosed in the four participating clinics that had a computerized registry of all subfertile women evaluated during 1980–1995. We attempted to frequency match the non-IVF comparison group according to the distribution of subfertility diagnoses in the IVF group. Most women in the non-IVF group registered for their first consultation in the 1980s (before IVF became a routine procedure) and underwent tubal surgery and/or hormonal treatments. The majority of those who registered after 1990 withdrew from the waiting list for IVF because they pursued other treatment options, reached the age of 40 years (the upper age limit for IVF at the time), became pregnant or decided to refrain from IVF for various reasons, such as divorce. When the non-IVF group was compared with the IVF group, it turned out that 911 women selected into the non-IVF comparison group subsequently received IVF. These women had subfertility treatments other than IVF in one centre and subsequently received IVF in a second centre. In the description of the cohort, these women are included in the IVF group (Table 1) (see also section 'Statistical analysis').

Based on names, birth dates and addresses at the time of subfertility treatment all cohort members were traced. Given that the subjects' last visit to the fertility clinic could date back to 1980, extensive tracing techniques were required to obtain current addresses of all women (Klip *et al.*, 2001; Klip, 2002; de Boer *et al.*, 2003), using the municipal population offices that fully cover the Netherlands. From the initial 26 465 women, 4.2% was not approached (the OMEGA cohort study, Fig. 1).

### Risk factor questionnaire

Between 1997 and 1999, 25 353 women received a risk factor questionnaire, a study information letter, and a brochure. Each participant was asked written informed consent for medical record data abstraction and future linkage with disease registries. The study information letter was signed by the treating gynaecologist or, if he/she had left, the current head of the IVF department. In the study information letter as well as in the brochure, women were informed about the purpose, the design and the privacy aspects of the study. The purpose of the study was stated as follows: 'to examine whether women who underwent an IVF treatment more frequently report gynaecological health problems compared with women who did not have an IVF treatment'. After 4–6 weeks, non-responders were sent a reminder. Non-responders to the second letter were approached by telephone. The 23 page questionnaire ascertained information on the women's reproductive histories, subfertility treatment, use of exogenous hormones, lifestyle factors and family history of cancer.

A total of 16 343 women returned the questionnaire (response rate 65.2%). The response rate was substantially lower in the non-IVF group (48.7%) than in the IVF group (71.1%).

### Medical records

Trained abstractors collected information on cause of subfertility and all fertility treatments. Cause of subfertility was classified as tubal, male factor, endometriosis, ovarian disorders, cervical factor, uterine abnormalities or unexplained. Multiple causes of subfertility were registered if applicable.

For each IVF and insemination cycle, we recorded date, dosage and type of FDs used in each phase of the menstrual cycle (hMG, FSH, clomiphene, hCG, GnRH and progesterone), number of oocytes collected and outcome. For FDs used prior to inseminations/IVF, we also coded date, dosage and type of FDs used per cycle. We made special attempts to collect information on subfertility treatments provided outside the participating IVF

**Table 1** Population characteristics of the OMEGA cohort by exposure status.

	IVF group (n = 19 146)		Non-IVF group (n = 6006)		Total (n = 25 152)	
	n	%	n	%	n	%
Year of birth						
≤1953	2527	13.2	1711	28.5	4238	16.8
1954–1957	4991	26.1	1440	24.0	6431	25.6
1958–1960	5995	31.3	1506	25.1	7501	29.8
≥1961	5633	29.4	1349	22.5	6982	27.8
Age at first IVF treatment or visit (years)						
≤26	1425	7.4	1159	19.3	2584	10.3
27–29	3015	15.7	1233	20.5	4248	16.9
30–32	4929	25.7	1339	22.3	6268	24.9
33–35	4711	24.6	1152	19.2	5863	23.3
≥36	5066	26.5	1123	18.7	6189	24.6
Subfertility diagnosis <sup>a,b</sup>						
Tubal	6025	31.5	1938	32.3	7963	31.7
Endometriosis	1970	10.3	349	5.8	2319	9.2
Male factor	5492	28.7	809	13.5	6301	25.1
Hormonal factor <sup>c</sup>	1287	6.7	409	6.8	1696	6.7
Unexplained	3412	17.8	537	8.9	3949	15.7
Other factors	912	4.8	360	6.0	1272	5.1
Missing	3309	17.3	2388	39.8	5697	22.7
Number of IVF treatments <sup>b</sup>						
1–2 cycles	6304	32.9				
3–4 cycles	6271	32.8				
5 or more cycles	3352	17.5				
Missing	3219	16.8				
Time since first treatment or visit (years)						
≤5 years	493	2.6	31	0.5	524	2.1
5–9 years	689	3.6	147	2.4	836	3.3
10–14 years	10 343	54.0	1526	25.4	11 869	47.2
≥15 years	7621	39.8	4302	71.6	11 923	47.4
Median years of follow-up	14.3		16.4			

<sup>a</sup>Women could have more than one cause of subfertility, except for unexplained and missing, which were unique classifications.

<sup>b</sup>Information based on medical records; for women without medical record data, information was added from health questionnaire survey.

<sup>c</sup>Included ovulation disorders, polycystic ovary syndrome and premature menopause.

clinics, by screening intake forms and letters from other treating physicians. Due to limited funding, we could only complete medical record abstraction for 9 out of 12 centres, i.e. 13 807 women (76% of women in the IVF group) (Klip et al., 2001; Klip, 2002; de Boer et al., 2003).

## Incidence of ovarian malignancies

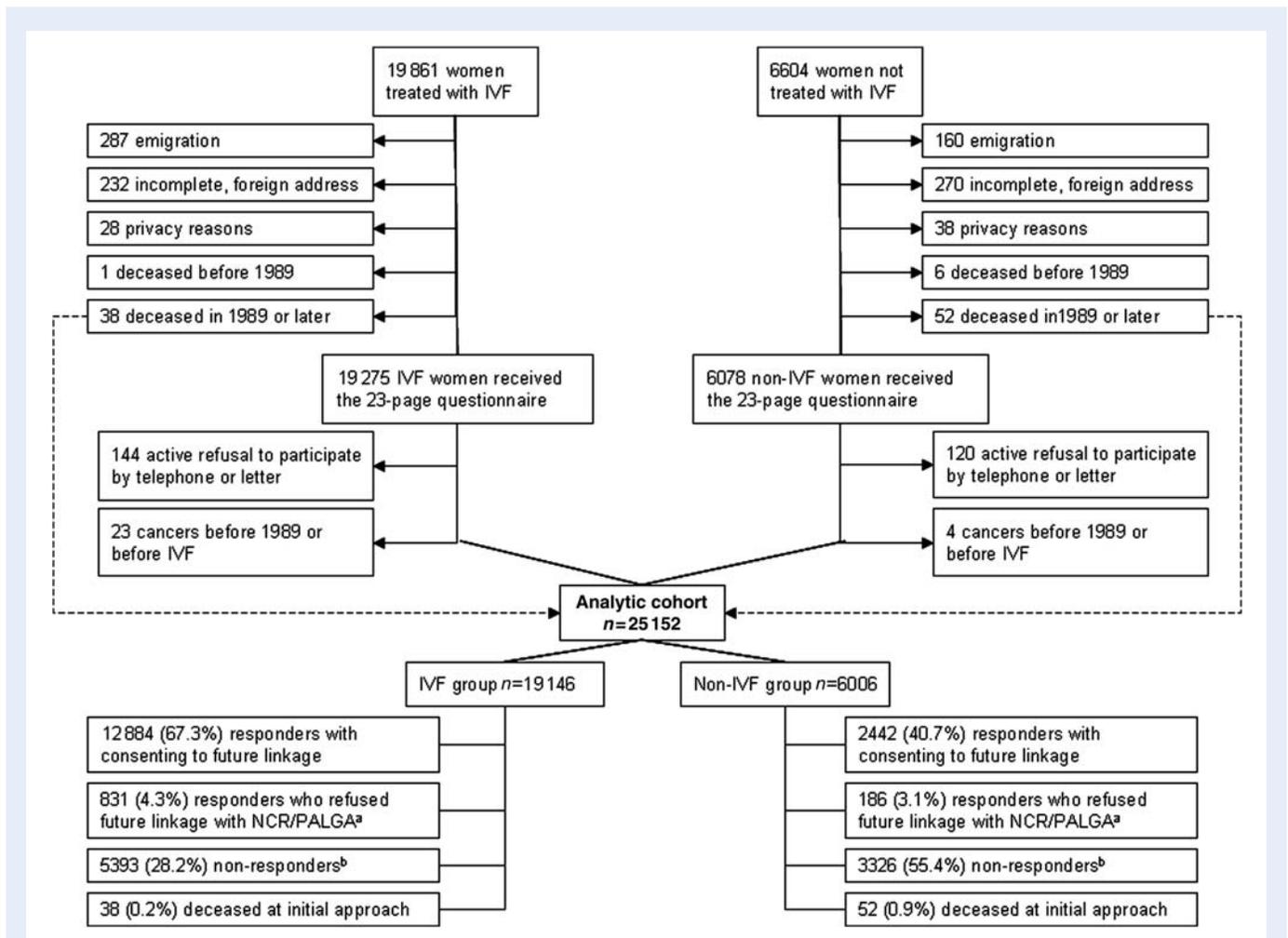
Cancer incidence in the period 1989–2003 was ascertained through linkage with the population-based Netherlands Cancer Registry (NCR)

(International Agency for Research on Cancer, 2003), and incidence of ovarian malignancies (including borderline ovarian tumours) through June 2007 was ascertained through linkage with the Dutch nationwide network and registry of histo- and cytopathology (PALGA). PALGA contains records of all histological diagnoses made in the Netherlands, with computerized data submission by all pathology laboratories, and nationwide coverage since 1989 (Casparie et al., 2007). We linked with PALGA since the NCR had incomplete data on borderline ovarian tumours; in addition PALGA case ascertainment is complete till 2 weeks prior to linkage, while the NCR lags a few years behind. We used a record linkage protocol developed previously (van den Brandt et al., 1990), which was based on the first four characters of the family name, gender and date of birth. All positive matches were checked for administrative twins by place of birth, postal code at cancer diagnosis and first initial. The NCR and PALGA granted us permission to not only link responders who gave permission, but also non-responders and deceased women, under additional privacy regulations. Only women who explicitly refused future linkage with disease registries (n = 1017; 4.0% of all women) were excluded from linkage. For each ovarian malignancy, we received information on date of diagnosis and morphology. Vital status as of June 2007 was obtained by linkage with the Central Bureau for Genealogy, which keeps computerized records of all deceased persons in the Netherlands since 1994.

## Statistical analysis

The analytic study cohort consisted of 25 152 women; 19 146 women in the IVF group and 6006 women in the non-IVF group (Fig. 1). Because the NCR and PALGA did not fully cover the Netherlands before 1989, the observation time for each participant started on 1 January 1989 or the date of first IVF treatment (IVF group), or clinic visit for subfertility evaluation (non-IVF group), whichever came last. Person-years of observation were calculated to the date PALGA follow-up ended (June 2007), date of ovarian cancer diagnosis or date of death, whichever came first. Women selected into the non-IVF comparison group who subsequently received IVF contributed person-time to the non-IVF group until the date of first IVF treatment, and switched to the IVF group after this date, according to standard cohort methodology regarding time-dependant allocation of person-years in case of changing exposure (Breslow and Day, 1987). Women diagnosed with ovarian cancer before entering the cohort (n = 14) or before 1989 (n = 13), were excluded from the analysis.

First, we compared ovarian cancer incidence in the IVF group and non-IVF group with incidence in the general population. We determined the standardized incidence ratio (SIR) as the ratio of the observed (O) and expected (E) number of cancers in the cohort. Expected numbers were based on age- and calendar period-specific reference rates for invasive ovarian cancer and borderline ovarian tumours from the NCR and PALGA, respectively (International Agency for Research on Cancer, 2003). Incidence rates for borderline ovarian tumours were calculated by the authors (T.M.M. and F.E.v L.), based on annual numbers of borderline ovarian tumour diagnoses obtained from PALGA. In all analyses, the subfertility cause(s) and treatments were preferably based on the medical records, and only derived from the woman's questionnaire if the records had not been abstracted. Information on reproductive factors was derived from the women's questionnaires, since these variables could change after IVF treatment. For non-responding women information from hospital databases was added when available. Previous FD use was defined as a combined variable relating to FD use during inseminations and FD use prior to inseminations/IVF, and was based on information from the medical records combined with the risk factor questionnaire.



**Figure 1** Identification of the OMEGA study cohort. <sup>a</sup>Women in this category contributed person time till date of questionnaire completion. <sup>b</sup>Including women who returned an empty questionnaire ( $n = 66$ ) and questionnaires that were returned to sender ( $n = 656$ ).

Cox proportional hazards models were used to compare cancer risk between the IVF group and the non-IVF group, adjusting for age and potential confounders such as parity and subfertility cause. Forward stepwise confounder selection, in which the effect of adding one confounder at a time was evaluated, was based on a  $> 10\%$  change in the risk estimate of the exposure variable of interest, irrespective of significance values.

In all analyses missing values were included as a separate category. Data were analysed with SPSS software (SPSS Inc., Chicago, IL, USA).

## Results

### Population characteristics

Characteristics of 19 146 IVF-treated women and 6006 women not treated with IVF are presented in Table 1. Women in the non-IVF group had a slightly longer median duration of follow-up than women in the IVF group (16.4 versus 14.3 years) and they were also older at the end of follow-up (mean age 49.4 versus 47.5 years). These differences reflect the initial inclusion criteria for the IVF and the non-IVF groups, with an over-representation of women in the non-IVF group seeking subfertility treatment in the years

before IVF treatment became a routine procedure. Cause of subfertility was related to tubal problems in 32% of women, 25% had male-factor subfertility, 9% endometriosis, 7% hormonal subfertility, 16% unexplained subfertility and 23% was missing (percentage add up to  $> 100\%$  due to multiple causes of subfertility). A total of 42% of the cohort was nulliparous at questionnaire completion. In the IVF group, 40% of women had one to two stimulated IVF cycles, 39% had three to four cycles and 21% received five or more cycles. IVF stimulation regimens used in the cohort have been described in detail previously (de Boer *et al.*, 2004). In brief, clomiphene/hMG or FSH/hMG stimulation protocols were used till 1988–1989, whereas stimulation with GnRH agonists became common after 1990 (from 20% in 1986 to about 90% after 1990). Furthermore, from 1984 to 1994, the number of ampoules of gonadotrophins strongly increased, as did the number of retrieved oocytes at the first IVF cycle (from 5.4 in 1986 to 10.7 in 1994) (de Boer *et al.*, 2004).

### Comparisons with external reference rates

After a median follow-up time of 14.7 years, 77 ovarian malignancies were observed in the full cohort [SIR = 1.43; 95% confidence interval

(CI) = 1.12–1.78]; 42 invasive ovarian cancers and 35 borderline ovarian tumours (Table II). Sixty-one ovarian malignancies were observed in the IVF group (SIR = 1.59; 95% CI = 1.21–2.04) and 16 in the non-IVF group (SIR = 1.02; 95% CI = 0.59–1.66). Compared with the general population rates, we observed a significantly increased risk for borderline ovarian tumours in the IVF group (SIR = 1.93; 95% CI = 1.31–2.73) and no increase in the non-IVF group (SIR = 0.67; 95% CI = 0.18–1.71). The SIRs for invasive ovarian cancer were not significantly raised in either IVF-treated women (1.35; 95% CI = 0.91–1.92) or non-IVF women (1.24; 95% CI = 0.64–2.17). The morphologies of the invasive ovarian cancers were serous (60%), mucinous (7%), clear-cell (7%), endometrioid (21%) and other (5%). Of the borderline ovarian tumours, 63% were serous and 37% were mucinous. Serous borderline ovarian tumours and invasive ovarian cancers occurred more frequently in the IVF group than in the non-IVF group ( $P = 0.04$ ).

The SIRs in both the IVF group and non-IVF group were strongly increased in the first year of follow-up (3- to 18-fold), possibly related to work-up for subfertility diagnosis and treatment. When we excluded the first year of follow-up, the SIR for all ovarian malignancies was 1.49 (95% CI = 1.12–1.94) in the IVF group and 0.85 (95% CI = 0.45–1.45)

in the non-IVF group. After 15 or more years, the SIR for invasive ovarian cancer in the IVF group was 3.54 (95% CI = 1.62–6.72,  $P$  for trend = 0.02), whereas the SIR in the non-IVF group was close to unity (Table II). No clear increase with longer follow-up was seen for borderline ovarian tumours ( $P$  for trend = 0.49).

Within the IVF group, SIRs of ovarian malignancy did not increase with a greater number of IVF cycles or ampoules of gonadotrophins (Table III). The mean number of oocytes harvested per stimulated cycle and the maximum number over all treatment cycles were used as a proxy for a woman's responsiveness to ovarian stimulation; the total number of oocytes collected over all cycles was used as a proxy for the amount of damage to the ovarian epithelium. The SIRs did not appear to be associated with any of these variables. FD use prior to IVF treatment was not associated with an increased SIR for all ovarian malignancies combined; for invasive ovarian cancer the SIR was non-significantly increased (SIR = 1.69; 95% CI = 0.95–2.79), while for borderline ovarian tumours the SIR was increased for women who did not use FDs prior to IVF treatment (SIR = 2.93; 95% CI = 1.71–4.69). These observations must be interpreted with caution since information on previous FD use was missing for 27% of women.

**Table II** Incidence of ovarian malignancies by years of follow up and exposure status.

Follow-up	IVF group				Non-IVF group				Total			
	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All ovarian malignancies												
< 1 years	6	1.52	3.94	1.44–8.57	3	0.31	9.55	1.97–27.91	9	1.84	4.90	2.24–9.30
1–4 years	9	7.52	1.20	0.55–2.27	1	1.74	0.57	0.01–3.20	10	9.27	1.08	0.52–1.98
5–9 years	16	12.41	1.29	0.74–2.09	3	3.58	0.84	0.17–2.45	19	15.99	1.19	0.72–1.86
10–14 years	18	13.22	1.36	0.81–2.15	4	4.63	0.86	0.23–2.21	22	17.85	1.23	0.77–1.87
≥ 15 years	12	3.73	3.22	1.66–5.62	5	5.36	0.93	0.30–2.18	17	9.08	1.87	1.09–3.00
All intervals	61	38.41	1.59	1.21–2.04	16	15.63	1.02	0.59–1.66	77	54.03	1.43	1.12–1.78
All intervals excl. first year	55	36.88	1.49	1.12–1.94	13	15.31	0.85	0.45–1.45	68	52.20	1.30	1.01–1.65
Invasive ovarian cancer												
< 1 years	2	0.78	2.57	0.31–9.26	3	0.16	18.35	3.79–53.60	5	0.94	5.30	1.72–12.37
1–4 years	5	3.94	1.27	0.41–2.96	1	0.93	1.07	0.03–5.97	6	4.88	1.23	0.45–2.68
5–9 years	4	6.90	0.58	0.16–1.48	2	2.03	0.99	0.12–3.56	6	8.93	0.67	0.25–1.46
10–14 years	10	8.13	1.23	0.59–2.26	2	2.85	0.70	0.09–2.54	12	10.98	1.09	0.56–1.91
≥ 15 years	9	2.54	3.54	1.62–6.72	4	3.68	1.09	0.30–2.79	13	6.22	2.09	1.11–3.57
All intervals	30	22.30	1.35	0.91–1.92	12	9.65	1.24	0.64–2.17	42	31.95	1.31	0.95–1.78
All intervals excl. first year	28	21.52	1.30	0.86–1.88	9	9.48	0.95	0.43–1.80	37	31.01	1.19	0.84–1.64
Borderline ovarian tumours												
< 1 years	4	0.74	5.38	1.46–13.77	0	0.15	0	0.00–24.59	4	0.89	4.47	1.21–11.45
1–4 years	4	3.58	1.12	0.03–2.86	0	0.81	0	0.00–4.55	4	4.39	0.91	0.25–2.33
5–9 years	12	5.51	2.18	1.13–3.81	1	1.55	0.64	0.02–3.59	13	7.06	1.84	0.98–3.15
10–14 years	8	5.09	1.57	0.68–3.10	2	1.79	1.12	0.14–4.04	10	6.87	1.45	0.70–2.68
≥ 15 years	3	1.18	2.53	0.52–7.40	1	1.68	0.60	0.02–3.32	4	2.86	1.40	0.38–3.58
All intervals	31	16.10	1.93	1.31–2.73	4	5.98	0.67	0.18–1.71	35	22.08	1.59	1.10–2.20
All intervals excl. first year	27	15.36	1.76	1.16–2.56	4	5.83	0.69	0.19–1.76	31	21.19	1.46	0.99–2.08

Obs, observed; Exp, expected; SIR, standardized incidence ratio; CI, confidence interval.

**Table III** Incidence of ovarian malignancies in IVF-treated women, according to IVF treatment characteristics, subfertility and parity.

IVF group	Person years	All ovarian malignancies				Invasive ovarian cancer				Borderline ovarian tumours			
		Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
Total number of IVF cycles <sup>a,b</sup>													
1–2 cycle(s)	82 599	21	13.99	1.50	0.93–2.29	11	8.12	1.35	0.68–2.42	10	5.87	1.70	0.97–3.74
3–4 cycles	84 025	22	14.46	1.52	0.95–2.30	10	8.43	1.19	0.57–2.18	12	6.04	1.99	1.22–4.14
≥5 cycles	47 661	12	8.43	1.42	0.74–2.49	7	4.97	1.41	0.57–2.90	5	3.45	1.45	0.47–3.38
Subfertility diagnosis <sup>b,c,d</sup>													
Tubal	84 822	35	14.96	2.34	1.63–3.25	15	8.90	1.69	0.94–2.78	20	6.06	3.30	2.02–5.10
Endometriosis	26 853	14	4.59	3.05	1.67–5.12	10	2.68	3.73	1.79–6.86	4	1.90	2.10	0.57–5.38
Male factor	70 793	16	11.53	1.39	0.79–2.25	11	6.58	1.67	0.83–2.99	5	4.95	1.01	0.33–2.36
Hormonal factor <sup>e</sup>	16 873	3	2.64	1.14	0.23–3.32	2	1.49	1.34	0.16–4.84	1	1.15	0.87	0.02–4.86
Unexplained	45 846	5	7.97	0.63	0.20–1.46	3	4.67	0.64	0.13–1.88	2	3.30	0.61	0.07–2.19
Other factors	12 005	4	2.02	1.98	0.54–5.07	2	1.17	1.71	0.21–6.19	2	0.85	2.35	0.28–8.48
Previous FD use <sup>c,f</sup>													
No	95 782	26	14.15	1.84	1.20–2.69	9	8.35	1.08	0.49–2.05	17	5.8	2.93	1.71–4.69
Yes	109 149	20	15.41	1.30	0.79–2.01	15	8.88	1.69	0.95–2.79	5	6.52	0.77	0.25–1.79
Missing	49 297	9	7.33	1.23	0.56–2.33	4	4.29	0.93	0.25–2.38	5	3.03	1.65	0.53–3.85
Parity <sup>a</sup>													
Nulliparous	86 058	24	12.82	1.87	1.20–2.79	9	7.58	1.19	0.54–2.25	15	5.24	2.86	1.60–4.72
Parous	123 242	21	17.38	1.21	0.75–1.85	14	10.03	1.40	0.76–2.34	7	7.35	0.95	0.38–1.96
Missing	44 928	10	6.68	1.50	0.72–2.75	5	3.91	1.28	0.41–2.98	5	2.77	1.81	0.59–4.22
Total no. of ampoules hMG/FSH <sup>g</sup>													
1–40 ampoules	48 033	10	6.85	1.46	0.70–2.69	5	3.99	1.25	0.41–2.93	5	2.86	1.75	0.57–4.08
41–80 ampoules	49 345	11	7.08	1.55	0.78–2.78	5	4.12	1.21	0.39–2.83	6	2.96	2.03	0.74–4.42
≥81 ampoules	57 749	14	8.60	1.63	0.89–2.73	8	5.06	1.58	0.68–3.11	6	3.54	1.69	0.62–3.69
Missing	99 101	20	14.35	1.39	0.85–2.15	10	8.35	1.20	0.57–2.20	10	6.00	1.67	0.80–3.07
Total no. of oocytes <sup>g</sup>													
0–19 oocytes	89 929	20	13.84	1.45	0.88–2.23	10	8.27	1.21	0.58–2.22	10	5.57	1.80	0.86–3.30
≥20 oocytes	79 186	16	10.63	1.50	0.86–2.44	7	6.01	1.16	0.47–2.40	9	4.62	1.95	0.89–3.70
Missing	85 113	19	12.42	1.53	0.92–2.39	11	7.24	1.52	0.76–2.72	8	5.18	1.55	0.67–3.05
Mean no. of oocytes <sup>g</sup>													
0–3 oocytes	21 468	6	3.81	1.57	0.58–3.43	3	2.40	1.25	0.26–3.65	3	1.41	2.12	0.44–6.20
4–6 oocytes	46 899	14	7.31	1.91	1.05–3.21	7	4.39	1.60	0.64–3.29	7	2.92	2.39	0.96–4.93
≥7 oocytes	100 747	15	13.35	1.12	0.63–1.85	6	7.50	0.80	0.29–1.74	9	5.85	1.54	0.70–2.92
Missing	85 113	20	12.41	1.61	0.98–2.49	12	7.24	1.66	0.86–2.90	8	5.17	1.55	0.67–3.05
Maximum no. of oocytes <sup>g</sup>													
0–5 oocytes	33 819	9	5.75	1.56	0.72–2.97	5	3.57	1.40	0.45–3.27	4	2.19	1.83	0.50–4.69
6–10 oocytes	58 581	13	8.75	1.49	0.79–2.54	5	5.16	0.97	0.31–2.26	8	3.59	2.23	0.96–4.39
≥11 oocytes	76 714	13	9.97	1.30	0.69–2.23	6	5.56	1.08	0.40–2.35	7	4.41	1.59	0.64–3.27
Missing	85 113	20	12.41	1.61	0.98–2.49	12	7.24	1.66	0.86–2.90	8	5.17	1.55	0.67–3.05

Obs, observed; Exp, expected; SIR, standardized incidence ratio; CI, confidence interval.

