Association Between Assisted Reproductive Technology Conception and Autism in California, 1997–2007

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Objectives. We assessed the association between assisted reproductive technology (ART) and diagnosed autistic disorder in a population-based sample of California births.

Methods. We performed an observational cohort study using linked records from the California Birth Master Files for 1997 through 2007, the California Department of Developmental Services autism caseload for 1997 through 2011, and the Centers for Disease Control and Prevention's National ART Surveillance System for live births in 1997 through 2007. Participants were all 5 926 251 live births, including 48 865 ART-originated infants and 32 922 cases of autism diagnosed by the Department of Developmental Services. We compared births originated using ART with births originated without ART for incidence of autism.

Results. In the full population, the incidence of diagnosed autism was twice as high for ART as non-ART births. The association was diminished by excluding mothers unlikely to use ART; adjustment for demographic and adverse prenatal and perinatal outcomes reduced the association substantially, although statistical significance persisted for mothers aged 20 to 34 years.

Conclusions. The association between ART and autism is primarily explained by adverse prenatal and perinatal outcomes and multiple births. (*Am J Public Health.* 2015;105:963–971. doi:10.2105/AJPH.2014.302383)

Autism spectrum disorder (ASD) is a serious developmental disability characterized by deficits of communication and social interaction and often accompanied by restricted or repetitive behaviors. Recent surveillance efforts estimate ASD to occur in 1 in 68 US children aged 8 years, with rapid increases in identified incidence over the past decade.[1] Numerous studies have investigated the causes and correlates of autism and ASD, commonly finding male gender[2,3]; prenatal and perinatal factors,[4–6] such as preterm birth, low birth weight, and gestational diabetes; and parental characteristics, such as higher socioeconomic status[5] and education,[2,5] older parental age,[2,3,7,8] White race,[2,5] and history of psychiatric conditions[4] to be associated with autism. In addition, there is a large but complex genetic component with a subset of inherited familial autism cases as well as an important role for rare and common copy number variations.[9–11]

As autism diagnoses have risen, the use of assisted reproductive technology (ART), defined as in vitro fertilization (IVF) and similar procedures in which both egg and sperm are handled, has increased rapidly.[12] In a typical ART procedure, fresh or frozen egg and sperm from donors or from 1 or both of the parents are combined in a laboratory for fertilization and cultured for several days before implantation in a woman's uterus.[13] Often hormonal medication is used to stimulate or regulate ovulation. There are several variations, including IVF with intracytoplasmic sperm injection, in which the egg is fertilized by injecting the sperm directly into the egg, and less common procedures, in which the fertilized embryo, or a mixture of sperm and eggs, is placed in the fallopian tubes rather than the uterus (zygote intrafallopian transfer and gamete intrafallopian transfer, respectively). Often, multiple embryos are transferred to maximize the probability of implantation and pregnancy, producing a high rate of twin and higher-order multiple births from ART. In recent years, the Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine have issued voluntary guidelines regarding the number of embryos to transfer for various patient types, which has resulted in a reduction in multiple births.[14]

ART-originated pregnancies share many of the correlates of autism, including parents who are older and have higher levels of education and multiple births, preterm delivery, pregnancy and labor complications, low birth weight, and other birth defects and developmental disabilities. Further, the use of ART contributes to the preexisting trend of older parents by pushing on the upper boundary of the fertile age range.

A few studies have investigated the relationship between ART conception and the risk of developmental disabilities and autism diagnoses; however, results are mixed and inconclusive.[15–25] For example, some studies have found no differences between children originated with ART and control groups of children with regard to congenital malformation or developmental delay,[26] but others have found increased risks of emotional disturbances,[27] lower cognitive and language skills,[28] and cerebral palsy.[20] In a recent review of the evidence on the ART–autism association 8 studies, many with design limitations including short follow-up periods and insufficient sample sizes, showed inconsistent results.[20] Most studies that have examined ART outcomes have failed to collect data from participants older than 2 years[29]; this is a problem because autism is often diagnosed when a child is older than 4 years.[1] One recent case-control study found no association, although there was some evidence of a link between less severe forms of ASD and artificial insemination and ovulation-induction treatment among older mothers.[23] A study of children with ASD found no evidence of increased copy number variations or other autism-related genetic events among children originated with ART.[30]

