Early Autism Spectrum Disorders in Children Born to Fertile, Subfertile, and ART-Treated Women

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Abstract

Introduction We examined the prevalence of autism spectrum disorders (ASDs) in Massachusetts (MA) comparing children born via assisted reproductive technology (ART) and children born to women with indicators of subfertility but no ART (Subfertile), to children born to women with neither ART nor indicators of subfertility (Fertile). We assessed the direct, indirect, and total effects of ART and subfertility on ASD among singletons.

Methods This study included 10,147 ART, 8072 Subfertile and 441,898 Fertile MA resident births from the MA Outcome Study of ART (MOSART) database linked with Early Intervention program participation data. ART included fresh in vitro fertilization (IVF), fresh intracytoplasmic sperm injection (ICSI), and frozen embryo transfer. We estimated the prevalence of ASD by fertility group. We used logistic regression to assess the natural direct effect (NDE), natural indirect effect (NIE) through preterm birth, and total effects of each fertility group on ASD.

Results The NDE indicated that, compared to the Fertile group, the odds of ASD were not statistically higher in the ART (OR_{NDE} 1.07; 95% CI 0.88–1.30), Subfertile (OR_{NDE} 1.11; 95% CI 0.89–1.38), IVF (OR_{NDE} 0.91; 95% CI 0.68–1.22), or ICSI (OR_{NDE} 1.13; 95% CI 0.84–1.51) groups, even if the rate of preterm birth was the same across all groups. The total effect (product of NDE and NIE) was not significant for ART ($OR_{Total\ Effect}$ 1.08; 95% CI 0.89–1.30), Subfertile ($OR_{Total\ Effect}$ 1.11; 95% CI 0.89–1.38), IVF ($OR_{Total\ Effect}$ 0.92; 95% CI 0.69–1.23), or ICSI ($OR_{Total\ Effect}$ 1.13; 95% CI 0.84–1.52).

Conclusion Compared to children born to Fertile women, children born to ART, ICSI, or IVF, or Subfertile women are not at increased risk of receiving an ASD diagnosis.

Keywords Assisted reproductive technology · In vitro fertilization · Intracytoplasmic sperm injection · Autism spectrum disorders · Subfertile · MOSART

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Significance

What is Already Known About the Subject?

Increased risk of autism following infertility and/or infertility treatment has been suggested by various studies; however, the effect of assisted reproductive technology (ART) and maternal subfertility on autism spectrum disorders (ASDs) among children aged 0–3 years has not been clearly established in population-based studies in the US.

What This Study Adds?

Compared to singleton children born to Fertile women, the odds of ASD were not higher among children born via ART and children born to women with subfertility. This study

shows the degree to which intervening on preterm birth using a mediation model could better estimate ASD.

Introduction

The use of ART procedures has steadily risen over the last decades. The proportion of births attributable to ART in Massachusetts (MA) reached 4.8% compared to 1.6% for the national average in 2013 (Sunderam et al. 2013). Parallel to this increase, the prevalence of ASDs in the US and other developed countries has increased significantly (Newschaffer et al. 2005; Fombonne et al. 2006; Parner et al. 2008; Hvidtjorn et al. 2011). In MA, the incidence of ASDs increased from 56 per 10,000 children in 2001 to 93 per 10,000 in 2005. Data from the Massachusetts Department of Public Health (MDPH) Early Intervention (EI) program indicate a 50% increase in ASDs incidence from 94 in 2006 to 141 per 10,000 in 2012, or 1 in 71 births (unpublished data, MDPH).

Previous research showed that children conceived via ART and those born to women with indicators of subfertility but no ART ("Subfertile" in this study, defined from hospital records and birth certificate data indicating prior treatment for conditions related to infertility), are more likely to have adverse perinatal outcomes including multiple birth, preterm birth, low birthweight, and small for gestational age, even among singletons (Wright et al. 2003; Schieve et al. 2004; Declercq et al. 2015; Luke et al. 2016a, b). These same adverse outcomes have been associated with a higher likelihood of ASDs (Manning et al. 2011). Past research also indicated that regardless of preterm status, singletons born to women who used ART and Subfertile women had higher odds of being enrolled in EI programs, a proxy for developmental delays (Diop et al. 2016).

Concerns about the risk of autism following infertility and/or infertility treatment have been suggested by various studies. In a population-based study in the US, the authors found that intracytoplasmic sperm injection (ICSI) is associated with an increased incidence of autism diagnosis. However, this study was restricted to ART-conceived children and concluded that additional studies are needed to explain the increased risk and safety of ICSI (Kissin et al. 2015). A Danish population-based study found that the risk of infantile autism was lower among children born to women who used in vitro fertilization (IVF) and ICSI (Maimburg and Vaeth 2007). A nested case–control study did not find an association between ART and history of infertility and ASD, but suggested an increased risk with ovulation inducing drug and artificial insemination (Lyall et al. 2012). A systematic review of seven studies concluded that ART did not appear to be a risk factor for ASDs, but suggested that large and high quality studies are needed to further explain the association between ART and ASDs (Conti et al. 2013).

