ORIGINAL ARTICLE

Reproductive Technologies and the Risk of Birth Defects

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ABSTRACT

BACKGROUND

The extent to which birth defects after infertility treatment may be explained by underlying parental factors is uncertain.

METHODS

We linked a census of treatment with assisted reproductive technology in South Australia to a registry of births and terminations with a gestation period of at least 20 weeks or a birth weight of at least 400 g and registries of birth defects (including cerebral palsy and terminations for defects at any gestational period). We compared risks of birth defects (diagnosed before a child's fifth birthday) among pregnancies in women who received treatment with assisted reproductive technology, spontaneous pregnancies (i.e., without assisted conception) in women who had a previous birth with assisted conception, pregnancies in women with a record of infertility but no treatment with assisted reproductive technology, and pregnancies in women with no record of infertility.

RESULTS

Of the 308,974 births, 6163 resulted from assisted conception. The unadjusted odds ratio for any birth defect in pregnancies involving assisted conception (513 defects, 8.3%) as compared with pregnancies not involving assisted conception (17,546 defects, 5.8%) was 1.47 (95% confidence interval [CI], 1.33 to 1.62); the multivariate-adjusted odds ratio was 1.28 (95% CI, 1.16 to 1.41). The corresponding odds ratios with in vitro fertilization (IVF) (165 birth defects, 7.2%) were 1.26 (95% CI, 1.07 to 1.48) and 1.07 (95% CI, 0.90 to 1.26), and the odds ratios with intracytoplasmic sperm injection (ICSI) (139 defects, 9.9%) were 1.77 (95% CI, 1.47 to 2.12) and 1.57 (95% CI, 1.30 to 1.90). A history of infertility, either with or without assisted conception, was also significantly associated with birth defects.

CONCLUSIONS

The increased risk of birth defects associated with IVF was no longer significant after adjustment for parental factors. The risk of birth defects associated with ICSI remained increased after multivariate adjustment, although the possibility of residual confounding cannot be excluded. (Funded by the National Health and Medical Research Council and the Australian Research Council.)

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Consistent evidence from individual studies, including registry-based cohort studies[1,2] and meta-analyses, has linked assisted conception involving in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) with an increased risk of birth defects.[3-8] The associations between the use of these techniques and birth defects have appeared to be stronger for singleton births than for multiple births.[9,10] It is unclear whether the excess of birth defects after IVF or ICSI may be attributable to patient characteristics related to infertility,[8] rather than to the treatment, and whether the risk is similar across assisted reproductive technologies and related therapies.[3,11,12] Available studies have been limited by small numbers of participants,[13,14] the pooling of exposure groups,[15] or, specifically for case–control studies, retrospective collection of data and questionable appropriateness of controls.[3,10,13]

We performed a population-wide cohort study, examining births and pregnancies terminated because of birth defects, to assess the risks of defects from pregnancy to a child's fifth birthday across a range of methods for treating infertility as compared with the risk associated with pregnancy that was not assisted by reproductive technology (spontaneous pregnancy). We also assessed the risks of birth defects associated with spontaneous pregnancy among women with a previous birth with assisted conception and women with a documented history of infertility but no treatment at assisted-reproductive-technology clinics.

METHODS

DATA SOURCES

Records of Patients with Infertility

Details of treatment with assisted reproductive technology as defined by the National Health and Medical Research Council[16] were provided by the two clinics in South Australia (a state with a population of 1.6 million) that were registered to provide infertility treatment involving embryo manipulation. Both clinics (operated by the University of Adelaide and Flinders University) provided data for all infertility treatments from January 1986 through December 2002. A description of the infertility groups and detailed information on demographic characteristics by mode of conception or treatment type are available in the Supplementary Appendix, available with the full text of this article at NEJM.org. More than 99.99% of births resulting from assisted conception were linked to the state birth registry (described below), indicating minimal loss to follow-up.

Perinatal Outcomes

Any birth resulting from assisted conception is recorded in the South Australian Perinatal Statistics Collection, which by law requires notification of all live births and stillbirths of at least 20 weeks' gestation or with a birth weight of at least 400 g in South Australia with the use of a standardized notification form. Maternal preexisting medical conditions and conditions in pregnancy, as documented in the labor-ward records, are also recorded on the notification form. Approximately 20,000 births are recorded annually in South Australia. Notifications of all medical terminations of pregnancy are also required by state law, and those that are induced at 20 weeks' gestation or later are included in the perinatal data collection. For completeness, we did not exclude the 1916 births to women with unknown or out-of-state addresses (0.6% of the entire sample).

