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Infertility treatment and autism risk using the Modified Checklist for Autism in Toddlers (M-CHAT)

S.L. Robinson[1], T. Parikh[2], T. Lin[6], E.M. Bell[3], E. Heisler[1], H. Park[1], C. Kus[4], J.E. Stern[5], and E.H. Yeung[1,*]

[1]Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development, Bethesda, MD, USA [2]Program in Reproductive Endocrinology and Gynecology, *Eunice Kennedy Shriver* National Institutes of Child Health and Human Development, Bethesda, MD, USA [3]Departments of Environmental Health Sciences and Epidemiology and Biostatistics, University at Albany School of Public Health, Albany, NY, USA [4]Division of Family Health, New York State Department of Health, New York, NY, USA [5]Department of Obstetrics and Gynecology, Geisel School of Medicine, Dartmouth College, Lebanon, NH [6]Glotech, Inc., Rockville, MD, USA

*Correspondence address. 6710B Rockledge Dr, MSC 7004, Bethesda, MD 20817, USA. Tel: +1 301-435-6921; E-mail: edwina.yeung@nih.gov

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STUDY QUESTION: Are toddlers conceived by fertility treatment at higher risk of failing a screening tool for autism spectrum disorders (ASD) than toddlers not conceived by treatment?

SUMMARY ANSWER: Compared with children not conceived by infertility treatment, children conceived by any infertility treatment, ovulation induction with or without intrauterine insemination (OI/IUI), or assisted reproductive technologies (ART) appeared to have had higher odds of failing an ASD screening; however, results were inconclusive and need replication.

WHAT IS KNOWN ALREADY: Although most of the studies which have examined risk of ASD after ART show no association, the results are mixed. Thus, further studies are needed to clarify this association.

STUDY DESIGN SIZE, DURATION: The Upstate KIDS Study is a population-based, prospective cohort study of children born in New York State between 2008 and 2010. Children were screened for ASD using the Modified Checklist for Autism in Toddlers (M-CHAT) at ages 18 and 24 months.

PARTICIPANTS/MATERIALS, SETTING, AND METHODS: The New York State live-birth registry was used to identify newborns conceived with and without fertility treatment with a 1:3 ratio, frequency matched on region of birth. At 18 and 24 months, 3183 and 3063 mothers, respectively, completed the M-CHAT questionnaire. The current analysis included 2586 singletons and 1296 twins with M-CHAT information at 18 and/or 24 months. Multivariable logistic regression with generalized estimating equations (GEE) was used to estimate odds ratios (aOR) and 95% confidence intervals (CI) after adjustment for covariates such as maternal age, education and plurality.

MAIN RESULTS AND THE ROLE OF CHANCE: We found that 200 (5.2%) and 115 (3.0%) children failed the M-CHAT at 18 and 24 months, respectively. The associations between use of infertility treatment and failing the M-CHAT at 18 and/or 24 months were positive but inconclusive as they failed to exclude no association (18 months aOR 1.71, 95% CI: 0.81–3.61; 24 months aOR 1.78, 95% CI: 0.66–4.81; and both 18 and 24 months aOR 1.53, 95% CI: 0.78–2.99). The relationships between OI/IUI and ART with M-CHAT failure at 18 and/or 24 months were similar to those of using any fertility treatment. *In vitro* fertilization with intracytoplasmic sperm injection was not consistently positively or inversely associated with M-CHAT failure at each time point (18 months aOR 1.20, 95% CI: 0.51–2.83; 24 months aOR 0.93, 95% CI: 0.37–2.31; and both 18 and 24 months aOR 1.09, 95% CI: 0.50–2.60).

LIMITATIONS REASONS FOR CAUTION: The M-CHAT is a screening tool used for ASD risk assessment, and therefore, M-CHAT failure does not indicate ASD diagnosis. In addition, we did not have power to detect associations of small magnitude. Finally, non-response to follow-up may bias the results.

WIDER IMPLICATIONS OF THE FINDINGS: Despite lack of precision, the positive associations between ART and M-CHAT failure suggest that larger population-based studies with longer follow-up are needed.

STUDY FUNDING/COMPETING INTEREST(S): Supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD; contracts HHSN275201200005C, HHSN267200700019C). The sponsor played

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Key words: autism spectrum disorder / Modified Checklist for Autism in Toddlers (M-CHAT) / assisted reproductive technologies (ART) / infertility / in vitro fertilization (IVF) / intracytoplasmic sperm injection (ICSI) / ovulation induction with or without intrauterine insemination (OI/IUI)

Introduction

The increasing use of fertility treatments, specifically ART, has helped many couples become parents. As ART gains popularity, concerns remain regarding the potential health and developmental consequences of controlled ovarian hyperstimulation, IVF and ICSI often used to assist in achieving conception (Agarwal *et al.*, 2005; Catford *et al.*, 2017; Conti *et al.*, 2013). It has been reported, for example, that ART use is associated with increased risk of congenital malformations and nervous systems defects (Qin *et al.*, 2015).