While results from past studies on the risk of autism after ART have been mixed, few have examined the risk of autism among children born to parents with a history of infertility. In a case–control study, the authors found that several infertility indicators were associated with an increased risk of ASD among multiples. Infertility indicators were defined as having documented infertility medications from pharmacy records dispensed to women any time in the past or around the time of the index pregnancy or ICD-9-CM (International Classification of Diseases, Ninth Revision, Clinical Modification) codes for female or male infertility. However, they concluded that their findings should be interpreted with caution due to insufficiently detailed data on treatments associated with ART and small study sample (n=21 cases and n=54 controls) (Grether et al. 2013). Recent research suggests that with few exceptions, the excess morbidity for women and children results from the underlying cause of infertility rather than ART treatment parameters alone (Luke et al. 2016a, b).

ASDs are serious lifelong neurodevelopmental disorders characterized by impairments in social function, communication, and social behaviors (First 2013) and can be diagnosed as early as 1–2 years of age (Kotsopoulos 2015). The Healthy People 2020 goal is to have children with ASD be evaluated by age 36 months and community-based support and services initiated by age 48 months, for improved developmental and educational outcomes (Rogers et al. 2014; Christensen et al. 2016).

The association of ART, subfertility and ASDs among children aged 0–3 years has not been clearly established in population-based studies in the US. Our objectives were to (1) examine the prevalence of, and characteristics associated with ASD diagnosis comparing children conceived via ART, children born to women with indicators of subfertility but no ART (Subfertile), and children born to women with neither ART nor indicators of subfertility (Fertile), (2) conduct a sub-analysis of specific types of ART, and (3) assess the direct, indirect (through preterm birth), and total effects of ART and subfertility on early ASD among singletons using a mediation approach. Prior studies have adjusted for preterm birth as a confounder, which is "not only unnecessary", but "it can also be harmful" for estimation of total effects (Ananth and Schisterman 2017). To our knowledge, this is the first population-based study in the US that used a mediation approach to show the degree to which intervening on preterm could better estimate ASD.

Methods

Study Design and Setting

We conducted a longitudinal cohort study of live births in MA from July 1, 2004 through December 31, 2010 and

children who participated in EI programs between July 1, 2004 and December 31, 2013.

Data Sources

This study linked the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS), the MA Pregnancy to Early Life Longitudinal (PELL), and EI program participation data. All ART clinics in MA during our study period contributed data to SART CORS, including patient demographic characteristics, medical history, infertility diagnoses, specific ART treatments and pregnancy outcomes. SART CORS has been described elsewhere (Luke et al. 2012).

PELL is a population-based data system that links delivery records to hospital discharge records for both women and children. This core dataset is longitudinally linked probabilistically to non-birth-related inpatient admissions, observational stays, and emergency room visits. More than 99% of MA deliveries from 1998 to 2010 have been linked in PELL. PELL has been described elsewhere (Nannini et al. 2011). PELL allows for uniquely identifying deliveries and children born to the same woman, and multiple hospitalization records belonging to the same women or children to be classified as such.

EI program participation data are collected by MDPH; EI provides services at no cost to children aged 0–3 years with established developmental delays, social/emotional, and environmental factors. MA children at risk for ASD are screened using the Modified Checklist for Autism in Toddlers (Snow and Lecavalier 2008) administered by an interdisciplinary team, including a pediatrician, child psychologist, behavioral specialist, child neurologist, and child psychiatrist, or licensed mental health counselor. This interdisciplinary team evaluates young children to establish the diagnosis of ASD before children are enrolled in autism specialty services (Manning et al. 2011). Once children are determined to be eligible for ASD services, an Individualized Family Service Plan (IFSP) is developed by EI providers and parents. Treatment data for ASDs are obtained through statewide coordinated EI services (Clements et al. 2006).

Data Linkage and Study Sample

The Massachusetts Outcomes Study of Assisted Reproductive Technology (MOSART) database was created using a five-phase algorithm to link PELL and SART CORS, via women's first and last name, father's last name, and women's and baby's dates of birth and yielding an overall linkage rate of 89.7% (Kotelchuck et al. 2014). The MOSART database from July 1, 2004 through December 31, 2010 included

474,784 deliveries resulting in 486,075 live births and fetal deaths, linked to 70,086 ART cycles from SART CORS.