Birth Defects

Congenital abnormalities detected at birth or in the neonatal period (within 28 days after birth) are reported by doctors to the South Australian Pregnancy Outcome Unit with the use of a standardized congenital-abnormality form. The South Australian Birth Defects Register includes information on birth defects (including cerebral palsy) obtained from the Pregnancy Outcome Unit and South Australian abortion statistics collection as well as notifications from multiple sources up to the child's fifth birthday. Cases of "acquired" cerebral palsy (i.e., those attributed to events occurring after the perinatal period) were not included. Birth defects were coded by registry staff independently of birth-defect notifications; clinical observers who issued notifications may have been aware of the mode of conception. Previous assessment of the same reporting method in an adjacent jurisdiction revealed no significant reporting bias.[4]

Terminations of pregnancy for congenital abnormalities before 20 weeks of gestation are reported by law to the state department of health and are included in the state birth-defects registry. Diagnoses of birth defects are validated by cross-referencing of medical records before the reports are registered, and the diagnoses are coded according to the British Paediatric Association (BPA) modification of the *International Classification of Diseases, 9th Revision* (ICD-9), including struc-

tural abnormalities, biochemical abnormalities, and those that are chromosomal or otherwise genetic (www.wch.sa.gov.au/services/az/other/phru/birthdefect.html). Minor defects are generally excluded from the registry, with the exception of those that require treatment or are disfiguring. Linkage of the records of patients with a history of infertility treatment was performed with the use of probabilistic matching software (AutoMatch, MatchWare Technologies) and manual matching of patient identifiers and birth outcome data. The birth-defect data were linked to the perinatal-outcomes collection and to the pregnancies resulting from assisted conception by a unique accession number for each birth; manual matching was used to resolve inconsistencies in the patient or birth data between the files, such as a change in the mother's family name.

The study was approved by the ethics committees of the South Australian Department of Health, the University of Adelaide, and Flinders University. Individual patient consent was not required by the ethics committees.

STATISTICAL ANALYSIS

The prevalence of birth defects was compared among the following groups: births as a result of each method of infertility treatment, including spontaneous conception during periods of observation and between, or subsequent to, treatment cycles; births as a result of spontaneous conception in women with a previous birth with assisted conception; births to women with a history of infertility on their perinatal outcomes record and no history of treatment with assisted reproductive technology; and births to women in the general population with no recorded history of infertility or treatment.

Odds ratios were calculated by comparing the prevalence of birth defects between groups, with the use of two-tailed P values and SAS statistical software, version 9.2 (SAS Institute). No adjustment for multiple births was made, except in sensitivity analyses to assess model robustness, because multiple gestations may be considered to be on the causal pathway between exposure to assisted reproductive technology and birth defects.[4] Information on the zygosity of twins was not available.

The crude estimates included minimal adjustment for the effect of clustering of births within women, with the use of logistic generalized estimating equations. The adjusted analyses included a priori confounders of maternal age (categorized in 5-year age groups), parity, fetal sex, year of birth, maternal race or ethnic group, maternal country of birth, maternal conditions in pregnancy (preexisting hypertension, pregnancy-induced hypertension, preexisting diabetes, gestational diabetes, anemia, urinary tract infection, epilepsy, and asthma), maternal smoking during pregnancy, socioeconomic disadvantage on the basis of the postal code of the mother's residence (according to the Socio-economic Indexes for Areas[17]), and maternal and paternal occupation, coded according to the Australian Standard Classification of Occupations.[18] We also prespecified subgroup analyses for singleton and multiple births and used prespecified contrasts to test the effects of treatment method (including fresh vs. frozen embryos) with the use of the same analytic strategy. There was no adjustment for multiple comparisons. Multiple pregnancies included twins and higher-order multiple pregnancies, because the latter are uncommon in South Australia, even for pregnancies resulting from assisted conception, owing to longstanding restrictions on the transfer of three or more embryos.

RESULTS

MATERNAL AND BIRTH CHARACTERISTICS

The available data set contained a total of 327,420 births and terminations of pregnancy. After exclusion of births to mothers younger than 20 years of age (among whom there were only 2 births resulting from assisted conception), there were 308,974 births for analysis. As compared with women who conceived spontaneously, women who used assisted reproductive technology were older and were more likely to be nulliparous and white, and they resided in less disadvantaged postal-code areas (Table 1). Women in the assisted-conception group were also more likely to have a stillbirth and to deliver by cesarean section and at a gestation of less than 37 weeks or less than 32 weeks and were less likely to have a male singleton (Table 2). In addition, their children had a lower mean birth weight than the children of women in the spontaneous-conception group.

RISK OF BIRTH DEFECTS ASSOCIATED WITH ASSISTED CONCEPTION

Births after any assisted conception were associated with a significantly increased risk of any birth defect (513 defects [8.3%]), as compared with births to fertile women that did not involve assisted con-

Table 1. Characteristics of Births and Terminations of Pregnancy According to Mode of Conception.