The best evidence comes from several well-designed Scandinavian studies using large population-level registry databases. A Danish study found an elevated risk of autism for infants originated through ART, although the difference was not statistically significant after adjustments.[18] However, this study did find elevated risks for certain subgroups, notably girls and those born after ovarian stimulation treatment. Another Danish registry study found no increased risk of childhood and adolescent psychological disorders resulting from IVF, but they did find a slightly elevated risk of autism and several other disorders arising from ovulation induction and assisted insemination.[25] Similarly, a recent Swedish study found no elevated risk of autism from IVF, but it did find a significant risk from certain subtypes of IVF procedures as well as an elevated risk of intellectual disability. Both studies suggest the importance of further research on large population-level data sets.

Because of the increasing use of ART as well as the increasing incidence and uncertain etiology of autism, it is important to explore whether these phenomena are associated. We assessed the possible association between ART and diagnosed autism in a 10-year cohort of California children.

METHODS

We constructed the data set from 3 sources: the California Birth Master Files for 1997–2007, the California Department of Developmental Services (DDS) autism caseload records for 1997–2011, and the Centers for Disease Control and Prevention's National ART Surveillance System for live births for 1997–2007.

The variables we extracted from the California birth records included maternal and paternal age at birth; maternal education, race/ethnicity, and birthplace; infant gender and birth weight and the duration of gestation; plurality; parity; pregnancy and birth complications; mode of delivery; source of payment for labor and delivery; and quantity of prenatal care. We derived variables known to be associated with autism and ART, including preterm birth (less than 37 weeks) and small for gestational age (weight below the 10th percentile for the gestational age).

The DDS system coordinates diagnoses, services, and support for persons with developmental disabilities in California, including patients with autistic disorder (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*[31] [*DSM-IV*] code 299.0) but generally not those with other ASDs such as Asperger's syndrome. The vast majority of persons with autism in California are enrolled in the DDS, making it the largest administrative source of data on autism diagnoses.[32] We classified children enrolled in the DDS autism caseload as having a diagnosis of autism on the date of enrollment. We did not identify autism cases through population surveillance, so incidence may underestimate the true incidence rate. When we use the term "incidence" we refer specifically to incidence of DDS-identified autistic disorder as diagnosed per the *DSM-IV*.

The Division of Reproductive Health of the Centers for Disease Control and Prevention collects and maintains the National ART Surveillance System, a registry of all ART cycles initiated in US fertility clinics. Reporting is mandatory,[33] and it is estimated that at least 95% of all ART cycles are represented in the database.[34] The registry collects data for all ART procedures in which both gametes are handled (i.e., IVF, gamete intrafallopian transfer, intrafallopian transfer) but not procedures such as intrauterine insemination or ovulation-induction treatment.

The Society for Assisted Reproductive Technology collected the information on each ART procedure from ART clinics for the years 1997 through 2003 and Westat collected it for the years 2004 through 2007.

Linking Procedures

We selected the subset of ART procedures that led to a live birth and were performed at an ART clinic in California. All types of ART procedures, including IVF with and without intracytoplasmic sperm injection, intrafallopian transfer, and gamete intrafallopian transfer, are included in this group. We found ART births in the California Birth Master Files using Link Plus 2.0 software,[35] on the basis of mother's date of birth, infant's date of birth, plurality, mother's zip code, and gravidity. We manually reviewed uncertain matches, and we used infant gender, maternal race, and infant birth weight to resolve duplicate or uncertain matches. We successfully linked 90% of ART births to a California birth, which is similar to that of previous links of ART surveillance data with birth certificate data previously conducted and validated with high reliability as part of the States Monitoring ART Collaborative project in other states.[36]

We linked autism cases from the DDS probabilistically to the California Birth Master Files on first and last names, middle initial, date of birth, gender, race/ethnicity, and maternal zip code. We manually reviewed uncertain matches. On average, we linked 86% of eligible children with autism in the DDS database to a birth record. Typically, unmatched DDS records were of children born outside California who moved into the state after birth.[37]

Statistical Analysis

We calculated frequencies and percentages summarizing the demographic composition and selected autism risk factors for ART-originated and naturally originated infants. We have reported P values for the χ^2 test of association between each variable and ART use. We analyzed 2 different samples: (1) total 1997 to 2007 California resident birth cohort, and (2) an analysis subset of children designed to exclude children of mothers who are very different from the typical ART patient and to eliminate ascertainment bias stemming from differences in the use of medical care.[38]