Children enrolled in the EI program are linked to PELL using a unique child ID. A probabilistic algorithm comprised of nine steps was used to match EI program data with PELL using LinkPro software (InfoSoft, Inc., Winnipeg, Manitoba, Canada) and multiple permutations of child's first and last names, mother's last name, child's and mother's date of birth, child's sex, and zip code. Overall 86% of EI children linked to a PELL record. Linked PELL–EI data including children born from July 1, 2004 through December 31, 2010 (n = 486,075) and children with any referral in EI between July 1, 2004 and December 31, 2013 (n = 130,201) were merged with MOSART. After excluding fetal and infant deaths (n = 4500) and multiple births (n = 21,458), our final study sample included 460,117 singletons, which were classified according to maternal fertility group (10,147 ART, 8072 Subfertile and 441,898 Fertile). ART is defined as all treatments in which both eggs and sperm are manipulated in vitro and includes fresh IVF, fresh ICSI, and frozen embryo transfer (FET). For FET cycles the method of insemination (IVF vs. ICSI) is not recorded in SART CORS. After excluding FET and missing insemination method, we conducted a sub-analysis of IVF (n = 4834) and ICSI (n = 3904). Figure 1 illustrates our study sample.
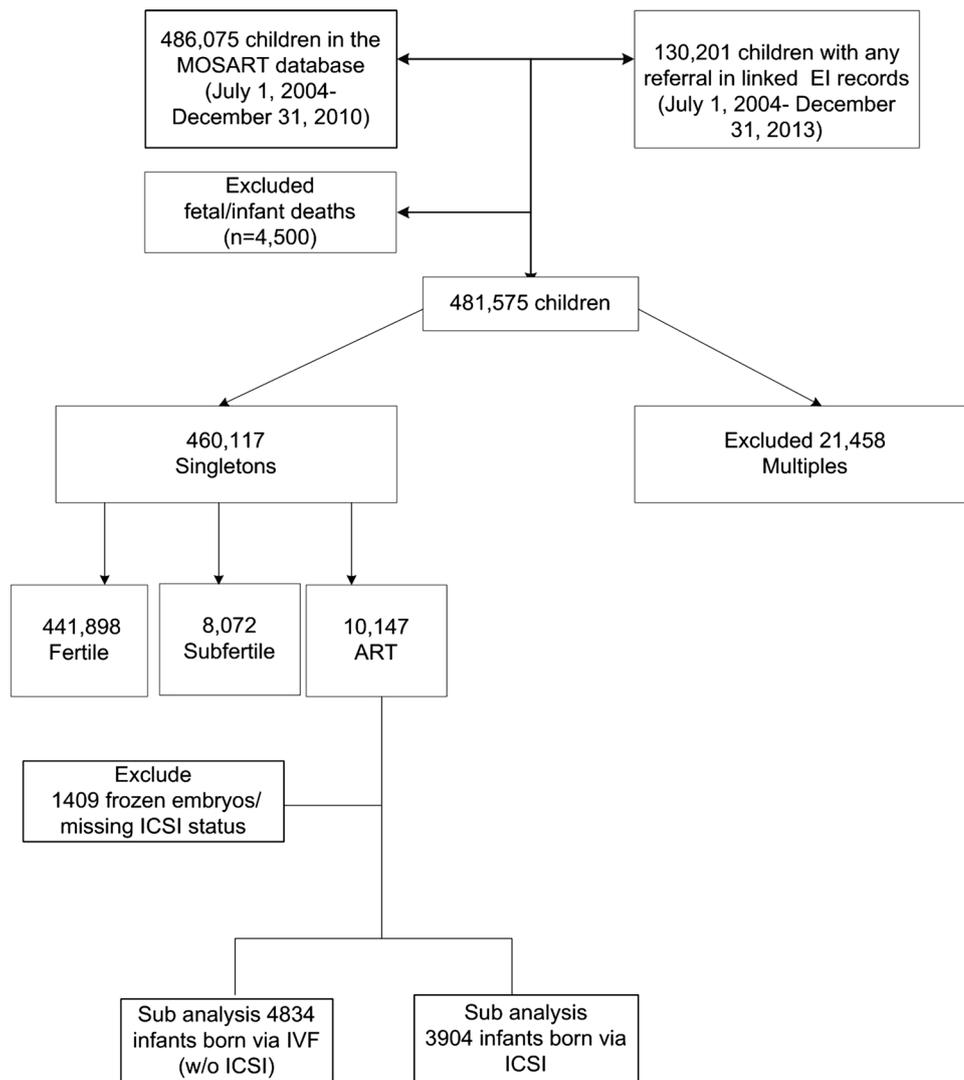
Dependent Variable

Our dependent variable was an ASD diagnosis among children aged 0–3 years, defined as receiving autism-related services in the EI specialty services program with a diagnosis of autistic disorder (ICD-9-CM code 299.00), Asperger's disorder (299.80), pervasive disorders not otherwise specified (299.90), or ASD or childhood disintegrative disorder (299.10).

Independent Variables and Mediator

Our main independent variable was maternal fertility status (ART, Subfertile, or Fertile group) for the delivery of the index child; our mediator was preterm birth defined as a delivery at < 37 weeks gestation. To assess the direct, indirect and total effects of maternal fertility group on ASDs, we conducted separate analyses comparing children born to Fertile women with (1) children born via ART, (2) children born to Subfertile women, (3) children born via IVF, and (4) children born via ICSI.

Our subfertility measure (Subfertile) was derived from the MOSART database and was defined as having (1) at least one of the two fertility-treatment questions [ART (e.g., artificial insemination, IVF) or Fertility Drug (e.g., Clomid, or Pergonal)] on the MA birth certificate checked as "yes" for the index delivery, (2) a prior hospital encounter for a

Fig. 1 Flow diagram of study sample with inclusion and exclusion criteria

Covariates

Covariates were selected based on known risk factors associated with ASDs and ART and included parental demographics (maternal and paternal age, race, education, marital status, and nativity), insurance, smoking, prenatal care, parity, gender, method of delivery, gestational and chronic hypertension and diabetes, and breech presentation.

condition specifically related to infertility (ICD-9-CM codes 628.0, 628.2, 628.3, 628.8, 628.9, V230), or (3) if a woman had an ART cycle or other fertility treatment indicated on the birth certificate in the 5 years prior to the index delivery but did not have an ART cycle associated with the current index delivery. We removed those cases where a woman received ART for the current index delivery and the remaining deliveries were classified as Subfertile (Declercq et al. 2014).