Characteristic	Assisted Conception (N=6163)	Spontaneous Conception (N=302,811)	P Value
	no. of births (%)		
Age			<0.001
20–24 yr	133 (2.2)	62,981 (20.8)	
25–29 yr	1367 (22.2)	114,074 (37.7)	
30–34 yr	2736 (44.4)	88,924 (29.4)	
35–39 yr	1612 (26.2)	31,728 (10.5)	
≥40 yr	315 (5.1)	5,104 (1.7)	
Socioeconomic status: lowest quartile*	1621 (26.3)	104,267 (34.4)	<0.001
White race†	5968 (96.8)	283,169 (93.5)	<0.001
Nulliparous	4023 (65.3)	113,489 (37.5)	<0.001
Paternal occupation: manager or professional‡	2374 (38.5)	82,217 (27.2)	<0.001
Smoked during pregnancy§	1021 (18.1)	29,727 (26.5)	<0.001
Singleton birth	4333 (70.3)	295,220 (97.5)	<0.001
Baby's sex¶			0.03
Male	3104 (50.4)	155,723 (51.4)	
Female	3052 (49.5)	146,803 (48.5)	
Diseases in pregnancy‖			
Any diabetes**	364 (5.9)	9,140 (3.0)	<0.001
Hypertension	96 (1.6)	3,410 (1.1)	0.01
Pregnancy-induced hypertension	770 (12.5)	26,496 (8.8)	<0.001
Urinary tract infection	344 (5.6)	14,940 (4.9)	0.08
Asthma	257 (4.2)	12,771 (4.2)	0.98
Epilepsy	41 (0.7)	1,604 (0.5)	0.13
Anemia	795 (12.9)	18,257 (6.0)	<0.001

* Socioeconomic status was determined with the use of the Index of Disadvantage in the Socio-economic Indexes for Areas, Australian Bureau of Statistics, 2006, on the basis of the postal code of the mother's residence.
† Race was determined from the maternity records on the basis of self-report by mothers.
‡ Paternal occupation was categorized according to the Australian Standard Classification of Occupations, Australian Bureau of Statistics, 1990.
§ For smoking during pregnancy, the total number of patients was 5648 in the assisted-conception group and 112,150 in the spontaneous-conception group. Smoking was recorded routinely starting in 1998.
¶ Sex could not be assigned at birth by hospital staff on the basis of the appearance of the genitalia in 7 births (0.1%) in the assisted-conception group and in 285 births (0.1%) in the spontaneous-conception group.
‖ Maternal conditions in pregnancy were recorded in the labor-ward summary and transcribed onto the standard perinatal-outcomes form for state reporting.
** Any diabetes included gestational diabetes and preexisting diabetes, which were included as separate variables in the multivariate analysis.

ception (17,546 defects [5.8%]; unadjusted odds ratio, 1.47; 95% confidence interval [CI], 1.33 to 1.62); this risk was attenuated but remained significant after multivariate adjustment (adjusted odds ratio, 1.28; 95% CI, 1.16 to 1.41). Ignoring the effect of clustering within the mother had a minimal effect on the crude odds ratios for any defect (unadjusted odds ratio, 1.48; 95% CI, 1.35 to 1.62) (data not shown). All additional reported results have been adjusted for the clustering of births within the mother. After the subsequent exclusion of persons with cerebral palsy, the crude odds ratio for any birth defect was 1.42 (95% CI, 1.31 to 1.58). All other reported results include cerebral palsy as an outcome (Table 3). The risk of any birth defect was significantly higher among births resulting from assisted conception than among spontaneous births for singleton births

Table 2. Characteristics of Births after Assisted Conception or Spontaneous Conception, According to Multiplicity.