Of particular interest is the potential relation between ART use and autism spectrum disorder (ASD). ASD is a neurodevelopmental condition often diagnosed within the first few years of childhood characterized by impaired social interaction or communication, together with restricted and repetitive behaviors (American Psychiatric Association, 2013). According to the Centers for Disease Control and Prevention, 1 in 59 children have ASD, with an observed increase in the prevalence of ASD diagnoses over the past two decades (Baio *et al.*, 2018; Christensen *et al.*, 2016). An increase in diagnosis only partially accounts for the rise in ASD, which highlights the importance of understanding what factors may contribute to this trend (Polyak *et al.*, 2015).

Although the exact causes of ASD are unknown, both environmental and genetic factors have been associated with ASD. Specific to ART, exposures associated with increased infertility such as older paternal age, sperm quality and maternal infertility diagnoses have been related to risk of ASD in the offspring (Grether *et al.*, 2013; Jenkins *et al.*, 2014; Schieve *et al.*, 2017; Sharma *et al.*, 2015). General use of ART has been associated with ASD diagnosis in a large retrospective cohort study in California, in a case-control study in India and among a subgroup of children with mothers older than 35 years in the USA (Lyall *et al.*, 2012; Mamidala *et al.*, 2013; Fountain *et al.*, 2015). However, results from additional studies are mixed, with several studies finding null associations (Hvidtjorn *et al.*, 2011; Lehti *et al.*, 2013; Lyall *et al.*, 2013; Diop *et al.*, 2019) and one (Maimburg and Vaeth, 2007) finding an inverse association. Further, there is evidence of a stronger association in twins (Grether *et al.*, 2013; Fountain *et al.*, 2015) compared to singletons. Additionally, researchers have studied the associations of specific infertility interventions and procedures with ASD, as summarized in two recent systematic reviews (Conti *et al.*, 2013; Catford *et al.*, 2017). In short, two studies found ICSI use during IVF treatment to be associated with increased risk of ASD compared with IVF treatment without ICSI (Sandin *et al.*, 2013; Kissin *et al.*, 2015). Hormonal interventions involved in ovulation induction with or without intrauterine insemination (OI/IUI) have been related to ASD in some studies (Lyall *et al.*, 2012; Grether *et al.*, 2013; Davidovitch *et al.*, 2018) but not in others (Hvidtjorn *et al.*, 2011; Lyall *et al.*, 2013; Mamidala *et al.*, 2013; Schieve *et al.*, 2017). Given the wide use of ART nationally and conflicting results from previous studies, it is important to further examine these associations.

Early detection and intervention in ASD can improve developmental and behavioral outcomes (Daniel *et al.*, 2009). Although age at ASD diagnosis has decreased since the mid-1990s in the USA (Parner *et al.*, 2008), most children are still diagnosed later in childhood, with median age of diagnosis being 50 months (Christensen *et al.*, 2018). Early ASD diagnosis is more common among children of women with higher socioeconomic status (Jo *et al.*, 2015), which may reflect the ability of these women to better recognize early symptoms and navigate the health care system rather than population-level differences in ASD prevalence. Indeed, there is evidence that the ART and ASD association could be influenced by systematic differences in ASD diagnosis by socioeconomic status (Schieve *et al.*, 2015). For these reasons, accessible screening assessment tools that aid in early detection and follow-up of children presenting with developmental findings concerning for ASD are essential and studies using these instruments may help address diagnostic biases which could have influenced previous investigations. Currently, several such autism-specific screening tools exist. For young children, the most prominently used is the Modified Checklist for Autism in Toddlers (M-CHAT), which utilizes a parent-based questionnaire to yield risk of autism development in a child at ages 18 and 24 months.

Given the prior evidence that children conceived with infertility treatment may be at high risk for the development of ASD, we sought to examine whether conception with infertility treatment was related to increased risk of failing the M-CHAT, an early ASD screening tool, within the Upstate KIDS Study, a prospective cohort study which oversampled children of mothers who used infertility treatments. Additionally, we assessed whether the association with ASD screening results varied by mode of infertility treatment, such as OI/IUI or ART with or without ICSI. We hypothesize that the use of ART and other fertility treatments may be associated with increased M-CHAT failures.

Materials and Methods

Study setting and participants

The Upstate KIDS Study is a population-based, prospective cohort study of children born in New York State (except for five boroughs of New York City) between 2008 and 2010 (Buck Louis *et al.*, 2014). The New York State live-birth registry was used to identify study participants. The study was initially created to examine the role of infertility treatments on child development. All infants whose birth certificate indicated the use of fertility treatment were invited to participate, as were multiples regardless of mode of conception. Frequency matching on region of delivery of infants conceived with and without fertility

treatment was performed at a 1:3 ratio. In total, 5034 mothers and 6171 children were recruited.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The New York State Department of Health and the University at Albany Institutional Review Board (IRB) approved the study and served as the IRB designated by the National Institutes of Health for this study under a reliance agreement. All participants provided written informed consent. This article does not contain any studies with animals performed by any of the authors.

Measurements

Positive ASD screening, as detected by the M-CHAT

The M-CHAT is one of the most widely used ASD screening instruments both in the USA and internationally for children as young as 18 months (Robins *et al.*, 2014). In our study, mothers of 3242 children completed the questionnaire at 18 months postpartum, and 3135 completed it at 24 months. Of these, mothers of 2419 children completed the M-CHAT on both occasions and mothers of 2213 children completed one M-CHAT.