Mediation Approach

We used a mediation approach which assumes no unmeasured confounding to account for the known associations of ASD and prematurity and prematurity and ART (Ananth and VanderWeele 2011). We assumed that adjusting for the covariates listed above would be satisfactory to control for confounding of (1) exposures–outcome (fertility groups–ASD), (2) mediator–outcome (preterm–ASD), and (3) exposures–mediator (fertility groups–preterm). The mediation approach is used when a variable of interest is on the causal pathway between the exposure and the outcome (MacKinnon et al. 2007; Ananth and VanderWeele 2011). Prior studies have adjusted for preterm birth as a confounder, which is "not only unnecessary," but "it can also be harmful" for estimation of total effects (Ananth and Schisterman 2017). In this study, our mediator, preterm birth, is associated with higher odds of ASD (Manning et al. 2011; Diop et al. 2016), and ART and subfertility are associated

with higher odds of preterm birth (Schieve et al. 2004; Luke et al. 2015). The mediation approach allowed us to split the total effect of each of our exposure groups on early ASDs into a natural direct effect (NDE) and a natural indirect effect (NIE; Valeri and Vanderweele 2013). Our theoretical causal framework for mediation is shown in Fig. 2.

Statistical Analysis

We examined maternal and infant characteristics among singleton ART, Subfertile, Fertile, IVF, and ICSI, and estimated the prevalence of early ASD using χ^2 statistics ($\alpha = 0.05$). We used logistic regression to estimate crude and adjusted odds ratios (aORs) and 95% confidence intervals (CIs) to verify the three relationships for determining mediation. Based on our mediation framework, we estimated the NDE and NIE of the ART–ASD relationship by fitting a model for ASD (Y), conditional on ART (X), preterm birth (M), and ART–preterm birth interaction (X × M) and the covariates (C). We then fit a model for preterm birth (M), conditional on ART (X) and the covariates (C). We conducted similar analyses using Subfertile, IVF, and ICSI as exposures. We ruled out the possibility of effect modification of preterm birth on the association of ART, IVF, ICSI and Subfertile and ASD after we examined the interaction terms in our models. The mediation analysis was conducted using a SAS macro (Valeri and Vanderweele 2013). All analyses were conducted using SAS software version 9.3 (SAS Institute, Inc., Cary, NC, USA). This study was approved by the Institutional Review Boards of the MDPH and Dartmouth College and the SART Research Committee.

Results

Compared to Fertile, women in the ART, Subfertile, IVF, and ICSI groups were generally older, with a higher proportion giving birth at or after age 35. Compared to Fertile (66.3%), the proportion of White non-Hispanic women was higher in the ART (85.1%), Subfertile (84.4%), IVF (84.4%), and ICSI (85.7%) groups. In addition, women in the ART, Subfertile, IVF, and ICSI groups were more likely to be US born, highly educated, married, on private insurance, with less inadequate prenatal care, deliver via primary

cesarean, with higher proportion of pregnancy and chronic hypertension, gestational diabetes and chronic diabetes, preterm birth, and breech delivery (Table 1). Table 2 shows the prevalence of early ASD by maternal, paternal, and infant characteristics. The prevalence of ASD was higher among children born to women and fathers aged 38 years or more, Hispanic and Black non-Hispanic women, women with some college or associate degree, hypertension and diabetes (chronic and pregnancy), cesarean delivery, on public insurance, male children, and children born preterm or via breech. Table 3 shows the prevalence of early ASDs by fertility group. Compared to the Fertile group, the prevalence of ASD was 1.2% (p < 0.05) in the ART group, 1.1% (p = 0.35) in the Subfertile group, 1.0% (p = 0.85) in the IVF, and 1.3% (p = 0.07) in the ICSI group.

Crude and aORs used to assess our mediation conditions are presented in Table 4. In the adjusted models, compared to the Fertile group, the relationship between ART and ASD was not significant (aOR 1.08; 95% CI 0.89–1.31). Similarly, the relationships between Subfertile, IVF, ICSI, and ASD were not significant. However, ART was significantly associated with preterm birth (aOR 1.51; 95% CI 1.40–1.63) and preterm birth was significantly associated with ASD (aOR 1.31; 95% CI 1.17–1.47). Likewise, when compared to the Fertile group, Subfertile (aOR 1.17; 95% CI 1.06–1.28), IVF (aOR 1.50; 95% CI 1.35–1.67), and ICSI (aOR 1.42; 95% CI 1.27–1.60) were all significantly associated with preterm birth, and preterm birth was significantly associated with ASD in the Subfertile (aOR 1.34; 95% CI 1.20–1.50), IVF (aOR 1.33; 95% CI 1.18–1.49), and ICSI (aOR 1.32; 95% CI 1.17–1.48) groups.