Birth Characteristic	Assisted Conception			Spontaneous Conception		
	Singleton Births (N=4333)	Multiple Births (N=1830)	All Births (N=6163)	Singleton Births (N=295,220)	Multiple Births (N=7591)	All Births (N=302,811)
Pregnancy terminated because of defect — no. (%)	29 (0.7)	4 (0.2)	33 (0.5)	1,492 (0.5)	21 (0.3)	1,513 (0.5)
Stillborn — no. of births (%)	45 (1.0)*	44 (2.4)	89 (1.4)*	1,549 (0.5)	154 (2.0)	1,703 (0.6)
Liveborn — no. of births (%)†	4259 (98.3)	1782 (97.4)	6041 (98.0)	292,179 (99.0)	7416 (97.7)	299,595 (98.9)
Mode of delivery — no. of births (%)‡						
Vaginal	2709 (63.6)	626 (35.1)	3335 (55.2)	225,277 (77.1)	3683 (49.7)	228,960 (76.4)
Cesarean	1550 (36.4)*	1156 (64.9)	2706 (44.8)*	66,900 (22.9)	3733 (50.3)	70,633 (23.6)
Child's sex — no. of births (%)§						
Male	2123 (49.8)¶	923 (51.8)‖	3046 (50.4)	150,580 (51.5)	3642 (49.1)	154,222 (51.5)
Female	2136 (50.2)	859 (48.2)	2995 (49.6)	141,595 (48.5)	3773 (50.9)	145,368 (48.5)
Birth weight — g**	3259±641*	2240±661*	2958±796*	3,399±553	2407±620	3,375±576
Gestation — no. of births (%)*						
<32 wk	86 (2.0)	253 (14.2)	339 (5.6)	2,495 (0.9)	654 (8.8)	3,149 (1.1)
32–36 wk	337 (7.9)	813 (45.6)	1150 (19.0)	13,577 (4.6)	2999 (40.4)	16,576 (5.5)
37–40 wk	3402 (79.9)	714 (40.1)	4116 (68.1)	236,526 (81.0)	3759 (50.7)	240,285 (80.2)
>40 wk	434 (10.2)	2 (0.1)	436 (7.2)	39,581 (13.5)	4 (0.1)	39,585 (13.2)

* P<0.001 for the comparison between the assisted-conception group and the spontaneous-conception group.
† For mode of delivery, child's sex, and gestation, the denominator is the number of live births.
‡ Mode of delivery was unknown for two spontaneous births (both singleton).
§ Sex could not be assigned at birth by hospital staff on the basis of the appearance of the genitalia in five spontaneous births (four singleton and one multiple).
¶ P=0.03 for the comparison between the assisted-conception group and the spontaneous-conception group.
‖ P=0.05 for the comparison between the assisted-conception group and the spontaneous-conception group.
** Plus–minus values are means ±SD.

but not for twins. However, the relative risk of birth defects associated with assisted conception did not differ significantly between singletons and twins (P=0.44).

Births after assisted conception were associated with significantly increased adjusted odds ratios for any defect and multiple defects classified according to ICD-9 codes (740 through 759) and for subcategories of cardiovascular, musculoskeletal, urogenital, and gastrointestinal abnormalities and cerebral palsy. For multiple births, the only defect category for which there was a significantly increased risk was respiratory defects, whereas for singleton births, there were significant associations between the use of assisted reproductive technology and risks of multiple defects, congenital abnormalities, cardiovascular defects, musculoskeletal defects, urogenital defects, and cerebral palsy.

There were no significant associations between assisted conception and the risk of any recorded syndrome (Down's, Edwards's, Patau's, Pierre Robin's, Turner's, or Klinefelter's syndromes), although these conditions were rare.

TYPE OF ASSISTED CONCEPTION

Births after IVF and ICSI combined were associated with a significantly increased risk of any birth defect, as compared with births to fertile women that did not involve assisted conception (unadjusted odds ratio, 1.43; 95% CI, 1.26 to 1.62); this risk was attenuated but remained significant after multivariate adjustment (adjusted odds ratio, 1.24; 95% CI, 1.09 to 1.41) (data not shown). When we looked in detail at IVF and ICSI, the odds ratio for birth defects associated with IVF (165 defects [7.2%]) was 1.26 (95% CI, 1.07 to 1.48) in unadjusted analyses and 1.07 (95% CI, 0.90 to 1.26) after multivariate adjustment; corresponding odds ratios associated with ICSI (139 [9.9%]) were 1.77

Table 3. Odds Ratio for Birth Defects According to Category of Defect and Multiplicity.*

