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Association between infertility treatment and children's neurodevelopment: the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study

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Abstract

Objectives: This study aims to examine the association between infertility treatment and neurodevelopment in children at 2 and 3.5 years of age.

Design: Prospective cohort study

Setting: Pregnant women were recruited in obstetric clinics or hospitals and their children were followed up by the questionnaire.

Participants: The study population consisted of mother-child pairs who participated in the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study.

Primary and secondary outcome measures: The children's neurodevelopmental outcomes were assessed at 2 and 3.5 years of age using the Ages and Stages Questionnaire, third edition (ASQ-3), which consists of questions on five developmental domains. We performed a multivariate logistic regression analysis of the association between infertility treatment (including ovulation induction [OI], artificial insemination with husband's sperm [AIH], and assisted reproductive technology [ART]) and the clinical range of ASQ-3.

Results: Of 9,655 mother-child pairs, 273 (2.8%) and 487 (5.0%) were conceived through OI/AIH and ART, respectively. The odds of having developmental delays at 2 years of age were higher in children conceived through OI/AIH [odds ratio (OR), 1.36; 95% confidence interval

(95% confidence interval [CI]), 1.00-1.85] and ART (OR, 1.36; 95% CI, 1.07-1.72) than in those conceived naturally. Additionally, OI/AIH and ART were significantly associated with communication (OR, 1.93; 95% CI, 1.25-2.98) and gross motor (OR, 1.50; 95% CI, 1.08-2.09) delays, respectively. In contrast, infertility treatments were not associated with any domains of the ASQ-3 in children aged 3.5 years.

Conclusion: In this study, we found a significant association between infertility treatment and children's neurodevelopment at 2 years of age, whereas no statistically significant differences were found at 3.5 years of age.

Keywords: Ages and Stages Questionnaire, Cohort study, Infertility treatment, Neurodevelopment, Assisted reproductive technology.

Word count: 2399

Strengths and limitations of this study

- The large sample size in this study allowed us to investigate the association between each type of infertility treatment and neurodevelopmental outcomes.
- We evaluated children's neurodevelopment at two points—2 and 3.5 years of age.

- ASQ-3 is a screening tool (not a diagnostic tool) for developmental delays.
- The number of children born through specific forms of infertility treatment, such as fresh embryo transfer, was insufficient to allow sub-analysis of fresh or frozen embryo transfer.

Introduction

With the rapid progress in infertility treatment, the number of women who use infertility treatment is increasing, and more than 10% of the childbearing population has resorted to assisted reproductive technology (ART) for conception.[1] In recent years, the number of fertility treatments in Japan has also been on the increase. In 2018, 56,000 new-borns were conceived through ART, including in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). This accounts for approximately 6% of all live births in the same year.[2] Because conception using ART involves several processes (such as physical manipulation of gametes and exposure of embryos to hormones and culture media) that differ greatly from those in a natural conception, there is potential for disturbance of normal early developmental processes.[3, 4] Therefore, many studies have investigated the association between ART and neurodevelopmental outcomes. A systematic review of neurodevelopmental disorders,[5] neuromotor, cognitive, language, and behavioural outcomes of children born after ART showed that ART had no significant effect on children's neuromotor and cognitive development.[6] A

previous hospital-based cohort study in Japan found that at 2 years of age, no significant difference in neurodevelopment existed between children conceived through ART and those conceived naturally.[7] In contrast, the Danish National Birth Cohort study showed that children conceived through ART had a slight delay in motor and cognitive development at 1.5 years of age compared to children conceived naturally.[8]

The findings have been almost entirely consistent in showing that singleton children conceived through ART and born at term are no different neurodevelopmentally from those born following natural conception. However, the evidence remains equivocal,[8–11] and another review stated that possible associations between infertility treatment and developmental delay require further assessment in larger studies.[12] The inconsistent results in the previous studies may have been due to the small sample size (fewer than 1000 children),[3] and a longitudinal study with a larger sample size is needed.

This study aims to investigate the association between infertility treatment and children's neurodevelopment at 2 and 3.5 years of age in a Japanese birth cohort.

Methods

Study setting and participants

This study was based on data obtained by the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study (TMM BirThree Cohort Study). The TMM BirThree Cohort Study is a prospective cohort study based in Miyagi Prefecture, Japan, and has been published elsewhere.[13, 14] Pregnant women and their family members were contacted in obstetric clinics or hospitals from 2013 to 2017, and 23,406 pregnant women participated in the study. Written informed consent was obtained from all participants. All participants were free to decline consent to participate in the research and were told that there was no disadvantage or risk involved with their refusal to participate. The TMM BirThree Cohort Study protocol was approved by the Tohoku University Tohoku Medical Megabank Organization's internal review board (2013-1-103-1).

Infertility treatments

We extracted infertility treatment types from maternal medical records, and they included ovulation induction (OI), artificial insemination (AIH), IVF, and ICSI; we refer to IVF and ICSI as ART in this study.