Table 5 shows the results of the mediation analysis. The NDE indicated that, compared to the Fertile group, the odds of early ASD were not meaningfully higher in the ART (OR_{NDE} 1.07; 95% CI 0.88–1.30), Subfertile (OR_{NDE} 1.11; 95% CI 0.89–1.38), IVF (OR_{NDE} 0.91; 95% CI 0.68–1.22) and ICSI (OR_{NDE} 1.13; 95% CI 0.84–1.51) groups, even if the rate of preterm birth is the same across all groups. The NIEs (odds of early ASD among the exposure groups under their observed preterm rates) are statistically significant; however, the ORs were equal to 1.00 for the Subfertile (OR_{NIE} Subfertile vs. Fertile 1.00; 95% CI 1.00–1.00) and ICSI (OR_{NIE} ICSI vs. Fertile 1.00; 95% CI 1.00–1.01) groups, and nearly equal to 1.00 for both ART (OR_{NIE}

Fig. 2 Mediation framework used to assess whether preterm is a mediator of ART or subfertility in autism spectrum disorders
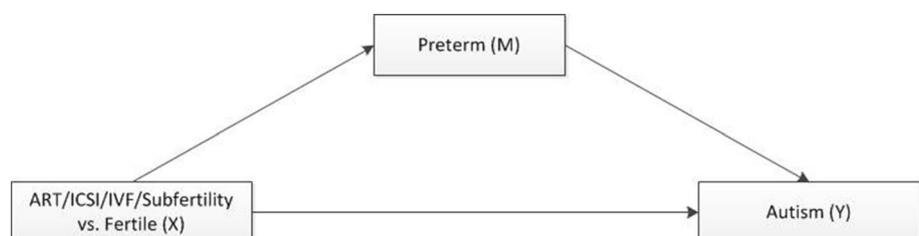
Table 1 Characteristics of study sample, by fertility groups, Massachusetts birth July 1, 2004–December 31, 2010

Demographic characteristics	Fertile	Subfertile		ART		ICSI		IVF	
n	441,898	8072		10,147		3904		4834	
	%	%	*p* value	%	p-value	%	p-value	%	p-value
Mother's age									
≤ 30	55.0	16.4	< 0.0001	11.9	< 0.0001	10.9	< 0.0001	13.7	< 0.0001
31–34	24.2	28.9		27.2		26.8		27.9	
35–37	12.5	26.2		24.8		25.1		24.1	
38–40	6.1	18.3		20.4		21.3		20.2	
41–42	1.5	6.7		8.7		9.6		8.4	
43+	0.6	3.5		6.9		6.2		5.8	
Race/ethnicity									
Hispanic	14.9	4.5	< 0.0001	3.4	< 0.0001	3.1	< 0.0001	3.8	< 0.0001
Non-Hispanic White	66.3	84.4		85.1		85.7		84.4	
Non-Hispanic Black	8.9	3.1		3.1		2.6		3.4	
Asian/Pacific Islands	7.7	6.9		7.4		7.7		7.3	
American Indians and others	2.2	1.1		1.0		0.9		1.1	
Education									
< High school or HS/GED	37.8	11.8	< 0.0001	9.4	< 0.0001	8.6	< 0.0001	10.4	< 0.0001
Some college/associate	21.5	17.7		15.4		14.9		16.3	
BA degree or post-graduate	40.7	70.5		75.2		76.5		73.3	
Marital status									
Married	65.1	94.3	< 0.0001	95.6	< 0.0001	95.5	< 0.0001	95.8	< 0.0001
Not married	34.9	5.7		4.4		4.5		4.2	
Prenatal care									
Inadequate/intermediate	16.8	9.0	< 0.0001	7.2	< 0.0001	7.0	< 0.0001	7.0	< 0.0001
Adequate	45.9	43.9		43.7		44.2		42.3	
Adequate plus	37.3	47.1		49.2		48.7		50.7	
Parity									
1	45.8	37.9	< 0.0001	62.4	< 0.0001	65.9	< 0.0001	65.2	< 0.0001
2	34.1	40.2		30.0		27.5		28.9	
3+	20.1	21.9		7.5		6.6		5.9	
Method of delivery									
Vaginal	67.5	53.9	< 0.0001	52.9	< 0.0001	54.8	< 0.0001	53.8	< 0.0001
VBAC	2.0	3.2		1.4		1.4		1.2	
Primary C-section	18.0	20.5		32.1		32.4		32.6	
Repeated C-section	12.6	22.4		13.6		11.4		12.3	
Father's age									
≤ 30	38.9	10.3	< 0.0001	7.1	< 0.0001	6.9	< 0.0001	7.8	< 0.0001
31–34	25.0	23.2		21.9		23.2		21.1	
35–37	15.6	24.3		23.1		23.8		22.1	
38–40	10.1	20.0		19.8		20.8		18.5	
41–42	4.0	8.5		9.4		9.5		9.3	
43+	6.4	13.8		18.7		15.8		21.2	
Chronic hypertension									
No	98.4	97.4	< 0.0001	96.9	< 0.0001	97.0	< 0.0001	97.3	< 0.0001
Yes	1.6	2.6		3.1		3.0		2.7	
Pregnancy hypertension									
No	91.4	89.8	< 0.0001	87.4	< 0.0001	88.5	< 0.0001	87.4	< 0.0001
Yes	8.6	10.2		12.6		11.5		12.6	