The M-CHAT was the screening tool originally used in data collection for the Upstate KIDS Study; however, an updated version, the M-CHAT-R/F, was released in 2009 (after the initiation of study follow-up) with a two-stage assessment system. In the scoring system for the M-CHAT-R/F, cut-points for a positive screen for ASD included a M-CHAT score $\geq$3 on initial assessment and $\geq$2 on follow-up assessment. In addition, the M-CHAT/R-F eliminated three ineffective questions, refining each question's wording, and clarifying the cutoff range for 'failing' (Robins *et al.*, 2014). Overall, the M-CHAT-R/F halves the number of missed cases while significantly increasing the sensitivity of the instrument (Robins *et al.*, 2014). Although the toddlers in the study were administered the M-CHAT, we rescored the tests to reflect the M-CHAT R/F revision guidelines; the sensitivity and specificity of the M-CHAT R/F for ASD diagnosis using the $\geq$3 cutoff is 73 and 89%, respectively (Robins *et al.*, 2014).

Infertility treatment and type: maternal self-report on baseline questionnaire

Mothers enrolled in the study completed a baseline questionnaire regarding their current health status and medical history at 4 months postpartum. Ninety-seven percent of enrolled mothers ($n = 4886$) completed the baseline questionnaire. In this questionnaire, mothers reported on their use of fertility treatment and specified if they had used OI/IUI, IVF or ICSI. Participants with a history of infertility were further categorized as using ART with or without ICSI or non-ART techniques (OI/IUI). Maternal report of primary exposure of infertility treatment was validated through linkage to the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) database. Sensitivity and specificity of maternal self-report as it relates to assessment by the SART database was 0.93 and 0.99, respectively (Buck Louis *et al.*, 2015). Maternal self-report has been shown to be equivalent and at times superior, as it enables the use of information not gathered through the SART database (Liberman *et al.*, 2014); therefore, we considered self-reported treatment as our exposure of interest.

Covariates

A priori selected covariates were acquired from vital records and maternal-report questionnaire at 4 months postpartum. Demographic covariates included maternal age, race/ethnicity, education, marital status and type of insurance. Health behaviors during pregnancy such as maternal smoking and alcohol use were also elicited at that time. Covariates pertaining to maternal health status consisted of gestational hypertension, pre-pregnancy diabetes, gestational diabetes, pre-pregnancy body mass index (BMI; kg/m^2) and history of polycystic ovary syndrome (PCOS). Paternal characteristics included father's age at the child's birth and BMI. Covariates pertaining to the child included infant sex, plurality, gestational age at delivery, Apgar score at 5 min, Townsend index and birthweight.

Maternal age and insurance type, paternal age, child sex, plurality, gestational age, Apgar score at 5 minutes and birthweight were obtained from vital records. Maternal BMI was calculated using pre-pregnancy height and weight obtained from birth certificates and from the baseline questionnaire if missing. Maternal race/ethnicity was obtained from maternal report, with vital records used when questionnaire data was unavailable. Gestational hypertension, pre-pregnancy diabetes and gestational diabetes were identified by combining information from maternal-report at baseline, birth certificate information and linkage with in- and out-patient hospital data from the Statewide Planning and Research Cooperative System. The Townsend index, a measure of economic deprivation, was calculated using census information, with increasing scores denoting greater deprivation based on neighborhood characteristics (Townsend *et al.*, 1988; Eibner and Sturm, 2006). Other covariates (i.e. maternal education, marital status, smoking and alcohol use during pregnancy and PCOS status; and paternal age, height and weight) were reported by mothers in the baseline questionnaire at 4 months post-partum. Paternal BMI was calculated from maternal report of paternal height and weight at baseline.

Statistical analysis

Singletons and twins with information on infertility treatment status and at least one completed M-CHAT questionnaire were included in the analysis. Higher-order multiples were excluded from the analysis due to small numbers ($n = 76$; 72 triplets and 4 quadruplets). The final sample included 2586 singletons and 1296 twins.

We first compared baseline characteristics between children who failed the M-CHAT (at 18, 24, either 18 or 24 and 18 and 24 months) and children who did not using the χ^2 or Kruskal–Wallis test. For such baseline descriptive statistics, we limited our sample to singletons and one twin of a pair to avoid correlations within families. Multivariable logistic regression with generalized estimating equations (GEE) was used to calculate the adjusted odds ratios (aOR) and 95% confidence intervals (CI) between modalities of conception and M-CHAT failures at 18 and 24 months of age, separately, and at either 18 or 24 months. We applied sampling weights in all models to account for the over-sampling of infants conceived with infertility treatment and twins (Buck Louis *et al.*, 2014). Estimates were made for any infertility treatment (yes/no) as well as specific interventions (ART, OI/IUI and ICSI). In all analyses, children who were not conceived by infertility treatment served as the comparison group. Both unadjusted and adjusted models were examined. We considered four different adjustment strategies to enhance comparability with previously published literature. In Model