<sup>a</sup>Information based on health questionnaire survey; for non-responding women information was added from the medical records.

<sup>b</sup>Missing values of this variable were retrospectively completed for all cases; among non-cases with missing values, we distributed person time according to the distribution of person-years over categories of this variable.

<sup>c</sup>Information based on medical records; for women without medical record data, information was added from health questionnaire survey.

<sup>d</sup>Women may contribute person-years to more than one type of subfertility except for the categories unexplained and missing, which were unique classifications.

<sup>e</sup>Hormonal factors included ovulation disorders, polycystic ovary syndrome and premature menopause.

<sup>f</sup>Previous FD use was defined as a combined variable relating to FD use during inseminations and FD use prior to inseminations/IVF.

<sup>g</sup>Information based solely on medical records; no data abstraction could be done for 24% of the cohort that did give informed consent to do so.

**Table IV** Adjusted HRs for cancer risk in IVF group versus non-IVF group.

Cancer site	Overall		≥ 1 year follow-up		≥ 10 years follow-up	
	HR	95% CI	HR	95% CI	HR	95% CI
All ovarian malignancies <sup>a</sup>	2.05	1.10–3.82	2.14	1.07–4.25	2.08	0.86–5.00
Invasive ovarian cancer <sup>b</sup>	1.14	0.54–2.41	1.51	0.65–3.54	2.26	0.78–6.55
Borderline ovarian tumours <sup>c</sup>	6.38	2.05–19.84	4.23	1.25–14.33	2.26	0.46–11.05

HR, hazard ratio; CI, confidence interval.

<sup>a</sup>Adjusted for age at end of follow-up, endometriosis, tubal problems.

<sup>b</sup>Adjusted for age at end of follow-up, endometriosis.

<sup>c</sup>Adjusted for age at end of follow-up, tubal problems, parity.

Endometriosis was associated with significantly increased risk of invasive ovarian cancer, whereas tubal problems significantly increased the SIR for borderline ovarian tumours.

### Comparisons within the cohort

Direct comparison of the IVF group with the non-IVF group (Table IV) yielded an adjusted hazard ratio (HR) for all ovarian malignancies of 2.14 (95% CI = 1.07–4.25), excluding the first year of follow-up. The adjusted HRs for invasive ovarian cancer and borderline ovarian tumours were 1.51 (95% CI = 0.65–3.54) and 4.23 (95% CI = 1.25–14.33), respectively. No trends emerged with number of IVF cycles or other IVF treatment characteristics, but numbers in subcategories were small. Clomiphene use prior to IVF was not associated with increased risk of ovarian malignancies (HRs for all malignancies, invasive ovarian cancer and borderline ovarian tumours were 0.89 (95% CI = 0.45–1.77), 1.22 (95% CI = 0.50–2.99) and 0.62 (95% CI = 0.21–1.83), respectively). Finally, we compared the risk of all ovarian malignancies between the IVF group and women in the non-IVF group who never used FDs (HR = 1.83; 95% CI = 0.70–4.82, based on five cases in 2115 unexposed women).

### Discussion

This large nationwide cohort study with a median follow-up of 15 years shows that women treated with ovarian stimulation for IVF have a 2-fold increased risk of ovarian malignancies compared with subfertile women not treated with IVF. The excess risk was mostly due to borderline ovarian tumours, but 15 or more years after IVF treatment we also observed a SIR of 3.5 for invasive ovarian cancer.

Surprisingly, we observed that a high proportion (46%) of all ovarian malignancies in the IVF group concerned borderline ovarian tumours, whereas in the general population (below the age of 50 years) borderline ovarian tumours account only for 15–30% (Hart, 2005) of epithelial ovarian malignancies. So far only few studies examined FD use in relation to risk of borderline ovarian tumours, related to the fact that most population-based cancer registries do not record borderline ovarian tumours. Our cohort study is the first one examining

the risk of borderline ovarian tumours following IVF treatment. Strikingly, the few case–control studies that examined the risk of borderline ovarian tumours after FD use found 2- to 4-fold increased risks (Harris et al., 1992; Rossing et al., 1994; Shushan et al., 1996; Parazzini et al., 1998; Ness et al., 2002), though based on small numbers. In a case–cohort study (Rossing et al., 1994) reporting an 11-fold risk increase of ovarian malignancies after 12 or more cycles of clomiphene, 5 of the 11 ovarian tumours were borderline ovarian tumours. Although screening for ovarian tumours in IVF-treated women has never been recommended in the Netherlands, we considered whether the increased risk of borderline ovarian tumours in the IVF group might be due to increased medical surveillance. We sent a questionnaire about diagnostic procedures to the gynaecologists of all case subjects with a borderline ovarian tumour who had given permission to approach their physician ( $n = 18$ ). We received information for 14 subjects; in all cases, the diagnosis was made subsequent to complaints for which the woman visited her gynaecologist, rendering surveillance bias an unlikely explanation of our findings. Remarkably, we observed a high proportion of serous borderline ovarian tumours (63%), which was also seen in one case–control study (Ness et al., 2002). Mucinous borderline ovarian tumours are more frequent in the general population (Verbruggen et al., 2009).

Risk of borderline ovarian tumours was particularly strongly elevated in the first year after IVF, which is in line with several case reports of borderline ovarian tumours developing during or shortly after ovarian stimulation treatments (Atlas and Menczer, 1982; Goldberg et al., 1992; Nijman et al., 1992), providing support for speculations that ovarian stimulation may induce growth in existing highly differentiated tumours (Brinton et al., 2005). We excluded ovarian tumours occurring in the first year after IVF, because of concern that their diagnosis might be related to diagnostic and treatment procedures for infertility. The early increase in risk was followed by a SIR close to unity in the 1–4 year follow-up interval; subsequently, risk of borderline ovarian tumours remained elevated up to more than 15 years after first IVF treatment. Hence, our data suggest that IVF treatment may be causally related to a prolonged increase of the risk of highly differentiated tumours. The natural history of borderline ovarian tumours is unclear and it is unknown which part of borderline ovarian tumours, if undetected, would develop into invasive ovarian cancer (Singer et al., 2003; Sherman et al., 2004; Shih and Kurman, 2004).

A concerning finding of our study is the increased SIR of invasive ovarian cancer in the IVF group after more than 15 years of follow-up, which was not observed in the non-IVF group. We cannot compare this result with findings from others since our study is the first reporting on cancer risk more than 10 years after IVF treatment. However, Brinton et al. (2004) followed a large cohort of 12 193 women treated for infertility prior to the IVF era. After 15 or more years of follow-up they reported non-significantly elevated rate ratios of ovarian cancer, 1.48 (95% CI = 0.7–3.2) for clomiphene and 2.46 (95% CI = 0.7–8.3) for gonadotrophins (when compared with never use of these drugs). Sanner et al. (2009) reported on a Swedish cohort treated for infertility in the 1960s–1970s, with a median follow-up of 33 years. Gonadotrophins were associated with increased risk of invasive ovarian cancer (relative risk = 5.28, 95% CI = 1.70–16.47) but clomiphene was not (when compared with never use of these drugs) (Sanner et al., 2009). Ovulation stimulating drugs such as clomiphene were introduced in the late 1960s and IVF treatment with gonadotrophins,

resulting in much stronger ovarian stimulation, did not become widely available until the late 1980s. Consequently, women exposed to clomiphene have just recently reached the age range at which ovarian cancer frequently occurs (>70 years), while the oldest IVF-treated women have only recently reached their 50s. Since the induction period of ovarian cancer with respect to established risk factors amounts to 25 years or more (Risch, 1998), much longer follow-up is needed to fully evaluate the effects of gonadotrophins.

If ovarian stimulation were causally related to the risk of ovarian malignancy, we would expect increasing risks with greater number of IVF cycles or number of oocytes harvested. No such dose–response trends emerged. However, numbers in relevant dose categories were small, and data were missing for 17% of subjects, which reduced power for these analyses. In addition, the number of IVF cycles and number of harvested oocytes are only proxies for the number of ovarian punctures, which may have reduced the power to detect a dose–response relationship.

Case–control studies of the association between ovarian cancer risk and FD use have shown inconsistent results, with some studies reporting increased risks for subgroups (e.g. nulliparous women) (Ness *et al.*, 2002; Rossing *et al.*, 2004) and some suggesting a dose–response effect for clomiphene (Ness *et al.*, 2002; Rossing *et al.*, 2004). Treatment with hMG or FSH, as in IVF, may increase the number of ovulations to approximately six to nine times that of untreated women (Fishel and Jackson, 1989), which is a much stronger increase than the doubling of ovulations with clomiphene (Glasier, 1990; Derman and Adashi, 1994).

Nationwide cohort studies of IVF-treated women have only been reported from Australia (Venn *et al.*, 1999), Israel (Lerner-Geva *et al.*, 2003) and Sweden (Källén *et al.*, 2011). The first two cohort studies did not show increased risk of ovarian cancer in the IVF group compared with the general population (Venn *et al.*, 1999; Lerner-Geva *et al.*, 2003), while the recent Swedish study reported for parous women increased risk of ovarian cancer after IVF, compared with all other Swedish women who gave birth in the study period (HR = 2.09; 95% CI = 1.39–3.12) (Källén *et al.*, 2011). However, this study had no information on subfertility cause; therefore it is not clear whether the risk increase is attributable to IVF or subfertility. Of all cohort studies including IVF-treated women, our study includes the largest number of ovarian malignancies ( $n = 77$  versus 13, 3 and 26 cases in the cohort studies from Australia, Israel and Sweden) (Venn *et al.*, 1999; Lerner-Geva *et al.*, 2003; Källén *et al.*, 2011).

Our study design had several strengths and weaknesses. Advantages include the large size of our cohort and the long-term follow-up. Selection bias can be ruled out since we were able to link 96% of our cohort with the population-based cancer and pathology registries, enabling us to also evaluate the occurrence of borderline ovarian tumours. All ovarian malignancies were histologically confirmed. Furthermore, we collected reproductive variables after IVF directly from the participating women, whereas for the majority of women information on subfertility cause and treatment could be abstracted from the medical files. Our data also include information on FD use prior to IVF, although this was incomplete for 27% of women. A limitation of our study is, however, that the comparison group of women unexposed to IVF treatment was relatively small, and that a proportion of these women (40%), had used FDs (clomiphene) outside the IVF

setting (as did 54% of women in the IVF group), thus restricting the power for comparisons with a truly unexposed reference group. However, if multiple ovarian punctures rather than hormonal stimulation would induce ovarian malignancy, potential differences in FD use outside the IVF setting are not relevant.

Unfortunately, the response rate to the questionnaire was lower in the non-IVF group (49 versus 71% in the IVF group). Since we were allowed to link non-responders with the NCR and PALGA, differential non-response could not affect our overall risk estimates. However, the larger proportion of missing values for potential confounders (reproductive factors, cause of subfertility) among controls complicated our multivariable analyses. Adjustment for potential confounders did not materially affect our risk estimates, however.

We wondered whether the increased SIR of invasive ovarian cancer observed in the IVF group after 15 years might be due to less oral contraceptive (OC) use and/or lower parity in IVF-treated women. However, in the non-IVF group no increased SIR after long-term follow-up was seen. The proportion of long-term ( $\geq 7$  years) OC users was high in our cohort and very similar in the IVF group and the non-IVF group (39.2 and 38.1%, respectively). Dutch women start OC use early and have a late age at first birth (mean 1985–1995: 28 years (Statistics Netherlands; [www.cbs.nl](http://www.cbs.nl), 2011) and only 19.1% of the IVF group and 22.5% of the non-IVF group never used OC or used them <1 year. Consequently, OC use was not a confounder in our multivariable Cox analysis. IVF-treated women remained more often nulliparous than the non-IVF group (44 versus 35%), but adjustment for parity only affected our results for borderline ovarian tumours, not for invasive ovarian cancer.

Our study is the only IVF cohort including a comparison group of subfertile women not treated with IVF, in addition to a comparison with the general population. Such a comparison group is important since IVF-treated women differ from the general population with respect to several risk factors for ovarian malignancies, e.g. subfertility and nulliparity. We cannot exclude the possibility, however, that the severity of certain causes of subfertility in the IVF group was not the same as in the non-IVF group. Since adjustment for individual causes of subfertility only slightly affected our estimates of the risk associated with IVF (data not shown), residual confounding by severity of certain subfertility causes seems unlikely, however.

Another limitation of our study is that our results are based on IVF treatment protocols used until 1995, prior to the adoption of currently applied milder stimulation regimens.

In conclusion, our results suggest that ovarian stimulation for IVF may increase the risk of ovarian malignancies, especially borderline ovarian tumours. Knowledge about the magnitude of the risks associated with ovarian stimulation is important for women considering starting or continuing IVF treatment, as well as their treating physicians. Clearly, the outcome of weighing a wish to conceive against the potential risks associated with IVF may differ among couples considering fertility treatment. In the Netherlands the cumulative risk of ovarian malignancy (including borderline ovarian tumours) is small, i.e. 0.45% at the age of 55 years. If our results are true, we would estimate a 0.71% risk for women who underwent IVF. It should be explained to women opting for IVF treatment that a borderline ovarian tumour does not constitute a lethal disease, although it may require extensive surgery and cause substantial morbidity. Ovarian cancer, however, is a disease with a high case fatality rate, for which

effective screening methods are not available (Hermsen *et al.*, 2007). Although our findings give reason for some concern, they are still based on rather small numbers, no dose–response relationship was found and the risk increase for invasive ovarian cancer was not statistically significant in multivariable analyses. Even larger prospective cohort studies of IVF-treated women, with prolonged follow-up and a subfertile comparison group not treated with IVF, are needed to confirm or refute our findings and to conduct dose–response analyses with more power.

## Authors' roles

F.E.v L. and C.W.B. designed the OMEGA study and were principal investigators of the study. F.E.v L. also coordinated statistical analyses, contributed to interpretation of the data and drafted the paper. C.W.B. contributed to interpretation of the data and drafting of the manuscript. H.K. contributed to the design of the study, coordinated identification of the cohort and data collection, did statistical analyses and contributed to interpretation of data. T.M.M. coordinated data collection, did the statistical analyses, contributed to study design, interpretation of the data and drafting of the manuscript. A.M.G.vd S. contributed to data collection and statistical analysis. C.B.L., M.K., J.S.E.L., C.A.M.J., F.M.H., B.J.C., W.N.P., J.M.J.S., A.H.M.S., F.vd V., J.L.H.E., P.A.v D. and N.S.M. provided IVF patient data and contributed to interpretation of the data. All authors contributed to critical revisions of the draft manuscript. All authors saw and approved the final version of the report.

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## Conflict of interest

J.L.H.E. declares that he works in a department that has received unrestricted research grants from MSD and Ferring.

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# Spontaneous Abortion Among Pregnancies Conceived Using Assisted Reproductive Technology in the United States

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**OBJECTIVE:** To examine rates and risk factors for spontaneous abortion among pregnancies conceived using assisted reproductive technology (ART).

**METHODS:** Subjects were 62,228 clinical pregnancies resulting from ART procedures initiated in 1996–1998 in US clinics. Spontaneous abortion was based on ART clinic report and was defined as loss of the entire pregnancy. Spontaneous abortion rates for ART pregnancies were compared with spontaneous abortion rates from the National Survey of Family Growth, a population-based survey of US women 15–44 years.

**RESULTS:** The spontaneous abortion rate among ART pregnancies was 14.7%. This was similar to rates among pregnancies reported in the National Survey of Family Growth. Among pregnancies conceived with the patient's oocytes and freshly fertilized embryos, the spontaneous abortion risk ranged from 10.1% among women 20–29 years to 39.3% among women older than 43. Spontaneous abortion risk among pregnancies conceived with donor eggs was 13.1% with little variation by patient age. Spontaneous abortion risk was increased for pregnancies conceived with frozen and thawed embryos and decreased among multiple-gestation pregnancies. Spontaneous abortion risk was increased among women reporting previous spontaneous abortions and ART attempts, and among

women who used clomiphene or zygote intrafallopian transfer. Pregnancies conceived by young women, but gestated by a surrogate, were at increased risk for spontaneous abortion in comparison with young women who gestated their own pregnancies.

**CONCLUSION:** These findings suggest that ART does not pose a risk for spontaneous abortion. Factors related to oocyte or embryo quality are of primary importance in assessing spontaneous abortion risk. (*Obstet Gynecol* 2003;101:959–67. © 2003 by The American College of Obstetricians and Gynecologists.)

An estimated 10–15% of clinically recognized pregnancies end in spontaneous abortion.<sup>1–4</sup> In addition to advanced maternal age, factors that have been associated with spontaneous abortion include chromosomal abnormalities, single gene mutations, structural uterine abnormalities, endocrine abnormalities, immunologic factors, genital infections, cigarette smoking, alcohol use, and various environmental and occupational exposures.<sup>1–3</sup> Some of these, such as chromosomal abnormalities, are clearly related to the embryo and others, such as uterine abnormalities, are clearly related to the host. The mechanisms through which other risk factors operate are not all completely defined; additionally, a given factor, such as age, may act through both embryo and host mechanisms.

With the advent of in vitro fertilization and related assisted reproductive technologies (ART), several population-based reports suggested that pregnancies conceived using these treatments had higher than expected spontaneous abortion rates.<sup>5–10</sup> However, interpretation of findings to date is constrained by several methodological shortcomings: Clinical pregnancy and spontaneous abortion were inconsistently defined across studies; rates were sometimes reported for several years' data combined, thus encompassing changes in technology and drug protocols; adjustment for age and other factors, such as medical history, was lacking or inadequate.

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*The data used for this study were collected using the Society for Assisted Reproductive Technology reporting system, which was developed in 1986. Since 1995, data from this system have been used by the Centers for Disease Control and Prevention to calculate pregnancy success rates for assisted reproductive technology clinics operating in the United States. This system is jointly supported by the Society for Assisted Reproductive Technology; the American Society for Reproductive Medicine; the Centers for Disease Control and Prevention; and RESOLVE, the National Infertility Association.*

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Numerous small studies from ART clinic populations have also been reported,<sup>11-21</sup> some of which have assessed risk factors for spontaneous abortions with ART. However, with the exception of an association with maternal age, findings across studies have been inconsistent.

The US ART Registry includes data for over 180,000 ART procedures performed in 1996 through 1998. We used these data to calculate spontaneous abortion rates for ART pregnancies in the United States. We further examined potential risk factors for spontaneous abortion. Such analyses have implications for this specific population of ART pregnancies; additionally, analyses such as these on a large population-based sample of pregnancies with complete and early pregnancy diagnosis and complete data on spontaneous abortion contribute to the general knowledge base of the epidemiology of spontaneous abortion.

## MATERIALS AND METHODS

Clinics and medical practices in the United States are mandated to report data for every ART procedure to the Centers for Disease Control and Prevention.<sup>22</sup> The Society for Assisted Reproductive Technology creates a database of ART procedures performed annually in US clinics and provides these data to the Centers for Disease Control and Prevention. ART is defined as infertility treatments in which both oocytes and sperm are handled outside the body; these include in vitro fertilization-transcervical embryo transfer, gamete and zygote intrafallopian transfer, frozen embryo transfer, and donor embryo transfer. Data abstracted from patient records and submitted to the Centers for Disease Control and Prevention include patient demographics, medical history, and clinical information on the ART procedure and resultant pregnancies and births. However, 5-7% of clinics do not report data each year. Most of these are known to be small practices; thus, we estimate that the data reported represent more than 95% of all ART procedures performed.

Approximately 10% of reporting clinics are selected each year for data validation site visits, which include review of pregnancy outcomes reported. To date, error rates have been low (less than 2%).

For the present study, we selected ART embryo transfer procedures performed from January 1, 1996, through December 31, 1998, in which the patient was between 20 and 55 years ( $n = 181,340$ ). Of these, 63,855 (35.2%) resulted in a clinical pregnancy. We defined clinical pregnancy as documentation of one or more gestational sacs visible sonographically. An ectopic pregnancy was not considered a clinical pregnancy. Clinical pregnancies

were diagnosed and documented by the ART providers. They obtained the outcome for each pregnancy through active follow-up with either the patient or her obstetric provider. We excluded from analyses 1627 pregnancies (2.5%) for which outcome data were not available (ie, pregnancies lost to follow-up), bringing our final sample to 62,228 pregnancies. Approval for this study was obtained from the Institutional Review Board at the Centers for Disease Control and Prevention.

We defined spontaneous abortion as loss of the *entire* pregnancy. Pregnancies in which the number of fetuses was reduced, either spontaneously or through medical intervention, but the pregnancy continued and resulted in a birth, were thus not considered spontaneous abortions. For this analysis, only pregnancy losses in which spontaneous abortion was the specific outcome designated are considered; pregnancies reported as stillbirths (less than 1% each year) or induced abortions (less than 2% each year) were not counted as spontaneous abortions. (However, because induced abortions might have ended as spontaneous abortions if the pregnancy had not been terminated, we did perform selected sensitivity analyses in which we recalculated spontaneous abortion rates after recoding induced abortions to spontaneous.)

For 75% of the spontaneous abortions reported, completed weeks' gestation was reported as 20 or less. For 23%, date of pregnancy loss was not recorded. In rare cases (2%), a patient or provider specified that the pregnancy ended as a spontaneous abortion, but weeks' gestation was either greater than 20 or was implausible. For this analysis, we considered the latter two groups as having a spontaneous abortion, ie, we gave preference to the provider report that the pregnancy ended in spontaneous abortion. Likewise, we did not recode the rare cases in which the pregnancy outcome was reported as stillbirth, but the gestational age was less than 20 weeks.

We calculated spontaneous abortion rates as the number of clinical pregnancies ending in spontaneous abortion divided by the total number of clinical pregnancies. We present rates as per 100 clinical pregnancies.

We initially assessed variation in spontaneous abortion rates by patient age, pregnancy plurality, and ART treatment type. Two different pregnancy plurality variables were created and analyzed: one based on the number of gestational sacs visible sonographically, and a second based on the number of fetal hearts visible sonographically; because results were comparable, we only present findings based on gestational sacs here. Treatment type was classified according to: 1) whether embryos were fertilized during the current procedure (fresh) or had been previously fertilized and frozen until the current procedure, and 2) whether the source of the

**Table 1.** Percentage Distribution of Study Population by Patient and ART Treatment Factors

	<i>n</i> *	%
Patient age (y)		
20–29	8143	13.1
30–34	22,190	35.7
35–37	14,128	22.7
38–40	9948	16.0
41–43	4899	7.9
44–47	2372	3.8
48–55	548	0.9
Primary infertility diagnosis		
Endometriosis	8531	13.7
Tubal factor	15,450	24.8
Male factor	15,350	24.7
Ovulatory dysfunction	9716	15.6
Uterine factor	1201	1.9
Other infertility cause <sup>†</sup>	7089	11.4
Idiopathic	4886	7.9
Previous total pregnancies		
0	29,591	47.6
1	16,032	25.8
≥2	16,603	26.7
Previous spontaneous abortions		
0	37,531	60.3
1	13,962	22.4
≥2	10,724	17.2
Previous live births		
0	46,712	75.3
1	11,457	18.5
≥2	3873	6.2
Previous ART procedures		
0	33,066	54.9
1	13,867	23.0
≥2	13,347	22.1
Year of current ART procedure		
1996	17,131	27.5
1997	20,795	33.4
1998	24,302	39.1
Oocyte source		
Patient	54,458	87.5
Donor	7770	12.5
Type of ovarian stimulation medication <sup>‡</sup>		
Gonadotropins only	46,174	98.3
Clomiphene (± gonadotropins)	430	0.9
None (unstimulated)	37	0.1
Luteal support only	290	0.6
Embryo type		
Fresh	54,601	87.7
Frozen	7627	12.3
ART method <sup>‡</sup>		
IVF–ET	44,602	92.9
GIFT	1825	3.8
ZIFT	1087	2.3
Combination	509	1.1
Gestational surrogate used		
No	61,507	98.8
Yes	721	1.2
Assisted hatching used		
No	35,958	59.7
Yes	24,266	40.3

(continued)

**Table 1.** Continued

	<i>n</i> *	%
ICSI used <sup>‡</sup>		
No	26,468	58.0
Yes	19,131	42.0
Pregnancy plurality		
Singleton	34,983	56.4
Twin	18,391	29.7
Triplet or more	8634	13.9

ART = assisted reproductive technology; IVF–ET = in vitro fertilization–transcervical embryo transfer; GIFT = gamete intrafallopian transfer; ZIFT = zygote intrafallopian transfer; ICSI = intracytoplasmic sperm injection.

\* Sample size was reduced for some analyses because of missing values for covariates.

<sup>†</sup> Other causes of infertility include immunologic causes, chromosomal abnormalities, cancer chemotherapy, and serious illness.

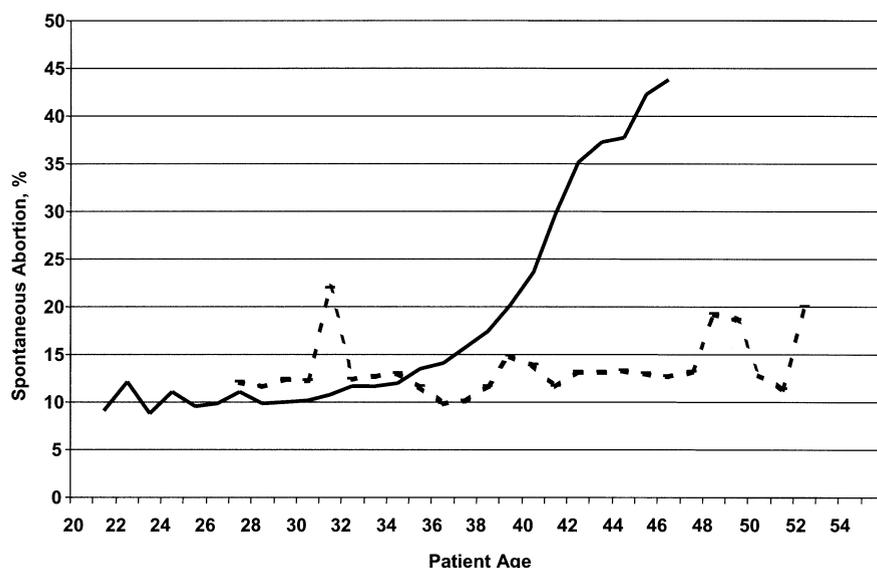
<sup>‡</sup> Percentage distribution based on ART procedures with patient oocytes and freshly fertilized embryos because data were not available for procedures that used donor oocytes or frozen embryos.

oocytes was the patient or a woman serving as an oocyte or embryo donor.