Table 1 (continued)

Demographic characteristics	Fertile	Subfertile		ART		ICSI		IVF	
n	441,898	8072		10,147		3904		4834	
	%	%	p value	%	p-value	%	p-value	%	p-value
Chronic diabetes									
No	98.8	98.3	< 0.0001	98.0	< 0.0001	98.0	< 0.0001	98.0	< 0.0001
Yes	1.2	1.7		2.0		2.0		2.0	
Gestational diabetes									
No	94.4	91.5	< 0.0001	91.8	< 0.0001	92.4	< 0.0001	91.4	< 0.0001
Yes	5.6	8.5		8.2		7.6		8.6	
Preterm									
No	93.8	92.5	< 0.0001	89.9	< 0.0001	90.2	< 0.0001	90.2	< 0.0001
Yes	6.2	7.5		10.1		9.8		9.8	
Breech									
No	97.0	96.3	< 0.01	94.2	< 0.0001	94.4	< 0.0001	94.4	< 0.0001
Yes	3.0	3.7		5.8		5.6		5.6	
Mother's nativity									
Non-US	28.0	18.3	< 0.0001	18.9	< 0.0001	17.7	< 0.0001	20.3	< 0.0001
US-born	72.0	81.7		81.1		82.3		79.7	
Infant gender									
Male	51.2	51.2	0.90	51.3	0.82	53.1	0.04	49.5	0.01
Female	48.8	48.8		48.7		46.9		50.5	
Payer									
Private	57.7	91.3	< 0.0001	96.7	< 0.0001	96.9	< 0.0001	96.5	< 0.0001
Public	42.3	8.7		3.3		3.1		3.5	

HS/GED high school/general educational development

ART vs. Fertile 1.01; 95% CI 1.00–1.01) and IVF (OR_{NIE} IVF vs. Fertile 1.01; 95% CI 1.00–1.01). The total effect which is the product of NDE and NIE was not significant for ART ($OR_{Total Effect}$ 1.08; 95% CI 0.89–1.30), Subfertile ($OR_{Total Effect}$ 1.11; 95% CI 0.89–1.38), ICSI ($OR_{Total Effect}$ 1.13; 95% CI 0.84–1.52), and IVF ($OR_{Total Effect}$ 0.92; 95% CI 0.69–1.23). The total effect was equal to the aORs in Table 4 across all exposure groups.

Discussion

As the proportion of ART infants remains significantly above national averages in MA, it is important to improve our understanding of the long-term health outcomes of these children. This study examined the likelihood of receiving an ASD diagnosis in the first 3 years of life among singletons. Compared to children born to Fertile women, the odds of early ASD were not higher among children born to ART, Subfertile, IVF, and ICSI groups through a direct mechanism (i.e., the NDE effects were not significant). Our findings are consistent with other case–control and prospective studies

in the US and Sweden (Lyall et al. 2012; Conti et al. 2013; Grether et al. 2013; Rogers et al. 2014).

A Danish population-based study found that the risk of infantile autism was lower among children born to women who used IVF and ICSI; the authors speculate that women who use assisted conception were more likely to interact with the health care system, and may have better behaviors and experiences early on during pregnancy. While this study raised an interesting question, it was limited by the small study sample as there were only 10 cases and 23 controls (Maimburg and Vaeth 2007).

A large population-based retrospective study in California found that the incidence of autism diagnosis was higher among children born to women who conceived via ICSI when compared to those who utilized traditional IVF. The study also found that compared to other types of infertility, there is reduced association of autism diagnosis among singletons born to parents with unexplained infertility and among multiples born to parents with tubal factors. While this study had a large sample size (n = 42,383), it was restricted to ART-conceived children and did not compare autism diagnosis among ART to non-ART conceived

Table 2 Prevalence of autism spectrum disorder among singletons, Massachusetts birth July 1, 2004–December 31, 2010

Demographic characteristics	Autism (%)	No autism (%)	p-value
Mother's age			
≤ 30	1.0	99.0	< 0.0001
31–34	0.9	99.1	
35–37	1.0	99.0	
38–40	1.2	98.8	
41–42	1.2	98.8	
43+	1.3	98.7	
Race/ethnicity			
Hispanic	1.2	98.8	< 0.0001
Non-Hispanic White	1.0	99.0	
Non-Hispanic Black	1.1	98.9	
Asian/Pacific Islands	0.8	99.2	
American Indians and others	0.9	99.1	
Education			
< High school or HS/GED	1.1	98.9	< 0.0001
Some college or associate	1.2	98.8	
BA degree or post-graduate	0.8	99.2	
Marital status			
Married	0.9	99.1	< 0.0001
Not married	1.1	98.9	
Prenatal care			
Inadequate/intermediate	1.0	99.0	0.0001
Adequate	0.9	99.1	
Adequate plus	1.1	98.9	
Parity			
1	1.1	98.9	< 0.0001
2	1.0	99.0	
3+	0.8	99.2	
Method of delivery			
Vaginal	0.9	99.1	< 0.0001
VBAC	0.7	99.3	
Primary C-section	1.3	98.7	
Repeated C-section	1.0	99.0	
Father's age			
≤ 30	1.0	99.0	< 0.0001
31–34	0.9	99.1	
35–37	0.9	99.1	
38–40	1.1	98.9	
41–42	1.2	98.8	
43+	1.2	98.8	
Chronic hypertension			
No	1.0	99.0	< 0.01
Yes	1.3	98.7	
Pregnancy hypertension			
No	1.0	99.0	< 0.0001
Yes	1.3	98.7	