1, we adjusted for potential sociodemographic confounders which included maternal age, race/ethnicity, education, marital status, smoking and private insurance. In Model 2, we added infant sex and plurality, which are typically included as adjustment variables, although plurality may be a consequence of infertility treatment. In Model 3, we adjusted for Model 1 covariates and potential mediators of the association between infertility treatment and M-CHAT failures (gestational age, birthweight, infant sex, Apgar score) to determine if these mediators explain any associations detected. In Model 4, we included variables in Model 1 and other potential confounders of the association which are not examined in all previous literature (maternal PCOS, paternal age and Townsend index). Paternal age was entered into the model as the difference between paternal and maternal age since parental ages are highly correlated. As Model 2 is the most comparable to results from other studies, this was considered our primary analysis. Paternal and maternal BMI did not meaningfully alter results and for parsimony, was not retained in the model. Multiple imputations of 50 datasets for missing covariate and outcome data were used to address missing covariate data and non-response to follow-up questionnaires in the primary analysis (van Buuren *et al.*, 2011). Covariates with missing information included maternal smoking during pregnancy ($n = 2$), drinking during pregnancy ($n = 2$), insurance status ($n = 3$), BMI ($n = 6$), pre-pregnancy diabetes ($n = 48$), marital status ($n = 151$), PCOS ($n = 280$); paternal age ($n = 196$) and BMI ($n = 384$); and child Apgar score ($n = 8$) and Townsend index ($n = 238$). At 18 and 24 months, 724 (18.65%) and 838 (21.59%) of children were missing data on the M-CHAT, respectively. As a sensitivity analysis, we present unimputed data. All statistical analyses were conducted with SAS 9.4 (SAS Institute, Inc., Cary, NC).

Results

Using the revised M-CHAT, 200 children failed the M-CHAT at 18 months and 115 children failed at 24 months. In Table I, the bivariate association between maternal, paternal and infant socio-demographic and behavioral factors and offspring M-CHAT failure is presented. At 18 months, children whose mothers had higher socioeconomic status markers were less likely to fail the M-CHAT as compared with their counterparts. Maternal smoking during pregnancy and male sex and plurality of the infant were positively associated with an M-CHAT fail at 18 and 24 months. Sociodemographic characteristics of mothers who completed the M-CHAT at either time point are compared with those of mothers who did not complete the M-CHAT in Supplementary Table SI. Mothers who completed the M-CHAT are older, have lower BMI and have higher socioeconomic status markers.

Table II presents the associations between infertility treatment and mode of treatment with M-CHAT failures at 18, 24 and both 18 and 24 months. Although infertility treatment was positively related to M-CHAT failure at 18, 24 and both 18 and 24 months, associations failed to exclude no association (aORs: 1.71, 95% CI: (0.81, 3.61); 1.78, 95% CI: 0.66–4.81); and 1.53, 95% CI: (0.78, 2.99), respectively) (Model 2). Unadjusted models show weaker associations whereas alternative models of adjustment for other sets of potential confounders (Models 1 and 4) or potential mediators (Model 3) show similar results. In Supplementary Table SII, the relations are presented without multiple imputations. Before adjustment for infant sex and plurality, infertility treatment was related to M-CHAT failure at 18 (aOR 2.15, 95% CI: 1.09, 4.27) and 24 months (aOR 2.76, 95% CI: 1.04, 7.32) (Model 1); however, these associations became attenuated with further adjustment (aORs 18 months: 1.92, 95% CI: 0.90–4.11; 24 months: 2.37, 95% CI: 0.83–6.71, Model 2) in models without imputation.

With regards to mode of treatment (non-ART versus ART), a positive association was detected at 18 (OI/IUI: aOR 1.84, 95% CI: (0.73, 4.65); ART: aOR 1.48, 95% CI: (0.63, 3.48)), 24 (OI/IUI: aOR 1.85, 95% CI: (0.57, 6.05); ART: aOR 1.61, 95% CI: (0.49, 5.33)), or both 18 and 24 (OI/IUI: aOR 1.59, 95% CI: (0.68, 3.74); ART: aOR 1.40, 95% CI: (0.67, 2.93)) months (Model 2) although the confidence limits failed to exclude no association. Unadjusted and alternative models of adjustment (Models 1, 3 and 4) showed similar results. Of note, associations were consistently positive, albeit not able to exclude no effect. Results are similar in models without imputation (Supplementary Table SII).

We found no association between ICSI and revised M-CHAT failure at 18 (aOR 1.20, 95% CI: (0.51, 2.83)), 24 (aOR 0.93, 95% CI: (0.37, 2.31)), or both 18 and 24 (aOR 1.09, 95% CI: (0.50, 2.39)) months (Model 2). Alternative models of adjustment (Models 1, 3 and 4) showed similar results. The results are similar in models with no imputations (Supplementary Table SII).

Discussion

In the present study, we found positive associations between infertility treatment and M-CHAT failure at 18, 24 and 18 or 24 months but we were unable to exclude the possibility of no association between infertility treatment and M-CHAT failure, regardless of adjustment for parental and child variables that may affect fetal development or subsequent neurological development. We also examined types of infertility treatment as an exposure. We saw positive associations for OI/IUI and ART treatment with M-CHAT failure after adjustment; however, these associations also failed to exclude no association. We did not see consistent associations between ICSI and M-CHAT failure at each time point (e.g. 18 and 24 months). This lack of precision underscores the need for larger population-based studies with longer follow-up as well as a more sensitive screening tool for ASD.