Neurodevelopmental assessments

The third edition of the Ages and Stages Questionnaire (ASQ-3) was used to evaluate child neurodevelopment.[15, 16] ASQ-3 is a comprehensive, reliable screening questionnaire that can be used for children aged 1 and 66 months. The guardians of children aged 2 and 3.5 years filled in the questionnaire. Each questionnaire contains 30 questions divided into five developmental domains: “communication”, “fine motor”, “gross motor”, “problem solving”, and “personal-social”. Each domain has a set of six items, and each item is given a score of 10, 5, and 0 corresponding to “yes”, “sometimes”, and “not yet”, respectively. The total score ranges from 0 to 60 for each domain. We defined “developmental delay” in a domain as when the score was greater than two standard deviations (SD) below the mean in that domain.[17] In this study, we used the validated Japanese translation of ASQ-3.[16]

Covariates

Considering previous studies and the characteristics of the population in this study, we included maternal age at delivery, parity, preterm birth (PTB), child sex, birth defects, multiple births, maternal education, and household income as covariates of developmental outcomes.[7, 18] Maternal age, gestational weeks, parity, child sex, birth defect, and multiple birth were obtained from medical records. Data on maternal level of education and household income were obtained using a self-report questionnaire.

Statistical analysis

Characteristics of mothers and children were compared in three groups: natural conception, OI/AIH, and ART. Continuous and categorical variables were described as mean (SD) and frequency or proportion, respectively. Differences in prevalence were analysed using the chi-square test. Logistic regression analyses were performed to determine the associations between infertility treatment and each of the five domains of ASQ-3 at 2 and 3.5 years of age after adjusting for possible confounding factors. Odds ratios (ORs) and confidence intervals (CIs) were calculated. Children who were conceived naturally were used as the reference group in all analyses. For subgroup analyses, participants were classified into five groups as follows: natural conception, OI, AIH, IVF, and ICSI. Furthermore, we compared developmental outcomes among children born following natural conception, fresh embryo transfer (ET), and frozen-thawed ET. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Patient and public involvement

No participants were involved in this study because we used existing dataset for the analysis.

Results

Characteristics of the participants

We analysed data from 9,655 eligible mother-child pairs who filled in ASQ-3 questionnaires (Figure 1). Of the 9,655 mother-child pairs, 273 (2.8%) were conceived through OI/AIH and 487 (5.0%) through ART. Maternal and child characteristics are shown in Table 1. Mothers who were exposed to infertility treatment were older and had higher levels of educational and household income. Children who were conceived following infertility treatment were likely to have a lower birth weight (LBW) and gestational age.

Infertility treatment and children's neurodevelopment at 2 years of age

In total, 1,437 children (14.9%) had developmental delay at 2 years of age when screened using the ASQ-3. The proportion of children with developmental delays at 2 years of age was 14.3%, 21.3%, and 22.5% for natural conception, OI/AIH, and ART, respectively. In the multivariable models, the odds of having developmental delays at 2 years of age were higher in children conceived through OI/AIH (OR, 1.36; 95% CI, 1.00-1.85) and ART (OR, 1.36; 95% CI, 1.07-1.72) than in those conceived naturally. OI/AIH and ART were significantly associated with communication (OR, 1.93; 95% CI, 1.25-2.98), and gross motor (OR, 1.50; 95% CI, 1.08-2.09)

delays, respectively (Table 2).

Infertility treatment and children's neurodevelopment at 3.5 years of age

In total, 1,257 children (13.0%) had developmental delay at 3.5 years of age when screened using the ASQ-3. The proportion of children with developmental delay at 3.5 years of age was 12.9%,14.7%, and 13.8% for natural conception, OI/AIH, and ART, respectively. In the multivariable models, the odds of having developmental delays at 3.5 years of age were higher in children conceived through OI/AIH (OR, 1.13; 95% CI, 0.79-1.61) and ART (OR, 1.03; 95% CI, 0.78-1.37) than in those conceived naturally. Moreover, children conceived through infertility treatment had no statistically significant differences in any domains at 3.5 years of age (Table 2).

Subgroups based on the 5 domains and embryo types were analysed and the results are shown in supplementary tables S1 to S4. Although the number of children conceived through each infertility treatment was less, the results, when compared within the five groups or types of ET, were similar to those within the three groups.

Discussion

We investigated the association between infertility treatment and children's neurodevelopment,

among 9,655 mother-child pairs. In multivariable models, a significant association between infertility treatment and neurodevelopment was observed among the children conceived through infertility treatment, at 2 years of age; no statistically significant differences were found in them at 3.5 years of age.

Children conceived through ART are known to be at risk of LBW, PTB, and birth defects,[19] which are risk factors for neurodevelopmental disorders.[20, 21] However, the data from previous studies among children aged 1–5 years suggest that there are no developmental differences between children conceived through ART and those conceived naturally, after adjusting for confounding variables.[3, 19, 22] Even when children with congenital anomalies or genetic syndromes and multiple births (which are known to affect development) were excluded from the study (n = 9,271), a significant association between infertility treatment and children's neurodevelopment was seen at 2 years of age, while no statistically significant differences were found at 3.5 years of age (Table S5). A previous study showed no significant difference in the neurodevelopmental scores between children conceived through ART and those conceived naturally; however, it showed a decrease in the score units of each scale among children conceived through ART.[23] The reason for the discrepancy in results at 2 years of age between our study and the previous study might be the proportion of children conceived through infertility treatment. Because infertility treatment has become widespread owing to technological