Table 2 (continued)

Demographic characteristics	Autism (%)	No autism (%)	p-value
Chronic diabetes			
No	1.0	99.0	< 0.0001
Yes	1.5	98.5	
Gestational diabetes			
No	1.0	99.0	< 0.0001
Yes	1.4	98.6	
Preterm			
No	1.0	99.0	< 0.0001
Yes	1.5	98.5	
Breech			
No	1.0	99.0	0.01
Yes	1.2	98.8	
Mother's nativity			
Non-US	0.8	99.2	< 0.0001
US-born	1.1	98.9	
Infant gender			
Male	1.6	98.4	< 0.0001
Female	0.4	99.6	
Payer			
Private	0.9	99.1	< 0.0001
Public	1.1	98.9	

HS/GED high school/general educational development

Table 3 Prevalence of autism spectrum disorder among singletons, by fertility groups, Massachusetts birth July 1, 2004–December 31, 2010

Demographic characteristics	Autism n (%)	No autism n (%)	p-value
Fertility group			
Fertile	4363 (1.0)	437,535 (99.0)	Ref.
Subfertile	88 (1.1)	7984 (98.9)	0.35
ART	120 (1.2)	10,027 (98.8)	< 0.05
ICSI	50 (1.3)	3854 (98.7)	0.07
IVF	49 (1.0)	4785 (99.0)	0.85

children and to children born to women with indicators of subfertility (Kissin et al. 2015).

Our study confirmed that singletons born to ART, Subfertile, IVF, ICSI groups were associated with a greater risk of preterm birth, which is consistent with prior studies (Perri et al. 2001; Pandey et al. 2012; Diop et al. 2016). We also found that compared to the Fertile group, the NIEs (odds of early ASD among children born to ART, Subfertile, IVF, and ICSI groups under their observed preterm birth rate) were significant, however, the ORs were close to 1, indicating that risk of ASD increased by only 1% or less through preterm birth. The total effect which is the

Table 4 Unadjusted and adjusted odds rations from logistic regression models for each of the criteria for the mediation analysis in singletons, Massachusetts birth July 1, 2004–December 31, 2010

Criteria for mediation	Unadjusted OR[a] (95% CI)	p-value	Adjusted OR[b] (95% CI)	p-value
ART versus Fertile (n = 404,604)				
(1) Relationship between ART and ASD	1.20 (1.00, 1.45)	0.05	1.08 (0.89, 1.31)	0.43
(2) Relationship between ART and preterm	1.80 (1.69, 1.93)	< 0.0001	1.51 (1.40, 1.63)	< 0.0001
(3) Relationship between preterm and ASD	1.57 (1.41, 1.75)	< 0.0001	1.31 (1.17, 1.47)	< 0.0001
Subfertile versus Fertile (n = 402,511)				
(1) Relationship between Subfertile and ASD	1.13 (0.91, 1.40)	0.26	1.11 (0.89, 1.39)	0.40
(2) Relationship between Subfertile and preterm	1.28 (1.17, 1.39)	< 0.0001	1.17 (1.06, 1.28)	< 0.01
(3) Relationship between preterm and ASD	1.61 (1.44, 1.79)	< 0.0001	1.34 (1.20, 1.50)	< 0.0001
ICSI versus Fertile (n = 398,564)				
(1) Relationship between ICSI and ASD	1.26 (0.94, 1.68)	0.12	1.13 (0.85, 1.53)	0.29
(2) Relationship between ICSI and preterm	1.75 (1.57, 1.95)	< 0.0001	1.42 (1.27, 1.60)	< 0.0001
(3) Relationship between preterm and ASD	1.59 (1.42, 1.77)	< 0.0001	1.32 (1.17, 1.48)	< 0.0001
IVF versus Fertile (n = 399,421)				
(1) Relationship between IVF and ASD	1.05 (0.79, 1.40)	0.74	0.92 (0.69, 1.23)	0.58
(2) Relationship between IVF and preterm	1.76 (1.59, 1.94)	< 0.0001	1.50 (1.35, 1.67)	< 0.0001
(3) Relationship between preterm and ASD	1.59 (1.43, 1.78)	< 0.0001	1.33 (1.18, 1.49)	< 0.0001

Data with missing covariates are excluded in all models

[a]For the first two assumptions, the unadjusted OR is the crude OR of the association. For the third assumption, the unadjusted OR is the association of ASD (outcome) and preterm (mediator), controlling only for fertility status (exposure)

[b]Models adjusted for maternal demographics (maternal paternal age, race, education, marital status, nativity), insurance, smoking, prenatal care, parity, gender, method of delivery, chronic and pregnancy hypertension, gestational and chronic diabetes, breech