The analysis subset excluded infants whose mothers were younger than 20 years, had less than a high school education, had prenatal care or delivery paid for by Medi-Cal or another public source, or had missing information on prenatal care or inadequate prenatal care[39] or started prenatal care in the third trimester. Each of these exclusion categories included a very small percentage of ART-originated children (Table 1), so sample restriction was preferable to statistical adjustment for these specific factors. Additionally, we eliminated observations missing values for any of the model covariates from the sample (about 4%). The large sample and small percentage of missing data made listwise deletion preferable to multiple imputation or other strategies.[40] This left a total of 2 420 330 children in the analysis subset; altogether, we excluded 3 505 921 mother–child pairs.

We calculated diagnosed autism incidence with robust 95% confidence intervals (CIs) adjusted for multiple deliveries, for ART and

TABLE 1—Percentage Distribution of Key Variables by ART and Autism Status: California 1997–2007

Variable	Non-ART (n = 5 877 386), %	ART (n = 48 865), %	P^a	No Autism (n = 5 893 329), %	Autism (n = 32 922), %	P^a
Birth year			≤ .001			≤ .001
1997	8.9	4.1		8.9	7.2	
1998	8.8	5.5		8.8	7.8	
1999	8.8	5.9		8.8	8.1	
2000	9.0	6.4		9.0	9.1	
2001	8.9	8.3		8.9	9.6	
2002	8.9	9.8		8.9	10.2	
2003	9.1	10.9		9.1	11.0	
2004	9.2	11.6		9.2	10.5	
2005	9.3	11.9		9.3	10.0	
2006	9.5	12.2		9.5	9.1	
2007	9.5	13.3		9.6	7.4	
Infant's gender			.335			≤ .001
Female	48.9	49.1		49.0	16.8	
Male	51.1	50.9		51.0	83.2	
Plurality			≤ .001			≤ .001
Singleton	97.5	47.3		97.1	94.8	
Twin	2.4	46.4		2.8	4.8	
Triplet or more	0.1	6.4		0.1	0.4	
Parity			≤ .001			≤ .001
Multiparous	61.3	49.6		61.3	56.6	
Primiparous	38.7	50.4		38.7	43.4	
Mother's age, y			≤ .001			≤ .001
< 20	10.2	0.0		10.1	5.1	
20–24	23.2	0.4		23.1	17.8	
25–29	26.5	6.5		26.3	25.4	
30–34	24.0	27.7		24.0	28.0	
35–39	13.0	37.4		13.1	18.5	
≥ 40	3.1	28.0		3.3	5.2	
Mother's race			≤ .001			≤ .001
Non-Hispanic White	30.6	67.7		30.9	34.4	
Black	6.3	2.4		6.2	7.4	
Hispanic	49.8	11.1		49.5	40.8	
Asian or Pacific Islander	11.9	16.2		11.9	16.0	
Native American	0.5	0.2		0.4	0.4	
Other or unknown	1.0	2.5		1.0	1.0	
Mother's birthplace			≤ .001			≤ .001
US and US territories	54.1	72.1		54.3	56.3	
Outside US	45.9	27.9		45.7	43.7	
Mother's education			≤ .001			≤ .001
< high school diploma	30.3	2.2		30.2	18.8	
High school diploma	27.8	9.5		27.6	26.2	
Some college	20.0	18.2		19.9	24.9	
≥ 4-y college graduate	21.9	70.1		22.3	30.1	
Small for gestational age			≤ .001			≤ .001
≥ 10th percentile	90.4	78.1		90.4	89.2	
< 10th percentile	9.6	21.9		9.6	10.8	