advancement, the proportion of children conceived through infertility treatment was higher in our study than in previous studies.[24–26] Therefore, statistical differences might be detectable in our study. In addition, frozen-thawed embryos have been used for most ARTs in Japan.[27] Frozen-thawed ET is associated with a significantly lower incidence of PTB and LBW, which are risk factors for neurodevelopmental disorders.[28] Among children with developmental delay at 2 years of age, the proportion with developmental delay at 3.5 years of age was 47.3%,46.6%, and 38.5% for natural conception, OI/AIH, and ART, respectively. The proportion of children with developmental delay at 3.5 years of age was low, especially in those conceived through ART. Our longitudinal study demonstrated that at 2 and 3.5 years of age, the neurodevelopmental status of children conceived through infertility treatment, especially ART, might eventually catch up with that of those conceived naturally. In Japan, the health check-up for children is done at 1.5 years of age, so the counsel provided by doctors and public health nurses, which is tailored towards individual development, might also help promote the children's development. A previous study in Japan stated that the developmental state of a child needs to be understood not only based on the characteristics of the child at one point but also on the progress of the child's development from the results of the health check-up at 1.5 years of age. In fact, it has been suggested that it is important to conduct a follow-up health check-up at 3 years of age in relation to the development at the previous age.[29]

Developmental delay in the communication domain was seen at age 2 years in children conceived through OI/AIH but not in those conceived through ART. A previous study showed that compared with children conceived naturally, those conceived through OI had lower verbal ability at 3 years of age, while those conceived through ART had higher verbal ability; children conceived through infertility treatment had higher verbal ability at 5 years of age than those conceived naturally.[30] Another study in Japan showed that children conceived through ART had significantly better language development than those conceived naturally.[7] Although direct comparisons with other studies assessing neurodevelopment in children conceived through infertility treatment are difficult because each researcher uses various assessment tools and control groups, the children conceived through ART might have higher verbal ability or better language development than those conceived through OI/AIH or naturally. Children conceived through infertility treatments generally enjoy advantageous socioeconomic benefits. It is possible that parents who utilise ART to conceive may invest more into rearing the children, which might have the greatest impact on language skills.[7, 30, 31]

Furthermore, ART was associated with developmental delays in the gross motor domain at 2 years of age. This association was not seen at 3.5 years of age. A hospital-based cohort study in Japan reported no significant difference in gross motor function (evaluated using the Ability for Basic Movement Scale for Children) at 12 months of age between the ART and

control groups.[32] In contrast, one study showed that infertility treatment, especially ICSI, may be associated with a slight delay in gross motor development at 1.5 years of age.[8] However, another study suggested no differences in motor development at 5 years of age among children conceived through ICSI, IVF, and natural conception.[33] These findings suggest that infertility treatment might be associated with children's development in the early stage, but the neurodevelopment of children conceived through infertility treatment might catch up with that of those conceived naturally.

This study had some limitations. The ASQ-3 is a screening tool (not a diagnostic tool) for developmental delays; however, it is considered to be highly reliable because it has been validated in many countries worldwide and has been used in a variety of studies.[18, 34, 35] Since we focused on children who filled in both questionnaires (at 2 and 3.5 years of age), there was the concern of bias due to the reduced number of participants and the deterioration of statistical power in the analysis. However, among the children who did not fill in the questionnaire at 3.5 years of age, the percentage of those who had neurodevelopmental delay at 2 years of age was 14.5%,10.8%, and 15.0% for natural conception, OI/AIH, and ART, respectively. Therefore, there was no bias such as more children with neurodevelopmental delay at 2 years of age among children who did not answer the questionnaire at 3.5 years old and the potential applicability of our results to national populations can be discussed. In the

subgroup analysis with detailed classification, the number of children conceived through specific forms of infertility treatment, such as fresh ET, was insufficient to compare the ET types. Research in this field is complex because of the need to collect various data to determine the effects of infertility treatment on offspring outcomes. As this was an observational study, residual confounding might have occurred. However, this study was a large longitudinal birth cohort with detailed information from the participants, and although continued follow-up of children born after infertility treatment is needed, this study helps to increase the understanding of the association between infertility treatment and neurodevelopmental outcomes in Japanese children.

Conclusion

In this study, we found a significant association between infertility treatment and neurodevelopment among children who were conceived through infertility treatments and those conceived naturally, at 2 years of age; however, no statistically significant differences were found at 3.5 years of age.

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Data availability statement: We share some of the identified data from the BirThree Cohort

Study in the Tohoku Medical Megabank Organization.

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Figure Legend

Figure 1. Flow chart of the exclusion criteria in the TMM BirThree Cohort Study

This flow chart describes the exclusion criteria and the number of total participants, excluded participants, and eligible participants. ASQ-3, Ages & Stages Questionnaires, Third Edition.