We also evaluated several other potential patient-, ART treatment-, and pregnancy-related risk factors for spontaneous abortion. Because we observed considerable variation in spontaneous abortion rates by ART treatment type, we limited these additional in-depth analyses to pregnancies conceived by a single (and by far the most common) treatment type, procedures using the patient's own oocytes, and freshly fertilized embryos. In all, 48,055 pregnancies (77%) were conceived using this treatment type. Risk factors evaluated included primary infertility diagnosis, previous total pregnancies, previous spontaneous abortions, previous live births, previous ART procedures, year the current ART procedure was performed, type of ovarian stimulation medication used, method of embryo transfer (in vitro fertilization–transcervical embryo transfer, gamete and zygote intrafallopian transfer), whether a woman other than the patient gestated the pregnancy (gestational carrier or surrogate), and use of specialized procedures in conjunction with the ART (assisted hatching and intracytoplasmic sperm injection).

We calculated odds ratios and 95% confidence intervals for the risk of spontaneous abortion by the factors described above. Because in our initial analyses maternal (ART patient) age and pregnancy plurality were found to be strongly related to spontaneous abortion risk, we evaluated all associations separately for singleton pregnancies and adjusted odds ratios for age using logistic regression.

Finally, we compared the rates of spontaneous abortion for ART pregnancies with rates of spontaneous abortion from the National Survey of Family Growth, a



**Figure 1.** Spontaneous abortion rates for assisted reproductive technology pregnancies conceived with freshly fertilized embryos by source of oocytes used and maternal age. The solid line indicates pregnancies conceived with the patient's oocytes, and the dashed line indicates pregnancies conceived with donor oocytes.

*Schieve. Spontaneous Abortion and ART. Obstet Gynecol 2003.*

population-based survey of women 15–44 years in the civilian noninstitutionalized population of the United States.<sup>23</sup> The most recent National Survey of Family Growth was conducted in 1995; 10,847 women were included in that survey. In-person interviews were conducted between January and October 1995. We calculated spontaneous abortion rates among women included in the National Survey of Family Growth who completed a pregnancy in 1993, 1994, or 1995 (partial year only). A sampling weight was assigned to each record to account for differing sampling rates within racial ethnic subgroups and nonresponse; therefore, rates were calculated as weighted national estimates.

## RESULTS

For over half the pregnancies included in this study, the patient was aged 35 years or older (Table 1). Tubal factor and male factor were the two most common infertility diagnoses. Although approximately one-half of the patients reported one or more previous pregnancies, about one-fourth reported a previous live birth. Nearly 40% reported a previous spontaneous abortion though, and 45% reported a previous ART procedure. The number of pregnancies included in the current study increased in each successive year (1996–1998). The vast majority of pregnancies were conceived with the patient's oocytes, ovarian stimulation with gonadotropins, freshly fertilized embryos, and uterine transfer (in vitro fertilization–transcervical embryo transfer). Only 1% of pregnancies were gestated by a surrogate, in 40% an assisted hatching technique was used, and in 42% intracytoplasmic sperm injection was used as part of the in

vitro fertilization. Nearly 44% of pregnancies were multiple gestations.

The spontaneous abortion rate among all clinical pregnancies in the study population was 14.7%. However, this rate varied greatly depending on the source of the oocyte, whether the embryos were freshly fertilized or had been frozen and thawed, and the patient's age. Among pregnancies that had been conceived using the patient's oocytes and freshly fertilized embryos, an increase in the spontaneous abortion rate with increasing age was observed beginning in the early 30s; this trend became more marked from the mid-30s onward (Figure 1). In contrast, there was little variation in spontaneous abortion rates by patient age among pregnancies that were conceived using donor oocytes and freshly fertilized embryos. These trends are further illustrated in Table 2. Women aged 20–29 who conceived using their own oocytes and freshly fertilized embryos had a 10.1% spontaneous abortion rate. The rates were more than tripled for women in their 40s. The spontaneous abortion rate for pregnancies conceived with donor oocytes and freshly fertilized embryos was 13.1%. It should be noted that data on the ages of the oocyte donors were not available; however, in many ART clinics, oocyte donors are limited to women in their 20s or early 30s. In contrast, patients using donor oocytes tend to be older, with 95% of all donor oocyte procedures performed on women aged 37 or older.

Pregnancies conceived with frozen and thawed embryos had higher rates of spontaneous abortion overall in comparison with pregnancies with freshly fertilized embryos (Table 2). A trend of increasing risk with in-

**Table 2.** Spontaneous Abortion Rates by Source of Oocytes Used in the ART Procedure, Status of the Embryos, and Patient Age

	All pregnancies				Singleton pregnancies			
	Pregnancies (n)	SAB (n)	SAB rate (%)	OR (95% CI)	Pregnancies (n)	SAB (n)	SAB rate (%)	OR (95% CI)
<b>Freshly fertilized embryos</b>								
Patient oocytes, age 20–29	7016	705	10.1	1.0 (Reference) <sup>†</sup>	3552	505	14.2	1.0 (Reference) <sup>†</sup>
Patient oocytes, age 30–34	18,846	2124	11.4	1.13 (1.04, 1.24)	9721	1572	16.2	1.16 (1.04, 1.30)
Patient oocytes, age 35–37	11,759	1683	14.3	1.50 (1.36, 1.64)	6608	1288	19.5	1.46 (1.31, 1.63)
Patient oocytes, age 38–40	7722	1543	20.0	2.23 (2.03, 2.46)	4862	1260	25.9	2.11 (1.88, 2.37)
Patient oocytes, age 41–43	2481	808	32.6	4.32 (3.86, 4.85)	1792	672	37.5	3.62 (3.17, 4.14)
Patient oocytes, age 44–47	219	86	39.3	5.79 (4.37, 7.68)	169	80	47.3	5.42 (3.95, 7.44)
Donor oocytes, all ages*	6546	860	13.1	1.35 (1.22, 1.51)	3130	649	20.7	1.58 (1.39, 1.79)
<b>Frozen and thawed embryos</b>								
Patient oocytes, age 20–29	921	144	15.6	1.0 (Reference) <sup>†</sup>	611	117	19.2	1.0 (Reference) <sup>†</sup>
Patient oocytes, age 30–34	2601	376	14.5	0.91 (0.74, 1.12)	1707	317	18.6	0.96 (0.76, 1.22)
Patient oocytes, age 35–37	1555	259	16.7	1.08 (0.86, 1.35)	1052	209	19.9	1.05 (0.81, 1.35)
Patient oocytes, age 38–40	905	203	22.4	1.56 (1.23, 1.98)	650	175	26.9	1.56 (1.19, 2.03)
Patient oocytes, age 41–43	286	88	30.8	2.40 (1.76, 3.26)	218	80	36.7	2.45 (1.74, 3.44)
Patient oocytes, age 44–47	104	24	23.1	1.62 (0.99, 2.64)	75	21	28.0	1.64 (0.95, 2.83)
Donor oocytes, all ages*	1224	229	18.7	1.24 (0.99, 1.56)	808	189	23.4	1.29 (0.99, 1.67)

ART = assisted reproductive technology; SAB = spontaneous abortion; OR = odds ratio; CI = confidence interval.

\* Age indicates the age of the patient undergoing the ART procedure, ie, not the age of the oocyte donor. Patients older than 47 y who used their own oocytes were not included in this analysis ( $n = 43$ ). The donor oocyte categories, however, include patients of all ages up to 55 y combined.

<sup>†</sup>  $P < .001$ , test for trend for increasing odds of spontaneous abortion with increasing patient age, among patients using their own oocytes.

creasing age was still observed for pregnancies conceived with the patient's oocytes; however, it was much less pronounced. For frozen embryo pregnancies, findings are based on patient age at the time of embryo transfer; age of the patient at the time of oocyte retrieval and fertilization was not available.

For all treatment types, pregnancy plurality was found to be highly associated with spontaneous abortion. Overall, spontaneous abortion rates were 20.4%, 7.5%, and 5.0% for singleton, twin, and triplet or greater pregnancies, respectively. We, therefore, repeated all analyses after restricting our sample to singleton pregnancies. In all age and treatment type categories, spontaneous abortion rates were higher among singleton pregnancies (Table 2). However, the same patterns of increasing risk with age were observed.

Several other factors were also associated with spontaneous abortion in this ART population (Table 3). After restricting to singleton pregnancies and adjusting for patient age, an increased risk for spontaneous abortion was observed among patients who had previous pregnancies and spontaneous abortions, had previously undergone ART, whose study pregnancy was conceived with ART in 1997 or 1998, who used clomiphene for ovarian stimulation, and who used zygote intrafallopian transfer rather than in vitro fertilization–transcervical embryo transfer. Decreased spontaneous abortion rates were observed among patients diagnosed with endometriosis.

The association between use of a gestational surrogate and spontaneous abortion varied according to patient age (Table 4). Among women younger than 35, pregnancies carried by a gestational surrogate had an increased risk for spontaneous abortion. Among women older than 40, use of a gestational carrier appeared to be protective. Although statistical power was low within individual age strata, the age-specific odds ratios showed a linear trend. Also, the effects for the youngest and oldest age groups were in opposite directions.

Spontaneous abortion rates among ART patients who conceived using their own oocytes and freshly fertilized embryos were comparable to, or even lower than, the spontaneous abortion rates of similarly aged women included in the National Survey of Family Growth (Table 5). The rates remained comparable after sensitivity analyses in which the ART sample was restricted to singleton pregnancies and induced abortions were recorded as spontaneous.

## DISCUSSION

The spontaneous abortion rate for this US population-based sample of ART pregnancies was comparable to rates previously reported for naturally conceived clinical pregnancies<sup>1–4</sup> and rates calculated from a representative sample of US women included in the National Survey of Family Growth. Thus, our findings suggest that ART as it is currently performed does not pose an

**Table 3.** Associations Between Patient, Pregnancy, and ART Treatment Factors and Spontaneous Abortion Among Pregnancies Conceived With the Patient's Oocytes and Freshly Fertilized Embryos

	All pregnancies	Singleton pregnancies	Singleton pregnancies
	SAB rate (%)	SAB rate (%)	Age-adjusted OR* (95% CI)
Total	14.5	20.1	
Primary infertility diagnosis			
Endometriosis	13.2	17.8	0.87 (0.78, 0.96)
Tubal factor	14.6	20.3	Reference
Male factor	14.0	19.4	0.96 (0.89, 1.04)
Ovulatory dysfunction	15.7	22.0	1.09 (0.99, 1.22)
Uterine factor	17.6	24.2	1.09 (0.89, 1.34)
Other infertility cause <sup>†</sup>	16.3	22.4	0.99 (0.87, 1.11)
Idiopathic	14.2	20.7	0.93 (0.82, 1.04)
Previous total pregnancies			
0	13.4	18.6	Reference
1	14.6	20.3	1.03 (0.96, 1.11)
≥2	16.5	23.0	1.11 (1.03, 1.19)
Previous spontaneous abortions			
0	13.4	18.7	Reference
1	15.4	21.1	1.08 (1.00, 1.16)
≥2	17.3	24.0	1.19 (1.10, 1.29)
Previous live births			
0	14.4	19.9	Reference
1	14.3	20.7	0.94 (0.87, 1.02)
≥2	15.0	20.9	0.91 (0.80, 1.04)
Previous ART procedures			
0	13.7	19.0	Reference
1	14.7	20.7	1.04 (0.96, 1.12)
≥2	16.9	23.4	1.15 (1.07, 1.25)
Year of current ART procedure			
1996	13.1	18.4	Reference
1997	15.1	21.1	1.22 (1.13, 1.32)
1998	14.9	20.5	1.18 (1.09, 1.27)
Type of ovarian stimulation medication			
Gonadotropins only	14.4	20.0	Reference
Clomiphene (± gonadotropins)	20.5	27.0	1.37 (1.06, 1.77)
None (unstimulated)	18.9	19.4	0.85 (0.34, 2.13)
Luteal support only	14.8	21.4	0.94 (0.62, 1.43)
ART method			
IVF-ET	14.4	20.0	Reference
GIFT	15.1	20.6	0.93 (0.80, 1.08)
ZIFT	17.1	22.7	1.22 (1.01, 1.47)
Combination	13.4	20.2	0.96 (0.72, 1.28)
Assisted hatching used			
No	13.4	18.5	Reference
Yes	15.8	22.1	1.04 (0.97, 1.11)
ICSI, used			
No	14.4	20.1	Reference
Yes	14.5	20.0	1.03 (0.97, 1.10)

Abbreviations as in Tables 1 and 2.

\*Odds ratios calculated from logistic regression models. For each factor, a model was constructed that included that factor and patient age as independent variables. These analyses were limited to singleton pregnancies.

<sup>†</sup>Other causes of infertility include immunologic causes, chromosomal abnormalities, cancer chemotherapy, and serious illness.

increased risk for subsequent spontaneous abortion. Further, although a potentially higher-risk subset of the population may use ART, their risk for spontaneous abortion is either no different from the risk in the general population or is mitigated by the ART procedure.

Our findings also provide insight into the contribution of oocyte and host factors in the etiology of spontaneous abortion. Several of the findings by subgroup implicate oocyte factors. First, factors that are thought to be associated with increased oocyte and embryo quality—young

**Table 4.** Association Between Use of a Gestational Surrogate and Spontaneous Abortion by Patient Age

	Singleton pregnancies (n)*	SAB rate (%)	OR (95% CI)
Age 20–35			
Patient-gestated pregnancy	13,172	15.6	
Gestational carrier used	101	22.8	1.60 (1.00, 2.55)
Age 35–40			
Patient-gestated pregnancy	11,377	22.2	
Gestational carrier used	93	23.7	1.09 (0.67, 1.76)
Age 41–47			
Patient-gestated pregnancy	1935	38.4	
Gestational carrier used	26	34.6	0.85 (0.38, 1.92)

Abbreviations as in Table 2.

\* Sample is singleton pregnancies conceived using patient's oocytes and freshly fertilized embryos. Pregnancies for which age was greater than 47 were excluded.

maternal age and use of donor oocytes among older women—were associated with a decreased risk for spontaneous abortion. Likewise, factors that may be associated with decreased oocyte and embryo quality were associated with an increased spontaneous abortion risk. These include older maternal age, use of previously frozen embryos, use of clomiphene (potentially related to increased endogenous luteinizing hormone surge and

consequent adverse effects on oocyte quality), and use of zygote intrafallopian transfer (because embryos selected for zygote intrafallopian transfer are at an earlier stage and thus embryo quality is more difficult to judge). Additionally, the marked decline in spontaneous abortion rates for multiple gestation pregnancies suggests that each embryo's individual risk for loss must be considered in addition to the total risk for loss associated with the uterine environment. For this study, we defined spontaneous abortion as loss of the entire pregnancy, rather than evaluating the loss rate according to each implanted embryo. However, further analysis of multiple-gestation pregnancies that progressed to delivery indicates that often one or more embryos/fetuses were lost before delivery (data not shown). We were not able to distinguish between spontaneous and medically induced fetal reductions.

Albeit seemingly less important, uterine or host factors were also implicated in this study. Although the risk for spontaneous abortion was decreased when donor oocytes were used, the risk for older women using donor oocytes was still moderately elevated when compared with younger women using their own oocytes. The differential effects observed among pregnancies carried by a gestational surrogate provide further insights. Among women over 40, use of a gestational surrogate was associated with a protective effect, suggesting that host-related risk factors were mitigated for these pregnancies. The opposite effect was noted among younger women who used a gestational surrogate. One possible explanation relates to the differential ages between the patient and the woman serving as the gestational surrogate. Although we did not have data on the age of the gestational surrogate, these women are typically older than 30. Thus, the higher risk for spontaneous abortion among pregnancies conceived by younger women, but gestated by women somewhat older in comparison with

**Table 5.** Comparison of Spontaneous Abortion Rates From ART Pregnancies With Spontaneous Abortion Rates From Pregnancies Reported in the 1995 National Survey of Family Growth\*

	SAB (%)			
	Age 20–29	Age 30–34	Age 35–37	Age 38–40
ART pregnancies	10.1	11.3	14.3	20.0
Patient oocytes				
Freshly fertilized embryos				
ART pregnancies	14.2	16.2	19.5	25.9
Patient oocytes				
Freshly fertilized embryos				
Singleton pregnancy				
ART pregnancies	15.9	17.5	21.6	29.3
Patient oocytes				
Freshly fertilized embryos				
Singleton pregnancy				
Induced abortion recoded as spontaneous				
NSFG	14.1	16.7	23.0	27.6
Pregnancies ending 1993–1995				

NSFG = National Survey of Family Growth (public-use files); all other abbreviations as in Table 2.

\* The NSFG included a component in which data were obtained for each pregnancy reported by NSFG respondents. Spontaneous abortion rates were calculated based on total clinical intrauterine pregnancies (ie, ectopic pregnancies were not included). Data are not presented for women older than 40 because of insufficient sample size in NSFG.

young women who gestated their own pregnancies, again points to a host effect.

Our finding of an increased risk for current spontaneous abortion among women with one or more prior spontaneous abortions is consistent with previous studies. The increased risk for spontaneous abortion among procedures performed after 1996 is difficult to interpret; this may be related to an increasingly heterogeneous ART patient population as innovations offer increased hope for patients with more severe infertility disorders.

Because current ART treatment typically includes progesterone supplementation for luteal support, it is feasible that the risk for spontaneous abortion among this ART population is actually lower than the theoretical risk for this population had they conceived naturally. Previous studies indicate progesterone supplementation in early pregnancy may be efficacious in preventing spontaneous abortion among women with recurrent pregnancy loss<sup>24</sup>; however, double-blinded randomized trials have not been reported, and the issue continues to be debated.<sup>25</sup>

The spontaneous abortion rate reported here is similar to the rate reported among ART pregnancies in the United Kingdom,<sup>26</sup> but is lower than rates from several other population-based ART registries and surveys.<sup>5-10</sup> It is difficult to speculate on the possible reasons for these differences across populations because rates were not typically presented according to maternal age, plurality, and ART treatment characteristics in the registry reports and publications. We cannot discount that differences in ART patient populations across countries and years of treatment accounted for some of the differences. It is also possible that differing definitions for clinical pregnancy and spontaneous abortion and varying methodologies for collecting and reporting data contributed to these differences. It is highly probable that in all populations, ART pregnancies are diagnosed earlier and followed more closely than pregnancies conceived naturally. In the United States, preclinical pregnancies (ie, those defined based on a positive  $\beta$ -human chorionic gonadotropin only) are often recorded among women receiving ART treatment; however, only clinical pregnancies (based on ultrasound confirmation) are reported in the US registry. This provides some comparability with naturally conceived pregnancies.

The US ART registry has a number of other strengths that are desirable for an analysis of spontaneous abortion rates. The system is estimated to capture 95% of all ART procedures performed in the United States. Data are reported for each individual ART procedure (as opposed to aggregate statistical data reporting systems used in some countries). Reporting of ART pregnancies and pregnancy outcomes is very complete.

Methodological limitations should also be considered when interpreting these findings. Karyotype data were not available, nor were other specific clinical data pertaining to embryo quality. Thus, although we were able to generally gauge that oocyte factors were of primary importance in this population, we were not able to precisely estimate their contribution. Because these data were collected according to ART procedure and women may undergo multiple procedures, some women may be represented more than once in this sample. It is not possible to link procedures performed on the same patient in the current dataset, and thus we could not calculate true per-patient rates or adjust statistical tests for the lack of independence. However, women most likely to have contributed more than one pregnancy to the study population are those most likely to have suffered a pregnancy loss. Thus, the actual "per-patient" spontaneous abortion rates are likely slightly lower than the rates presented here.

Despite some limitations, this registry provided an opportunity to examine spontaneous abortion on a large, population-based sample of ART pregnancies, with complete ascertainment of pregnancies and pregnancy outcomes. The findings presented here should reassure women undergoing ART that their risk for a spontaneous abortion does not appear to be increased; however, older women using their own eggs still need to consider that their risk is quite high, even if not increased in comparison with women who conceive spontaneously. The findings that oocyte factors appear to play a large role in these losses provide potential future direction for screening and intervention. Although high-order embryo transfer does appear to offer some protection against pregnancy loss, this is not a satisfactory solution as the risks posed by multiple-gestation pregnancies to both the mother and the surviving infants are great.<sup>27-31</sup>

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## Spontaneous embryonic loss rates in twin and singleton pregnancies after transfer of top- versus intermediate-quality embryos

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**Objective:** To determine whether embryo quality is associated with early spontaneous loss rates in twin and singleton pregnancies after IVF/intracytoplasmic sperm injection (ICSI).

**Design:** Retrospective, single center analysis.

**Setting:** The Center of Reproductive Medicine, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy.

**Patient(s):** Women undergoing IVF/ICSI and two- or three-embryo transfer of intermediate- and top-quality embryos.

**Intervention(s):** First-trimester sonography at 6 to 7 weeks to determine number of embryos with positive heartbeat. Number of embryos lost was calculated from a second-trimester sonogram.

**Main Outcome Measure(s):** Rates of total pregnancy loss, as related to embryo quality, initial number of embryos, maternal age <35 or ≥35 years, and IVF procedure.

**Results:** A total of 94 losses (23.1% of 407 pregnancies) were counted, with similar proportions in pregnancies after transfer of intermediate- or top-quality embryos. Neither the mode of IVF procedure nor the number of transferred embryos affected the loss rate. In contrast, the loss rate was significantly higher in older mothers after transfer of intermediate-quality embryos (odds ratio [OR 2.4], 95% confidence interval [CI] 1.1–5.5). Losses among singletons were significantly higher compared with losses among twins (OR 2.5, 95% CI 1.1–6.0), but this was observed in top-quality embryos only.

**Conclusion(s):** Top-quality but not intermediate-quality ETs are associated with lower early spontaneous loss rates among twin pregnancies after IVF/ICSI. (Fertil Steril® 2005;84:1602–5. ©2005 by American Society for Reproductive Medicine.)

**Key Words:** IVF, ICSI, embryo quality, twins, embryonic loss, maternal age

Results from three recent case–control retrospective studies suggest that complete pregnancy loss occurs less frequently in twin compared with singleton pregnancies after IVF/intracytoplasmic sperm injection (ICSI) (1–3). One potential explanation for this counterintuitive and somewhat surprising finding is an excessive loss of singleton embryos in these studies. This argument, however, seems less plausible because similar singleton embryo loss rates after IVF were separately reported from European (1, 4) and American (5) series. Further analyses suggested that the improved survival of early twin gestations is related neither to maternal age (3, 6) nor to the mode of assisted conception and the number of embryos transferred in a given cycle (7).

Another explanation of why twin embryos after IVF/ICSI might have a better survival potential than singleton embryos assumes that embryo quality is higher in cycles resulting in twin pregnancies, leading to a different embryologic potential for successful development. This hypothesis corroborates with that of Zegers-Hochschild et al. (2), who suggested that women with high reproductive efficacy exposed to assisted reproductive technologies (ART) produce better embryos and have a higher chance of implantation (i.e., having multiple gestations).

Previous studies that examined early pregnancy losses included cases in which a mixture of embryos with different quality was transferred. To further test the embryologic potential for successful development, we set the null hypothesis that transfer of embryos of similar quality should result in similar loss rates of twins and singleton embryos. We therefore conducted a secondary analysis of the Reggio Emilia ART database to compare embryonic loss rates in

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twins and singletons after transfer of embryos of the highest quality, stratified only by the number of transferred embryos, method of IVF, and by maternal age.

## MATERIALS AND METHODS

We analyzed the dataset of clinical pregnancies resulting from IVF/ICSI during the period from January 1, 1992, to December 31, 2004 in our non-private clinic at Arcispedale Santa Maria Nuova, at Reggio Emilia, Italy. The ART procedures were done in a predominantly (>95%) Italian population. All women (with a few exceptions) were nulliparous, with a mean ( $\pm$ SD) age of  $34.2 \pm 4.0$  years. In our service we transfer only day 2 to 3 embryos, without assisted hatching. According to the ART protocols in Reggio Emilia, cryopreservation and disposal of excess embryos are not permitted, and all transferable embryos were transferred in each cycle.

The data were prospectively collected and regularly updated. After biochemical diagnosis of pregnancy, all pregnant patients underwent first-trimester transvaginal sonography at 4 to 5 weeks after ovum pick-up to count the number of embryos with positive heartbeat. The number of embryos lost was calculated from a second-trimester sonogram.

This study focused on the loss of the entire pregnancy in embryos of similar quality. We excluded cases with single embryonic loss in twins and transfers of mixed-quality embryos. A single operator (A.N.) assessed embryo quality, using inverted microscopy. Quality of embryos was defined by regularity of the blastomeres in terms of shape and size, absence or presence of cytoplasmic fragmentation, characteristics of the cytoplasm in terms of density and homogeneity,

and by adequate cleaving rate (8). To avoid the potentially confounding effect of high number of transferred embryos (7) and for sample size considerations, we restricted the analysis to pregnancies after transfer of either two or three intermediate-quality embryos or after transfer of two or three top-quality embryos.

In the initial analysis, we compared the number of pregnancies between cases conceived by IVF with vs. without ICSI, in cases with transfer of two vs. three embryos, and in mothers aged <35 vs.  $\geq 35$  years. We then compared total pregnancy losses. We used commercial software (True Epistat Software; Math Archives, Round Rock, TX) to compare frequencies by the Fisher's exact tests. We derived odds ratios (OR) and Cornfield's 95% confidence intervals (CI). The ethics committee (institutional review board) of the hospital approved the study. The authors do not have any conflict of interest in performing this study.

## RESULTS

A total of 407 pregnancies with documented heartbeats met the inclusion criteria (123 after transfer of intermediate-quality embryos and 284 after transfer of top-quality embryos). Table 1 shows the characteristics of both groups. Intermediate- and top-quality ETs were performed in similar proportion after IVF with or without ICSI. As expected from selecting unmixed transfers only, the availability of top-quality embryos was inversely related to the number of ETs. A greater proportion of young mothers was found in the intermediate-quality embryo cohort. All in all, there were more singleton pregnancies in the intermediate-quality transfers.