Continued

TABLE 1—*Continued*

Preterm			≤ .001			≤ .001
≥ 37 wk	89.5	63.8		89.3	86.6	
< 37 wk	10.5	36.2		10.7	13.4	
Hypertension or preeclampsia			≤ .001			≤ .001
None recorded	97.5	92.8		97.5	96.6	
Present	2.5	7.2		2.5	3.4	
Maternal diabetes			≤ .001			≤ .001
None recorded	98.0	96.9		98.0	97.0	
Present	2.0	3.1		2.0	3.0	
Mode of delivery			≤ .001			≤ .001
Vaginal	73.7	35.5		73.4	65.3	
Cesarean	26.3	64.5		26.6	34.7	
Trimester prenatal care begun						≤ .001
None	0.6	0.1		0.6	0.3	
First	84.8	96.7		84.9	88.8	
Second	12.2	3.0		12.1	9.3	
Third	2.4	0.2		2.4	1.6	
Payment source for prenatal care			≤ .001			≤ .001
Private insurance or payment	54.3	97.0		54.6	64.6	
Public source	45.7	3.0		45.4	35.4	
Payment source for delivery			≤ .001			≤ .001
Private insurance or payment	53.9	97.0		54.2	64.3	
Public source	46.1	3.0		45.8	35.7	
Adequacy of prenatal care			≤ .001			≤ .001
Missing	3.0	2.3		3.0	2.4	
Inadequate	9.6	1.2		9.6	6.8	
Intermediate	12.7	4.7		12.6	11.2	
Adequate	41.3	22.2		41.1	40.3	
Adequate plus	33.5	69.6		33.7	39.3	
DDS autism status			≤ .001			≤ .001
No autism	99.4	98.8				
Autism	0.6	1.2				
Originated using ART?						≤ .001
No				99.2	98.2	
Yes				0.8	1.8	

Note. ART = assisted reproductive technology; DDS = California Department of Developmental Services.
[a]We derived *P* values from the χ^2 test for association between categorical predictor variable and ART conception or autism status.

natural conceptions, for all children by plurality group, and for the analysis subset. We estimated hazard risk ratios (HRRs), with robust sandwich SEs to adjust for clustering on family ID, for the hazard of autism diagnosis among children originated with ART compared with those originated without ART.[41] Children born in later years were observed for a shorter period, potentially introducing ascertainment bias; we adjusted for this difference statistically by including indicator variables for birth years.

We estimated 3 sets of adjusted models on the analysis subset. Model 1 included demographic factors only: year of birth, infant gender, maternal education (college graduate or not), and maternal race (non-Hispanic White, Black, Asian, Hispanic, and other). Model 2 was the main adjusted model and it included all variables in model 1 plus factors associated with both ART and autism that might have an underlying biological impact on pregnancy health and fetal development: maternal age (20–34 years or ≥ 35 years) and parity and an interaction between maternal age and ART.

Model 3 included all variables in model 2 plus adverse perinatal outcomes previously associated with both ART and autism: multiple birth, preterm delivery (< 37 weeks), small for gestational age (< 10th percentile), maternal diabetes, pregnancy hypertension or preeclampsia, and cesarean delivery. These are outcomes that occur after conception and thus

are not properly considered confounders but rather potential pathways or mechanisms through which ART and autism may be associated. The HRR for the effect of ART on autism in model 3 represents only any direct effect, not the indirect association created because ART pregnancies and deliveries tend to have more complications and include more multiple births.[38,42–48] This is a simple way of exploring the effect of mediators but is valid only if the confounders of the relationship between response and exposure are properly accounted for, an assumption that is difficult to confirm.[49] However, we used our exclusion strategy, combined with controls for confounders such as age and education, to accomplish this as much as possible with observational data.

Additionally, we estimated adjusted HRRs within selected strata to assess possible effect modifications. Stratified HRRs adjusted for all variables in model 2 except the stratification variable, and we conducted interaction tests for all stratification variables. We conducted all analyses using SAS, version 9.2 (SAS Institute, Cary, NC).