Table 1. Characteristics of the study population

	Total		Infertility treatment						p
			Natural conception		OI/AIH		ART		
	n=9,655	%	n=8,895	%	n=273	%	n=487	%	
Mother's age, mean (SD), y	32.7	4.7	32.4	4.6	34.3	3.9	37.2	3.8	<0.0001
Pre-pregnancy BMI (kg/m2)									
< 18.5	1,365	14.1	1,279	14.4	36	13.2	50	10.3	0.0707
18.5 -< 25.0	7,204	74.6	6,636	74.6	196	72.1	372	76.3	
≧ 25.0	1,083	11.2	977	11.0	41	15.0	65	13.4	
Educational level									
High school graduate or less	2,711	28.1	2,565	28.8	49	18.0	97	19.9	<0.0001
Junior or vocational college graduate	3,513	36.4	3,215	36.1	100	36.6	198	40.7	
University graduate or above	2,722	28.2	2,457	27.6	102	37.4	163	33.5	
Household income (JPY/year)									
< 4,000,000	3,117	32.3	2,974	33.4	63	23.1	80	16.4	<0.0001
4,000,000 to < 6,000,000	3,063	31.7	2,810	31.6	86	31.5	167	34.3	
≥ 6,000,000	2,992	31.0	2,658	29.9	111	40.7	223	45.8	
Cigarette smoking									
Never	6,194	64.6	5,675	64.3	195	71.4	324	66.7	<0.0001
Stopped before pregnancy	2,291	23.9	2,075	23.5	60	22.0	156	32.1	
Stopped after pregnancy	950	9.9	928	10.5	17	6.2	5	1.0	
Smoked during early pregnancy	152	1.6	150	1.7	1	0.4	1	0.2	
Alcohol consumption									

Drinking at early pregnancy	1,917	20.0	1,766	20.0	57	21.0	94	19.3	0.5426
Former	3,273	34.1	3,023	34.2	93	34.2	157	32.3	
Never	3,871	40.3	3,568	40.4	105	38.6	198	40.7	
Cannot drink because of constitution	536	5.6	482	5.5	17	6.3	37	7.6	
Parity									
Nullipara	4,564	47.3	4,024	45.2	202	74.0	338	69.4	<0.0001
Multipara	5,091	52.7	4,871	54.8	71	26.0	149	30.6	
Child's sex									
Male	4,958	51.4	4,581	51.5	137	50.2	240	49.3	0.5874
Female	4,697	48.7	4,314	48.5	136	49.8	247	50.7	
Gestational age									
≥37	9,017	93.4	8,338	93.7	245	89.7	434	89.1	<0.0001
< 37	638	6.6	557	6.3	28	10.3	53	10.9	
Birth weight (g)									
≥2,500	8,689	90.0	8,037	90.4	229	83.9	423	86.9	0.0004
< 2,500	952	9.9	844	9.5	44	16.1	64	13.1	
Birth defect									
No	9,477	98.3	8,740	98.4	269	98.5	468	96.1	0.0033
Yes	169	1.8	146	1.6	4	1.5	19	3.9	
Multiple birth									
No	9,440	97.8	8,746	98.3	244	89.4	450	92.4	<0.0001
Yes	215	2.2	149	1.7	29	10.6	37	7.6	

ASQ-3（2Y）										
	> Mean -2SD	8,218	85.1	7,626	85.7	215	78.8	377	77.4	<0.0001
	≤ Mean -2SD	1,437	14.9	1,269	14.3	58	21.3	110	22.6	
ASQ-3（3.5Y）										
	> Mean -2SD	8,398	87.0	7,746	87.1	233	85.4	419	86.0	0.5749
	≤ Mean -2SD	1,257	13.0	1,149	12.9	40	14.7	68	14.0	
Father's age, mean (SD), y		n=4,183		n=3,784		n=142		n=257		
		34.5	5.7	34.2	5.6	35.1	4.8	38.8	5.2	<0.0001

Standard deviation: SD, Body mass index: BMI, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH.

Assisted reproductive technology: ART, Ages & Stages Questionnaires, Third Edition: ASQ-3.

Table 2. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,655)

		2 years								3.5 years							
				Crude			Adjusted					Crude			Adjusted		
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI	
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper
Total score																	
Natural conception	8,895	1,269	14.27	ref			ref			1,149	12.92	ref			ref		
OI/AIH	273	58	21.25	1.62	1.21	2.18	1.36	1.00	1.85	40	14.65	1.16	0.82	1.63	1.13	0.79	1.61
ART	487	110	22.59	1.75	1.41	2.19	1.36	1.07	1.72	68	13.96	1.09	0.84	1.42	1.03	0.78	1.37
Communication																	
Natural conception	8,895	445	5.00	ref			ref			482	5.42	ref			ref		
OI/AIH	273	27	9.89	2.09	1.39	3.14	1.93	1.25	2.98	17	6.23	1.16	0.70	1.91	1.13	0.68	1.90
ART	487	27	5.54	1.12	0.75	1.66	0.95	0.62	1.45	29	5.95	1.11	0.75	1.63	1.04	0.69	1.56
Gross motor																	
Natural conception	8,895	483	5.43	ref			ref			367	4.13	ref			ref		
OI/AIH	273	20	7.33	1.38	0.87	2.19	1.15	0.72	1.85	16	5.86	1.45	0.86	2.42	1.26	0.74	2.13
ART	487	49	10.06	1.95	1.43	2.65	1.50	1.08	2.09	25	5.13	1.26	0.83	1.91	1.03	0.67	1.60
Fine motor																	
Natural conception	8,895	389	4.37	ref			ref			513	5.77	ref			ref		
OI/AIH	273	17	6.23	1.45	0.88	2.40	1.28	0.76	2.13	19	6.96	1.22	0.76	1.97	1.20	0.74	1.96
ART	487	34	6.98	1.64	1.14	2.36	1.27	0.86	1.86	31	6.37	1.11	0.76	1.62	1.05	0.71	1.56
Problem solving																	
Natural conception	8,895	354	3.98	ref			ref			505	5.68	ref			ref		