**TABLE 1**

**Comparison of pregnancies after transfer of intermediate- and top-quality embryos by mode of conception, number of transferred embryos, maternal age, and plurality.**

Variable	Intermediate-quality (n = 123)	Top-quality (n = 284)	OR (95% CI)
Mode of conception			
IVF	57 (46.3)	158 (55.6)	0.7 (0.4–1.1)
ICSI	66 (53.7)	126 (44.4)	
No. of transferred embryos			
3	94 (76.4)	175 (61.6)	2.0 (1.2–3.4)
2	29 (23.6)	109 (38.4)	
Maternal age (y)			
<35	87 (70.7)	153 (53.9)	2.1 (1.3–3.3)
$\geq 35$	36 (29.3)	131 (46.1)	
Plurality			
Singletons	106 (86.2)	215 (75.7)	2.0 (1.1–3.7)
Twins	17 (13.8)	69 (24.3)	

Note: Pregnancy data are presented as n (%).

La Sala. Embryo quality and early pregnancy loss. *Fertil Steril* 2005.

TABLE 2

Comparison of loss rates of the entire pregnancy after transfer of intermediate- and top-quality embryos by mode of conception, number of transferred embryos, maternal age, and plurality.

Variable	Intermediate-quality (n = 123)	Top-quality (n = 284)	OR (95% CI)
Total	33 (26.8)	61 (21.5)	1.3 (0.8–2.2)
Mode of conception			
IVF	18/57 (31.6)	34/158 (21.5)	1.7 (0.8–3.5)
ICSI	15/66 (22.7)	27/126 (21.4)	1.1 (0.5–2.3)
Comparison, OR (95% CI)	1.6 (0.7–3.8)	1.0 (0.5–1.8)	
No. of transferred embryos			
2	8/29 (27.6)	20/109 (18.3)	1.7 (0.6–4.8)
3	25/94 (26.6)	41/175 (23.4)	1.2 (0.6–2.2)
Comparison, OR (95% CI)	1.0 (0.4–2.9)	0.7 (0.4–1.4)	
Maternal age (y)			
<35	17/87 (19.5)	28/153 (18.3)	1.1 (0.5–2.2)
≥35	16/36 (44.4)	33/131 (25.2)	2.4 (1.1–5.5)
Comparison, OR (95% CI)	0.3 (0.1–0.8)	0.7 (0.4–1.2)	
Plurality			
Singletons	29/106 (27.4)	53/215 (24.6)	1.1 (0.6–2.0)
Twins	4/17 (23.5)	8/69 (11.6)	2.3 (0.5–10.0)
Comparison, OR (95% CI)	1.0 (0.3–4.9)	2.5 (1.1–6.0)	

Note: Pregnancy loss presented as n (%).

La Sala. Embryo quality and early pregnancy loss. *Fertil Steril* 2005.

A total of 94 early losses of the entire pregnancy were counted (23.1%), with similar proportions in pregnancies after transfer of intermediate-quality embryos (33 of 123, 26.8%) and in those after transfer of top-quality embryos (61 of 284, 21.5%). Table 2 shows that mode of ART and number of transferred embryos did not affect the loss rate. In contrast, the loss rate was significantly higher in older mothers after transfer of intermediate-quality embryos. Finally, losses among singletons (53 of 215, 24.6%) were significantly greater compared with losses among twins (8 of 69, 11.6%) (OR 2.5, 95% CI 1.1–6.0), but this effect was observed in top-quality embryos only.

## DISCUSSION

The starting point of this study was the finding of recent comparative observations indicating a lower complete pregnancy loss of IVF/ICSI twin gestations compared with singletons pregnancies (1–3, 6, 7). This observation suggests that the embryologic potential for early development is not the same for twins and singletons, with twins having an advantage over singletons. We have also observed that this effect is by and large independent of maternal age, mode of conception, and number of transferred embryos (3, 6, 7).

In the common scenario, the best embryo(s) are chosen for transfer. However, this does not mean that all transferred embryos are of the same quality, and in fact transferred embryos might often be of different quality. This reality does

not permit a simple evaluation of the potential effect of embryo quality on early development, and the possible effect of embryo quality could not be excluded (6, 7).

In the present study, we controlled for the effect of embryo quality by using transfers of either intermediate- or top-quality embryos. In studying the effect of embryo quality, it is important to remove inherent selection bias produced by choosing only top-quality embryos from a wide selection of embryos of different qualities. The unique ET protocol in Reggio Emilia—transfer of all available embryos—eliminates any selection of embryos for transfer. Moreover, because we evaluated unmixed quality embryos, the fertilization rate and transfer rate per cycle in our study are much the same and enable evaluation of the net effect of embryo quality on early losses. Although this methodology reduces the number of cases enrolled for analysis, three conclusions are apparent from the analysis.

First, loss rates were not related to whether ICSI was or was not performed, nor were they related to whether two or three embryos were transferred. Second, loss rates were influenced by maternal age in pregnancies after transfer of intermediate-quality embryos but not among the top-quality embryo subgroup. Finally, loss rates of singletons and twins were not affected by embryo quality, but there was a 2.5-fold increased loss of singletons when top-quality embryos were transferred. In simple terms, our data indicate that pregnan-

cies resulting from transfer of top-quality embryos have different, plurality-dependent loss rates.

In a previous analysis, we speculated that the similar implantation rates per transfer (i.e., development of more than one live embryo) might represent a better capacity of the uterus for early embryonic development and/or better embryonic quality (6, 7). Following this study, we might speculate that when less than “top”-quality embryos are transferred, the fact that two embryos have implanted has no beneficial effect on early embryonic survival. In contrast, the implantation of two top-quality embryos significantly increases the survival of the entire pregnancy, and we might speculate that early twin gestations resulting from top-quality embryos only have a better utero–fetal interaction.

A good candidate explanation for this interaction is a superior hormonal support of the uterine milieu, produced by the well-recognized greater placental mass observed in early twin pregnancies (7). However, this does not seem to be enough because the advantage related to the co-presence of two embryos is demonstrated in pregnancies after transfer of top-quality embryos but not after intermediate-quality embryos. In other words, the related advantage should precede embryonic development and should certainly last beyond the stages of positive heartbeat.

We acknowledge the subjective nature of embryonic quality assessment, as well as the fact that morphologic classification of embryo quality is but one of several strategies adopted to improve the accuracy for selecting viable embryos for transfer (9). The other strategies are excluding anomalous embryos by preimplantation genetic diagnosis and excluding less viable embryos that will undergo developmental arrest by extending the duration of culture to the blastocyst stage (9). However, Volpes et al. (10) recently studied the predictive value for implantation and pregnancy rates of the number of good-quality embryos on day 3 and found that the number of good-quality embryos available is a strong predictive value for both pregnancy rate and implantation rate. Furthermore, in the model of single top-quality ETs (11), the data show that embryo selection was an excellent tool for prediction of pregnancy but not for prediction of embryonic loss.

It is unknown to what extent our observation represents the situation in spontaneous conceptions and to what extent it represents the situation when gestational sacs rather than

heartbeats are considered (12). It is also unknown whether the advantage of twins remains the same in various regimens of hormonal support used in IVF protocols, or when transfers are performed at the blastocyst stage or with frozen–thawed embryos. Finally, it is unknown why some twin pregnancies are totally lost whereas others result in the “vanishing twin” syndrome (13). Nonetheless, our findings reiterate the fact that our intuitive view about the gloomy outcome of early twin gestations should be reappraised.

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# The effect on human sex ratio at birth by assisted reproductive technology (ART) procedures – an assessment of babies born following single embryo transfers, Australia and New Zealand, 2002–2006

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**Objective** To assess the effect on the human sex ratio at birth by assisted reproductive technology (ART) procedures.

**Design** Retrospective population-based study.

**Setting** Fertility clinics in Australia and New Zealand.

**Population** The study included 13 368 babies by 13 165 women who had a single embryo transfer (SET) between 2002 and 2006.

**Methods** Logistic regression was used to model the effect on the sex ratio at birth of ART characteristics [*in vitro* fertilisation (IVF) or intracytoplasmic sperm insemination (ICSI) SET, cleavage-stage or blastocyst SET, and fresh or thawed SET] and biological characteristics (woman's and partner's age and cause of infertility).

**Main outcome measures** Proportion of male births.

**Results** The crude sex ratio at birth was 51.3%. Individual ART procedures had a significant effect on the sex ratio at birth. More males were born following IVF SET (53.0%) than ICSI SET (50.0%), and following blastocyst SET (54.1%) than cleavage-stage SET (49.9%). For a specific ART regimen, IVF blastocyst SET produced more males (56.1%) and ICSI cleavage-stage SET produced fewer males (48.7%).

**Conclusions** The change in the sex ratio at birth of SET babies is associated with the ART regimen. The mechanism of these effects remains unclear. Fertility clinics and patients should be aware of the bias in the sex ratio at birth when using ART procedures.

**Keywords** ART births, blastocyst, cleavage-stage embryo, ICSI, IVF, logistic regression, sex ratio at birth, single embryo transfer.

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## Introduction

The sex ratio at birth, also known as the secondary sex ratio (SSR), can be defined as the proportion of males in all live births. Variation in SSR within most human populations is common,<sup>1–3</sup> with the SSR deviating from the equality ratio of males to females (50% males) in the population.<sup>4</sup> Assisted reproductive technology (ART) to treat infertility has developed rapidly since the first *in vitro* fertilisation (IVF) baby was born in 1978. The SSR among babies born after ART treatment varies as in any other population.<sup>5–7</sup> However, whether the variation in SSR

among ART babies is a result of natural causes (i.e. environmental or biological causes) or of the effect of ART is debatable. Concerns about whether ART might alter SSR have led researchers to study SSR in ART babies.

In 1989, Thatcher *et al.*<sup>8</sup> reported that the SSR of babies born following *in vitro* fertilisation and embryo transfer (IVF-ET) was significantly higher than normal (64.1% males). In 2000, Ghazzawi *et al.*<sup>9</sup> observed a significantly higher proportion of female births (61.7%) following the transfer of embryos fertilised by intracytoplasmic sperm injection (ICSI). Other researchers<sup>10,11</sup> have also observed a trend of a higher SSR in babies born after IVF, and lower

SSR after ICSI, although the findings were nonsignificant between the observed SSR and the general population. The reduction in SSR after ICSI has often been attributed to male infertility, because ICSI is predominantly used to treat male infertility.<sup>12</sup>

Both advances in the development of embryo culture media and evidence of higher pregnancy rates by blastocyst transfer (BT)<sup>13–16</sup> have provided fertility clinics with the opportunity to offer BT to patients for treatment. In 1994, Pergament *et al.*<sup>17</sup> observed that the transfer of fast growth embryos resulted in more male births than female births. Other studies that have found skewed SSR in favour of males after BT<sup>18–20</sup> have attributed the excess of male births to the selection for transfer of fast growth male embryos. The suggestion is that, because there is sex-related differentiation in embryo development, and male embryos show, on average, more blastomeres at the time of transfer, more male embryos may be selected for transfer. Other researchers,<sup>21–25</sup> however, have not found a significant association between higher SSR and BT.

One of the limitations of SSR studies in ART babies is the small sample size, which may explain the lack of association between ART procedures and SSR.<sup>26,27</sup> The lack of association could also be related to the use of different analytical techniques.<sup>28</sup>

Another common limitation is the inclusion of multiple births. In the SSR calculation, monozygotic multiples can add additional weight to a particular sex, which may distort the relationship between specific ART procedures and SSR. For example, in a population study, it is not possible to distinguish a set of same-sexed twins as a result of a single embryo (monozygotic) or two embryos (dizygotic) if two or more embryos are transferred. Consequently, a single embryo resulting in a set of monozygotic twins contributes twice in the calculation of a particular sex towards a particular ART procedure, whereas each embryo resulting in a set of dizygotic twins contributes only once in the calculation.

The aims of this study were to address a number of these limitations using ART population data from Australia and New Zealand, and to investigate whether ART procedures alter the SSR of ART babies.

## Methods

### Data sources

Data for this retrospective population-based study were supplied from the Australia and New Zealand Assisted Reproductive Database (ANZARD), a repository of ART treatment cycles and their outcomes from all fertility clinics in Australia and New Zealand. The study population included 13 368 babies born to 13 165 women, who had SET between 2002 and 2006. The size of this study popula-

tion is sufficiently large to detect differences in SSR between the groups using different ART procedures. As in most studies investigating human SSR, the deviation from the general population SSR is usually small but significant, by about 1–2%.<sup>29</sup> For the detection of a difference of 1.5% (two-sided test) with a statistical significance of 0.05, a power of 90% requires a sample size of about 12 000 (SAS Power and Sample Size 3.1; SAS Institute Inc., Cary, NC, USA).

To eliminate the potential bias on the SSR calculation by monozygosity in relation to the ART procedures, we restricted our study population to babies born following SET. ANZARD does not have information on zygosity. For the purpose of this study, the following assumptions were made: that babies in the sets of same-sexed multiples were monozygotic and that no spontaneous conception occurred. Therefore, we included only one baby in each set of multiple births in the SSR calculation and analysis. In the study population, multiples accounted for about 2.9% of total SET babies. After selecting one baby from each set of multiples, the proportion of babies born as multiples in the study population reduced to 1.4%.

### Study variables

The data variables included in the study were the baby's sex, fertilisation procedure (IVF or ICSI), stage of embryo development at transfer (blastocyst or cleavage-stage embryo), type of embryo (fresh or thawed), woman's and partner's age at the time of transfer, and cause of infertility. BT was defined as the transfer of a day-4 or older embryo, as specified in the ANZARD collection. Cleavage-stage embryo transfer was a transfer of a day-2 or day-3 embryo.

Sex selection is banned in Australia. In particular, three states, Victoria, South Australia and Western Australia, have specific legislation on the use of ART which bans sex selection.<sup>30–32</sup> Furthermore, the Guidelines of the National Health and Medical Research Council (NHMRC)<sup>33</sup> in Australia prohibit sex selection in Australia. Fertility clinics in Australia to be accredited must comply with the NHMRC ART guidelines. The sex of the babies analysed in this study was not subject to the process of sex selection.

### Statistical analysis

The proportion of male live births is used in this study as the SSR. Data were stratified by categorical variables. Logistic regression analysis with backward stepwise (likelihood ratio) method was used to model the SSR as the dependent variable and other variables as predictor variables. In the tables, the odds ratios (ORs) and 95% confidence intervals (CIs) were given by stratification or by predictors.  $P < 0.05$  was considered to be significant. SPSS v17 (SPSS Inc., Chicago, IL, USA) was used for analysis.

## Results

The SSR for babies born following SET was 51.3%, which did not differ from the Australian population SSR of 51.5% ( $P = 0.655$ ).<sup>34</sup> The majority of women who gave birth(s) following SET were aged between 30 and 39 years (75.1%). The proportion of babies born following ICSI SET increased from 51.5% in 2002 to 59.2% in 2006. Similarly, the proportion of babies born following single blastocyst transfer (SBT) also increased from 26.3% in 2002 to 34.7% in 2006. Table 1 shows the SSRs, crude ORs and 95% CIs, the distribution of babies and their parental mean ages by categorical variables. Among these SET babies, 3.2% were born following the transfer of either a donor embryo or donor oocyte.

Comparing the SSRs between the categorical variables, we observed a significant difference between IVF and ICSI groups ( $P = 0.001$ ), with less male babies born following ICSI SET and more male babies born following IVF SET. We also observed that SSR was significantly higher in

babies born following SBT than in babies born following cleavage-stage SET ( $P < 0.001$ ).

There was a statistically significant univariate association ( $P = 0.032$ ) between fresh/thawed embryo transfer and SSR. However, this association was not significant after adjusting for other variables in logistic regression analysis. Logistic regression analysis did not detect any contributions to the change in SSR by the partner's age and the cause of infertility.

Table 2 presents the results from logistic regression analysis. The ICSI procedure, SBT and woman's age at the time of transfer were the significant factors affecting the probability of having a male birth. Among these factors, SBT was the most significant contributor to the change in SSR, whereas ICSI was the second most significant factor. Although significant, the effect of the woman's age was much smaller than that of the SBT and ICSI procedure.

To further assess the effect of the fertilisation procedure and stage of embryo development on SSR, we stratified the data by the ART treatment regimen: transfer of IVF

**Table 1.** Sex ratio at birth by individual assisted reproductive technology (ART) characteristics of single embryo transfer (SET) babies, Australia and New Zealand, 2002–2006

Characteristics	Number and %	Mean age (years) at the time of treatment (mean $\pm$ SD)		SSR (%)*	OR (95% CI)**
		Woman	Partner		
<b>SET babies</b>	13 368	33.2 $\pm$ 4.3	36.3 $\pm$ 6.1	51.3	
2002	1165	33.5 $\pm$ 4.4	36.8 $\pm$ 6.2	51.4	
2003	1561	33.1 $\pm$ 4.4	36.4 $\pm$ 6.0	51.2	
2004	2448	33.0 $\pm$ 4.3	36.2 $\pm$ 6.2	51.9	
2005	3446	33.2 $\pm$ 4.1	36.3 $\pm$ 6.1	50.5	
2006	4748	33.2 $\pm$ 4.3	36.3 $\pm$ 6.1	51.6	
<b>Fertilisation procedure</b>					
IVF	44.6%	33.4 $\pm$ 4.2	35.6 $\pm$ 5.4	53.0	1
ICSI	55.4%	33.0 $\pm$ 4.3	36.9 $\pm$ 6.6	50.0	0.89 (0.83, 0.95)
<b>Stage of embryo development at transfer</b>					
Cleavage-stage SET	67.1%	33.2 $\pm$ 4.4	36.4 $\pm$ 6.2	49.9	1
Blastocyst SET	32.9%	33.2 $\pm$ 4.1	36.2 $\pm$ 6.0	54.1	1.18 (1.10, 1.27)
<b>Type of embryo</b>					
Fresh SET	69.1%	32.8 $\pm$ 4.2	36.0 $\pm$ 6.2	51.9	1
Thawed SET	30.9%	34.0 $\pm$ 4.4	37.2 $\pm$ 6.3	49.9	0.92 (0.86, 0.99)
<b>Cause of infertility</b>					
Male only	40.6%	32.8 $\pm$ 4.2	36.9 $\pm$ 6.7	50.6	1
Female only	16.3%	33.3 $\pm$ 4.1	35.2 $\pm$ 5.2	51.2	1.02 (0.93, 1.13)
Male and female	7.1%	33.3 $\pm$ 4.4	36.2 $\pm$ 5.8	51.1	1.02 (0.89, 1.18)
Unexplained	29.3%	33.6 $\pm$ 4.4	36.2 $\pm$ 5.7	52.0	1.06 (0.97, 1.15)
Other***	6.6%	33.6 $\pm$ 4.8	36.4 $\pm$ 6.4	53.0	1.10 (0.95, 1.27)

ICSI, intracytoplasmic sperm insemination; IVF, *in vitro* fertilisation.

\*Sex ratio at birth or secondary sex ratio (SSR) – proportion of males of all live born babies.

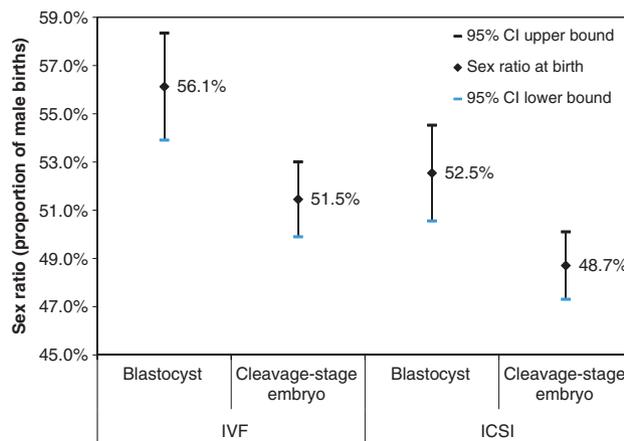
\*\*Logistic regression analysis for predicting the odds ratio (OR) and 95% confidence interval (CI) for a male birth by single factor. 1, reference category.

\*\*\*Other includes missing cause of infertility.

**Table 2.** Predictors of secondary sex ratio (SSR) of single embryo transfer (SET) babies, Australia and New Zealand, 2002–2006

Predictor	Wald $\chi^2$	P value	OR (95% CI)
Fertilisation procedure – intracytoplasmic sperm insemination	13.60	<0.001	0.88 (0.82, 0.94)
Stage of embryo development at transfer – blastocyst	21.07	<0.001	1.19 (1.11, 1.28)
Woman's age	8.97	0.003	0.99 (0.98, 1.00)

Logistic regression model for predicting the odds ratio (OR) and 95% confidence interval (CI) for a male birth after adjusting for other factors. Wald  $\chi^2$  indicates the statistical significance of each predictor in the model.



**Figure 1.** Sex ratio at birth of babies born following single embryo transfer by specific assisted reproductive technology (ART) regimens, Australia and New Zealand, 2002–2006. CI, confidence interval; ICSI, intracytoplasmic sperm insemination; IVF, *in vitro* fertilisation.

blastocyst (IVF SBT), transfer of IVF cleavage-stage embryo (IVF cleavage-stage SET), transfer of ICSI blastocyst (ICSI SBT) and transfer of ICSI cleavage-stage embryo (ICSI cleavage-stage SET). We then calculated the SSRs for the four groups (Figure 1). The highest SSR (56.1% males) was in the IVF SBT group and the lowest SSR (48.7% males) was in the ICSI cleavage-stage SET group. The SSR in the IVF cleavage-stage SET group was similar to the Australian population SSR of 51.5%. As a result of the opposing effect of the ICSI procedure and SBT, SSR in the ICSI SBT group did not differ significantly from that of the Australian population.

## Discussion

This first population-based retrospective study confirms that a specific ART regimen can alter the SSR of babies

born following SET. It shows that the use of ICSI is associated with a reduction in SSR, whereas BT is associated with an increase in SSR. In particular, our results show that an ART regimen for a combination of ART procedures can alter SSR significantly, with the highest SSR among babies born following IVF SBT and the lowest SSR in babies born following ICSI cleavage-stage SET.

A low SSR in ICSI babies has been observed previously by Ghazzawi *et al.*<sup>9</sup> in 2000, Bonduelle *et al.*<sup>10</sup> in 2002, Scott and Ryan<sup>11</sup> in 2006 and Hentemann *et al.*<sup>35</sup> in 2009. The effect of decreased SSR by ICSI was first demonstrated by Luke *et al.*<sup>36</sup> in 2009. They reported that there was a 10% reduction in SSR by ICSI in babies born following BT, compared to IVF. In our study, a similar reduction (12%) in SSR was also observed. Our findings are also consistent with the result by Luke *et al.*<sup>36</sup> that a diagnosis of infertility does not contribute to the change in SSR. Although the underlying mechanism of reduction in SSR by ICSI remains unclear, one hypothesis by Luke *et al.*<sup>36</sup> is that the cause may be potentially iatrogenic, because the underlying ratio of X- and Y-bearing sperm is almost equal (50.3% Y-bearing sperm).<sup>37,38</sup>

The higher proportion of male births after BT seen in our study is consistent with the literature.<sup>35,39,40</sup> There are a number of possible reasons. One is the morphological selection criteria. More male blastocysts may be selected for transfer because male embryos cleave faster than female embryos from day 2 and up to blastocyst stage.<sup>17,18,22,41,42</sup> Another reason is that male embryos have a faster preimplantation development rate than female embryos.<sup>20</sup> However, two recent studies by Weston *et al.*<sup>24</sup> and Csokmay *et al.*<sup>25</sup> assessing retrospectively the mean number of cells and embryo grades of male and female babies, found no difference in growth and delivery rates between male and female embryos. The authors concluded that BT and the selection of higher grade embryos for transfer did not contribute to the increase in SSR.

Although some early studies observed a trend of higher male births following BT,<sup>21–23,43</sup> this higher proportion of male births was not significant. One explanation for a lack of an association between BT and an increase in male births is an underpowered study design. This was evident in the studies by Weston *et al.*<sup>24</sup> (435 births, 51.3% males) and Csokmay *et al.*<sup>25</sup> (498 embryos and 120 births, 51.7% males). To overcome the problem of an underpowered study using data from a single clinic, meta-analyses using data from several similar studies have been employed to assess the effect of BT on SSR by some researchers. For example, Milki *et al.*<sup>22</sup> in 2003 analysed data from seven published articles comparing SSRs between BT ( $n = 1391$ ) and cleavage-stage ET ( $n = 1909$ ). Chang *et al.*<sup>40</sup> in 2009, using data from four single centres, assessed the effect of BT on SSR ( $n = 2711$ ). Both meta-analytic review studies

found that, by combining published data, SSR was significantly higher in babies born following BT than in babies born following cleavage-stage ET. One of the difficulties in drawing conclusions from meta-analytic review studies is the heterogeneity of the pooled data used in the analysis, which may lead to bias in assessing the effect of the intervention or treatment.<sup>44,45</sup> The data used in Chang *et al.*<sup>40</sup> were obtained from 1995 to 2005. During this period, the technology of BT has evolved dramatically. In particular, the sequential medium for blastocyst culture, which was developed in the late 1990s, has improved the pregnancy and implantation rates in BT.<sup>46–48</sup> The effect of BT on SSR in 1995 may differ from that in 2005. Our population-based retrospective study, however, used individual record data from the same clinics in Australia and New Zealand between 2002 and 2006. With an overall power of >90% ( $n = 13\,165$ ), our study has confirmed that, at a population level, BT affects SSR significantly, with a higher probability of male births.

Another possible explanation for the inability to detect a significant change in SSR following BT is the unknown proportion of ICSI embryos involved in most earlier studies. As our results suggest, ICSI has the effect of reducing SSR, which may cancel out the effect associated with BT of increasing SSR. Over the 5-year period of this study, the proportions of SET babies born following ICSI and BT have increased by 15% and 32%, respectively. Consequently, the overall SSR for individual years during the study period did not change (Table 1) because of the opposing effects of ICSI and BT on SSR. Although ICSI was developed initially to treat male infertility, it is now also used to treat patients with other infertilities. In Australia and New Zealand, ICSI cycles increased from 57.6% in 2002 to 59.5% in 2006,<sup>5</sup> whereas, in European countries, 66.5% of all cycles were ICSI in 2006.<sup>49</sup> In the USA, the use of ICSI increased significantly from 11.0% in 1995 to 57.5% in 2005.<sup>50</sup> Around the world, ICSI cycles increased from 47.6% in 2000 to 56.6% in 2002.<sup>51</sup> The higher utilisation of ICSI may potentially reduce the overall SSR in ART babies, unless a parallel increase in the use of BT also occurs.