RESULTS

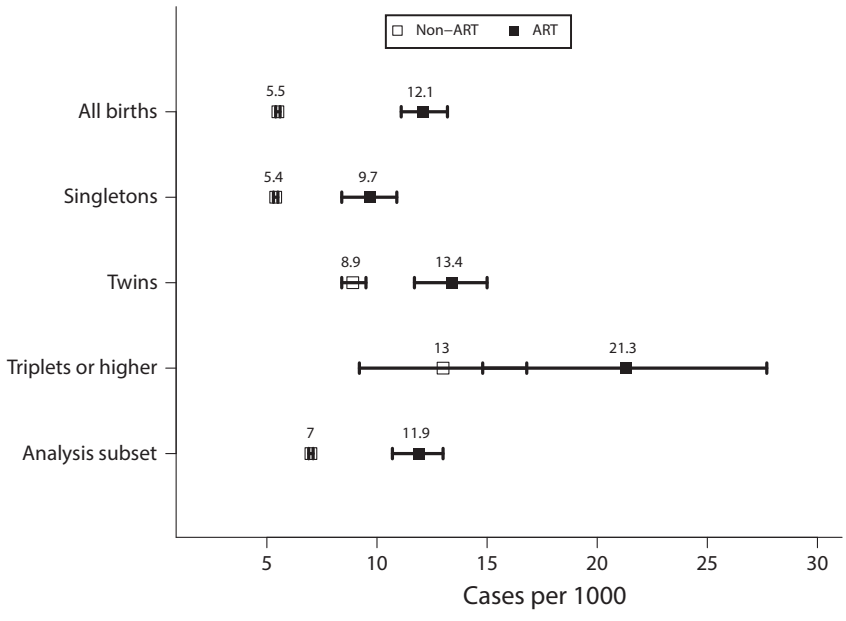
Of the 5 926 251 children born in California from 1997 to 2007, 48 865 (0.83%) were originated through ART and 32 922 (0.56%) were diagnosed with autism and enrolled in the DDS caseload by June 2011. In bivariate analyses, there were statistically significant differences between ART- and non–ART-originated children for all variables except infant gender (Table 1). ART-originated children were more likely to be born to highly educated, older, non-Hispanic White, primiparous mothers. Furthermore, ART-originated infants had mothers with higher levels of prenatal care and were more likely to be twins or higher-order multiples, to be born small for gestational age, and to have mothers who had complications of pregnancy and labor. ART-originated infants were also more likely to be diagnosed with autism. There were statistically significant differences between children with autism and those without on all variables; in particular, children with autism were more likely to be boys; born in multiple births; born to older, White, and more educated mothers; and born to mothers who have experienced complications of pregnancy and delivery.

Risk of Autism

ART-originated children had higher incidence of DDS-diagnosed autism than did children originated without ART. The groups composed of triplets and higher-order multiples had the highest incidence within each conception group (Figure 1). In the total study population of California births, diagnosed autism incidence was about twice as high for ART conceptions as for non–ART-originated children (12.1 and 5.5, respectively, per 1000 births). In the analysis subset the pattern was similar although attenuated: the incidence for ART conceptions was 11.9 per 1000 births, whereas the incidence for non-ART conception was 7.0 per 1000 births.

Assisted Reproductive Technology, Autism, and Additional Risk Factors

Table 2 shows the detailed results for the covariates in several adjusted models on the basis of the analysis subset. Other variables associated with autism included infant gender, maternal age and race, and parity. Complications of labor and pregnancy that were associated with ART were also correlated with eventual autism diagnosis. In the total study population, children originated with ART had elevated incidence of subsequent autism diagnosis (Figure 2; HRR = 2.32; 95% CI = 2.12, 2.54). This incidence was diminished but still elevated and statistically significant in the analysis subset (HRR = 1.79; 95% CI = 1.6, 2.0). Adjusting for demographic factors (model 1) reduced the HRR further: 1.71 (95% CI = 1.55, 1.89). Adjustment for maternal age and parity (model 2) resulted in HRRs for risk of autism of 1.74 (95% CI = 1.46, 2.07) for children born to mothers aged 20 to 34 years and 1.37 (95% CI = 1.21, 1.54) for children with mothers aged 35 years or older (Table 2). The inclusion of adverse perinatal outcomes occurring after conception (model 3) decreased the HRRs for ART to a relatively small effect size; whereas it remained marginally statistically significant for the younger maternal age group (HRR = 1.21; 95% CI = 1.01, 1.45), the effect was reduced to null for the older mothers (HRR = 1.00; 95% CI = 0.88, 1.14; Table 2).



Note. ART = assisted reproductive technology.

FIGURE 1—Unadjusted prevalence of autism with 95% confidence intervals for all births by plurality and, for the analysis subset, by ART conception status: California 1997–2007.