Birth-Defect Category	Singleton Births			
	Assisted Conception (N=4333)	Spontaneous Conception (N=295,220)	Unadjusted Odds Ratio	Adjusted Odds Ratio†
	no. of births (%)			
Any defect	361 (8.3)	16,989 (5.8)	1.48 (1.32–1.65)	1.30 (1.16–1.45)
Multiple defects	95 (2.2)	4,690 (1.6)	1.38 (1.13–1.70)	1.24 (1.00–1.54)
Congenital abnormalities: ICD-9 codes 740–759	335 (7.7)	15,372 (5.2)	1.52 (1.35–1.70)	1.32 (1.17–1.48)
Cardiovascular abnormalities: BPA codes 74500–74799	78 (1.8)	3,472 (1.2)	1.54 (1.22–1.93)	1.36 (1.08–1.72)
Musculoskeletal abnormalities: BPA codes 75400–75699	130 (3.0)	4,776 (1.6)	1.87 (1.57–2.24)	1.50 (1.24–1.80)
Urogenital abnormalities: BPA codes 75200–75399	95 (2.2)	4,872 (1.7)	1.34 (1.09–1.65)	1.25 (1.01–1.55)
Gastrointestinal abnormalities: BPA codes 74900–75199	34 (0.8)	1,832 (0.6)	1.26 (0.89–1.78)	1.18 (0.83–1.68)
Central nervous system abnormalities: BPA codes 74000–74299	22 (0.5)	1,104 (0.4)	1.37 (0.89–2.09)	1.34 (0.86–2.07)
Respiratory abnormalities: BPA codes 74800–74899	3 (0.1)	455 (0.2)	0.41 (0.12–1.40)	0.36 (0.11–1.18)
Chromosomal abnormalities: BPA codes 75800–75899	23 (0.5)	1,088 (0.4)	1.43 (0.94–2.17)	0.87 (0.57–1.33)
Metabolic abnormalities: BPA codes 24390–27790	3 (0.1)	379 (0.1)	0.59 (0.19–1.79)	0.53 (0.16–1.74)
Hematologic abnormalities: BPA codes 28200–28699	5 (0.1)	225 (0.1)	1.38 (0.56–3.35)	1.61 (0.61–4.23)
Cerebral palsy	17 (0.4)	496 (0.2)	2.35 (1.45–3.81)	2.22 (1.35–3.63)

* All odds ratios are for assisted conception as compared with spontaneous conception, with adjustment for clustering of births within the mother. BPA denotes British Paediatric Association, and ICD-9 *International Classification of Diseases, 9th Revision.*

† Analyses were adjusted for maternal age, parity, fetal sex, year of birth, maternal race or ethnic group, maternal country of birth, maternal conditions in pregnancy, maternal smoking during pregnancy, socioeconomic status, and maternal and paternal occupation.

(95% CI, 1.47 to 2.12) and 1.57 (95% CI, 1.30 to 1.90) (Table 4). As compared with ICSI, IVF was associated with a reduced risk of any birth defect (odds ratio, 0.68; 95% CI, 0.53 to 0.87). Assisted conception that did not involve gamete manipulation (specifically excluding IVF, ICSI, and gamete intrafallopian transfer), reflecting presumably less invasive types of assisted reproductive technology, was also associated with an increased risk of any birth defect (adjusted odds ratio, 1.24; 95% CI, 1.08 to 1.43).

When embryo-transfer cycles were subdivided according to whether the embryos were fresh or frozen, births after IVF fresh-embryo cycles were associated with a significantly increased unadjusted risk of any birth defect as compared with births to fertile women that did not involve assisted conception, but this risk was no longer significant after multivariate adjustment (adjusted odds ratio, 1.09; 95% CI, 0.89 to 1.33). ICSI fresh-embryo cycles were associated with an elevated risk of any birth defect that remained significant after multivariate adjustment (adjusted odds ratio, 1.66; 95% CI, 1.35 to 2.04).

Accordingly, IVF fresh-embryo cycles were associated with a lower risk than were ICSI fresh-embryo cycles (odds ratio, 0.64; 95% CI, 0.49 to 0.86). There was no significant increase in the risk of any birth defect among births resulting from IVF and ICSI (combined) frozen-embryo cycles as compared with births to fertile women that did not involve assisted conception (adjusted odds ratio, 1.10; 95% CI, 0.85 to 1.41) or births resulting from IVF frozen-embryo cycles as compared with those resulting from ICSI frozen-embryo cycles (adjusted odds ratio, 0.79; 95% CI, 0.46 to 1.34). However, a direct comparison of the relative risks for fresh-embryo cycles versus frozen-embryo cycles, for IVF and ICSI combined or for either procedure individually, showed no significant differences (data not shown).

Table 4 summarizes the risks of any birth defects among births involving other types of assisted conception as compared with births to fertile women that did not involve assisted conception. Births after gamete intrafallopian transfer, intrauterine insemination, or the use of clomiphene citrate at home were associated with significantly increased risks of any birth defect in adjusted analyses, whereas donor insemination and clini-