A possible confounder for the difference in sex-related embryo development is the *in vitro* culture conditions.<sup>52</sup> Ray *et al.*<sup>42</sup> have suggested that male embryos have higher metabolic activity than female embryos and, in turn, show significantly higher pyruvate and glucose uptake and lactate production. With the different embryo culture media used, the growth rate of embryos may be slightly different across fertility clinics. How this difference in growth rate affects the selection and implantation of embryos and, in turn, SSR is not clear. There remains a lack of clinical evidence on the alteration of SSR in relation to the embryo culture media.

The use of ART treatment around the world has increased. In Australia and New Zealand, 56 817 ART treatment cycles were started in 2007, an increase of 12.5% from 2006.<sup>53</sup> According to the latest report from the International Committee for Monitoring Assisted Reproductive Technology,<sup>51</sup> over 601 243 cycles were performed in 53 countries in 2002. It is estimated that between 219 000 and 246 000 ART babies were born following ART treatment in that year. ART babies accounted for between 1% and 4% of total births in some countries. In Australia, about 3.1% of total births were ART babies.<sup>53</sup> In 2007, 1.8% babies were born following ART treatment in the UK,<sup>54</sup> and over 1% were born in the USA.<sup>55</sup> Evidence has shown that one in six couples may experience difficulties in getting pregnant<sup>56</sup> in their reproductive lives, and these couples may require ART treatment at some stage of their reproductive lives. The proportion of ART babies born per population will probably continue to increase because of the unmet need for fertility treatment, the falling fertility rate and the development of low-cost IVF in many countries.<sup>57–59</sup> In parallel, there is an increasing trend for high-income countries to adopt SET, with the ICSI procedure and BT.<sup>49,51,53,55</sup> To date, there has been minimal impact on the SSR at the population level. However, the greater use of ART treatment and the increased use of the ICSI procedure and BT may have a major public health impact on SSR, dependent on future treatment regimens. At the individual patient level, the results from this study may provide an indication of the likelihood of having a male or female baby through the ART procedure proposed or used by clinicians.

The strength of this study is the large sample size with individual record data provided by the same ART clinics in the same period. We are able to demonstrate that the bias towards male births by BT and the bias towards female births by ICSI were statistically significant. However, by using registration data, such as ANZARD, for a retrospective study, we may have potentially introduced some variability in the selection criteria, culture media, and laboratory and clinical parameters. The importance of these factors in relation to the change in SSR has yet to be demonstrated with large multicentre clinical studies or from other regions or countries. The use of SET babies in this study has clearly identified the effect of ART procedures on SSR, and has set a common ground for comparison in SSR studies from other regions or countries.

## Conclusions

On the basis of this retrospective population study, we conclude that ART procedures have a significant effect on human SSR. Further investigation of both biological and environmental factors may take into account the effect of

ART factors on SSR. However, in the short term, fertility clinics and patients should be aware of the potential increase in SSR with the transfer of IVF blastocysts and decrease in SSR with the transfer of ICSI cleavage-stage embryos.

### Disclosure of interest

All authors report no potential conflicts of interest. MGC is a fertility specialist. He is a director of IVF Australia Pty Ltd. and Clinical Director at St George Hospital Kogarah, Sydney, Australia.

### Contribution to authorship

JHD conceived and designed the study, analysed the data and wrote the manuscript. MGC discussed the core ideas, provided clinical insight into the data and revised the manuscript. EAS discussed the core ideas, provided critical comments and revised the manuscript. All authors approved the final version of the manuscript.

### Details of ethics approval

Ethics approval for this study was granted by the Human Research Ethics Committee of the University of New South Wales, Australia, and the Ethics Committee of the Australian Institute of Health and Welfare.

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# The risk for four specific congenital heart defects associated with assisted reproductive techniques: a population-based evaluation

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**STUDY QUESTION:** Are the risks of hypoplastic left heart syndrome, transposition of great arteries, tetralogy of Fallot (TOF) and coarctation of the aorta increased in infants conceived by different assisted reproductive techniques (ARTs)?

**STUDY ANSWER:** ARTs, and particularly intracytoplasmic sperm injection (ICSI), are specifically associated with a higher risk of TOF.

**WHAT IS ALREADY KNOWN:** ARTs are associated with an increase in the overall risk of birth defects. The risk for congenital heart defects (CHDs) associated with ARTs has been evaluated as a whole but there is limited information on the risks for specific CHDs.

**STUDY DESIGN, MATERIAL AND METHODS:** We conducted a case–control study using population-based data from the Paris registry of congenital malformations for the period 1987–2009 and a cohort study of CHD (EPICARD) on 1583 cases of CHDs and 4104 malformed controls with no known associations with ARTs. ARTs included ovulation induction only, IVF and ICSI.

**RESULTS:** Exposure to ARTs was significantly higher for TOF than controls (6.6 versus 3.5%,  $P = 0.002$ ); this was not the case for the other three CHDs. ARTs (all methods combined) were associated with a 2.4-fold higher odds of TOF after adjustment for maternal characteristics, paternal age and year of birth [adjusted odds ratios (OR): 2.4, 95% confidence interval (CI): 1.5–3.7] with the highest risk associated with ICSI (adjusted OR: 3.0, 95% CI: 1.0–8.9). No statistically significant associations were found for the other CHDs.

**LIMITATIONS:** Our study cannot disentangle to what extent the observed associations between the risk of TOF and ARTs are due to causal effects of ARTs and/or the underlying infertility problems of couples who conceive following ART.

**IMPLICATIONS:** The developmental basis of the specific association between the risk of TOF and ARTs need to be further investigated.

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**COMPETING INTERESTS:** None.

**Key words:** assisted reproductive techniques / intracytoplasmic sperm injection / congenital heart defects / tetralogy of Fallot / epidemiology

## Introduction

Assisted reproductive techniques (ARTs) are known to be associated with a modest increase in the overall risk of congenital anomalies (Wennerholm et al., 2000; Hansen et al., 2002, 2005; Koivurova et al., 2002; Klemetti et al., 2005; Olson et al., 2005; Schieve et al., 2005). Relatively, little specific information exists on the risk of congenital heart defects (CHDs) for fetuses conceived following ARTs (Anthony et al., 2002; Hansen et al., 2002; Katalinic et al., 2004; Lie et al., 2005; Zhu et al., 2006; Reefhuis et al., 2009; Tararbit et al., 2011). Available evidence suggest an overall risk for CHDs in relation to ARTs that is comparable to that found for all congenital anomalies combined [odds ratios (OR)  $\sim 1.4$ – $1.5$ ] (Hansen et al., 2005; Tararbit et al., 2011). Specific associations between different methods of ARTs and categories of CHDs have also been reported by our group (Tararbit et al., 2011) using a subset of the data used in the present study.

However, previous studies have mostly examined the risk of CHDs in relation to ARTs for all CHDs combined or for broad categories of CHDs rather than for specific CHDs. Moreover, the associations between different methods of ARTs and specific CHDs have not been examined. Assessment of such specific associations is important as known teratogens are generally associated with the risk of one or a few specific malformations. Furthermore, specific associations between types of CHDs and ARTs may provide clues about the underlying mechanism of the higher risk of congenital malformations in fetuses conceived following ARTs.

Using population-based data from the Paris registry of congenital malformations and a cohort study of children with CHDs (the EPICARD study), we estimated the risks for four major specific CHDs: hypoplastic left heart syndrome (HLHS), transposition of great arteries (TGA), tetralogy of Fallot (TOF) and coarctation of the aorta (CoA) in relation to different methods of ARTs.

## Materials and Methods

### Data sources

Two sources of data were used for this study: (i) the Paris registry of congenital malformations and (ii) the EPICARD study (epidemiological study on the outcomes for congenital heart diseases). These two sources of data are briefly described below.

#### *The Paris registry of congenital malformations*

Since 1981, the Paris registry of congenital malformations registers all cases of birth defects and chromosomal anomalies among live births, stillbirths ( $\geq 22$  weeks of gestation) and pregnancy terminations. The registry covers the population of women who live in the Greater Paris area (Paris and its surrounding suburb) and deliver or have a termination of pregnancy for fetal anomaly in a Parisian maternity unit. The annual number of deliveries in our population is about 38 000.

The Paris registry is a member of the European network of registries of congenital malformations (European Surveillance of Congenital Anomalies, EUROCAT) and of the International clearinghouse for birth defects surveillance and research (Eurocat Special Report, 2009; Cocchi et al., 2010; Greenlees et al., 2011; Khoshnood et al., 2011). The registry follows the EUROCAT methodology and the quality of data is routinely monitored by both the EUROCAT and the French National Committee of Registries. Review of procedures regarding confidentiality of data is overseen by both the National Committee of Registries and the National

Committee of Informatics and Freedom (CNIL). Data are based on medical records and are collected from several sources including maternity units, neonatology wards, cytogenetic and pathology services.

In the present study, data from the registry corresponded to the period 1 January 1987 to 31 December 2009 as the first case of a malformation with exposure to IVF occurred in 1987 and 2009 was the last year for which data were available at the time of the study.

#### EPICARD

The EPICARD study is an on-going prospective cohort study of all children with a CHD (Khoshnood et al., 2012) born to women living in the Greater Paris area (Paris and its surrounding suburbs) between 2005 and 2008 regardless of the place of delivery ( $n = 317\,538$  births). The principal objectives of the study are to use population-based data from a large cohort of patients with CHDs to: (i) estimate the total and live birth prevalence, (ii) examine timing of diagnosis and assess medical and surgical management of children with CHDs, (iii) evaluate neonatal mortality and morbidity and neuro-developmental outcomes of children with CHDs and (iv) identify the factors associated with their health outcomes, especially the role of events during the neonatal period and of the initial medical and surgical management. All cases (live births, pregnancy terminations, fetal deaths) diagnosed in the prenatal period or up to 1 year of age in the birth cohorts between 1 May 2005 and 30 April 2008 were eligible for inclusion. The total number of cases of CHDs included in the study was 2867, including 2348 live births (82%), 466 pregnancy terminations (16.2%) and 53 fetal deaths (1.8%). Diagnoses were confirmed in specialized paediatric cardiology departments and for the majority of pregnancy terminations and fetal deaths by a foetopathologist examination. For others in which a pathology examination could not be done, the diagnoses were confirmed by consensus by a paediatric cardiologist and a specialist in echocardiography in the study group based on the results of prenatal echocardiography examination.

## Methods

A case–control study with malformed controls was performed. Cases were fetuses/children with HLHS, TGAs, TOF and CoA. Cases included in both the Paris registry and the EPICARD study were counted once. Malformed controls were isolated congenital defects other than CHDs for which no evidence of an association with ARTs was found in the literature. As recommended by Hook (1993), we selected a wide spectrum of heterogeneous birth defects as controls in order to decrease the risk of selection bias due to shared aetiological factors between cases and controls (Swan et al., 1992; Lieff et al., 1999). The malformations in the control group comprised cases of club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation in the Paris registry.

The risk (odds) of each CHDs in relation to ARTs was the main outcome measure. Data on exposure to ARTs were obtained from medical records. The same procedure for data collection and coding was used for information on ARTs in the two data sets (Paris registry and EPICARD) used in this study. Exposure to ARTs included the following categories: ovulation induction (OI) only, IVF and ICSI. Exposure to ARTs was assessed as follows: (i) a binary variable (ART yes/no), (ii) a variable in four categories (no ART, OI, IVF, ICSI) and (iii) a variable combining IVF and ICSI (IVF + ICSI) in a single category.

Potential confounding factors considered were maternal characteristics (age, occupation and geographic origin), paternal age and year of birth (or pregnancy termination). Although their exact relations to the risk for specific CHDs are not well known, these factors are associated with both exposure to ARTs and prevalence of birth defects in general (Vrijheid et al., 2000). Maternal occupation was coded in five categories (professional, intermediate, administrative/public service, other and none) following

the French National Institute of Statistics and Economic Studies classification. Geographic origin was coded in four categories: French, North African, Sub-Saharan African and other countries.

## Statistical analysis

The odds of each of the four specific CHDs versus controls in relation to ARTs was estimated using logistic regression models, after taking into account year of birth, maternal characteristics (age, occupation and geographic origin) and paternal age. Paternal age was missing for 20.6% of the study population. We used multiple imputation (Little and Rubin, 2002) for missing data on paternal age. Paternal age was imputed in 20 sets of data for each CHD separately using the case/control status, exposure to ARTs, maternal age and year of birth/termination. The pooled (over the 20 data sets) adjusted ORs for the association between ARTs and risk of each specific CHD were estimated using the method described by Little and Rubin (2002). In order to explore the possible role of multiple pregnancies in the association between ARTs and CHDs, we also conducted analyses with further adjustment for multiple pregnancies and tested for any interaction effect between multiple pregnancies and ARTs.

The statistical significance level was set at  $\alpha = 0.05$  and all tests were two-sided.

Analyses were done with Stata 11 software (Statacorp, Texas, USA).

## Ethics approval

No specific ethical approval was needed for this particular analysis. The French CNIL has authorized the surveillance and research activities of the registry using anonymous data and has approved the EPICARD study.

## Results

### Study population

After excluding cases with missing data on ARTs (3% of cases), the study population comprised 353 cases of HLHS, 444 cases of TGA, 395 cases of TOF and 391 cases of CoA. Approximately 14% of cases of HLHS, 3% of TGA, 20% of TOF and 10% of CoA were associated with chromosomal anomalies. The study population included 4104 malformed controls with complete information on ARTs, which comprised 1436 with congenital hip dislocation, 824 with club-foot, 782 with polydactyly, 517 with angioma, 381 with skin abnormality and 164 with syndactyly with complete information on ARTs; 3% of controls had missing data on ARTs.

Table 1 summarizes the results of the comparison of the maternal, paternal and pregnancy characteristics of cases of CHDs (all four specific CHDs combined) and controls. Overall, mothers of cases of CHDs were older, more likely to be from North Africa and in the occupational category 'none' when compared with mothers of controls. Stillbirths and terminations of pregnancy for fetal anomaly were more frequent for cases of CHDs than controls.

When comparisons of the characteristics of cases and controls were done for the four defects separately (detailed results not shown—available from authors), for CHDs other than TOF, the characteristics of cases and controls were for the most part comparable, except that mothers of cases of CoA were more likely to be from North Africa than controls. Most sociodemographic characteristics were different between cases of TOF and controls. Mothers of cases of TOF were significantly older and more likely to be from North Africa than controls. Mothers of cases of TOF were also

**Table 1** Associations between predictor variables and case/control status.

Characteristics	Controls		Cases		P
	n	% <sup>a</sup>	n	% <sup>a</sup>	
Mother					
Age (years)					
Mean (SD)	30.4 (5.2)		30.9 (5.5)		
Median (p25–p75)	30 (27–34)		31 (27–35)		
<20	59	1.4	22	1.4	0.011
20–29	1809	42.8	654	40.1	
30–34	1434	33.9	531	32.6	
35–39	722	17.1	316	19.4	
≥40	203	4.8	107	6.6	
Missing <sup>b</sup>	23	0.5	12	0.7	
Geographic origin					
France	2412	57.9	882	54.5	<0.001
North Africa	433	10.4	247	15.3	
Sub-Saharan Africa	550	13.2	163	10.1	
Other	770	18.5	327	20.2	
Missing <sup>b</sup>	85	2.0	23	1.4	
Occupation					
None	1083	26.3	440	29.7	<0.001
Professional	997	24.2	343	23.2	
Intermediate	856	20.8	263	17.8	
Administrative/public service	852	20.7	249	16.8	
Other	330	8.0	185	12.5	
Missing <sup>b</sup>	132	3.1	162	9.9	
Father					
Age (years)					
Mean (SD)	33.9 (6.6)		34.4 (6.7)		
Median (p25–p75)	33 (29–38)		33 (30–38)		
<20	5	0.1	3	0.2	0.133
20–29	890	25.8	277	22.6	
30–34	1198	34.7	422	34.4	
35–39	734	21.3	281	22.9	
≥40	623	18.1	244	19.9	
Missing <sup>b</sup>	800	18.8	415	25.3	
Pregnancy					
Multiplicity					
Singletons	2768	96.1	1382	95.8	0.756
Twins	103	3.6	57	4.0	
Triplets	8	0.3	3	0.2	
Outcome					
Stillbirths	7	0.2	46	2.8	<0.001
Live births	4231	99.6	1074	65.4	
Pregnancy terminations	12	0.3	522	31.8	

<sup>a</sup>% calculated with the total number of cases or controls without missing data as a denominator.

<sup>b</sup>% of missing data calculated with the total number of cases or controls as a denominator.

more likely to be in the occupational category 'none' than controls (data not shown).

## Risk of CHDs associated with ARTs

### All cases

Exposure to ARTs (all methods combined, Table II) was significantly higher for cases of TOF than controls (6.6 versus 3.5%,  $P = 0.002$ ). Exposure to the different methods of ARTs (data not shown) was also significantly different between cases of TOF and controls, in particular 2.5% of TOF were born following IVF versus 1.3% of controls and 1.3% of TOF were born following ICSI versus 0.3% of controls ( $P = 0.004$ ). Exposure to ARTs was not associated with a significantly higher risk of other CHDs.

Exposure to ART was associated with a 2.4-fold increase in the maternal characteristics and year of birth-adjusted odds of TOF (adjusted OR = 2.4, 95% CI: 1.5–3.7) (Table III). In contrast, ARTs were not associated with statistically significant increases in the risks of HLHS, TGA or CoA and the ORs were generally close to the null value (Table III). All three methods of ARTs were associated with significantly higher odds of TOF (Table IV). In particular, ICSI was associated with a 3-fold higher odds of TOF after adjustment for maternal characteristics and year of birth (adjusted OR = 3.0, 95% CI: 1.0–8.9). There was no evidence that IVF was associated with a higher odds of TOF when compared with OI (for IVF: adjusted OR = 2.0, 95% CI: 1.0–4.2; for OI: adjusted OR = 2.5, 95% CI: 1.3–4.8). For the other three specific CHDs, no statistically significant associations were observed. Further adjustment for paternal age using the multiple imputation estimates did not modify appreciably the above estimates (data not shown).

**Table II** Numbers of cases and controls and proportions of fetuses conceived after ARTs.

	<i>n</i>	% exposed to ART	<i>P</i> <sup>b</sup>
Controls <sup>a</sup>	4104	3.5	
All cases			
HLHS	353	2.8	0.491
TGA	444	2.7	0.363
TOF	395	6.6	0.002
CoA	391	3.3	0.831
Cases without chromosomal anomalies			
HLHS	303	2.6	0.413
TGA	430	2.8	0.423
TOF	315	7.3	0.001
CoA	350	3.7	0.860

CoA, coarctation of the aorta; HLHS, hypoplastic left heart syndrome; TGA, transposition of the great arteries; TOF, tetralogy of Fallot.

<sup>a</sup>The following malformations were used as controls: club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation.

<sup>b</sup>Comparison of the proportion of children/fetuses conceived after ART between the specific CHD and the malformed controls.

### Cases without associated chromosomal anomalies

Tables III and V show the results of the analyses for the associations between the risks of the four CHDs and ARTs (all methods combined, Table III) and separately for different methods of ARTs (Table V) for the subset of cases without associated chromosomal anomalies. All estimates were essentially the same as those found for all cases combined (i.e. when cases of each specific CHD with and without associated chromosomal anomalies were analysed together).

Results of the analyses, which included further adjustment for multiple pregnancies, were essentially the same as those found without adjustment for multiple pregnancies (data not shown). We found no statistically significant interaction effects between ARTs and multiple pregnancies for any of the four CHDs (data not shown).

## Discussion

Using population-based data on nearly 1600 cases of specific CHDs, we assessed the risk of four specific CHDs in relation to ARTs. We found that ARTs (all methods combined) were associated with a 2.4-fold increased risk of TOF, after taking into account maternal age, occupation, geographic origin, paternal age and year of birth. In particular, ICSI was associated with a 3-fold higher adjusted odds of TOF. In contrast, we did not find any statistically significant increases in the risk of CHDs in relation to ARTs for the other CHDs in our study, i.e. HLHS, transposition of the great arteries (TGA) and CoA. Risk estimates were comparable when cases with chromosomal anomalies were excluded, suggesting that the associations between ARTs and TOF are not due to the association of the latter with chromosomal anomalies. Further adjustment for multiple pregnancies did not substantially modify our results.

On the basis of our findings, we calculated attributable risk fractions, which would represent the proportion of cases of TOF that may be caused by ARTs, or equivalently, the proportion of cases of TOF that would be avoided were the exposure to ARTs removed *ceteris paribus*, 'if' the association we found between the risk of TOF and ARTs can be assumed to represent a causal relation (this may of course not be the case in part for reasons that are discussed further below). The attributable risk fraction estimates suggested in particular that around 6.5% of the TOF may have been caused by ARTs (all methods combined) and 2% by ICSI.

Our study has certain limitations. We had limited power to detect OR lower than 2 in the association between ARTs (for all methods combined) and specific CHDs and three in the case of the different methods of ARTs. Therefore, our study may have had insufficient power to detect statistically significant associations for other CHDs.

The models used to estimate the ORs for the different defects in relation to ARTs were not nested (i.e. were separate models) and we did not formally test the statistical significance of differences in the ORs for one defect versus another. The associations were not statistically significant for any of the defects except for TOF, whereas the numbers of cases for the other CHDs were comparable to those of TOF.

A potential source of bias in our study is related to the use of malformed controls (Swan et al., 1992; Lieff et al., 1999). The main advantage of using malformed controls is to reduce the risk of recall or other sources of information bias. But malformed controls may also be a source of selection

**Table III** Logistic regression analyses of the associations between assisted reproductive technologies (ART, all methods combined) and four specific CHDs.

	CHDs	ART	Unadjusted OR <sup>a</sup>	95% CI	Maternal Adjusted <sup>b</sup> OR <sup>a</sup>	95% CI
All cases	HLHS	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.8	0.4–1.5	0.8	0.4–1.8
	Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.8	0.4–1.4	0.7	0.4–1.4
	TOF	None	1.0	Ref.	1.0	Ref.
		All methods combined	1.9	1.3–3.0	2.4	1.5–3.7
CoA	None	1.0	Ref.	1.0	Ref.	
	All methods combined	0.9	0.5–1.7	1.1	0.6–2.0	
Cases without chromosomal anomalies	HLHS	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.7	0.4–1.5	0.8	0.3–1.7
	Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
		All methods combined	0.8	0.4–1.4	0.7	0.4–1.4
	TOF	None	1.0	Ref.	1.0	Ref.
		All methods combined	2.2	1.4–3.4	2.6	1.6–4.2
CoA	None	1.0	Ref.	1.0	Ref.	
	All methods combined	1.1	0.6–1.9	1.2	0.6–2.2	

<sup>a</sup>Odds ratios (OR) represent the odds of a birth (including live births, stillbirths and pregnancy terminations) with congenital heart defect (cases) relative to the odds of a birth with one of the malformed controls (club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation).

<sup>b</sup>Adjusted for maternal age, geographic origin, occupation and year of birth.

**Table IV** Logistic regression analyses of the associations between the different methods of ARTs and four specific CHDs.

CHD	ART	Unadjusted OR <sup>a</sup>	95% CI	Maternal Adjusted <sup>b</sup> OR <sup>a</sup>	95% CI
HLHS	None	1.0	Ref.	1.0	Ref.
	OI only	0.7	0.3–1.9	0.9	0.3–2.5
	IVF	0.6	0.2–2.0	0.5	0.1–2.3
	ICSI	1.8	0.4–7.9	1.6	0.3–7.2
	IVF + ICSI	0.8	0.3–2.1	0.8	0.3–2.3
Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
	OI only	0.6	0.2–1.5	0.6	0.2–1.7
	IVF	1.2	0.5–2.6	1.0	0.4–2.5
	ICSI	—	—	—	—
	IVF + ICSI	—	—	—	—
TOF	None	1.0	Ref.	1.0	Ref.
	OI only	1.5	0.8–2.9	2.5	1.3–4.8
	IVF	2.0	1.0–3.9	2.0	1.0–4.2
	ICSI	4.1	1.5–11.6	3.0	1.0–8.9
	IVF + ICSI	2.4	1.3–4.2	2.3	1.2–4.2
CoA	None	1.0	Ref.	1.0	Ref.
	OI only	0.7	0.3–1.7	1.0	0.4–2.6
	IVF	1.0	0.4–2.4	1.1	0.4–2.9
	ICSI	2.4	0.7–8.5	1.2	0.2–5.6
	IVF + ICSI	1.2	0.6–2.6	1.1	0.5–2.6

<sup>a</sup>Odds ratios (OR) represent the odds of a birth (including live births, stillbirths and pregnancy terminations) with congenital heart defect (cases) relative to the odds of a birth with one of the malformed controls (club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation).

<sup>b</sup>Adjusted for maternal age, geographic origin, occupation and year of birth.

bias if malformations included as controls are either directly or indirectly associated with ARTs. Risks could be under (over)-estimated if malformations included in the control group occur more (less) frequently in fetuses

conceived following ARTs. By selecting a heterogeneous group of malformations with no known association with ARTs, as recommended by Hook (1993), we aimed to minimize such bias. However, the possibility

**Table V** Logistic regression analyses of the associations between ARTs and four specific CHDs without associated chromosomal anomalies.