Most studies in the USA (Lyall *et al.*, 2013; Schieve *et al.*, 2017; Diop *et al.*, 2019) have not reported an association between general use of infertility treatment and ASD diagnosis. There are some notable exceptions (Lyall *et al.*, 2012; Fountain *et al.*, 2015). The largest study in the USA, a retrospective linkage study using records from California (Fountain *et al.*, 2015), found a positive association between ART use and ASD diagnosis (adjusted hazard ratio = 1.71, 95% CI: 1.56–1.89). Although imprecise, a similar odds ratio was reported in a subgroup analysis of women with maternal age ≥35 years from a case-control study nested within the Nurses' Health Study II (aOR: 1.58, 95% CI: 0.91–2.75) (Lyall *et al.*, 2012). This study further found that non-ART treatments (ovulation induction drugs and intrauterine insemination) were associated with reported risk of Asperger's in children. We were unable to examine the association of infertility treatment with positive ASD screening among those with advanced maternal age due to small numbers, and thus, we cannot directly compare our findings to those of the Nurses' Health Study II. However, our point estimates for the association comparing women with any use of infertility treatment to those without treatment are similar to estimates from these studies.

Table I Descriptive characteristics of the Upstate KIDS study population by Modified Checklist for Autism in Toddlers (M-CHAT) Outcome.

	Total	M-CHAT (18 months)			M-CHAT (24 months)			M-CHAT (18 and/or 24 months)*			
		Pass N (%)	Fail N (%)	P value	Pass N (%)	Fail N (%)	P value	Pass both N (%)	Fail one N (%)	Fail both N (%)	P value
Total (primary cohort)	3235 (100.0)	2497 (100.0)	153 (100.0)		2454 (100.0)	85 (100.0)		3007 (100.0)	184 (100.0)	27 (100.0)	
Maternal characteristics											
Age at child's birth, years; Mean ± SD	31.2 ± 5.9	31.4 ± 5.8	30.9 ± 6.6		31.6 ± 5.7	29.0 ± 7.2	0.0016	31.3 ± 5.8	29.8 ± 7.1	31.7 ± 5.7	0.0210
Race/ethnicity				<0.0001			<0.0001				<0.0001
White	2700 (83.5)	2125 (85.1)	106 (69.3)		2106 (85.8)	52 (61.2)		2550 (84.8)	114 (62.0)	22 (81.5)	
Non-White	535 (16.5)	372 (14.9)	47 (30.7)		348 (14.2)	33 (38.8)		457 (15.2)	70 (38.0)	5 (18.5)	
Education				<0.0001			<0.0001				<0.0001
<High school	132 (4.1)	78 (3.1)	21 (13.7)		59 (2.4)	17 (20.0)		92 (3.1)	32 (17.4)	3 (11.1)	
High school or GED equivalent	322 (10.0)	212 (8.5)	27 (17.7)		207 (8.4)	19 (22.4)		279 (9.3)	34 (18.5)	6 (22.2)	
Some college	888 (27.5)	673 (27.0)	47 (30.7)		642 (26.2)	24 (28.2)		823 (27.4)	57 (31.0)	7 (25.9)	
College	804 (24.9)	631 (25.3)	33 (21.6)		645 (26.3)	13 (15.3)		761 (25.3)	34 (18.5)	6 (22.2)	
Advanced degree	1089 (33.7)	903 (36.2)	25 (16.3)		901 (36.7)	12 (14.1)		1052 (35.0)	27 (14.7)	5 (18.5)	
Marital status, married	2853 (91.4)	2235 (92.3)	120 (83.3)	0.0001	2209 (92.4)	57 (76.0)	<0.0001	2685 (92.1)	137 (81.1)	20 (80.0)	<0.0001
Private insurance	2609 (80.7)	2066 (82.7)	94 (61.8)	<0.0001	2057 (83.9)	49 (57.7)	<0.0001	2468 (82.1)	115 (62.8)	14 (51.9)	<0.0001
Smoking during pregnancy	343 (10.6)	237 (9.5)	24 (15.7)	0.0126	213 (8.7)	19 (22.4)	<0.0001	300 (10.0)	37 (20.1)	3 (11.1)	<0.0001
Drinking during pregnancy	436 (13.5)	342 (13.7)	14 (9.2)		352 (14.3)	10 (11.8)		411 (13.7)	22 (12.0)	1 (3.7)	
Infertility treatment	1080 (33.4)	855 (34.2)	53 (34.6)		865 (35.2)	23 (27.1)		1014 (33.7)	54 (29.4)	11 (40.7)	
Type of infertility treatment											
OI/IUI	559 (17.3)	443 (17.8)	25 (16.3)		451 (18.4)	10 (11.8)		527 (17.5)	27 (14.7)	4 (14.8)	
ART	520 (16.1)	411 (16.5)	28 (18.3)		414 (16.9)	13 (15.3)		486 (16.2)	27 (14.7)	7 (25.9)	
Gestational hypertension	343 (10.6)	250 (10.0)	21 (13.7)		261 (10.6)	10 (11.8)		314 (10.4)	25 (13.6)	3 (11.1)	
Pre-pregnancy diabetes	37 (1.2)	26 (1.1)	4 (2.7)		28 (1.2)	2 (2.4)		33 (1.1)	2 (1.1)	2 (7.7)	
Gestational diabetes	336 (11.2)	217 (8.7)	23 (15.0)	0.0080	242 (9.9)	11 (12.9)		283 (9.4)	22 (12.0)	6 (22.2)	0.0449
BMI, kg/m^2; Mean ± SD	26.8 ± 6.6	26.6 ± 6.5	27.2 ± 6.8		26.6 ± 6.5	28.8 ± 7.7	0.0110	26.7 ± 6.6	27.5 ± 7.4	28.8 ± 6.5	
Gestational age, weeks; Mean ± SD	38.1 ± 2.4	38.2 ± 2.3	37.3 ± 3.0	<0.0001	38.2 ± 2.3	36.6 ± 3.3	<0.0001	38.2 ± 2.3	37.3 ± 3.0	36.2 ± 3.4	<0.0001
Townsend index; Mean ± SD	−2.2 ± 1.5	−2.3 ± 1.4	−1.5 ± 2.0	<0.0001	−2.3 ± 1.3	−1.2 ± 2.0	<0.0001	−2.3 ± 1.4	−1.4 ± 2.1	−1.5 ± 2.1	<0.0001
PCOS	277 (11.2)	265 (11.3)	12 (8.8)		264 (11.5)	9 (12.2)		319 (11.4)	13 (8.2)	4 (15.4)	