Table 5 Estimates of natural direct, natural indirect and total effect of the association between ART, Subfertility, ICSI, IVF and ASD mediated through preterm birth, in singletons, Massachusetts birth July 1, 2004–December 31, 2010

	Natural direct effect		Natural indirect effect		Total effect	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
ART versus Fertile	1.07 (0.88, 1.30)	0.49	1.01 (1.00, 1.01)	< 0.01	1.08 (0.89, 1.30)	0.46
Subfertile versus Fertile	1.11 (0.89, 1.38)	0.35	1.00 (1.00, 1.00)	0.01	1.11 (0.89, 1.38)	0.34
ICSI versus Fertile	1.13 (0.84, 1.51)	0.43	1.00 (1.00, 1.01)	< 0.01	1.13 (0.84, 1.52)	0.41
IVF versus Fertile	0.91 (0.68, 1.22)	0.53	1.01 (1.00, 1.01)	< 0.01	0.92 (0.69, 1.23)	0.56

Models adjusted for maternal demographics (maternal paternal age, race, education, marital status, nativity), insurance, smoking, prenatal care, parity, gender, method of delivery, chronic and pregnancy hypertension, gestational and chronic diabetes, breech. Data with missing covariates were excluded in all models

product of NDE and NIE was not significant for ART, Subfertile, IVF, or ICSI.

Our study has limitations. The study population was limited to MA and may not be generalizable to other states or countries. Children born in MA who received EI services outside of MA were not captured. While the American Academy of Pediatrics (AAP) recommends routine developmental screening to identify young children at risk of developmental delays (Duby et al. 2006), prior research has shown that ASD is often identified later in life (Maenner et al. 2013). In our study, ASD diagnosis was only captured among children 0–3 years of age; the median age at diagnosis was 19 months. Screening at 18 months is essential,

as symptoms of ASD often materialize by this age and can be "reliably" diagnosed, thus enabling EI; rescreening at 24 months is also critical for children who retrogress or could be missed at earlier ages (Dai et al. 2019).

Additionally, children with early ASD not enrolled in EI were not captured in this study since identification of ASD cases was based on participation and receipt of autism-related services in the EI specialty services dataset. However, the EI program in MA is very active and most children with ASD are believed to be in the program. Finally, we have likely underestimated the number of women in the Subfertile group, since it is based on a complex multi-factor algorithm, in which we were deliberately

conservative in our definitions. Given that women with fertility problems can be treated in outpatient settings, it is possible that we may have misclassified the Subfertile group, and the Fertile group may contain a small proportion of women who were actually Subfertile. However, since the Fertile group is much larger than the Subfertile group, it is unlikely this small number is sufficient to affect our findings.

Our study also has several strengths. MOSART is a large, population-based database which includes detailed ART treatment, post birth hospital utilization, and EI program participation data. Most ART studies in the US have relied on the national database and clinic-based data, which do not allow investigators to follow children post birth. To identify women with a history of infertility but no ART, we used a previously described algorithm that examined hospital discharge records available through our longitudinally linked MOSART database, rather than reports of infertility diagnosis by women. In addition, categorization of ART, IVF, and ICSI groups were based on SART CORS, the gold standard for ART treatment cycles obtained directly from all ART clinics in MA, and subsequently linked to in-state delivery records for resident women. About 95% of eligible ART treatment records were linked to a delivery record suggesting that the vast majority of ART births in MA were correctly classified.

Finally, our study used the mediation approach to disentangle the direct and indirect (through preterm birth) effects of ART, Subfertile, IVF, and ICSI on ASD, while prior studies have adjusted for preterm birth as a confounder, which is detrimental for estimation of total effects (Ananth and Schisterman 2017). Our study showed that independent of preterm birth there was no direct effect of ART, Subfertile, IVF, and ICSI on ASD diagnosis. Our study improves upon the previous literature because it allowed us to show the degree to which intervening on preterm could better estimate ASD among children born to women in the ART, Subfertile, IVF, and ICSI groups. The NIEs (odds of early ASD among the exposure groups under their observed preterm rates) are statistically significant.

As the prevalence of ASD has continued to increase, the search for its causes has continued to evolve; from the strong genetic origins (Muhle et al. 2004), to environmental exposures which may have teratogenic effect on the central nervous system early in pregnancy and play an important role in gene expression without DNA modification (Johnson and Myers 2007). Given that older parental age has been found to be associated with ASD due to alteration in genetic imprinting (Croen et al. 2007; Reichenberg et al. 2010), and the use ART, IVF, ICSI, and subfertility are more prevalent among older women who have elevated adverse birth outcomes, there is a legitimate concern about the potential risk of ASD among children born to these women.

Conclusion

We have found that children born to women in the ART, Subfertile, IVF, and ICSI groups are not at increased risk of receiving an early ASD diagnosis when compared to children born to Fertile women. Although there was no causal association between ART/subfertility and ASD we confirmed an association between preterm birth and ASD and between ART and preterm birth. Long term studies are needed to further elucidate the causes of ASD, while research in addtional areas potentially underlying child health sequelae due to parental infertility and its treatment (beyond ASD) must continue.

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