CHD	ART	Unadjusted OR <sup>a</sup>	95% CI	Maternal Adjusted <sup>b</sup> OR <sup>a</sup>	95% CI
HLHS	None	1.0	Ref.	1.0	Ref.
	OI only	0.7	0.3–1.9	0.8	0.2–2.5
	IVF	0.5	0.1–2.0	0.3	0.0–2.4
	ICSI	2.1	0.5–9.2	1.8	0.4–8.4
	IVF + ICSI	0.8	0.3–2.2	0.7	0.2–2.3
Transposition of the great arteries	None	1.0	Ref.	1.0	Ref.
	OI only	0.6	0.2–1.5	0.6	0.2–1.8
	IVF	1.2	0.5–2.7	1.1	0.5–2.6
	ICSI	—	—	—	—
	IVF + ICSI	—	—	—	—
TOF	None	1.0	Ref.	1.0	Ref.
	OI only	1.6	0.8–3.2	2.3	1.1–4.8
	IVF	2.2	1.1–4.5	2.5	1.2–5.2
	ICSI	5.2	1.8–14.7	3.7	1.3–10.9
	IVF + ICSI	2.8	1.6–5.0	2.8	1.5–5.2
CoA	None	1.0	Ref.	1.0	Ref.
	OI only	0.8	0.3–1.9	1.1	0.4–2.8
	IVF	1.1	0.4–2.7	1.3	0.5–3.3
	ICSI	2.7	0.8–9.6	1.3	0.3–6.1
	IVF + ICSI	1.4	0.7–2.9	1.3	0.6–2.9

<sup>a</sup>Odds ratios (OR) represent the odds of a birth (including live births, stillbirths and pregnancy terminations) with congenital heart defect (cases) relative to the odds of a birth with one of the malformed controls (club-foot, angioma, skin abnormality, polydactyly, syndactyly and congenital hip dislocation).

<sup>b</sup>Adjusted for maternal age, geographic origin, occupation and year of birth.

of residual bias due to shared aetiologies between cases and malformed controls cannot be excluded.

A differential misclassification bias for exposure assessment cannot be excluded if exposure to ARTs is ascertained in a different way for cases and controls. However, we have no reason to believe that ARTs may have been ascertained differentially for cases of TOF versus the other CHDs examined in our study.

We had a relatively high proportion of missing data on paternal age. The latter is known to be associated with ARTs and more specifically with ICSI. Estimates for ICSI could therefore be biased if the distribution of paternal age was different for subjects with missing data. We used multiple imputations for imputing missing paternal age using case/control status, exposure to ARTs, maternal age and year of birth, and adjustment for paternal age did not appreciably change our results. However, residual bias due to other paternal characteristics cannot be excluded.

The question of multiple pregnancies and its association with both ARTs and the risk of congenital anomalies is an important issue to consider. There is evidence suggesting that multiple pregnancies may be associated with a higher risk of congenital anomalies (Mastroiacovo et al., 1999; Glinianaia et al., 2008). This may specifically be the case for CHDs, although relatively little, and at times contradictory, information exists on the associations between multiple pregnancies and CHDs (Manning and Archer, 2006; Bahtiyar et al., 2007; Campbell et al., 2009). Moreover, it is not clear to what extent any association between multiple pregnancies and CHDs may in fact be due to ARTs. Our results remained similar after further adjustment for multiple pregnancies and

we did not find any statistically significant interaction effects between ARTs and multiple pregnancies for any of the CHDs, although this may have been due to limited power of our study for detecting interaction effects. In any case, none of the above precludes the possibility that multiple pregnancies may be on the causal pathway between ARTs and CHDs. It is worth noting, however, that the public health impact of ARTs on the risk for birth defects, including that of TOF found in our study, includes all (singleton and multiple) pregnancies.

Specific associations between ARTs and certain categories of CHDs, particularly the so-called conotruncal defects, which include TOF, have been reported (Tararbit et al., 2011; Reefhuis et al., 2009). In a recent study (Tararbit et al., 2011), the risk of CHDs associated with ARTs was also shown to vary more generally for different methods of ARTs and categories of CHDs defined based on anatomic and clinical criteria (Houyel et al., 2011). In particular, the authors found a stronger association between ICSI and the category ‘Malformations of the outflow tracts and ventriculoarterial connections’ that comprised, among other CHDs, the conotruncal defects.

The developmental origins of TOF are complex and not fully understood but they may involve abnormal development of neural crest cells. None of the other three CHDs studied is known to be of cardiac neural crest origin. In particular, TGA which is a defect of the outflow tract does not belong to the group of the conotruncal defects (Houyel et al., 2011) and migration/proliferation of neural crest cell appear to be normal in this condition (Bajolle et al., 2006). In order to further investigate, the hypothesis of the involvement of neural crest cells in the association between TOF and ARTs, we

assessed the risk for other, rarer CHDs thought to be of neural crest origin (TOF with pulmonary atresia, TOF with absent pulmonary valve and common arterial trunk). We found an increased overall risk associated with ARTs (data not shown) but the CIs were wide due to small sample sizes.

Given the uncertainties about both the developmental origins of cardiac defects and possible effects of ARTs on fetal development, the hypothesis of a potential implication of neural crest cells in the association between ARTs and TOF must be regarded as very tentative and no more than a reasonable speculation. Future observational and experimental studies using other designs (e.g. animal studies, genetic studies, fundamental research in biology of reproduction/ARTs as well as additional epidemiological studies) are needed to both further assess our observations and in order to understand the possible underlying mechanisms of the association between the risk of TOF and ARTs.

In conclusion, we found that cases of TOF were more likely to have been conceived following ARTs when compared with controls. ARTs were associated with a 2.4-fold higher risk of TOF after adjustment for maternal age, occupation, geographic origin, paternal age and year of birth; ICSI was specifically associated with a 3-fold higher risk of TOF. In contrast, we did not find statistically significant associations between ARTs and HLHS, TGA or CoA and most ORs were close to the null value. Our study cannot disentangle to what extent the observed associations between the risk of TOF and ARTs may be due to any causal effects of ARTs and/or the underlying infertility problems of couples who conceive following ARTs. Nevertheless, the developmental basis of the specific association between risk of TOF and ARTs, particularly ICSI, and the potential implication of neural crest cells in this association, need to be further investigated.

## Authors' roles

B.K. conceived the study. K.T. conducted the main statistical analyses and wrote the first draft of the manuscript with B.K. N.L. and A-C.T. assisted with statistical analysis. L.H., D.B. and F.G. contributed to the conceptualization of ideas and made suggestions about the required analyses. L.H. and D.B. provided expertise as paediatric cardiologists. All of the authors contributed to the interpretation of findings and revisions of the article.

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## Conflict of interest

None declared.

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## THE RISK OF MAJOR BIRTH DEFECTS AFTER INTRACYTOPLASMIC SPERM INJECTION AND IN VITRO FERTILIZATION

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### ABSTRACT

**Background** It is not known whether infants conceived with use of intracytoplasmic sperm injection or in vitro fertilization have a higher risk of birth defects than infants conceived naturally.

**Methods** We obtained data from three registries in Western Australia on births, births after assisted conception, and major birth defects in infants born between 1993 and 1997. We assessed the prevalence of major birth defects diagnosed by one year of age in infants conceived naturally or with use of intracytoplasmic sperm injection or in vitro fertilization.

**Results** Twenty-six of the 301 infants conceived with intracytoplasmic sperm injection (8.6 percent) and 75 of the 837 infants conceived with in vitro fertilization (9.0 percent) had a major birth defect diagnosed by one year of age, as compared with 168 of the 4000 naturally conceived infants (4.2 percent;  $P < 0.001$  for the comparison between either type of technology and natural conception). As compared with natural conception, the odds ratio for a major birth defect by one year of age, after adjustment for maternal age and parity, the sex of the infant, and correlation between siblings, was 2.0 (95 percent confidence interval, 1.3 to 3.2) with intracytoplasmic sperm injection, and 2.0 (95 percent confidence interval, 1.5 to 2.9) with in vitro fertilization. Infants conceived with use of assisted reproductive technology were more likely than naturally conceived infants to have multiple major defects and to have chromosomal and musculoskeletal defects.

**Conclusions** Infants conceived with use of intracytoplasmic sperm injection or in vitro fertilization have twice as high a risk of a major birth defect as naturally conceived infants. (N Engl J Med 2002;346:725-30.)

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In vitro fertilization was introduced into practice with little formal evaluation of its effects on the health of the children conceived with this procedure. When intracytoplasmic sperm injection was introduced in 1992, earlier concern reemerged that infants conceived with the use of assisted reproductive technology might have an increased risk of birth defects.<sup>1-4</sup>

In general, studies have not shown an increased risk of major birth defects in children conceived with either intracytoplasmic sperm injection or standard in vitro fertilization.<sup>5</sup> Much of this research, however, has had methodologic problems, including inadequate sample sizes and a lack of appropriate data for comparison. Moreover, the definitions of major birth defects used for infants conceived with assisted reproductive technology were different from those used for infants conceived naturally; this difference may have led to an underestimation of the relative prevalence of birth defects among infants conceived with assisted reproductive technology.<sup>6</sup>

Treatment with assisted reproductive technology is provided by three private clinics in Western Australia. Treatment is regulated by the Human Reproductive Technology Act 1991, which established the statutory Reproductive Technology Register that contains information on all procedures performed with assisted reproductive technology in Western Australia since April 1993.<sup>7</sup> We compared the prevalence of major birth defects among infants conceived with such procedures with that in a random sample of naturally

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conceived infants, using the same system of classification for all birth defects.

**METHODS**

**Collection of Data**

We used data from the Reproductive Technology Register to identify all pregnancies of at least 20 weeks' gestation resulting from intracytoplasmic sperm injection or standard in vitro fertilization treatment undertaken between 1993 and 1997 and all terminations of such pregnancies because of fetal abnormalities (regardless of the length of gestation). The three private clinics performed 719, 1191, and 2931 cycles of embryo transfer, respectively, during this period.

The Midwives' Notification System collects information on all infants delivered in Western Australia at 20 weeks' gestation or later.<sup>8</sup> A random sample of 4000 infants born in Western Australia between 1993 and 1997 was selected after the exclusion of the infants conceived with assisted reproductive technology.

The Western Australian Birth Defects Registry collects information on birth defects occurring in liveborn and stillborn infants delivered in Western Australia, and on pregnancies terminated because of fetal malformations.<sup>9</sup> For the purposes of the registry, birth defects are defined as abnormalities that are probably of prenatal origin, including structural, chromosomal, and genetic defects. The classification system of the British Paediatric Association, based on the *International Classification of Diseases, 9th Revision (ICD-9)*, is used to code each defect, and all defects are classified as major or minor according to a method devised by the Centers for Disease Control and Prevention.<sup>9</sup> Most minor defects (listed in Supplementary Appendix 1, available with the full text of this article at <http://www.nejm.org>) are excluded from the registry; however, defects on the exclusion list that require treatment or are disfiguring are included. Approximately 90 percent of cases in the registry involve at least one major defect (with or without minor defects); the remainder involve minor defects only.<sup>9</sup> Birth defects diagnosed prenatally and in children up to six years of age are included. Cases are reported by multiple statutory and voluntary sources with a high level of ascertainment and accuracy.<sup>10</sup>

Automatch (probabilistic matching software)<sup>11</sup> was used to link the records of the three registers. When linkage was complete, birth records were available for all infants in the study; records of birth defects were available for those for whom a link was found within the Birth Defects Registry.

To assess the potential effects of differential surveillance according to mode of conception, a list of all birth defects reported for each child was prepared without identification of whether conception was assisted or natural. An independent pediatrician examined the list and identified, on the basis of clinical experience, defects that might have been diagnosed because of closer surveillance and might not otherwise have been detected in a child less than one year of age.

Approval for the study was obtained from the appropriate institutional ethics committee.

**Prevalence of Birth Defects**

The prevalence of major birth defects diagnosed by one year of age was calculated for the intracytoplasmic-sperm-injection, in-vitro-fertilization, and natural-conception groups. We compared the groups by calculating odds ratios for major birth defects and exact 95 percent confidence intervals on the basis of prevalence. The use of these odds ratios rather than relative risks facilitated the comparison with the odds ratios that were subsequently calculated by logistic regression. Two-tailed P values were calculated with the use of SPSS software.<sup>12</sup>

Multiple logistic-regression analysis was used to assess the effect of maternal age and parity and the sex of the infant on the odds-ratio estimates. Generalized-estimating-equation analyses were per-

formed with the use of Stata software<sup>13</sup> to examine the effect of potential correlations of risk between siblings in the data set.

Although our study dealt primarily with birth defects diagnosed at or after birth, it is possible that the rates of termination of pregnancy because of fetal anomalies might have differed between the assisted-conception groups and the natural-conception group and that investigating only births may have led to a biased result. We identified all terminations of pregnancy after the prenatal diagnosis of birth defects in the assisted-conception groups; there were four such terminations among the women who underwent in vitro fertilization and none among the women who underwent intracytoplasmic sperm injection. In Western Australia, there are 3.5 terminations of pregnancy because of fetal anomalies per 1000 total births. For the sake of comparison, we conducted a secondary analysis including 14 pregnancies that had resulted from natural conception and that had been terminated because of birth defects; these pregnancies were randomly selected from the Birth Defects Registry and added to the 4000 births for this analysis.

**RESULTS**

The study included 301 infants conceived with intracytoplasmic sperm injection, 837 infants conceived with standard in vitro fertilization, and 4000 naturally conceived infants. As compared with the mothers of the natural-conception group of infants, the women who had undergone treatment with assisted reproductive technology were, on average, older and less likely to have had a child previously (Table 1). They were more likely to be married or cohabiting, to be white, and to live in the metropolitan area of Perth. As compared with the infants in the natural-conception group the infants conceived with assisted repro-

**TABLE 1. CHARACTERISTICS OF 4916 WOMEN WHO CONCEIVED WITH INTRACYTOPLASMIC SPERM INJECTION, WITH IN VITRO FERTILIZATION, OR NATURALLY.\***

CHARACTERISTIC	INTRACYTOPLASMIC-SPERM-INJECTION GROUP (N=240)	IN-VITRO-FERTILIZATION GROUP (N=676)	NATURAL-CONCEPTION GROUP (N=4000)
Age — yr	32.6±4.0†	34.1±4.6†	28.2±4.4
Parity — no. (%)			
0	183 (76)†	454 (67)	1612 (40)
≥1	57 (24)	222 (33)	2388 (60)
Married or cohabiting — no. (%)	237 (99)†	664 (98)†	3564 (89)
Ethnic group — no. (%)			
White	230 (96)†	639 (95)†	3500 (88)
Aboriginal or Torres Strait Islander	1 (≤1)	3 (≤1)	220 (6)
Other	9 (4)	34 (5)	280 (7)
Place of residence — no. (%)‡			
Metropolitan Perth	197 (82)†	557 (82)†	2884 (72)
Rural area	43 (18)	119 (18)	1112 (28)
Unknown	—	—	4 (<1)

\*Plus-minus values are means ±SD. Numbers of mothers do not match numbers of infants because of multiple births.

†P<0.001 for the comparison with the natural-conception group.

‡Data were missing for four mothers of infants in the natural-conception group.

ductive technology were more likely to be delivered by cesarean section, to have low birth weight, and to be born before term (Table 2). When only singleton infants were considered, low birth weight and delivery by cesarean section were significantly more common in both the in-vitro-fertilization group and the intracytoplasmic-sperm-injection group than in the natural-conception group, and preterm birth was significantly more common in the in-vitro-fertilization group than in the natural-conception group.

In a total of 26 of the infants conceived with intracytoplasmic sperm injection (8.6 percent [95 percent confidence interval, 5.7 to 12.4 percent]), 75 of the infants conceived with in vitro fertilization (9.0 percent [95 percent confidence interval, 7.1 to 11.1 percent]), and 168 of the naturally conceived infants (4.2 percent [95 percent confidence interval, 3.6 to 4.9 percent]), a major birth defect was diagnosed by one year of age ( $P < 0.001$  for the comparisons between the natural-conception group and the assisted-conception groups). There were no significant differences in prevalence among the clinics (data not shown). When all the infants were considered, those conceived with assisted reproductive technology were more than twice as likely as naturally conceived infants to have a major birth defect diagnosed by one year of age (Table 3). The results were similar and remained significant when only singleton infants were considered, when the analyses were further restricted to singletons born at term (at least 37 weeks of gestation), and when the analyses were adjusted for maternal age and parity, the sex of the infant, and

correlation of the risk of birth defects between siblings (Table 3).

About two thirds of the major defects were diagnosed during the first week of life (Fig. 1), and more than 90 percent were diagnosed by six months of age. The defects in three infants in the natural-conception group (all renal defects), four infants in the intracytoplasmic-sperm-injection group (two with renal defects and two musculoskeletal defects), and one infant in the in-vitro-fertilization group (a musculoskeletal defect) were identified by the independent pediatrician as possibly having been diagnosed early because of close surveillance. When these infants were excluded from the analysis, the odds ratio for a major birth defect diagnosed by one year of age as compared with the natural-conception group was 1.8 (95 percent confidence interval, 1.1 to 2.9) in the intracytoplasmic-sperm-injection group and 2.2 (95 percent confidence interval, 1.7 to 3.0) in the in-vitro-fertilization group.

When pregnancies terminated because of fetal abnormalities were included in the analysis, the overall prevalence of major birth defects was 4.5 percent in the natural-conception group and 9.4 percent in the in-vitro-fertilization group; it was unchanged at 8.6 percent in the intracytoplasmic-sperm-injection group. When the nine infants with known inherited conditions and the seven with metabolic disorders were excluded from the analysis, the overall prevalence of birth defects was 8.0 percent in the intracytoplasmic-sperm-injection group, 8.5 percent in the in-vitro-fertilization group, and 4.0 percent in the natural-

TABLE 2. MODE OF DELIVERY AND CHARACTERISTICS OF INFANTS CONCEIVED WITH INTRACYTOPLASMIC SPERM INJECTION, WITH IN VITRO FERTILIZATION, OR NATURALLY.\*

VARIABLE	ALL INFANTS					SINGLETONS ONLY				
	ICSI (N=301)	P VALUE	IVF (N=837)	P VALUE	NATURAL CONCEPTION (N=4000)	ICSI (N=186)	P VALUE	IVF (N=527)	P VALUE	NATURAL CONCEPTION (N=3906)
Delivered by cesarean section — no. (%)	95 (32)	<0.001	365 (44)	<0.001	816 (20)	48 (26)	0.05	183 (35)	<0.001	776 (20)
Male sex — no. (%)	165 (55)		454 (54)		2048 (51)	102 (55)		286 (54)		2000 (51)
Stillborn — no. (%)	2 (1)		17 (2)	<0.001	26 (1)	0		6 (1)		25 (1)
Birth weight — g	2847±799	0.02	2806±844	0.005	3345±592	3271±552	0.02	3182±686	<0.001	3368±571
Preterm delivery (<37 wk) — no. (%)	93 (31)	<0.001	265 (32)	<0.001	273 (7)	16 (9)		73 (14)	0.001	225 (6)
Multiple birth — no. (%)	115 (38)	<0.001	310 (37)	<0.001	94 (2)	—		—		—
Low birth weight — no. (%)										
<1500 g	18 (6)	<0.001	65 (8)	<0.001	51 (1)	2 (1)		19 (4)	<0.001	41 (1)
<2500 g	75 (25)	<0.001	188 (22)	<0.001	196 (5)	12 (6)		38 (7)	0.002	163 (4)
Gestational age — wk	37.0±3.3	0.004	36.7±3.8	0.002	39.0±2.1	38.6±2.2		38.0±3.0	0.03	39.1±2.0

\*Plus-minus values are means ±SD. P values are for the comparisons with the natural-conception group and are not significant if not shown. ICSI denotes intracytoplasmic sperm injection, and IVF in vitro fertilization.

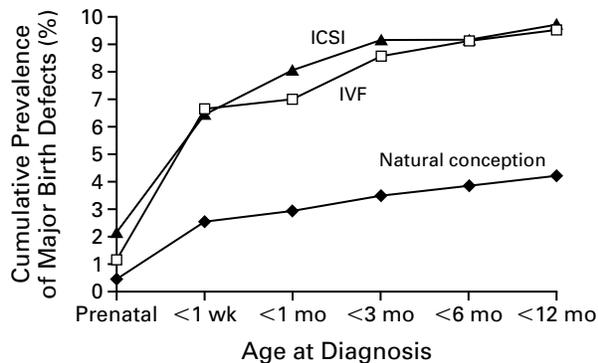
**TABLE 3.** PREVALENCE OF MAJOR BIRTH DEFECTS DIAGNOSED BY ONE YEAR OF AGE.\*

GROUP	NO. OF INFANTS	PREVALENCE no. (%)	UNADJUSTED	ADJUSTED
			ODDS RATIO (95% CI)	ODDS RATIO (95% CI)†
All infants				
Natural conception	4000	168 (4.2)	1.0	1.0
Intracytoplasmic sperm injection	301	26 (8.6)	2.2 (1.3–3.3)	2.0 (1.3–3.2)
In vitro fertilization	837	75 (9.0)	2.6 (1.7–3.0)	2.0 (1.5–2.9)
All singletons				
Natural conception	3906	164 (4.2)	1.0	1.0
Intracytoplasmic sperm injection	186	18 (9.7)	2.4 (1.4–4.1)	2.2 (1.3–3.9)
In vitro fertilization	527	50 (9.5)	2.4 (1.7–3.4)	2.2 (1.5–3.2)
Term singletons‡				
Natural conception	3681	149 (4.0)	1.0	1.0
Intracytoplasmic sperm injection	170	15 (8.8)	2.3 (1.2–4.0)	2.2 (1.2–4.0)
In vitro fertilization	454	38 (8.4)	2.2 (1.5–3.2)	2.1 (1.4–3.2)

\*CI denotes confidence interval.

†The odds ratios were adjusted for maternal age and parity, the sex of the infant, and correlation between siblings.

‡Term was defined as at least 37 weeks of gestation.



**Figure 1.** Cumulative Prevalence of Diagnosed Major Birth Defects in Singleton Infants, According to Age at Diagnosis. ICSI denotes intracytoplasmic sperm injection, and IVF in vitro fertilization.

conception group. The odds ratios for a major birth defect associated with assisted conception in these analyses remained similar to those calculated in the primary analysis (data not shown). All the infants in our study with unilateral undescended testis or hypospadias had undergone surgery and were therefore included in our primary analyses. Nevertheless, in some systems of classification of birth defects, these defects would be regarded as minor. When infants

with these conditions were excluded from the analysis, the odds ratio for a major birth defect was 2.5 (95 percent confidence interval, 1.6 to 4.0) in the intracytoplasmic-sperm-injection group and 2.2 (95 percent confidence interval, 1.6 to 3.0) in the in-vitro-fertilization group.

As compared with infants conceived naturally, a significantly greater proportion of those conceived with assisted reproductive technology had musculoskeletal and chromosomal defects (Table 4). Those conceived with in vitro fertilization, but not those conceived with intracytoplasmic sperm injection, had a significantly greater prevalence of cardiovascular, urogenital, and other defects. Some, but not all, of these findings persisted when the analysis was restricted to singletons (Table 4). We also compared the proportions of infants with multiple major defects, defined as two or more defects affecting different systems. Six of the infants conceived with intracytoplasmic sperm injection (2.0 percent), 13 of those conceived with in vitro fertilization (1.6 percent), and 20 of the naturally conceived infants (0.5 percent) had multiple major defects. Overall, the infants conceived with assisted reproductive technology were significantly more likely to have multiple major defects than the naturally conceived infants (odds ratio associated with intracytoplasmic sperm injection, 4.1 [95 percent confidence interval, 1.6 to 10.2]; odds ratio associated with in vitro fertilization, 3.1 [95 percent confidence interval, 1.6 to 6.3]). A complete list of the birth de-

**TABLE 4.** PREVALENCE OF MAJOR BIRTH DEFECTS ACCORDING TO THE ORGAN SYSTEM AFFECTED.\*

TYPE OF MAJOR DEFECT	ALL INFANTS					SINGLETONS ONLY				
	ICSI (N=301)	P VALUE	IVF (N=837)	P VALUE	NATURAL CONCEPTION (N=4000)	ICSI (N=186)	P VALUE	IVF (N=527)	P VALUE	NATURAL CONCEPTION (N=3906)
	no. (%)		no. (%)		no. (%)	no. (%)		no. (%)		no. (%)
Any	26 (8.6)	<0.001	75 (9.0)	<0.001	168 (4.2)	18 (9.7)	<0.001	50 (9.5)	<0.001	164 (4.2)
Cardiovascular	4 (1.3)		15 (1.8)	<0.001	24 (0.6)	3 (1.6)		7 (1.3)		24 (0.6)
Urogenital	7 (2.3)		22 (2.6)	0.01	54 (1.4)	5 (2.7)		14 (2.7)	0.03	52 (1.3)
Musculoskeletal	10 (3.3)	0.004	28 (3.3)	<0.001	45 (1.1)	5 (2.7)		20 (3.8)	<0.001	44 (1.1)
Gastrointestinal	3 (1.0)		5 (0.6)		25 (0.6)	2 (1.1)		2 (0.4)		24 (0.6)
Central nervous system	0		3 (0.4)		6 (0.2)	0		2 (0.4)		6 (0.2)
Chromosomal	3 (1.0)	0.05	6 (0.7)	0.03	9 (0.2)	3 (1.6)	0.02	3 (0.6)		9 (0.2)
Metabolic	1 (0.3)		2 (0.2)		4 (0.1)	0		1 (0.2)		4 (0.1)
Other†	2 (0.7)		21 (2.5)	<0.001	25 (0.6)	2 (1.1)		15 (2.8)	<0.001	25 (0.6)

\*If an infant had more than one major birth defect diagnosed by one year of age and the defects affected different organ systems, the infant appears more than once in the table. If an infant had two unrelated major defects affecting the same organ system, the infant appears only once in the table. P values are for the comparisons with the natural-conception group.

†Other major birth defects included major defects of the respiratory system, Klippel–Trénaunay–Weber syndrome, Holt–Oram syndrome, infantile Marfan’s syndrome, and nonimmune hydrops fetalis, among others.

fects is provided in Supplementary Appendix 2 (available with the full text of this article at <http://www.nejm.org>).

Although minor birth defects were not the primary focus of this study, the Birth Defects Registry collects details of defects that would otherwise be considered minor but are disfiguring or require treatment (e.g., polydactyly). Such defects were diagnosed by one year of age in 1 infant in the intracytoplasmic-sperm-injection group (0.3 percent), 7 infants in the in-vitro-fertilization group (0.8 percent), and 25 infants in the natural-conception group (0.6 percent).