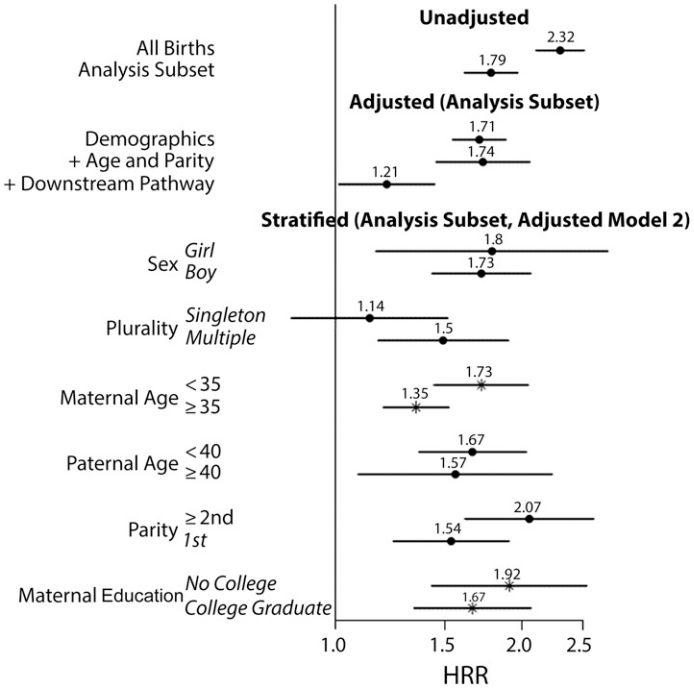
TABLE 2—Risk Factors for Autism Diagnosis and Associations With ART and All Covariates for Unadjusted and 3 Adjusted Models Estimated on Analysis Subset (n = 2 420 330): California 1997–2007

	Crude: Unadjusted, HRR (95% CI)	Model 1: Adjusted for Demographics, HRR (95% CI)	Model 2: Model 1 + Mother's Age, Parity, HRR (95% CI)	Model 2 + Pathway Factors, HRR (95% CI)
ART effect	1.790 (1.636, 1.959)	1.713 (1.551, 1.892)		
For mothers aged 20–34 y			1.736 (1.457, 2.069)	1.210 (1.011, 1.448)
For mothers aged ≥ 35 y			1.365 (1.209, 1.541)	1.002 (0.881, 1.139)
Birth year				
1997		0.666 (0.619, 0.717)	0.668 (0.621, 0.719)	0.679 (0.631, 0.731)
1998		0.709 (0.660, 0.761)	0.711 (0.662, 0.764)	0.721 (0.671, 0.774)
1999		0.771 (0.719, 0.827)	0.773 (0.720, 0.828)	0.782 (0.729, 0.838)
2000		0.850 (0.794, 0.909)	0.851 (0.795, 0.910)	0.858 (0.802, 0.918)
2001		0.914 (0.855, 0.977)	0.915 (0.856, 0.979)	0.919 (0.860, 0.983)
2002 (Ref)		1.000	1.000	1.000
2003		1.049 (0.983, 1.121)	1.046 (0.979, 1.117)	1.044 (0.978, 1.115)
2004		1.038 (0.970, 1.110)	1.033 (0.966, 1.105)	1.027 (0.960, 1.098)
2005		1.072 (1.001, 1.147)	1.066 (0.996, 1.141)	1.054 (0.984, 1.128)
2006		1.080 (1.006, 1.160)	1.070 (0.996, 1.149)	1.064 (0.990, 1.143)
2007		1.187 (1.101, 1.281)	1.177 (1.092, 1.270)	1.171 (1.085, 1.263)
Male infant		4.710 (4.524, 4.904)	4.710 (4.524, 4.904)	4.678 (4.493, 4.871)
Mother is college graduate		1.085 (1.050, 1.121)	1.009 (0.976, 1.0430)	1.013 (0.980, 1.047)
Mother's race/ethnicity				
Non-Hispanic White (Ref)		1.000	1.000	1.000
Black		1.431 (1.341, 1.527)	1.470 (1.378, 1.568)	1.416 (1.327, 1.511)
Asian		1.114 (1.062, 1.168)	1.124 (1.072, 1.180)	1.118 (1.065, 1.173)
Hispanic		1.012 (0.970, 1.055)	1.062 (1.018, 1.107)	1.053 (1.010, 1.098)
Other		1.012 (0.970, 1.055)	1.249 (0.999, 1.560)	1.232 (0.986, 1.540)
Mother is foreign-born		1.143 (1.099, 1.189)	1.129 (1.086, 1.174)	1.129 (1.085, 1.1740)
Primiparous			1.338 (1.297, 1.380)	1.335 (1.294, 1.3780)
Complications of pregnancy and delivery on the causal pathway				
Multiple birth				1.418 (1.304, 1.5410)
Small for gestational age				1.110 (1.057, 1.1660)
Preterm (< 37 wk)				1.181 (1.124, 1.240)
Maternal diabetes				1.276 (1.173, 1.3870)
Hypertension or preeclampsia				1.115 (1.027, 1.2110)
Cesarean delivery				1.272 (1.231, 1.3140)
-2Log L	498 555.79	490 104.63	489 471.22	488 900.48
AIC	498 557.79	490 140.63	489 513.22	488 954.48

