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Assisted reproductive technology and major structural birth defects in the United States†

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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

† 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)[a]	ART (n = 51)[a]	*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 ($n = 4741$)[a]	ART ($n = 51$)[a]	*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^2	278 (6.1%)	2 (3.9%)	
18.5–24.9 kg/m^2	2584 (56.9%)	29 (56.9%)	
25–29.9 kg/m^2	1003 (22.1%)	14 (27.5%)	
≥30 kg/m^2	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. [a]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[a], stratified by plurality (National Birth Defects Prevention Study, 1997–2003)

Birth defect categories	Singletons			Twins or higher		
	Unassisted conception	ART	Unadjusted OR (95% CI)[b]	Unassisted conception	ART	Unadjusted OR (95% CI)[b]
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)

[a]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; [b]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[a] AOR (95% CI)	Twins or higher[b] 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[c]	2.1 (1.1–4.0)	1.3 (0.6–2.8)
Perimembraneous VSD[c]	1.4 (0.6–3.3)	1.1 (0.4–2.8)
ASD secundum/NOS[c]	3.0 (1.5–6.1)	1.7 (0.7–3.9)
VSD and ASD[c]	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.
[a]Adjusted for maternal age, center (Massachusetts versus rest), family income, and parity; [b]Adjusted for: maternal age, family income, folic acid use, parity, and periconceptional alcohol use; [c]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 (Adegboye 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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