OI/AIH	273	13	4.76	1.21	0.68	2.13	0.90	0.50	1.60	18	6.59	1.17	0.72	1.91	1.20	0.73	1.98
ART	487	32	6.57	1.70	1.17	2.47	1.18	0.79	1.75	27	5.54	0.98	0.66	1.45	0.91	0.60	1.38
Personal-social																	
Natural conception	8,895	449	5.05	ref			ref			400	4.5	ref			ref		
OI/AIH	273	24	8.79	1.81	1.18	2.79	1.46	0.93	2.28	16	5.86	1.32	0.79	2.21	1.20	0.70	2.04
ART	487	42	8.62	1.78	1.28	2.47	1.25	0.88	1.77	28	5.75	1.30	0.87	1.92	1.05	0.69	1.60

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, Assisted reproductive technology: ART

Multivariable logistic models were adjusted for maternal age, parity, gestational week, child sex, birth defect, multiple birth, maternal education level, and household income.

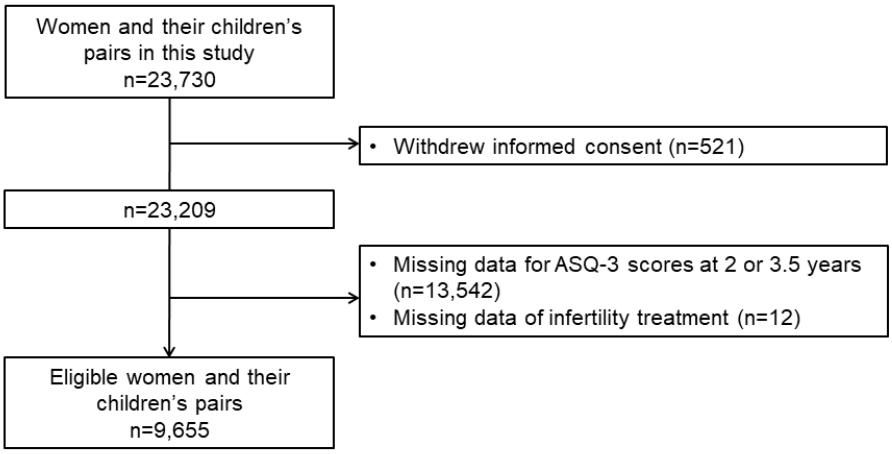

Figure 1. Flow chart of the exclusion criteria in the TMM BirThree Cohort Study

254x190mm (96 x 96 DPI)

Table S1. Characteristics of the study population

	Total		Infertility treatment										p
			Natural conception		OI		AIH		IVF		ICSI		
	n=9,655	%	n=8,895	%	n=147	%	n=126	%	n=342	%	n=145	%	
Mother's age, mean (SD), y	32.7	4.7	32.4	4.6	33.1	3.7	35.6	3.7	37.1	3.7	37.3	3.9	<0.0001
Pre-pregnancy BMI (kg/m^2)													
< 18.5	1,365	14.1	1,279	14.4	16	10.9	20	15.9	29	8.5	21	14.5	0.0283
18.5 to < 25.0	7,204	74.6	6,636	74.6	104	70.8	92	73.0	261	76.3	111	76.6	
≥ 25.0	1,083	11.2	977	11.0	27	18.4	14	11.1	52	15.2	13	9.0	
Educational level													
High school graduate or less	2,711	28.1	2,565	28.8	28	19.1	21	16.7	74	21.6	23	15.9	<0.0001
Junior college or vocational college graduate	3,513	36.4	3,215	36.1	51	34.7	49	38.9	135	39.5	63	43.5	
University graduate or above	2,722	28.2	2,457	27.6	56	38.1	46	36.5	111	32.5	52	35.9	
Household income (JPY/year)													
< 4,000,000	3,117	32.3	2,974	33.4	42	28.6	21	16.7	64	18.7	16	11.0	<0.0001
4,000,000 to < 6,000,000	3,063	31.7	2,810	31.6	37	25.2	49	38.9	120	35.1	47	32.4	
≥ 6,000,000	2,992	31.0	2,658	29.9	62	42.2	49	38.9	145	42.4	78	53.8	
Cigarette smoking													
Never	6,194	64.6	5,675	64.3	100	68.0	95	75.4	221	64.8	103	71.0	<0.0001
Stoped before pregnancy	2,291	23.9	2,075	23.5	31	21.1	29	23.0	115	33.7	41	28.3	
Stopped after pregnancy	950	9.9	928	10.5	15	10.2	2	1.6	5	1.5	0	0.0	
Smoking at early pregnancy	152	1.6	150	1.7	1	0.7	0	0.0	0	0.0	1	0.7	
Alcohol drinking													
Drinking at early pregnancy	1,917	20.0	1,766	20.0	25	17.0	32	25.6	75	22.0	19	13.1	0.0741
Former	3,273	34.1	3,023	34.2	55	37.4	38	30.4	112	32.8	45	31.0	
Never	3,871	40.3	3,568	40.4	54	36.7	51	40.8	128	37.5	70	48.3	
Cannot drink because of constitution	536	5.6	482	5.5	13	8.8	4	3.2	26	7.6	11	7.6	
Parity													
Nullipara	4,564	47.3	4,024	45.2	100	68.0	102	81.0	242	70.8	96	66.2	<0.0001
Multipara	5,091	52.7	4,871	54.8	47	32.0	24	19.1	100	29.2	49	33.8	
Child sex													
Male	4,958	51.4	4,581	51.5	84	57.1	53	42.1	158	46.2	82	56.6	0.0205
Female	4,697	48.7	4,314	48.5	63	42.9	73	57.9	184	53.8	63	43.5	
Gestational week													
≥37	9,017	93.4	8,338	93.7	130	88.4	115	91.3	302	88.3	132	91.0	<0.0001
< 37	638	6.6	557	6.3	17	11.6	11	8.7	40	11.7	13	9.0	
Birth weight (g)													
≥2,500	8,689	90.0	8,037	90.4	126	85.7	103	81.8	294	86.0	129	89.0	0.0039
< 2,500	952	9.9	844	9.5	21	14.3	23	18.3	48	14.0	16	11.0	
Birth defect													
No	9,477	98.3	8,740	98.4	145	98.6	124	98.4	330	96.5	138	95.2	0.005
Yes	169	1.8	146	1.6	2	1.4	2	1.6	12	3.5	7	4.8	
Multiple birth													
No	9,440	97.8	8,746	98.3	128	87.1	116	92.1	311	90.9	139	95.9	<0.0001
Yes	215	2.2	149	1.7	19	12.9	10	7.9	31	9.1	6	4.1	
ASQ-3（2Y）													
> Mean -2SD	8,218	85.1	7,626	85.7	119	81.0	96	76.2	265	77.5	112	77.2	<0.0001
≤ Mean-2SD	1,437	14.9	1,269	14.3	28	19.1	30	23.8	77	22.5	33	22.8	
ASQ-3（3.5Y）													
> Mean -2SD	8,398	87.0	7,746	87.1	127	86.4	106	84.1	295	86.3	124	85.5	0.8329
≤ Mean-2SD	1,257	13.0	1,149	12.9	20	13.6	20	15.9	47	13.7	21	14.5	
Father's age, mean (SD), y	n=4,183		n=3,784		n=74		n=68		n=172		n=85		
	34.5	5.7	34.2	5.6	34.3	4.7	35.9	4.7	38.5	5.4	39.6	4.9	<0.0001