Note. AIC = Aikake information criterion; ART = assisted reproductive technology; CI = confidence interval; HRR = hazard risk ratio. We calculated the effect of ART for all mothers aged ≥ 20 y for crude and adjusted model 1. Model 1 includes demographic factors: year of birth, infant's gender, and mother's education and race. The estimated effect of ART is reported separately for mothers aged 20–34 and ≤ 35 y for models 2 and 3 because of the presence of an interaction. Model 2 includes all variables in model 1 plus factors associated with both ART and autism that might have an underlying biological impact on pregnancy health and fetal development: maternal age and parity and an interaction between mother's age and ART. Model 3 includes all variables in model 2 plus perinatal outcomes previously associated with both ART and autism: multiple birth, preterm delivery, small for gestational age, maternal diabetes, pregnancy hypertension or preeclampsia, and cesarean delivery. Estimates for baseline categories are omitted.

Assisted Reproductive Technology and Autism in Selected Strata

Figure 2b presents several strata-specific adjusted HRRs for risk of autism. In each of these models, we included all variables from model 2 as potential confounders with the exception of the stratification variable, and only estimates for younger maternal ages are shown. The adjusted HRR for autism was statistically elevated for every subgroup except singletons. The ART–autism association was stronger among the subgroups that had a lower incidence of diagnosed autism, such as girls, second or greater births, and children whose mothers were younger than 35 years when the



Note. ART = assisted reproductive technology; CI = confidence interval; HRR = hazard risk ratio. The figure includes unadjusted, adjusted, and strata-specific estimates. Model 1 adjusts for demographics, including birth year, infant gender, and maternal race, education, and place of birth. Model 2 adjusts for all variables in model 1 plus maternal age and parity. Model 3 adjusts for model 2 variables plus adverse perinatal outcomes (multiple birth, preterm, small for gestational age, maternal diabetes, hypertension and preeclampsia, and cesarean delivery). Stratified results adjust for all model 2 variables, except the stratification variable. As the association was notably higher among the subgroup with younger maternal ages, we have presented all stratified results for the younger maternal age subgroup only, except when stratifying by maternal age. Because of missing data on paternal age, the sample size for that stratified model is n = 2 375 846. We conducted interaction tests by interacting the stratification variable with ART.
*$P < .05$.

FIGURE 2—HRR (95% CI) for autism among ART-originated and non–ART-originated children estimated for all births and the analysis subset: California, 1997–2007.

infant was born and had a lower education level. However, statistical tests for interaction showed that only the maternal age and education differentials were statistically significant.

DISCUSSION

We found an elevated incidence of diagnosed autism among children originated through ART in the overall population as well as among subgroups divided by plurality, parity, infant gender, maternal age and education, and paternal age. At the population level, children originated with ART in California had more than twice the incidence of autism of children originated without ART. After adjustment for demographics and other factors associated with both ART and autism, including maternal age and parity, this increased incidence remained statistically significant for children born to mothers younger than 35 years. The elevated incidence may be owing, primarily, to the higher incidence of adverse pregnancy and labor outcomes including multiple births.

Population-level studies in Denmark and Sweden failed to find a statistically significant difference in risk for autism from IVF, but they did find elevated risk in certain subgroups, for example, girls[18] and children resulting from certain types of IVF procedures.[22] We also found a higher point estimate for the female subgroup; however, it did not differ statistically from the estimate for boys. Another study found no association between multiple births and autism estimated prevalence (although it did show elevated estimated prevalence of cerebral palsy) among 1994 US births,[19] which contrasts with our finding of elevated rates of diagnosed autism among multiple births.