**DISCUSSION**

We found that infants conceived with assisted reproductive technology were more than twice as likely as naturally conceived infants to have major birth defects diagnosed during the first year of life and were also more likely to have multiple major defects. The increase in the risk of a major birth defect associated with assisted conception remained significant when only singleton or term singleton infants were considered, as well as after adjustment for maternal age and parity, the sex of the infant, and correlation between siblings. Furthermore, the estimates of the prevalence of defects reported to the registry by one year of age in the assisted-conception groups were well in excess of the 6 percent prevalence of major birth de-

fects reported by six years of age during the same period in the general population.<sup>9</sup> The risk of birth defects was similar among infants conceived with in vitro fertilization and those conceived with intracytoplasmic sperm injection.

We designed our study to address the major methodologic problems of previous research. We used the same source of data and the same birth-defect classification system for all three groups of infants. Furthermore, data on birth defects were collected without reference to the mode of conception. There is nevertheless a risk of differential diagnostic vigilance, given that infants conceived with assisted reproductive technology may be more closely examined than naturally conceived infants, because of either the history of their conception or a clinical condition associated with prematurity or multiple birth. If so, major birth defects might have been diagnosed earlier in the assisted-conception groups. However, the results were essentially unchanged when we excluded defects that might be more likely to be detected with closer surveillance. We also found that the excess risk remained when only term singletons were considered.

Pregnancies that result from treatment with assisted reproductive technology may be more closely monitored than those that result from natural conception. However, detailed ultrasonographic examinations of fetal anatomy are performed at 16 to 20 weeks of

gestation in almost all pregnancies in Western Australia. Furthermore, the majority of the defects diagnosed prenatally in the infants in both the assisted-conception groups and the natural-conception group would have been clinically obvious at birth. We also minimized the likelihood of differential diagnostic vigilance by including defects diagnosed up to one year after birth, by which time most major defects are likely to have been detected; in Western Australia 70 percent of all major birth defects are diagnosed by one year of age.<sup>9</sup> Increased diagnostic vigilance may also increase the rate of detection of more subtle defects; however, such vigilance is unlikely to explain the excess risk in the infants conceived with assisted reproductive technology, since the majority of the defects in this group were either visible (e.g., cleft lip and palate) or would have been clinically obvious at, or soon after, birth (e.g., tracheoesophageal fistula). Finally, although the likelihood of terminating a pregnancy because of fetal anomalies may vary with the mode of conception, the inclusion of pregnancies that were terminated because of birth defects had little effect on our findings.

An excess risk of major birth defects in infants conceived with assisted reproductive technology is plausible. Factors that may increase the risk of birth defects include the relatively advanced age of infertile couples; the underlying cause of their infertility; the medications used to induce ovulation or to maintain the pregnancy in the early stages; and factors associated with the procedures themselves, such as the freezing and thawing of embryos, the potential for polyspermic fertilization, and the delayed fertilization of the oocyte.<sup>14-17</sup> Although older maternal age and low parity did not appear to explain our results, it is not possible to separate the excess risk that may be associated with infertility treatment from the excess risk related to the underlying causes of infertility.

Recent data have suggested that there is an increased risk of birth defects in infants conceived with in vitro fertilization<sup>18</sup> or intracytoplasmic sperm injection,<sup>19</sup> but these results might have been attributed to conditions associated with multiple and preterm birth.<sup>19</sup> Our results cannot be explained by these factors, since they remained similar when we restricted our analyses to term singletons.

We found that there may be an excess occurrence of major cardiovascular, urogenital, chromosomal, and musculoskeletal defects associated with assisted conception. However, these findings regarding specific organ systems should be interpreted with caution, since they are based on small numbers of infants in each group. Although the prevalence of a specific defect is rarely reported for infants conceived with assisted reproductive technology, others have also sug-

gested that the prevalence of these defects is increased among such infants.<sup>6,16,19-21</sup>

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# The sex ratio of singleton offspring in assisted-conception pregnancies

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**Objective:** To evaluate the effect of intracytoplasmic sperm injection (ICSI) and male factor infertility on the sex ratio in births from assisted reproductive technology.

**Design:** Historic cohort study.

**Setting:** Clinic-based data.

**Patient(s):** The study population included 15,164 singleton live births in the Society for Assisted Reproductive Technology national database for 2005 from cycles using ejaculated sperm, categorized by the use of insemination or ICSI and the absence or presence of male factor infertility, and cleavage- versus blastocyst-stage embryo transfers (ETs).

**Intervention(s):** None.

**Main Outcome Measure(s):** The probability of a male infant with and without the use of ICSI and in the presence or absence of male factor infertility.

**Result(s):** The sex ratio for all U.S. live births in 2005 was 52.5%, versus 48.9% for cleavage-stage and 51.6% for blastocyst-stage embryos. With blastocyst-stage embryos, the sex ratios were 49.6% and 54.9% with and without ICSI and 52.6% and 50.0% with and without male factor infertility, respectively. With cleavage-stage embryos, the sex ratio was not significantly affected by ICSI or male factor infertility, singly or in combination.

**Conclusion(s):** The use of ICSI, particularly with blastocyst-stage embryos, is associated with a decrease in the sex ratio of male infants. (Fertil Steril® 2009;92:1579–85. ©2009 by American Society for Reproductive Medicine.)

**Key Words:** Sex ratio, ICSI, blastocyst-stage embryos transfers, male factor infertility

In humans there is a well-documented disproportionate loss of males after conception (the primary sex ratio), at birth (the secondary sex ratio), reaching gender equilibrium by the third or fourth decade of life (the tertiary sex ratio), and declining further into old age. In humans, the proportion is approximately 248:100 males to females among fetuses delivered after very short gestation duration (16–19 weeks), declining very steeply to about 130:100 among preterm births (20–36 weeks), and declining further at birth to be 105:100 (1–4). In the United States there has been a steady fall in the secondary sex ratio overall since the 1960s, with signifi-

cant declines among births to white women, despite increases among births to black women (5–7). In 2005, the secondary sex ratio for live births in the United States was 1049:1000, or 51.2% males (8).

A number of biological and environmental factors have been shown to reduce the secondary sex ratio, including older maternal and paternal ages (7, 9), higher maternal weight, stressors (i.e., war, earthquakes, and economic distress), and toxins (i.e., smoking, pollutants, and pesticides) (10–14). In addition, a higher sex ratio has been noted for certain countries due to social factors for male sex preference and sex selection (15, 16). Male reproductive factors, including subfertility, abnormal sperm characteristics, and subsequent development of testicular cancer, have also been associated with a lower secondary sex ratio (a higher proportion of female infants) (17, 18). The objective of this analysis was to evaluate the effect of method of insemination and male factor infertility on the sex ratio of singleton births from assisted reproductive technology (ART).

## MATERIALS AND METHODS

The data source for this study was the Society for Assisted Reproductive Technology Clinic Outcomes Reporting

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System (SART CORS) database, which contains comprehensive data from more than 90% of all clinics providing ART in the United States; for 2005, this included 345 ART clinics. A 1-year cross-sectional sample was chosen because of the large size of the data set (the sample size for even 1 year is quite large) and because it was thought that confounding stressors, such as currently available embryo growth media, that could influence results over a longer period would be minimized if analysis were restricted to 1 year. In addition, the 2005 data were the most recent data available for analysis. This database contains data collected and verified annually by SART. Data from this system are reported to the Centers for Disease Control in compliance with the Fertility Clinic Success Rate and Certification Act of 1992 (Public Law 102-493, October 24, 1992).

The study population was limited to pregnancies of  $\geq 22$  weeks' gestation of live-born singletons. The data were limited to pregnancies fertilized with ejaculated sperm only and categorized by the use of intracytoplasmic sperm injection (ICSI) versus insemination, male factor infertility (absent or present), and cleavage versus blastocyst stage of embryo at transfer. Blastocyst transfer was defined as transfer occurring on days 4, 5, 6, or 7. Actual embryo stage is not available in the database. The dependent variable was the likelihood of a male infant at birth.

Maternal demographic factors, reproductive history, ART cycle specific parameters, and pregnancy outcomes were compared between and within the ICSI versus insemination groups using  $\chi^2$ - and Student's *t*-test. Since there can be multiple diagnoses for each birth, only pregnancies with a single infertility diagnosis ( $\sim 2/3$  of the pregnancies) were used to compare percent male infants between diagnoses within each of the insemination and ICSI groups (Table 2). All other comparisons and analyses included all cycles with both single and multiple infertility diagnoses (Table 3).

Logistic regression was used to model the likelihood of a male infant, using the insemination and no male factor group as the reference, for the total study population and stratified by stage of embryo at transfer. The models were also adjusted for maternal age, gravidity, history of prior spontaneous abortions, infertility diagnoses, use of fresh or frozen oocytes, use of donor or autologous oocytes, assisted hatching, and use of partner or donor sperm. Adjusting for these additional factors did not significantly change the results, and therefore these factors were not retained in the final models. Data were analyzed using the Statistical Package for the Social Sciences, version 14.0 (SPSS, Inc., Chicago). The study was approved by the Committee for the Protection of Human Subjects at Dartmouth College and written in conjunction with the SART Research Committee.

## RESULTS

The study population included 15,164 singleton deliveries, including 5773 with insemination and 9391 with ICSI. The use of ICSI was associated with younger maternal age, lower

parity, a smaller proportion of patients with a diagnosis of some form of female factor infertility, reduced use of donor oocytes or donor semen, and use of assisted hatching (Table 1). Additionally, of note, there was no difference in the proportion of cycles using cleavage-stage versus blastocyst transfer between the ICSI and insemination groups (Table 1). Within the ICSI and insemination groups, the only significant difference in percent male infants was by embryo stage at transfer, with the highest proportion among pregnancies conceived with blastocyst-stage embryos and insemination (Table 2). As shown in Table 3, the use of ICSI, overall and with blastocyst-stage embryos, was associated with a significant reduction in the likelihood of a male infant singly or in combination with male factor infertility. With blastocyst-stage embryos, the reduction in the sex ratio with ICSI was greater, both singly and in combination with male factor infertility.

## DISCUSSION

In this paper, we show that the sex ratio of offspring after ART is reduced when ICSI rather than insemination is used to obtain fertilization using ejaculated sperm, particularly among births from blastocyst-stage embryos. The reduction in the proportion of males born after ICSI was not influenced by female age or diagnoses, ethnicity, or semen source. A diagnosis of male factor infertility alone was not associated with a significant change in the secondary sex ratio.

We do not believe it likely that the differences in demographic characteristics of the ICSI and insemination groups noted in Table 1 could account for the observed lower sex ratio with ICSI. The lower maternal age and the greater percentage of primiparous women in the ICSI group would both tend to increase the sex ratio and lead us to underestimate the effect of ICSI on reducing the sex ratio.

The human sex ratio at birth has long been known to be subject to a variety of preconception and intrapartum factors. Causes may range from genetics to differential survival of male fetuses in utero. Male gender is an independent risk factor for adverse perinatal outcomes, including stillbirth and prematurity, as well as higher neonatal and infant mortality at every gestational age (4, 19–21). Surprisingly, however, the proportion of males at birth (51.3%) is higher than the percentage of Y-bearing sperm found in human semen (50.3%) (22). Our study demonstrates a potential iatrogenic source of a decline in the sex ratio, as opposed to evolutionary antecedents, which may serve to address the greater mortality of males in the perinatal and young adult periods of life. We believe that the decrease in the sex ratio associated with ICSI may be iatrogenic, rather than due to male factor infertility itself, because of the decrease in sex ratio seen even in the absence of male factor as a diagnosis.

In this study, we found an overall sex ratio of 49.8% for the ART births evaluated. The sex ratios of births from insemination cycles (51.4%) and blastocyst-stage embryos (51.6%) were similar to the overall sex ratio for all U.S. live births

**TABLE 1****Characteristics of the study population.**

	<b>Both groups (N = 15,164)</b>	<b>Insemination (n = 5773)</b>	<b>ICSI (n = 9391)</b>	<b>P</b>
Maternal age, years $\pm$ SD	34.9 $\pm$ 4.9	35.3 $\pm$ 4.9	34.7 $\pm$ 4.9	< .0001
Maternal age range, %:				< .0001
<35	47.7	45.2	49.2	
35–37	23.2	23.4	23.1	
38–40	16.0	17.1	15.3	
41–42	6.6	6.7	6.6	
>42	6.5	7.5	5.9	
Ethnicity, %:				.62
Asian	8.1	8.0	8.1	
Black or African American	4.5	4.8	4.3	
Hispanic or Latino	6.3	5.9	6.6	
Other	0.5	0.5	0.5	
White	80.6	80.7	80.5	
Unknown/not stated	39.6	42.9	37.5	
Primigravida, %	44.4	39.3	47.6	< .0001
Unknown/not stated	0.2	0.3	0.1	
Any prior full-term birth, %	47.2	47.8	46.7	.32
Unknown/not stated	40.1	35.9	42.7	
Any prior spontaneous abortions, %	53.6	57.4	51.1	< .0001
Unknown/not stated	40.4	36.4	42.9	
Infertility diagnosis, % <sup>a</sup> :				
Male factor	35.6	8.1	52.4	< .0001
Endometriosis	14.0	16.7	12.4	< .0001
Polycystic ovarian syndrome	14.9	15.5	14.5	.13
Diminished ovarian reserve	20.9	21.2	20.8	.52
Tubal ligation	3.9	2.1	2.8	< .0001
Tubal hydrosalpinx	1.9	0.9	1.3	< .0001
Tubal other	19.8	10.7	14.2	< .0001
Uterine factors	4.3	4.6	4.2	.19
Other factors	13.4	13.5	13.4	.77
Unexplained factors	12.2	18.0	8.6	< .0001
Use of donor oocytes, %	13.7	14.9	12.9	.001
Assisted hatching, %:				< .0001
None	63.5	73.2	57.5	
All	36.5	26.8	42.5	
Semen source, %:				< .0001
Partner	96.4	95.5	97.0	
Donor	3.6	4.5	3.0	
Day of ET, %				.55
$\leq$ day 3 (cleavage stage)	67.1	67.5	66.9	
>day 3 (blastocyst stage)	32.9	32.5	33.1	
Length of gestation, weeks $\pm$ SD	38.3 $\pm$ 2.3	38.3 $\pm$ 2.3	38.4 $\pm$ 2.3	.13
Birthweight, grams $\pm$ SD	3,238 $\pm$ 625	3,227 $\pm$ 639	3,244 $\pm$ 616	.09
Birthweight Z-Score, Standard Deviation Units (SDU), SD	0.13 $\pm$ 1.03	0.11 $\pm$ 1.04	0.14 $\pm$ 1.03	.20

<sup>a</sup> Some patients have more than one diagnosis.

Luke. Sex ratios in assisted-conception pregnancies. *Fertil Steril* 2009.

**TABLE 2****Percent of male infants by method of fertilization<sup>a</sup>.**

	Insemination (n = 5773)	ICSI (n = 9391)
% Male infants	51.4	48.8
% Maternal age, years:		
<35	52.5	48.8
35–37	51.0	48.8
38–40	49.8	49.1
41–42	49.2	48.6
>42	52.2	49.2
<i>P</i>	.52	1.00
Ethnicity, %:		
Asian	51.3	49.2
Black or African American	45.6	47.4
Hispanic or Latino	52.8	48.6
Other	52.9	60.0
White	51.1	48.6
<i>P</i>	.70	.78
Primigravida, %	51.9	49.6
Any prior full-term birth, %	52.5	48.1
Any prior spontaneous abortions, %	50.5	47.8
Infertility diagnosis, %:		
Male factor	47.0	49.5
Endometriosis	51.8	48.2
Polycystic ovarian syndrome	52.6	47.2
Diminished ovarian reserve	51.8	50.7
Tubal ligation	47.3	54.1
Tubal hydrosalpinx	49.1	51.1
Tubal other	52.6	46.9
Uterine factors	54.5	49.2
Other factors	52.2	48.3
Unexplained factors	51.5	47.5
<i>P</i>	.99	.89
Use of donor oocytes, %	52.6	48.7
Assisted hatching, %		
None	52.2	49.3
All	49.0	48.6
<i>P</i>	.046	.72
Semen source, %		
Partner	51.5	49.0
Donor	50.2	45.1
<i>P</i>	.70	.21
Day of ET, %:		
≤ Day 3 (cleavage stage)	49.5	48.5
> Day 3 (blastocyst stage)	54.9	49.6
<i>P</i>	< .0001	.17

<sup>a</sup> Each parameter is analyzed individually.

Luke. Sex ratios in assisted-conception pregnancies. *Fertil Steril* 2009.

**TABLE 3****Percent and likelihood of male infants by male factor infertility and method of fertilization.**

Population	% Males	Odds ratio	95% Confidence interval	P
All:	49.8			
Insemination	51.4	1.00 (reference)		
ICSI	48.8	0.90	0.84–0.96	.002
Male factor absent	50.2	1.00 (reference)		
Male factor present	49.3	0.97	0.90–1.03	.29
Insemination, male factor absent	51.8	1.00 (reference)		
Insemination, male factor present	47.0	0.83	0.68–0.99	.045
ICSI, male factor absent	48.2	0.86	0.80–0.93	< .0001
ICSI, male factor present	49.5	0.91	0.84–0.98	.017
Cleavage-stage embryos:	48.9			
Insemination	49.5	1.00 (reference)		
ICSI	48.5	0.96	0.88–1.05	.37
Male factor absent	49.0	1.00 (reference)		
Male factor present	48.7	0.99	0.91–1.08	.84
Insemination, male factor absent	49.5	1.00 (reference)		
Insemination, male factor present	48.9	0.98	0.76–1.25	.84
ICSI, male factor absent	48.2	0.95	0.85–1.06	.33
ICSI, male factor present	48.7	0.97	0.88–1.07	.52
Blastocyst-stage embryos:	51.6			
Insemination	54.9	1.00 (reference)		
ICSI	49.6	0.81	0.71–0.92	.001
Male factor absent	52.6	1.00 (reference)		
Male factor present	50.0	0.90	0.80–1.02	.11
Insemination, male factor absent	55.8	1.00 (reference)		
Insemination, male factor present	46.2	0.68	0.48–0.96	.03
ICSI, male factor absent	48.7	0.75	0.64–0.88	< .0001
ICSI, male factor present	50.4	0.80	0.70–0.93	.003

Luke. Sex ratios in assisted-conception pregnancies. *Fertil Steril* 2009.

51.2% (8). The study population was similar to the general U.S. population, with a mean length of gestation of 38 weeks, but differed from the general fertile population in having a greater proportion of women  $\geq 35$  years old (see Table 1). Increasing maternal age has been shown to reduce the sex ratio (23).

ART has been previously reported to have a direct effect on the sex ratio. Our findings of a higher sex ratio with insemination and blastocyst-stage embryos confirm prior reports (24, 25). Fedder et al. (24) reported a sex ratio of 51.3% in the general Danish population and 53.1% in ART cycles using insemination. Luna et al. (25) reported the proportion

of males after culture to blastocyst stage to be higher (57.7%) than when cleavage-stage embryos were transferred (51.2%). The reasons for these differences were not determined, but the investigators suggest that male embryos may grow faster and are thus more often selected for transfer when culture is extended. If this is correct, our data suggest the possibility of additional differential growth rates between insemination and ICSI embryos. An additional explanation, however, is that there is differential death of male and female embryos in the early stages of embryogenesis.

Another factor that could affect the sex ratio in ART might be sperm separation procedures. Albumin gradients used to separate sperm by some laboratories have been reported to increase the proportion of X-bearing sperm (26). The type of media used to facilitate sperm capacitation has also been reported to influence the percent of X and Y sperm (27). Swim-up time, by contrast, may not result in a shift in sex ratio (28). ICSI specimens, particularly poor specimens, are often processed by a different methodology than specimens with good counts, since extensive processing to enrich for motile sperm decreases sperm recovery. Differences in processing may have contributed to our observed sex ratio findings in inseminated versus ICSI births.

Jacobsen et al. (18) found no difference in the proportion of males born to men with abnormal versus normal semen parameters. Count, motility, and morphology were evaluated separately, but none of these parameters affected outcome. These findings are in line with our results of no difference in the sex ratio between male factor and non-male factor patients. Male factor, as defined by SART for our study group, includes those men with semen parameters outside of the World Health Organization guidelines. This definition is inclusive of men with congenital abnormalities, hormonal deficiencies, environmental or medically induced causes, and varying parameters of abnormal sperm production. These different categories could not be separated for analysis.

Where male factor infertility was diagnosed in our study population, both insemination and ICSI resulted in a lower sex ratio compared with cycles in non-male factor patients using insemination. However, within this group of male factor patients, ICSI resulted in a significantly higher percentage of male offspring than did insemination (total study population, 49.5% vs. 47.0%; with blastocyst-stage embryos, 50.4% vs. 46.2%). One explanation for this could be a reduction in binding of Y-bearing sperm to eggs during a physiological fertilization process. This reduction might be the result of either abnormal binding sites on these sperm or a failure of some of the embryos to progress. This process might be partially overcome by the use of ICSI. Nevertheless, it is known that abnormal spermatozoa can fertilize an egg and produce embryos when ICSI is used (29), so it would be very surprising if the number of abnormalities in the male embryos were greater in the insemination group; this very possibility is suggested by our data. It is important to note, however, that the number of cycles with both male factor and insemination

was relatively small compared with the other groups and that it is likely that some of these cycles used donor sperm. Thus, these findings remain to be studied in future analyses before any definitive conclusion can be drawn.

Fedder et al. (24) showed that both ICSI and the source of sperm may affect the sex ratio. These investigators reported a 50.4% sex ratio with ICSI using ejaculated sperm and a 45.4% ratio with testicular biopsy or aspirated sperm, compared with a 53% sex ratio for ART insemination cycles. They suggest that Y-bearing sperm from patients with compromised sperm production are more likely to produce abnormal embryos than Y-bearing sperm from individuals with normal, ejaculated sperm parameters. In addition, they reported that the type of abnormality resulting in the need for biopsy contributed to further differences in the sex ratio. Testicular cancer has also been shown to reduce the proportion of male offspring (17), further supporting the hypothesis that the proportion of Y-bearing sperm may be depressed in cases of testicular malfunction. Since the proportion of aspirated and biopsy cycles in a single year of SART data is small, we chose to eliminate them from these analyses. We plan, however, to study these cycles further by using multiple years of SART data in a future study.

When ICSI is used in non-male factor patients, it is often for cases of unexplained infertility. A few clinics also use ICSI for all patients, regardless of diagnosis. Our study showed that, where there was no male pathology, the difference in the sex ratio between insemination and ICSI is clear and highly significant in both the total study population and among blastocyst-stage embryos. This observation strengthens our hypothesis that some component of the ICSI procedure is likely to cause the reduction in the sex ratio. The mechanism by which ICSI lowers the sex ratio at birth is not known. An increase in Y chromosome abnormalities that directly affect embryogenesis would be suspected. Previous studies have suggested a general disruption in genomic imprinting during the use of ICSI (30, 31), but whether this leads to an imbalance of Y-bearing embryos is not known. Embryogenesis may also be directly affected when sperm condition is compromised (32).

One limitation of our study is that reporting of diagnoses by clinics may not be completely accurate. We did not have reports of semen analyses or other diagnostic tests with which to make our own judgment about the accuracy of the clinics' diagnoses. Participating clinics are given diagnostic categories in an attempt to improve the consistency of reporting and exclusion of cycles. It is possible that by limiting some of the analysis to the 66% of cycles with a single diagnosis, we have missed the effect of combined factors (such as diminished ovarian reserve and male factor) on sex ratio. However, we are unaware of literature that would suggest that combined factors have a different effect on sex ratio than would single factors. It should also be emphasized that the only analysis that was limited to a single diagnosis was the analysis that compared sex ratio between diagnoses within

each of the insemination and ICSI groups (Table 2). All other comparisons and analyses included all cycles with both single and multiple infertility diagnoses (Table 3).

An additional limitation of the current SART database is that it is not possible to link multiple cycles to individual patients, as the data are deidentified. Data are currently reported per cycle, and a patient can contribute to the data set more than once. By restricting our analysis to live births in 1 year, the number of cycles per patient should be no more than one. It should be acknowledged that even though this study involves large numbers of cycles, it is theoretically possible that the reduction in sex ratio that we observed was due to chance. A follow-up study, with a larger sample size over several years, may be useful to perform, particularly when it becomes possible to link cycles to patients and determine the results for a course of treatment in an individual.

The use of ICSI in the United States has increased from 11.0% of ART cycles to 57.5% of cycles between 1995 and 2004 and has been performed in the majority of ART cycles since 2001, despite a stable proportion of diagnoses attributable to male factor infertility (33). Given that ART births still make up only about 1% of all live births in the United States and given the relatively small magnitude of the effect on the sex ratio, it is unlikely that our findings currently have any major implications for public health. However, our study shows that the use of ICSI may not be without unanticipated consequences, including a reduction in the sex ratio. The mechanisms for this effect on the sex ratio and its interactions with different male factor diagnoses must await additional studies. Because our findings suggest that ICSI may reduce the sex ratio, we recommend that ICSI only be done if medically necessary, in an effort to prevent this potential side effect.

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## Original Contribution

# Use of Fertility Drugs and Risk of Uterine Cancer: Results From a Large Danish Population-based Cohort Study

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Some epidemiologic studies have indicated that uterine cancer risk may be increased after use of fertility drugs. To further assess this association, the authors used data from a large cohort of 54,362 women diagnosed with infertility who were referred to Danish fertility clinics between 1965 and 1998. In a case-cohort study, rate ratios and 95% confidence intervals were used to assess the effects of 4 groups of fertility drugs on overall risk of uterine cancer after adjustment for potentially confounding factors. Through mid-2006, 83 uterine cancers were identified. Ever use of any fertility drug was not associated with uterine cancer risk (rate ratio (RR) = 1.10, 95% confidence interval (CI): 0.69, 1.76). However, ever use of gonadotropins (follicle-stimulating hormone and human menopausal gonadotropin) increased uterine cancer risk (RR = 2.21, 95% CI: 1.08, 4.50); the risk was primarily observed after 10 years of follow-up. Furthermore, uterine cancer risk increased with number of cycles of use for clomiphene (for  $\geq 6$  cycles, RR = 1.96, 95% CI: 1.03, 3.72) and human chorionic gonadotropin (for  $\geq 6$  cycles, RR = 2.18, 95% CI: 1.16, 4.08) but not for other gonadotropins. Use of gonadotropin-releasing hormone analogs was not associated with risk. Gonadotropins, and possibly clomiphene and human chorionic gonadotropin, may increase the risk of uterine cancer, with higher doses and longer follow-up leading to greater risk.

clomiphene; cohort studies; Denmark; fertility agents; gonadotropins; infertility; uterine neoplasms

Abbreviations: CI, confidence interval; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; RR, rate ratio.