Standard deviation: SD, Body mass index: BMI, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, In vitro fertilization: IVF, intracytoplasmic sperm injection: ICSI, Ages & Stages Questionnaires, Third Edition: ASQ-3.

Table S2. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,655)

	2 years									3.5 years								
				Crude			Adjusted					Crude			Adjusted			
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI		
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper	
Total score																		
Natural conception	8,895	1,269	14.27	ref			ref			1,149	12.92	ref			ref			
OI	147	28	19.05	1.41	0.93	2.14	1.22	0.79	1.87	20	13.61	1.06	0.66	1.71	1.01	0.62	1.64	
AIH	126	30	23.81	1.88	1.24	2.84	1.53	0.99	2.34	20	15.87	1.27	0.79	2.06	1.29	0.78	2.13	
IVF	342	77	22.51	1.75	1.35	2.27	1.34	1.02	1.76	47	13.74	1.07	0.79	1.47	1.02	0.73	1.42	
ICSI	145	33	22.76	1.77	1.20	2.62	1.41	0.94	2.11	21	14.48	1.14	0.72	1.82	1.07	0.66	1.74	
Communication																		
Natural conception	8,895	445	5	ref			ref			482	5.42	ref			ref			
OI	147	16	10.88	2.32	1.37	3.93	2.12	1.22	3.69	9	6.12	1.14	0.58	2.25	1.05	0.52	2.12	
AIH	126	11	8.73	1.82	0.97	3.40	1.71	0.88	3.30	8	6.35	1.18	0.58	2.44	1.23	0.59	2.59	
IVF	342	19	5.56	1.12	0.70	1.79	0.94	0.57	1.54	20	5.85	1.08	0.68	1.72	1.02	0.63	1.64	
ICSI	145	8	5.52	1.11	0.54	2.28	0.98	0.47	2.05	9	6.21	1.16	0.59	2.28	1.10	0.55	2.20	
Gross motor																		
Natural conception	8,895	483	5.43	ref			ref			367	4.13	ref			ref			
OI	147	7	4.76	0.87	0.41	1.87	0.76	0.35	1.64	8	5.44	1.34	0.65	2.75	1.19	0.57	2.48	
AIH	126	13	10.32	2.00	1.12	3.58	1.59	0.88	2.89	8	6.35	1.58	0.76	3.25	1.32	0.63	2.77	
IVF	342	32	9.36	1.80	1.24	2.62	1.38	0.93	2.05	14	4.09	0.99	0.58	1.71	0.80	0.45	1.40	
ICSI	145	17	11.72	2.31	1.38	3.87	1.81	1.07	3.07	11	7.59	1.91	1.02	3.56	1.64	0.87	3.12	
Fine motor																		
Natural conception	8,895	389	4.37	ref			ref			513	5.77	ref			ref			
OI	147	7	4.76	1.09	0.51	2.35	1.02	0.47	2.22	10	6.8	1.19	0.62	2.28	1.12	0.58	2.17	
AIH	126	10	7.94	1.89	0.98	3.63	1.55	0.80	3.03	9	7.14	1.26	0.63	2.49	1.29	0.64	2.62	
IVF	342	27	7.89	1.87	1.25	2.81	1.45	0.95	2.22	18	5.26	0.91	0.56	1.47	0.86	0.52	1.43	
ICSI	145	7	4.83	1.11	0.52	2.39	0.87	0.40	1.89	13	8.97	1.61	0.90	2.86	1.49	0.82	2.70	
Problem solving																		
Natural conception	8,895	354	3.98	ref			ref			505	5.68	ref			ref			
OI	147	6	4.08	1.03	0.45	2.34	0.80	0.34	1.84	9	6.12	1.08	0.55	2.14	1.12	0.56	2.23	
AIH	126	7	5.56	1.42	0.66	3.06	1.00	0.46	2.20	9	7.14	1.28	0.65	2.53	1.31	0.65	2.64	
IVF	342	22	6.43	1.66	1.06	2.59	1.13	0.70	1.80	20	5.85	1.03	0.65	1.64	0.98	0.60	1.57	
ICSI	145	10	6.9	1.79	0.93	3.43	1.31	0.67	2.55	7	4.83	0.84	0.39	1.81	0.76	0.35	1.66	
Personal-social																		
Natural conception	8,895	449	5.05	ref			ref			400	4.5	ref			ref			
OI	147	10	6.8	1.37	0.72	2.63	1.17	0.60	2.27	8	5.44	1.22	0.60	2.51	1.13	0.54	2.35	
AIH	126	14	11.11	2.35	1.34	4.13	1.77	0.99	3.19	8	6.35	1.44	0.70	2.97	1.28	0.60	2.70	
IVF	342	26	7.6	1.55	1.03	2.33	1.09	0.71	1.68	20	5.85	1.32	0.83	2.10	1.09	0.67	1.78	
ICSI	145	16	11.03	2.33	1.38	3.96	1.63	0.94	2.82	8	5.52	1.24	0.60	2.55	0.97	0.46	2.02	