Continued

Table I Continued

	Total	M-CHAT (18 months)			M-CHAT (24 months)			M-CHAT (18 and/or 24 months)*			
		Pass *N* (%)	Fail *N* (%)	*P* value	Pass *N* (%)	Fail *N* (%)	*P* value	Pass both *N* (%)	Fail one *N* (%)	Fail both *N* (%)	*P* value
Paternal characteristics											
Age at child's birth, years; Mean ± SD	33.7 ± 6.7	33.6 ± 6.5	33.7 ± 7.5		34.0 ± 6.6	33.1 ± 7.6		33.7 ± 6.6	32.9 ± 7.6	35.4 ± 7.1	
BMI, kg/m^2; Mean ± SD	28.3 ± 5.4	28.4 ± 5.4	28.5 ± 6.0		28.2 ± 5.2	30.3 ± 7.9		28.2 ± 5.3	28.2 ± 5.6	32.0 ± 9.0	
Child characteristics											
Plurality				0.0020			<0.0001				0.0001
Singleton	2586 (79.9)	2032 (81.4)	109 (71.2)		1981 (80.7)	53 (62.4)		2426 (80.7)	128 (69.6)	17 (63.0)	
Twin	649 (20.1)	465 (18.6)	44 (28.8)		473 (19.3)	32 (37.7)		581 (19.3)	56 (30.4)	10 (37.0)	
Infant sex				0.0158			0.0120				0.0074
Male	1661 (51.3)	1267 (50.7)	93 (60.8)		1248 (50.9)	55 (64.7)		1523 (50.7)	112 (60.9)	18 (66.7)	
Female	1574 (48.7)	1230 (49.3)	60 (39.2)		1206 (49.1)	30 (35.3)		1484 (49.4)	72 (39.1)	9 (33.3)	
Birth weight, grams; Mean ± SD	3210 ± 685	3238 ± 667	2951 ± 774	<0.0001	3236 ± 665	2830 ± 864	<0.0001	231 ± 669	2946 ± 827	2777 ± 741	<0.0001
Apgar score; Mean ± SD	8.9 ± 0.6	8.9 ± 0.6	8.8 ± 1.1	0.0133	8.9 ± 0.6	8.7 ± 1.1	0.0016	8.9 ± 0.6	8.8 ± 0.7	8.4 ± 1.8	0.0018

Data are *n* (%) unless stated otherwise. *P* value by chi-square or Kruskal–Wallis test, noted when $P < 0.05$.
Missing: 585 at M-CHAT at 18 months, 696 at 24 months.
GED, general educational development; OI, ovulation induction; PCOS, polycystic ovary syndrome.
*Seventeen study participants were missing M-CHAT data at 18 and 24 months. These subjects were included in the total column as their data was imputed in multivariable analyses.

Table II **Associations between infertility treatment and offspring Modified Checklist for Autism in Toddlers (M-CHAT) failure in the Upstate KIDS Study.**