The stratified analyses showed statistically significant associations between autism and ART in most subgroups; yet, in general, ART appeared to add less risk for groups whose pregnancies were already at higher risk for autism, such as male infants, older parents, primiparous mothers, and those with more education. There are many potential mechanisms through which ART could be associated with autism, including the biological factors related to the underlying fertility or quality of the germ cells, effects of the fertility hormones used during ART, other effects of the ART procedure, and the prenatal and perinatal complications associated with ART treatment.[24] We did not design this study to distinguish between most of these mechanisms. Yet on the basis of the evidence, we suspect that the elevated incidence we observed may come through the causal pathway of increasing adverse labor and pregnancy outcomes. That is, although ART may be associated with autism in part because mothers who use ART are older and more likely to be primiparous (indicating a possible role for underlying infertility), ART also may increase the chance of other, intermediate autism risk factors.

We found that multiple births played an important role in the association between ART and autism—in fact, the adjusted risk arising from ART was not significantly elevated among singletons (who make up less than half of ART-originated children). Twin and higher-order multiple births are a direct (although not necessary) consequence of ART procedures. Preterm and small for gestation age births, diabetes, hypertension, and cesarean deliveries also appear to be mediating factors in the ART–autism association; however, this effect may be indirect, by producing risky pregnancies that, without ART, would not have occurred. The mechanisms behind this association require further investigation.

A key advantage of our study is its size, which provides greater statistical power than any previous analysis. We included more than twice as many children, 7 times as many autism

diagnoses, and 6% more IVF exposures than did the largest previous study. Significantly, this means that these data included nearly 6 times as many children born from IVF and diagnosed with autism, which enabled us to produce more efficient estimates, particularly for adjusted models.

In addition, differences between the US and Scandinavian countries in procedures in which multiple embryos are transferred means that this study was better positioned to address the role of multiple births. In Sweden since 2003, the majority of ART procedures have involved single-embryo transfer, and the rate of multiple births has declined as a result.[50] Differences between this study and the Scandinavian studies suggest that the impact of ART on autism risk can vary by region, perhaps as a result of differing usage patterns, which highlights the need for more research. We did not examine the association between autism and specific types of ART procedures or infertility diagnoses, an obvious area for future research that will help us understand the mechanisms of association.

Limitations

Our study did have several limitations. We constructed our data through 2 imperfect linking procedures, which may have biased results. In addition, enrollment in the DDS is voluntary and so may omit some eligible children with autism. Although estimates of autism incidence as determined by the DDS are consistent with other population-based estimates,[1] at best this is incidence of diagnosed autism. Moreover, the DDS requirement for diagnosis of autistic disorder to receive services further limits inference to children with other ASDs and would miss any potential association between ART and milder conditions on the autism spectrum.

In our analysis, we could not distinguish between mechanisms arising from the ART procedure itself and any preexisting biological differences associated with infertility. Finally, as with all observational studies, it is possible that we did not include unmeasured demographic or parental characteristics in our limited socioeconomic status measures that may be responsible for the observed association.

Conclusions

We have provided evidence of an elevated incidence of diagnosed autism among children originated by ART, and we have found that multiple births and complications of pregnancy and delivery likely play mediating roles in the association. Increased incidence of autism persisted after adjustment for confounders as well as in most subgroups except singleton children. Adjusting for adverse prenatal and perinatal complications, including multiple births, reduced the association between ART and autism for younger mothers and eliminated it for older mothers. Although the precise mechanism is unclear, these results suggest that children born as a result of ART may have an elevated incidence of autism potentially because of the link between ART and adverse pregnancy and labor outcomes, especially multiple births. Although more research is needed, this suggests that single-embryo transfer,[13,51] when appropriate, may reduce the risk of autism among ART-originated infants. ■

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C. Fountain conducted data analysis and wrote the article. C. Fountain and Y. Zhang conducted the linking. C. Fountain and P. Bearman designed the study. All authors provided scientific input and article revisions.

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Human Participant Protection

This study was approved by the institutional review boards of Columbia University, the Centers for Disease Control and Prevention, and the California Committee for the Protection of Human Subjects.

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