Multiple Births				All Births			
Assisted Conception (N=1830)	Spontaneous Conception (N=7591)	Unadjusted Odds Ratio	Adjusted Odds Ratio†	Assisted Conception (N=6163)	Spontaneous Conception (N=302,811)	Unadjusted Odds Ratio	Adjusted Odds Ratio†
no. of births (%)				*no. of births (%)*			
152 (8.3)	557 (7.3)	1.14 (0.93–1.40)	1.16 (0.91–1.49)	513 (8.3)	17,546 (5.8)	1.47 (1.33–1.62)	1.28 (1.16–1.41)
55 (3.0)	188 (2.5)	1.21 (0.87–1.69)	1.10 (0.73–1.64)	150 (2.4)	4,878 (1.6)	1.51 (1.28–1.79)	1.33 (1.11–1.59)
124 (6.8)	490 (6.5)	1.05 (0.85–1.31)	1.03 (0.79–1.34)	459 (7.4)	15,862 (5.2)	1.45 (1.31–1.60)	1.25 (1.13–1.39)
30 (1.6)	142 (1.9)	0.87 (0.56–1.35)	0.99 (0.60–1.64)	108 (1.8)	3,614 (1.2)	1.47 (1.21–1.80)	1.33 (1.08–1.63)
25 (1.4)	102 (1.3)	1.01 (0.64–1.61)	0.92 (0.54–1.57)	155 (2.5)	4,878 (1.6)	1.58 (1.34–1.86)	1.26 (1.06–1.50)
50 (2.7)	173 (2.3)	1.21 (0.86–1.70)	1.10 (0.74–1.65)	145 (2.4)	5,045 (1.7)	1.43 (1.20–1.70)	1.30 (1.08–1.54)
23 (1.3)	88 (1.2)	1.07 (0.64–1.79)	1.13 (0.59–2.16)	57 (0.9)	1,920 (0.6)	1.45 (1.10–1.90)	1.36 (1.02–1.82)
10 (0.5)	42 (0.6)	0.96 (0.44–2.08)	1.08 (0.39–2.96)	32 (0.5)	1,146 (0.4)	1.37 (0.95–1.97)	1.36 (0.94–1.99)
10 (0.5)	14 (0.2)	3.03 (1.29–7.15)	2.47 (1.06–5.76)	13 (0.2)	469 (0.2)	1.31 (0.71–2.41)	1.10 (0.59–2.04)
6 (0.3)	14 (0.2)	1.74 (0.66–4.59)	1.34 (0.42–4.33)	29 (0.5)	1,102 (0.4)	1.28 (0.88–1.86)	0.82 (0.55–1.21)
5 (0.3)	5 (0.1)	3.17 (0.77–13.1)	3.09 (0.53–17.9)	8 (0.1)	384 (0.1)	0.98 (0.43–2.23)	0.93 (0.40–2.18)
1 (0.1)	3 (0.0)	1.52 (0.15–15.1)	0.86 (0.01–135.65)	6 (0.1)	228 (0.1)	1.24 (0.55–2.80)	1.34 (0.56–3.20)
16 (0.9)	50 (0.7)	1.32 (0.69–2.52)	1.39 (0.69–2.77)	33 (0.5)	546 (0.2)	2.97 (2.03–4.34)	2.66 (1.79–3.94)

cally supervised ovulation induction by various means were not; however, these analyses were limited by relatively small numbers of events.

SPONTANEOUS CONCEPTION IN WOMEN WITH A HISTORY OF INFERTILITY

As compared with births from spontaneous conception in fertile women, births from spontaneous conception in women who had had a previous birth with assisted conception were also associated with an increased overall risk of any birth defect, even after adjustment for confounders (adjusted odds ratio, 1.25; 95% CI, 1.01 to 1.56). A history of infertility without any treatment with assisted reproductive technology was associated with a similar, albeit borderline, significant increase in risk (odds ratio, 1.29; 95% CI, 0.99 to 1.68).

DISCUSSION

In this large observational study using detailed Australian databases with information on several potential confounders, we confirmed previous findings of an increased risk of birth defects among births conceived with assisted reproductive technology as compared with births from spontaneous conception.[4,5,10,15,19] After multivariate adjustment, the association between IVF and the risk of any birth defect was no longer significant, whereas the increased risk of any birth defect associated with ICSI remained significant.

These findings are consistent with the results of previous studies.[19,20] The strengths of the present study include the use of a single population registry with ascertainment of birth defects from pregnancy to a child's fifth birthday and information on multiple treatment methods. The possibility of treatment effects that are specific to ICSI is biologically plausible,[20,21] although differences in male infertility factors that lead to the use of ICSI may also underlie the association.[7] Information on paternal age was not available for the present study, although this variable is unlikely to be a major confounder, because the association between paternal age and birth defects is generally weak[22] and adjustment for maternal age may reduce the potential influence of paternal age, with which it correlates.