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, In vitro fertilization: IVF, intracytoplasmic sperm injection: ICSI.
Multivariable logistic models were adjusted for maternal age, parity, gestational week, child sex, birth defect, multiple birth, maternal education level, and household income.

Table S3. Characteristics of study population for ET

	Total		Natural conception		ART (n=485)						p
					Fresh ET		Frozen-thawed ET		Missing		
	n=9,382	%	n=8,895	%	n=46	%	n=314	%	n=127	%	
Mother's age, mean (SD), y	32.7	4.7	32.4	4.6	37.8	3.6	37.3	3.8	36.8	3.6	<0.0001
Pre-pregnancy BMI (kg/m^2)											
< 18.5	1,329	14.2	1,279	14.4	9	19.6	30	9.6	11	8.7	0.2997
18.5 to < 25.0	7,008	74.7	6,636	74.6	32	69.6	242	77.1	98	77.2	
≥ 25.0	1,042	11.1	977	11.0	5	10.9	42	13.4	18	14.2	
Educational level											
High school graduate or less	2,662	28.4	2,565	28.8	9	19.6	64	20.4	24	18.9	0.0018
Junior college or vocational college graduate	3,413	36.4	3,215	36.1	23	50.0	122	38.9	53	41.7	
University graduate or above	2,620	27.9	2,457	27.6	12	26.1	111	35.4	40	31.5	
Household income (JPY/year)											
< 4,000,000	3,054	32.6	2,974	33.4	6	13.0	53	16.9	21	16.5	<0.0001
4,000,000 to < 6,000,000	2,977	31.7	2,810	31.6	16	34.8	108	34.4	43	33.9	
≥ 6,000,000	2,881	30.7	2,658	29.9	22	47.8	144	45.9	57	44.9	
Cigarette smoking											
Never	5,999	64.4	5,675	64.3	24	52.2	209	66.8	91	71.7	<0.0001
Stoped before pregnancy	2,231	24.0	2,075	23.5	22	47.8	99	31.6	35	27.6	
Stopped after pregnancy	933	10.0	928	10.5	0	0.0	4	1.3	1	0.8	
Smoking at early pregnancy	151	1.6	150	1.7	0	0.0	1	0.3	0	0.0	
Alcohol drinking											
Drinking at early pregnancy	1,860	20.0	1,766	20.0	6	13.0	60	19.2	28	22.1	0.2181
Former	3,180	34.1	3,023	34.2	22	47.8	98	31.3	37	29.1	
Never	3,766	40.4	3,568	40.4	16	34.8	128	40.9	54	42.5	
Cannot drink because of constitution	519	5.6	482	5.5	2	4.4	27	8.6	8	6.3	
Parity											
Nullipara	4,362	46.5	4,024	45.2	35	76.1	212	67.5	91	71.7	<0.0001
Multipara	5,020	53.5	4,871	54.8	11	23.9	102	32.5	36	28.4	
Child sex											
Male	4,821	51.4	4,581	51.5	22	47.8	156	49.7	62	48.8	0.806
Female	4,561	48.6	4,314	48.5	24	52.2	158	50.3	65	51.2	
Gestational week											
≥37	8,772	93.5	8,338	93.7	38	82.6	285	90.8	111	87.4	<0.0001
< 37	610	6.5	557	6.3	8	17.4	29	9.2	16	12.6	
Birth weight (g)											
≥2,500	8,460	90.2	8,037	90.4	41	89.1	277	88.2	105	82.7	0.0814
< 2,500	908	9.7	844	9.5	5	10.9	37	11.8	22	17.3	
Birth defect											
No	9,208	98.2	8,740	98.4	45	97.8	302	96.2	121	95.3	0.0019
Yes	165	1.8	146	1.6	1	2.2	12	3.8	6	4.7	
Multiple birth											
No	9,196	98.0	8,746	98.3	42	91.3	287	91.4	121	95.3	<0.0001
Yes	186	2.0	149	1.7	4	8.7	27	8.6	6	4.7	
ASQ-3（2Y）											
> Mean -2SD	8,003	85.3	7,626	85.7	33	71.7	242	77.1	102	80.3	<0.0001
≤ Mean-2SD	1,379	14.7	1,269	14.3	13	28.3	72	22.9	25	19.7	
ASQ-3（3.5Y）											
> Mean -2SD	8,165	87.0	7,746	87.1	39	84.8	269	85.7	111	87.4	0.8594
≤ Mean-2SD	1,217	13.0	1,149	12.9	7	15.2	45	14.3	16	12.6	
Father's age, mean (SD), y	n=4,041		n=3,784		n=25		n=169		n=63		
	34.5	5.7	34.2	5.6	38.9	4.1	38.9	5.3	38.8	5.4	<0.0001