		Unadjusted		Model 1		Model 2		Model 3		Model 4	
	N (fail)	OR (95% CI)	*P*	aOR (95% CI)	*P*	aOR (95% CI)	*P*	aOR (95% CI)	*P*	aOR (95% CI)	*P*
M-CHAT fail at 18 m											
No treatment	2507 (172)	Reference		Reference		Reference		Reference		Reference	
Treatment	1375 (88)	1.31 (0.71, 2.41)	0.39	1.94 (0.98, 3.85)	0.06	1.71 (0.81, 3.61)	0.16	1.64 (0.80, 3.33)	0.18	1.82 (0.92, 3.60)	0.09
OI/IUI	690 (46)	1.39 (0.62, 3.12)	0.42	2.02 (0.82, 4.95)	0.13	1.84 (0.73, 4.65)	0.20	1.81 (0.74, 4.43)	0.20	1.87 (0.75, 4.63)	0.18
ART	684 (41)	1.16 (0.51, 2.65)	0.72	1.79 (0.84, 3.82)	0.13	1.48 (0.63, 3.48)	0.37	1.37 (0.59, 3.19)	0.46	1.74 (0.81, 3.74)	0.16
ICSI	440 (32)	0.98 (0.46, 2.07)	0.96	1.63 (0.71, 3.76)	0.25	1.20 (0.51, 2.83)	0.67	1.18 (0.49, 2.84)	0.71	1.57 (0.67, 3.67)	0.30
M-CHAT fail at 24 m											
No treatment	2507 (122)	Reference		Reference		Reference		Reference		Reference	
Treatment	1375 (43)	1.17 (0.53, 2.57)	0.70	2.19 (0.86, 5.60)	0.10	1.78 (0.66, 4.81)	0.25	1.95 (0.77, 4.93)	0.16	2.00 (0.68, 5.85)	0.21
OI/IUI	690 (18)	1.23 (0.45, 3.39)	0.68	2.17 (0.68, 6.87)	0.19	1.85 (0.57, 6.05)	0.31	1.98 (0.65, 6.06)	0.23	1.91 (0.49, 7.41)	0.35
ART	684 (24)	1.04 (0.37, 2.92)	0.94	2.18 (0.66, 7.14)	0.20	1.61 (0.49, 5.33)	0.43	1.84 (0.56, 6.03)	0.31	2.09 (0.62, 7.02)	0.23
ICSI	440 (12)	0.65 (0.29, 1.45)	0.29	1.57 (0.58, 4.23)	0.38	0.93 (0.37, 2.31)	0.87	1.25 (0.46, 3.38)	0.67	1.40 (0.49, 4.03)	0.53
EVER M-CHAT fail											
No treatment	2507 (245)	Reference		Reference		Reference		Reference		Reference	
Treatment	1375 (111)	1.10 (0.64, 1.90)	0.73	1.78 (0.96, 3.31)	0.07	1.53 (0.78, 2.99)	0.22	1.54 (0.82, 2.91)	0.18	1.67 (0.88, 3.16)	0.11
OI/IUI	690 (56)	1.15 (0.55, 2.38)	0.71	1.77 (0.77, 4.07)	0.18	1.59 (0.68, 3.74)	0.29	1.61 (0.70, 3.68)	0.26	1.62 (0.68, 3.85)	0.27
ART	684 (54)	1.02 (0.50, 2.08)	0.96	1.78 (0.91, 3.48)	0.09	1.40 (0.67, 2.93)	0.37	1.42 (0.69, 2.94)	0.34	1.73 (0.87, 3.41)	0.12
ICSI	440 (36)	0.82 (0.41, 1.61)	0.55	1.55 (0.72, 3.35)	0.27	1.09 (0.50, 2.39)	0.82	1.16 (0.51, 2.60)	0.72	1.46 (0.67, 3.20)	0.34

OR, odds ratio; aOR, adjusted odds ratio; OI, ovulation induction.
The no treatment group served as the comparison group for each of the exposure categories (i.e. any treatment, ART, OI/IUI, ICSI) in separate analyses and with the four covariate models described below. Information on infertility treatment type was not available for one study participant.
Model 1: adjusted for maternal age, race/ethnicity, education, marital status, smoking, private insurance
Model 2: adjusted as for Model 1 + infant sex + plurality (a potential mediator)
Model 3: adjusted as for Model 1+ gestational age, birthweight, infant sex, Apgar score (5 min) (potential mediators)
Model 4: adjusted as for Model 1+ PCOS, paternal age difference, Townsend index

These results contrast with results from other studies in the USA which did not observe such strong, positive associations between infertility treatment use and ASD. A population-based linkage study based in Massachusetts did not find an association between ART and ASD diagnosis (aOR: 1.08, 95% CI: 0.89–1.31) (Diop *et al.*, 2019). Additionally, smaller population-based case-control studies in California (Childhood Autism Risk from Genetics and the Environment—CHARGE, $n_{cases} = 513$, $n_{controls} = 388$) and across the USA (Study to Explore Early Development—SEED, $n_{cases} = 629$, $n_{controls} = 909$) found no association of ASD with infertility in crude (ORs: 1.1–1.2) or adjusted (aORs: 1.1–1.2) analyses (Lyall *et al.*, 2013; Schieve *et al.*, 2017). These studies may have been limited in detecting differences by therapy type.

International studies similarly show inconclusive results. The majority of prior cohort studies suggest that use of ART does not increase risk of adverse child outcomes such as ASD. In a retrospective cohort study in Denmark ($n = 588\,967$), one of the largest studies to date on infertility and ASD, there was a weak, positive association of ART with ASD diagnosis (RR 1.13, 95% CI: 0.97–1.31) following adjustment for maternal age, education level, parity, smoking, birthweight, and multiplicity. Of note, there was a positive association between ART and ASD diagnosis among girls in Denmark, but not among boys (Hvidtjorn *et al.*, 2011). Likewise, in Israel, no association between IVF treatment and ASD diagnosis was detected among over 110 000 male live births (Davidovitch *et al.*, 2018) in a retrospective national registry study. The association between ART and ASD diagnosis among females was not examined. Results from case-control studies vary. No association between conception with IVF and ASD diagnosis was found in a nested case-control study in Finland ($n_{cases} = 4164$, $n_{controls} = 16\,582$) (Lehti *et al.*, 2013). Smaller case-control studies in India and Israel have seen positive associations between ART and ASD diagnosis (Stein *et al.*, 2006; Mamidala *et al.*, 2013). In contrast, a population-based case-control study in Denmark found lower risk of ASD diagnosis among children born to ART (aOR: 0.37, 95% CI (0.14, 0.98)) (Maimburg and Vaeth, 2007).