In contrast to clinically managed induction of ovulation with the use of any of several drugs (e.g., clomiphene citrate, follicle-stimulating hormone, and human chorionic gonadotropin), the use of clomiphene citrate as a single agent at home was associated with an increased risk of birth defects. This finding is consistent with the results of previous case–control studies.[23-25] However, be-

Table 4. Odds Ratio for Any Birth Defects According to Type of Assisted Conception and Multiplicity.*

Type of Assisted Conception	Singleton Births		
	Defect	Unadjusted Odds Ratio	Adjusted Odds Ratio†
	no. of births with defect/ total no. of births		
Any	361/4333	1.45 (1.30–1.63)	1.28 (1.14–1.43)
IVF			
Fresh- or frozen-embryo cycles	105/1484	1.25 (1.02–1.52)	1.06 (0.87–1.30)
Fresh-embryo cycles	71/1005	1.25 (0.98–1.59)	1.05 (0.82–1.35)
Frozen-embryo cycles	34/479	1.24 (0.88–1.76)	1.08 (0.76–1.53)
ICSI			
Fresh- or frozen-embryo cycles	91/939	1.72 (1.38–2.15)	1.55 (1.24–1.94)
Fresh-embryo cycles	76/713	1.95 (1.53–2.48)	1.73 (1.35–2.21)
Frozen-embryo cycles	15/226	1.17 (0.70–1.97)	1.10 (0.65–1.85)
GIFT	34/319	1.98 (1.40–2.80)	1.73 (1.21–2.47)
Intrauterine insemination	54/580	1.67 (1.25–2.23)	1.46 (1.09–1.95)
Donor insemination	36/428	1.51 (1.08–2.11)	1.37 (0.98–1.92)
Ovulation induction	19/306	1.08 (0.68–1.74)	0.99 (0.62–1.59)
Clomiphene citrate at home	7/36	3.87 (1.58–9.51)	3.19 (1.32–7.69)
Other§	15/241	1.07 (0.63–1.82)	0.96 (0.56–1.63)
Spontaneous conception after previous birth from assisted reproductive technology	96/1306	1.27 (1.02–1.59)	1.26 (1.01–1.57)
Infertile but no history of treatment with assisted reproductive technology	52/600	1.54 (1.15–2.05)	1.37 (1.02–1.83)
No use of assisted reproductive technology and fertile	16,841/293,314	1.00	1.00

* The odds ratios are for the comparison with no use of assisted reproductive technology and fertile and have adjusted for clustering of births within the mother. GIFT denotes gamete intrafallopian transfer, ICSI intracytoplasmic sperm injection, and IVF in vitro fertilization.

† Analyses were adjusted for maternal age, parity, fetal sex, year of birth, maternal race or ethnic group, maternal country of birth, maternal conditions in pregnancy, maternal smoking during pregnancy, socioeconomic status, and maternal and paternal occupation.

‡ The 44 births resulting from donor insemination or treatment with "other" assisted reproductive technology involved no birth defects and were excluded from the analysis of the multiple-births subgroup.

§ Other includes births as a result of a pregnancy during an observational "tracking" cycle after an initial fertility assessment, timed intercourse, sperm–cervical mucus contact test, and administration of gonadotropin-releasing hormone analogues to control ovulation.

cause we cannot rule out residual confounding or chance as an explanation for the increased risk observed and given the small number of patients treated in this way, caution is warranted in interpreting this result.

The risks of birth defects associated with other forms of minimal treatment (e.g., timed intercourse, semen tests, or low-dose hormonal stimulation) were not significantly different from the risk with spontaneous conception. However, the numbers for these analyses were also relatively small, and the confidence intervals do not reliably exclude risks on the order of those associated with other treatments.

When we looked separately at births resulting from fresh-embryo cycles versus frozen-embryo cycles of IVF or ICSI as compared with births to fertile women, we found a significant increase in the risk of birth defects associated with fresh-embryo cycles but not with frozen-embryo cycles. The risk of birth defects in fresh-embryo cycles of IVF was also significantly lower than that in fresh-embryo cycles of ICSI. This is a more robust result than the finding in previous studies of a nonsig-