The etiology of uterine cancer is multifactorial, but excessive exposure to estrogens of both endogenous and exogenous origin is believed to be the key mechanism in uterine cancer carcinogenesis (1). Because of their influence on endogenous estrogen levels, nulliparity, late menopause, and late age at first birth are all well-established risk factors for uterine cancer (2–4). Concerning exogenous hormones, unopposed estrogen replacement therapy (estrogen-only pills) (5–7) and tamoxifen (8, 9) are known to increase uterine cancer risk. Furthermore, use of fertility hormones such as clomiphene and gonadotropins, which are effective for ovulation stimulation and have been widely used in fertility treatment during the last 3 decades, may also increase uterine cancer risk, since these exogenous hormones may increase estrogen levels during the follicular phase of ovulation induction cycles (10, 11).

However, in contrast to the fairly large number of studies that have investigated the association between fertility drugs and 2 other hormone-associated cancers, ovarian cancer and breast cancer (12), only a few epidemiologic studies have assessed the association between use of fertility drugs and uterine cancer: 10 cohort studies (13–22) and 1 case-control study (23). The majority of studies found no increased risk of uterine cancer after use of fertility drugs (13–17, 23), but most cohort studies suffered from methodological limitations, such as a small number of outcomes (2–12 uterine cancer cases), short and incomplete follow-up, and inability to control for potential confounders, whereas the case-control study had problems related to the validity of the reported use of drugs.

In contrast, 4 cohort studies (18–22), which involved a larger number of uterine cancer cases ( $n = 21–44$ ) and

had relatively long follow-up periods (>20 years), all indicated that fertility drugs may increase the risk of uterine cancer in some subgroups of users. An Israeli cohort study found a 2-fold nonsignificantly increased risk of uterine cancer among ever users of ovulation-stimulating drugs compared with never users (18, 19), while Althuis et al. (20), in a case-cohort study, found a 2-fold borderline-significantly increased risk of uterine cancer among infertile women who used clomiphene. Furthermore, in a cohort study of parous women, Calderon-Margalit et al. (21) found a 4.6-fold significantly increased risk of uterine cancer among ever users of clomiphene. Lastly, Silva et al. (22) found a dose-response gradient in uterine cancer risk, with infertile women who were provided with  $\geq 2,250$  mg of clomiphene having a 2.6-fold borderline-significantly increased risk compared with infertile women who were not treated with clomiphene.

To further assess the association between use of fertility drugs and risk of uterine cancer, we established a cohort of 54,362 Danish women who attended infertility clinics during the period 1965–1998. To our knowledge, this cohort represents the largest number of uterine cancer cases included in any infertility cohort study to date and comprises information about the women's fertility drug history and potential confounders such as reproductive factors. To further clarify the association between risk of uterine cancer and fertility drugs, we conducted a case-cohort study to evaluate the effects of different types of fertility drugs on uterine cancer risk.

## MATERIALS AND METHODS

### Cohort identification and data collection

The cohort has previously been described in detail in reports on the association between use of fertility drugs and risks of ovarian cancer (24), breast cancer (25), thyroid cancer (26), and malignant melanoma (27). Briefly, the cohort comprised women with infertility problems referred to Danish hospitals or private fertility clinics between 1965 and 1998. In addition, we included all women with an infertility diagnosis (*International Classification of Diseases*, Eighth Revision, code 628; *International Classification of Diseases*, Tenth Revision, code DN97) recorded in the National Patient Registry, a nationwide register of virtually all Danish hospital discharges for somatic conditions that have occurred since 1977. A total of 54,449 women with primary or secondary infertility were enrolled. All data were entered into a single database, with 1 record for each woman, including initial date of infertility evaluation, name of clinic, and the woman's civil registry number. To verify the personal identification number, the cohort was linked to the population-based civil registration system, with the civil registry number used as the key identifier. Of the 54,449 infertile women, all but 87 were found to have a valid civil registry number, which left 54,362 women in the cohort for analysis. The study was approved by the Danish Scientific Ethical Committee and the Danish Data Protection Agency.

### Identification of cases and subcohort

To determine uterine cancer status after enrollment in the study, we followed the cohort for uterine cancer occurrence from the date of the first infertility evaluation to the date of emigration, death, or hysterectomy or June 30, 2006, whichever occurred first. Using the civil registry number as the key identifier, we obtained information on date of emigration and date of death by linking the cohort to the civil registration system, while information on hysterectomies was gathered through linkage to the National Patient Registry. Information on uterine cancer status was obtained through cohort linkage to the Danish Cancer Registry and the Danish Registry of Pathology. The Danish Cancer Registry contains nationwide information on all incident cases of invasive cancer diagnosed in Denmark since 1943, and the Danish Registry of Pathology contains information on all pathology specimens collected from all Danish public and private pathology departments and is updated daily. The latter register was used to determine uterine cancer status in the cohort from January 1, 2004, onward, because the Danish Cancer Registry was updated only until December 31, 2003, at the time of analysis.

In the present study, uterine cancer cases were defined as either epithelial endometrial cancer or myometrial cancer. Uterine cancer cases in the Danish Cancer Registry were defined by *International Classification of Diseases*, Seventh Revision, code 172, whereas uterine cancer cases in the Danish Registry of Pathology were classified according to *International Classification of Diseases for Oncology*, First Edition, topography codes T82, T84, and T85 and relevant morphology codes. All incident uterine cancer cases identified through the 2 registries were classified according to the *International Classification of Diseases for Oncology*, First Edition, as either epithelial endometrial cancer (morphology codes M80703, M81403, M82103, M82603, M83803, M84413, M85603, and M85703) or myometrial cancer (morphology codes M88003 and M88903).

At the time of linkage, 101 women had been diagnosed with uterine cancer during the follow-up period. For comparison, a subcohort of 1,360 women was randomly selected from the infertility cohort in 4 strata according to age at entry into the cohort (18–26, 27–30, 31–36, or  $\geq 37$  years) and in 5 strata according to year of entry into the cohort (1965–1977, 1978–1984, 1985–1989, 1990–1996, or 1997–1998).

### Ascertainment of exposure and potential confounders

We collected hospital files and medical records on all infertility-related medical visits for all infertile women in whom uterine cancer developed and for members of the subcohort. For 18 cases, records could not be found, leaving 83 women with uterine cancer (82%) for analysis. In the subcohort, we excluded 78 women for whom the hospital files could not be found, 8 women for whom a diagnosis of infertility could not be confirmed, and 33 women for whom the cause of infertility was previous sterilization; this left 1,241 women in the subcohort (91%). Of the 1,241 subcohort members, 2 women were diagnosed with uterine cancer during the follow-up period; therefore, these women were

included both as cases and as members of the subcohort in the analyses.

We abstracted information on medical interventions for infertility, including the types of fertility drugs prescribed and the number of treatment cycles. For each treatment cycle, we abstracted the dates of starting and stopping use to define the windows of exposure to the drugs. We also intended to abstract information on the dosage of fertility drugs, but this information was recorded for only a minority of the women. We abstracted information about the causes of infertility and about any use of oral contraceptives from the medical records, but this information also was available for only a minority of the women. To obtain information on reproductive history for all included women, we linked the cohort to the Civil Registration System and the Danish National Birth Registry by civil registry number. The population-based National Birth Registry contains information about all births that have taken place in Denmark since 1973. Thus, information on reproductive history was obtained from this register from 1973 onwards, and reproductive history before 1973 was obtained from the Civil Registration System, since this register allows linkage of data on parents and children.

### Statistical analysis

According to the sampling strategy, we used the unweighted case-cohort approach (28, 29) to estimate rate ratios for uterine cancer in a Cox proportional hazards regression model stratified according to the sampling strata (age and year of enrollment). Age was used as the time scale to ensure that the estimates were based on comparisons of women of the same age. The analysis was corrected for delayed entry, such that women were considered at risk only from their age at the date of the first infertility evaluation. Rate ratios were estimated as suggested by Prentice (30), whereby all of the women in the subcohort contributed to all of the relevant risk sets until the end of the follow-up period due to diagnosis of cancer, death, migration, or censoring, whereas case women outside the subcohort entered only their own risk set. The 95% confidence intervals were based on robust estimates of the variance-covariance matrix of the Cox regression parameters.

Using this Cox model, we evaluated the effects of the following fertility drugs: follicle-stimulating hormone, human menopausal gonadotropin, clomiphene, human chorionic gonadotropin (hCG), and gonadotropin-releasing hormone (GnRH) analogs, all measured as any use, number of cycles of use, and years since first use. In the analyses, however, we combined follicle-stimulating hormone and human menopausal gonadotropin into 1 group called "gonadotropins," since they have identical modes of action.

The 83 uterine cancers included in the analyses were classified into 2 histologic types as either epithelial endometrial tumors ( $n = 79$ ) or myometrial tumors ( $n = 4$ ). Preliminary analyses showed that none of the risk estimates changed noticeably when we restricted the analyses to epithelial endometrial tumors only; thus, only risk estimates for all uterine cancers are presented. Potentially confounding factors investigated in the main analyses included parity

(nulliparous or parous), number of additional births, maternal age at the birth of the first child, and maternal age at the birth of the last child. All variables except maternal age at birth of the first child and maternal age at birth of the last child were entered as time-dependent covariates, which changed the values at the ages at which new events occurred (e.g., the birth of a child or the start of a new treatment cycle). Information about causes of infertility and any use of oral contraceptives was unavailable for most women; therefore, these factors were analyzed only in the subset of women for whom this information was available. All statistical analyses were performed in SAS/STAT, version 8.2 (SAS Institute Inc., Cary, North Carolina).

### RESULTS

The distributions of entry periods and ages at entry for the 54,362 women in the cohort have been described previously (25). The median year of entry was 1989. The median age at first evaluation of infertility was 30 years, whereas the median age at the end of follow-up was 47 years. The median length of follow-up was 16.0 years (range, 0.0–42.6 years), with 25% of the women being followed for more than 23 years. In total, the 54,362 women contributed 957,887 person-years of observation. The median time from entry to diagnosis of cancer was 19.5 years (range, 0.04–37.8 years), and the ages of the women at the time of diagnosis ranged from 28 years to 67 years (median, 50 years). Fertility drugs were used by 42 (51%) of the 83 women with uterine cancer and 615 (50%) of the 1,241 subcohort members. The most frequently used fertility drugs were hCG and clomiphene, being taken by 31 and 29 cases, respectively, and 413 and 415 subcohort members, respectively, followed by gonadotropins (used by 17 cases and 184 subcohort members) and GnRH analogs (used by 7 cases and 110 subcohort members).

Parous women had a significantly lower risk of uterine cancer than nulliparous women (rate ratio (RR) = 0.41, 95% confidence interval (CI): 0.26, 0.64). Risk decreased with number of additional births (RR = 0.52, 95% CI: 0.22, 1.28), maternal age at birth of the first child (RR = 0.89, 95% CI: 0.71, 1.12), and maternal age at birth of the last child (RR = 0.83, 95% CI: 0.65, 1.06) (Table 1).

After adjustment for parity and number of additional births, the overall risk of uterine cancer was not significantly affected by use of any fertility drug (RR = 1.10, 95% CI: 0.69, 1.76) (data not shown). We also assessed uterine cancer risk in relation to the specific types of fertility drugs (Table 2). Ever use of gonadotropins (follicle-stimulating hormone and human menopausal gonadotropin) increased uterine cancer risk (RR = 2.21, 95% CI: 1.08, 4.50), and the risk was primarily observed after 10 years of follow-up (i.e., 10 years since first use) (RR = 2.67, 95% CI: 1.21, 5.90). Furthermore, risks were also increased, though statistically nonsignificantly, after ever use of clomiphene (RR = 1.36, 95% CI: 0.83, 2.23) and hCG (RR = 1.36, 95% CI: 0.83, 2.23), whereas ever use of GnRH analogs was not associated with risk (RR = 1.09, 95% CI: 0.47, 2.52). The risks of uterine cancer after use of clomiphene, hCG, and GnRH

**Table 1.** Rate Ratios for Uterine Cancer According to Reproductive Factors, Denmark, 1965–2006<sup>a</sup>

Determinant	No. With Uterine Cancer	No. in Subcohort	RR <sup>b</sup>	95% CI
<b>Parity status</b>				
Nulliparous	48	426	1.00	Reference
Parous	35	817	0.41	0.26, 0.64
<b>No. of livebirths</b>				
1	28	388	0.66	0.40, 1.07
2	4	324	0.13	0.05, 0.35
≥3	3	105	0.33	0.10, 1.10
Risk per additional birth among parous women			0.52	0.22, 1.28
<b>Maternal age at birth of first child, years</b>				
<25	10	246	0.38	0.19, 0.76
25–29	12	237	0.52	0.26, 1.02
≥30	13	334	0.37	0.19, 0.70
Risk per 5 years among parous women			0.89	0.71, 1.12
<b>Maternal age at birth of last child, years</b>				
<30	19	272	0.65	0.38, 1.13
30–35	11	325	0.36	0.19, 0.69
≥35	5	220	0.21	0.08, 0.56
Risk per 5 years among parous women			0.83	0.65, 1.06

Abbreviations: CI, confidence interval; RR, rate ratio.

<sup>a</sup> All analyses were stratified according to calendar year (in categories) and age at start of follow-up (in categories).

<sup>b</sup> All rate ratios (except that for parity) were adjusted for parity (nulliparous/parous) and number of additional births (linear).

analogs were not associated with time since first use. However, a high number of treatment cycles increased uterine cancer risk significantly for both clomiphene (for ≥6 cycles, RR = 1.96, 95% CI: 1.03, 3.72) and hCG (for ≥6 cycles, RR = 2.18, 95% CI: 1.16, 4.08) (Table 2).

Potentially separate effects of fertility drugs on uterine cancer risk according to parity status were also analyzed (Table 3). For all 4 types of fertility drugs, however, the risk of uterine cancer was not markedly affected by parity status, and none of the interaction terms were statistically significant.

Because a substantial proportion of the women in the cohort had used more than 1 type of fertility drug, the main effect of each type of drug was assessed. The general results were not changed when the risk of uterine cancer was assessed only in those women who had used 1 type of drug compared with never users (data not shown). Finally, to evaluate the effects of various causes of infertility (anovulation, tubal disease, endometriosis, uterine disorder, cervical disorder, and male factors) and any use of oral contraceptives as potential confounders, we repeated all of the analyses for these 2 variables in the subsets of women for whom information was available. Data on causes of infertility were available for 47% of cases and 51% of

**Table 2.** Rate Ratios for Uterine Cancer According to Use of Fertility Drugs, Denmark, 1965–2006<sup>a</sup>

Use	Gonadotropins <sup>b</sup>				Clomiphene				Human Chorionic Gonadotropin				Gonadotropin-Releasing Hormone Analogs			
	No. With Uterine Cancer	No. in Subcohort	RR <sup>c</sup>	95% CI	No. With Uterine Cancer	No. in Subcohort	RR <sup>c</sup>	95% CI	No. With Uterine Cancer	No. in Subcohort	RR <sup>c</sup>	95% CI	No. With Uterine Cancer	No. in Subcohort	RR <sup>c</sup>	95% CI
Never	66	1,059	1.00		826	826	1.00		52	830	1.00		76	1,133	1.00	
Ever	17	184	2.21	1.08, 4.50	417	417	1.36	0.83, 2.23	31	413	1.36	0.83, 2.23	7	110	1.09	0.47, 2.52
<b>No. of cycles</b>																
<6	13	146	2.11	0.96, 4.62	256	256	1.01	0.54, 1.88	15	265	0.96	0.51, 1.81	6	105	0.96	0.40, 2.33
≥6	4	38	2.56	0.85, 7.75	161	161	1.96	1.03, 3.72	16	148	2.18	1.16, 4.08	1	5	4.76	0.53, 42.95
<b>Time since first use, years</b>																
<10	7	45	1.81	0.68, 4.83	34	34	2.35	0.92, 5.98	9	46	1.94	0.80, 4.69	2	39	0.47	0.11, 1.95
≥10	10	139	2.67	1.21, 5.90	383	383	1.28	0.74, 2.21	22	367	1.33	0.76, 2.31	5	71	2.46	0.89, 6.77

Abbreviations: CI, confidence interval; RR, rate ratio.

<sup>a</sup> All analyses were stratified according to calendar year (in categories) and age at start of follow-up (in categories).

<sup>b</sup> Follicle-stimulating hormone and human menopausal gonadotropin.

<sup>c</sup> Rate ratios were adjusted for parity (nulliparous/parous) and number of additional births (linear).

**Table 3.** Rate Ratios for Uterine Cancer in Nulliparous and Parous Women According to Use of Fertility Drugs, Denmark, 1965–2006<sup>a</sup>

Fertility Drug	Nulliparous Women				Parous Women				P for Interaction
	No. With Uterine Cancer	No. in Subcohort	RR <sup>b</sup>	95% CI	No. With Uterine Cancer	No. in Subcohort	RR <sup>b</sup>	95% CI	
Gonadotropins <sup>c</sup>									
Never use	37	352	1.00	Reference	29	707	1.00	Reference	
Ever use	11	74	2.30	0.94, 5.60	6	110	2.39	0.92, 6.20	0.95
Clomiphene									
Never use	32	277	1.00	Reference	22	549	1.00	Reference	
Ever use	16	149	1.30	0.68, 2.46	13	268	1.54	0.72, 3.29	0.74
Human chorionic gonadotropin									
Never use	28	286	1.00	Reference	24	544	1.00	Reference	
Ever use	20	140	1.65	0.87, 3.13	11	273	1.09	0.51, 2.35	0.41
Gonadotropin-releasing hormone analogs									
Never use	44	370	1.00	Reference	32	763	1.00	Reference	
Ever use	4	56	0.81	0.29, 2.27	3	54	2.25	0.61, 8.25	0.22

Abbreviations: CI, confidence interval; RR, rate ratio.

<sup>a</sup> All analyses were stratified according to calendar year (in categories) and age at start of follow-up (in categories).

<sup>b</sup> Rate ratios were adjusted for parity (nulliparous/parous) and number of additional births (linear).

<sup>c</sup> Follicle-stimulating hormone and human menopausal gonadotropin.

subcohort members, and data on any use of oral contraceptives were available for 46% of cases and 55% of subcohort members. However, none of the risk estimates changed noticeably after adjustment for these potential confounders (data not shown).

## DISCUSSION

Even though uterine cancer risk was not associated with ever use of any fertility drug, our results indicated that some fertility drug users may have an increased risk of uterine cancer. Ever use of gonadotropins increased uterine cancer risk, and the risk was primarily observed after 10 years of follow-up. Furthermore, a high number of cycles of clomiphene and hCG ( $\geq 6$  cycles of use) may increase the risk of uterine cancer. In contrast, no significantly increased risk was found after use of GnRH analogs, and none of the risk estimates were markedly changed when results were stratified by parity status or adjusted for causes of infertility or ever use of oral contraceptives.

The mechanisms for an association between fertility drugs and uterine cancer risk are not completely clear. However, high levels of unopposed estrogen have been linked to uterine cancer (31). Furthermore, since it is known that fertility drugs increase the levels of estrogen produced during the follicular phase of ovulation induction cycles and of progesterone produced during the simultaneous ovulation of multiple follicles (10, 32), it is likely that the use of fertility drugs indirectly increases uterine cancer risk by increasing estrogen levels during the menstrual cycle. Lastly, clomiphene might also affect uterine cancer risk by interacting directly with estrogen receptors in the uterus (33, 34),

whereas no direct effect on uterine cancer risk for gonadotropins, hCG, or GnRH analogs is known.

To our knowledge, relatively few epidemiologic studies have assessed the association between use of fertility drugs and uterine cancer risk (13–23). In line with our study, most studies failed to find a significantly increased risk of uterine cancer after use of any fertility drugs (13–19, 22). Only a large population-based historical cohort study by Calderon-Margalit et al. (21), which focused on 15,030 parous women in Jerusalem, Israel, between 1974 and 1976, found a significantly increased risk of uterine cancer among women treated with any fertility drugs (HR = 3.4, 95% CI: 1.3, 9.0); results were based on 44 uterine cancers. Furthermore, Calderon-Margalit et al. also found a significantly increased uterine cancer risk after exposure to clomiphene only (HR = 4.6, 95% CI: 1.6, 13.3) (21).

Recently, 2 other large cohort studies (20, 22) also assessed potential associations between uterine cancer risk and use of specific types of fertility drugs (clomiphene and gonadotropins). The cohort study by Althuis et al. (20) included 8,431 US women evaluated for fertility problems during 1965–1988 and 39 uterine cancer cases. Althuis et al. found a borderline-significant increase in the incidence of uterine cancer after use of clomiphene (RR = 1.8, 95% CI: 0.9, 3.3), with the highest risk being found for women with 6 or more treatment cycles (RR = 2.2, 95% CI: 0.9, 5.2) and among women who had first used clomiphene more than 20 years previously (RR = 2.5, 95% CI: 0.9, 7.2) (20). Additionally, in line with our results, Althuis et al. found no association between uterine cancer risk and clomiphene among women who remained nulliparous at follow-up (20). In the other cohort study, Silva et al. (22) followed

7,355 British women with ovulatory disorders during 1965–1999. On the basis of 31 uterine cancer cases, they found no association between ever use of clomiphene, time since first use of clomiphene, or number of treatment cycles and risk of uterine cancer, but the results showed a dose-response gradient in risk: Women who were provided with  $\geq 2,250$  mg of clomiphene had a more than 2-fold borderline-significant increased risk compared with women not treated with clomiphene (RR = 2.6, 95% CI: 0.9, 6.8) (22).

Concerning gonadotropins, results from our infertility cohort are the first known to suggest that this type of fertility drug may increase uterine cancer risk. Althuis et al. (20) and Silva et al. (22) also assessed the association between gonadotropins and uterine cancer risk but found no association; however, both of those studies were based on markedly fewer exposed cases than our analyses. In our study, the risk was primarily observed after 10 years of follow-up (10 years since first use), and this latency effect is in good concordance with the fact that uterine tumors generally grow slowly (31).

Our study had several strengths. First, the relatively long follow-up period (median, 16 years) in the present study was only matched by the follow-up periods in 4 cohort studies (19–22) which all had more than 20 years of follow-up, whereas all other cohort studies included fewer than 10 years of follow-up (13–17). In fact, the finding of latency effects in both our study and the study by Althuis et al. (20) may perhaps further help to explain why studies that included fewer than 10 years of follow-up failed to find an association between use of fertility drugs and uterine cancer, thus underscoring the importance of long-term follow-up of infertility cohorts. Second, only our study and the cohort studies by Althuis et al. (20) and Silva et al. (22) had a suitable reference group (i.e., a control group of infertile women who had not taken fertility drugs). Most other cohort studies (13–19) used standardized incidence ratios to compare the risk of uterine cancer in infertile women with that in the general population. This comparison controlled for the potential confounders age and calendar time but not for parity, causes of infertility, or oral contraceptive use. We showed previously that the infertile women in our cohort are not at higher risk of uterine cancer than women in the general Danish population, even after adjustment for parity (35). The case-cohort analysis used in the present study, however, showed a relatively strong association between uterine cancer risk and use of fertility drugs within the cohort. Our data therefore suggest that it is primarily the use of fertility drugs, not factors related to the diagnosis of infertility (e.g., genetic or biologic factors), that increases the risk of uterine cancer. Third, we had detailed information on the various types of fertility drugs used and the number of cycles used. This information allowed us to assess the specific effect on uterine cancer risk of the different types of fertility drugs, which is of great importance provided their potentially different effects. Finally, losses to follow-up were virtually absent in our study as a result of the precise linkage between our cohort and the Danish population-based registers, and uterine cancer diagnoses were completely ascertained through linkage with the Danish Cancer Registry and the Danish Registry of Pathology.

Our study did have some limitations. Even though the number of women with uterine cancer was markedly larger than in previous cohort studies, where the numbers of uterine cancer cases ranged from 2 to 44 (13–22), the total number of uterine cancers in our study was still not very large ( $n = 83$ ). Therefore, even though the precision of the risk estimates in our study was higher than in previous studies, our study may still have been somewhat underpowered and limited by risk estimates of relatively low precision, especially in subgroups of fertility drug users. In the present study, the follow-up period (16 years) was relatively long; however, the median age at the end of follow-up (47 years) was below the usual peak age (early 60s) of uterine cancer, which might have weakened our estimates. Data on potentially important risk factors, such as the cause of infertility and use of oral contraceptives, were available for only a minority of the women, and therefore these factors could be analyzed only in the subsets of women for whom we had this information. However, our results showed that adjustment for oral contraceptive use and causes of infertility did not change the risk estimates markedly. Furthermore, we found no association between oral contraceptive use and use of fertility drugs or between causes of infertility and use of fertility drugs (data not shown); this suggests that our results were not an artifact due to uncontrolled confounding by these factors. Lastly, our results might have been confounded by the fact that no information on other potential risk factors, such as obesity (a strong risk factor for both uterine cancer and infertility), menopausal status, and hormone therapy, was available.

In conclusion, even though uterine cancer risk was not associated with ever use of any fertility drug, the findings from our large, nationwide cohort study show that infertile women who are treated with gonadotropins, clomiphene, and hCG may have an increased risk of uterine cancer. Furthermore, the presence of both latency effects and dose-response effects adds further weight to our results and supports a causal relation. Since many of the women in our cohort have not yet reached the usual peak age for uterine cancer, we will continue to monitor their risk to establish a more definite link between use of fertility drugs and risk of uterine cancer. Finally, considering the large and increasing number of women being treated every year with fertility drugs (36), the unfavorable effects of fertility drugs such as increased risk of uterine cancer must always be put into perspective and balanced against the physical and psychological benefits of a pregnancy made possible only through the use of fertility drugs.

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