Previously, we found that following adjustment, type of infertility treatment (i.e. OI/IUI or ART) was not significantly associated with failing any developmental domains on the Ages and Stages Questionnaire (Yeung *et al.*, 2016). In this study, we observed a positive but inconclusive association with OI/IUI or ART and M-CHAT failure. Further, the point estimates were similar between those that used OI/IUI and ART. Results from other studies have been mixed. Many do not observe associations between any fertility medications or OI and ASD diagnosis (Hvidtjorn *et al.*, 2011; Lyall *et al.*, 2013; Mamidala *et al.*, 2013; Lyall *et al.*, 2012). Unfortunately, unlike some of the previous studies (Hvidtjorn *et al.*, 2011; Grether *et al.*, 2013; Schieve *et al.*, 2017; Davidovitch *et al.*, 2018), we were not able to examine the associations with specific infertility medications and thus cannot examine if certain medications are related to M-CHAT failure, as has been previously noted (Grether *et al.*, 2013; Davidovitch *et al.*, 2018).

ICSI has been hypothesized to relate to ASD risk as well. In performing ICSI, the sperm is preselected and physically injected into the egg. Either bypassing the physiological processes of fertilization or potential structural damage to the embryo could lead to possible undesired outcomes. The first coordinated study of mental development in ICSI children published in 1998 found children conceived with ICSI were at significantly increased risk for developmental delay at age 1 year compared with both *in vitro* fertilization (IVF) and naturally conceived control children (Bowen *et al.*, 1998). In 2004, a meta-analysis of ICSI and mental developmental outcomes with a total of 969 ICSI children and 828 controls (343 IVF, 485 naturally conceived) determined that ICSI alone is not a risk factor for abnormal mental development (Leslie, 2004). Our findings here are thus in agreement with the conclusions from this meta-analysis. Of note, more recently, studies suggest increased risk of ASD development when ICSI was used compared with conventional IVF (Sandin *et al.*, 2013; Kissin *et al.*, 2015; Catford *et al.*, 2017).

The strengths of this study include its population-based design with prospective follow-up. ART treatment was validated using the SART database to maintain accuracy of infertility diagnosis and treatment use. This enabled examination of the association with M-CHAT failure, a positive screening for ASD, by type of infertility treatment undertaken by participants. We adjusted for many potential confounders, which included sociodemographic as well as parent or child-related risk factors for ASD. Furthermore, there were a large number of participants surveyed, which strengthens the reliability of our findings.

We acknowledge that our study is limited by the use of the M-CHAT to screen for ASD risk instead of a diagnostic instrument of case status. Although failing the M-CHAT may lack specificity for ASD and may potentially suggest other disorders of developmental delay, the use of modified scoring for the M-CHAT-R/F attempts to enhance the specificity of this screening test. The use of M-CHAT assessment additionally provides an earlier screening tool and opportunity for intervention in children who may not yet have ASD diagnosis. Further, the use of the M-CHAT screening tool rather than a diagnostic instrument may circumvent biases inherent to ASD diagnosis (e.g. differential diagnosis rates by socioeconomic status) since M-CHAT failure was inversely related to higher socioeconomic status markers in our study. We also recognize that the number of participants using ICSI may be underpowered to detect small associations with ASD development. In addition, our study was underpowered to conduct subgroup analyses among children with preterm birth, low birthweight or who were born to mothers of advanced maternal age (≥35 years) due to small numbers of children with M-CHAT failures in these categories. In addition, residual confounding may bias our results. Specifically, confounding by indication is likely present as infertility may be associated with ASD diagnosis. Further, misclassification of confounders such as maternal PCOS status or smoking is possible and could lead to bias. Finally, although non-response to covariates at baseline and the M-CHAT questionnaire at 18 and 24 months was present, we accounted for the missing data using multiple imputation, which provides results that are generally less biased and more efficient when data is missing at random (Spratt *et al.*, 2010).

Conclusions

In conclusion, we found some positive but inconclusive associations between infertility treatment and screening for ASD in children up to 24 months of age, after adjustment for multiple factors. Given the importance of the question, more and larger studies should be done to evaluate the usefulness of screening of potentially high-risk children by the M-CHAT. Ongoing follow-up of children's developmental stage after 24 months of age is needed to ensure the absence of diagnosis and lack of association between infertility interventions and ASD.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

S.L.R. worked in substantial revisions to the manuscript. T.P. performed the literature review, assisted with data analysis and drafted the initial manuscript. E.B. and T.L. assisted in data analysis. E.Y. and C.K. conceptualized the research project and assisted in data analysis and manuscript review. E.Y. and E.B. supervised the data acquisition. J.E.S. assisted with linkage of SART CORS to Upstate KIDS Study. E.H. and H.P. were vital to editing the manuscript and the concepts presented. All authors critically reviewed the manuscript, participated in manuscript drafting and approved the final version as submitted.

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Conflict of interest

There are no conflicts of interest to declare.

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