Multiple Births			All Births		
Defect	Unadjusted Odds Ratio	Adjusted Odds Ratio†	Defect	Unadjusted Odds Ratio	Adjusted Odds Ratio†
no. of births with defect/ total no. of births			*no. of births with defect/ total no. of births*		
152/1786‡	1.15 (0.93–1.41)	1.17 (0.91–1.50)	513/6163	1.45 (1.32–1.60)	1.26 (1.14–1.40)
60/817	1.00 (0.74–1.35)	0.99 (0.71–1.37)	165/2301	1.26 (1.07–1.48)	1.07 (0.90–1.26)
50/642	1.07 (0.76–1.51)	1.05 (0.74–1.50)	121/1647	1.29 (1.06–1.57)	1.09 (0.89–1.33)
10/175	0.77 (0.41–1.44)	0.79 (0.39–1.59)	44/654	1.17 (0.87–1.59)	1.02 (0.75–1.39)
48/468	1.43 (1.03–1.99)	1.39 (0.96–2.01)	139/1407	1.77 (1.47–2.12)	1.57 (1.30–1.90)
40/398	1.40 (0.98–2.00)	1.35 (0.90–2.02)	116/1111	1.89 (1.54–2.31)	1.66 (1.35–2.04)
8/70	1.63 (0.74–3.60)	1.60 (0.71–3.58)	23/296	1.37 (0.89–2.11)	1.28 (0.83–1.99)
25/271	1.27 (0.77–2.08)	1.53 (0.92–2.56)	59/590	1.81 (1.37–2.41)	1.55 (1.16–2.07)
9/152	0.80 (0.42–1.53)	0.77 (0.39–1.53)	63/732	1.53 (1.18–1.99)	1.32 (1.01–1.73)
0/40‡			36/468	1.37 (0.98–1.91)	1.24 (0.89–1.73)
8/68	1.74 (0.73–4.18)	1.88 (0.84–4.22)	27/374	1.27 (0.83–1.93)	1.16 (0.76–1.75)
2/10	3.28 (0.87–12.33)	3.36 (0.81–13.98)	9/46	3.92 (1.84–8.38)	3.39 (1.61–7.13)
0/4‡			15/245	1.04 (0.61–1.78)	0.92 (0.54–1.58)
3/36	1.14 (0.35–3.72)	1.23 (0.36–4.27)	99/1342	1.27 (1.02–1.58)	1.25 (1.01–1.56)
15/207	1.00 (0.54–1.84)	0.82 (0.43–1.55)	67/807	1.47 (1.13–1.90)	1.29 (0.99–1.68)
539/7348	1.00	1.00	17,380/300,662	1.00	1.00

nificant tendency for frozen-embryo cycles to be at lower risk than fresh-embryo cycles,[26,27] although in those studies, as in the current study, the numbers for frozen-embryo cycles were smaller, and thus the power for these analyses lower, than for fresh-embryo cycles. Possible explanations for a reduced risk of birth defects with cryopreservation include a reduced likelihood that developmentally compromised embryos will survive the thawing process and the temporal separation of the developing embryo from exposure to hormonal stimulation drugs used early in treatment with assisted reproductive technology.[26-28]

The risk of a birth defect was increased among women with a history of infertility but no accompanying history of treatment with assisted reproductive technology, an observation that is consistent with the findings in a large Danish registry[8] and that implicates patient factors in this increased risk. Similarly, we found that "spontaneous" conception among women receiving treatment with assisted reproductive technology was also associated with an increased risk of birth defects. However, we cannot rule out the possibility that a proportion of the women with infertility received clomiphene citrate outside the infertility clinics licensed to manipulate gametes from medical practitioners treating anovulatory infertility, because we have previously reported that the use of clomiphene citrate as a sole therapy is common in this circumstance.[29] Treatment received by these patients would not be included in the registry of patients using assisted reproductive technology, and information on this practice was unavailable.

Significant associations between assisted conception and birth defects were evident for singleton births but not multiple births, a finding that is consistent with the results of other studies.[1,30] There was not a significant difference in risk between singletons and twins from assisted conception; however, the confidence intervals around risk

estimates were wide for multiple births. As noted previously, combining estimates of risk among singleton and multiple births may modify estimates of risk relative to births not resulting from assisted conception.[30,31] The absence of a significantly increased risk of birth defects for multiple births conceived by assisted reproductive technology may be explained, in part, by the fact that twins conceived by assisted reproductive technology are much more likely to be dizygotic (owing to transfer of more than one embryo) than twins conceived spontaneously; dizygotic twins are at lower risk for birth defects than are monozygotic twins.[31,32]

As reported in previous studies,[4,7,10,15,33] we observed associations of assisted conception with birth defects in analyses of single and multiple birth defects and in analyses that included or excluded cerebral palsy. Treatment with assisted reproductive technology was associated with increased risks of cardiovascular, musculoskeletal, urogenital, and gastrointestinal defects and cerebral palsy. The absence of an observed association with syndromes in our study is consistent with a review[34] of large studies but may also reflect the low frequency of these outcomes in South Australia. The increased risk of cerebral palsy that we observed in association with assisted conception is consistent with a report by Strömberg et al., who observed an increase in risk by a factor of 3.7 among children conceived by IVF and by a factor of 2.8 among singletons conceived by IVF.[33]

Although the large majority of births resulting from assisted conception were free of birth defects, treatment with assisted reproductive technology was associated with an increased risk of birth defects, including cerebral palsy, as compared with spontaneous conception. In the case of ICSI, but not IVF, the increased risk of birth defects persisted after adjustment for maternal age and several other risk factors. Although we cannot rule out the possibility that other patient factors contribute to or explain the observed associations, our findings can help provide guidance in counseling patients who are considering treatment for infertility.

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