Standard deviation: SD, Body mass index: BMI, Embryo transfer: ET, Assisted reproductive technology: ART, Ages & Stages Questionnaires, Third Edition: ASQ-3.

Table S4. Odds ratios (95% CIs) from logistic regression models for ET and children's neurodevelopment at the age of 2 and 3.5 years (n=485)

	2 years									3.5 years								
				Crude			Adjusted					Crude			Adjusted			
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI		
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper	
Total score																		
Natural conception	8,895	1,269	14.27	ref			ref			1,149	12.92	ref			ref			
Fresh ET	46	13	28.26	2.37	1.24	4.51	1.71	0.88	3.33	7	15.22	1.21	0.54	2.71	1.12	0.49	2.58	
Frozen-thawed ET	314	72	22.93	1.79	1.37	2.34	1.39	1.05	1.85	45	14.33	1.13	0.82	1.56	1.07	0.76	1.50	
Communication																		
Natural conception	8,895	445	5	ref			ref			482	5.42	ref			ref			
Fresh ET	46	4	8.7	1.81	0.65	5.07	1.43	0.49	4.19	1	2.17	0.39	0.05	2.82	0.33	0.04	2.42	
Frozen-thawed ET	314	16	5.1	1.02	0.61	1.70	0.88	0.52	1.50	23	7.32	1.38	0.89	2.13	1.28	0.81	2.02	
Gross motor																		
Natural conception	8,895	483	5.43	ref			ref			367	4.13	ref			ref			
Fresh ET	46	5	10.87	2.12	0.84	5.40	1.51	0.59	3.90	2	4.35	1.06	0.26	4.37	0.84	0.20	3.53	
Frozen-thawed ET	314	34	10.83	2.12	1.47	3.06	1.65	1.12	2.43	21	6.69	1.67	1.06	2.63	1.37	0.85	2.21	
Fine motor																		
Natural conception	8,895	389	4.37	ref			ref			513	5.77	ref			ref			
Fresh ET	46	3	6.52	1.53	0.47	4.94	1.06	0.32	3.47	3	6.52	1.14	0.35	3.69	1.11	0.34	3.67	
Frozen-thawed ET	314	21	6.69	1.57	1.00	2.47	1.21	0.75	1.94	19	6.05	1.05	0.66	1.69	1.01	0.62	1.65	
Problem solving																		
Natural conception	8,895	354	3.98	ref			ref			505	5.68	ref			ref			
Fresh ET	46	3	6.52	1.68	0.52	5.45	1.07	0.32	3.56	1	2.17	0.37	0.05	2.68	0.34	0.05	2.49	
Frozen-thawed ET	314	23	7.32	1.91	1.23	2.95	1.33	0.84	2.11	18	5.73	1.01	0.62	1.64	0.95	0.57	1.56	
Personal-social																		
Natural conception	8,895	449	5.05	ref			ref			400	4.5	ref			ref			
Fresh ET	46	3	6.52	1.31	0.41	4.25	0.82	0.25	2.74	2	4.35	0.97	0.23	4.00	0.75	0.18	3.19	
Frozen-thawed ET	314	36	11.46	2.44	1.70	3.49	1.76	1.20	2.58	17	5.41	1.22	0.74	2.00	0.99	0.59	1.66	

Confidence interval: CI, odds ratio: OR, Embryo transfer: ET.

Multivariable logistic models were adjusted for maternal age, parity, gestational week, child sex, birth defect, multiple birth, maternal education level, and household income.

Table S5. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,271)

		2 years								3.5 years							
				Crude			Adjusted					Crude			Adjusted		
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI	
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper
Total score																	
Natural conception	8,602	1,200	13.95	ref			ref			1,092	12.69	ref			ref		
OI/AIH	239	48	20.08	1.55	1.12	2.14	1.36	0.98	1.89	35	14.64	1.18	0.82	1.70	1.20	0.83	1.75
ART	430	90	20.93	1.63	1.28	2.08	1.33	1.03	1.71	57	13.26	1.05	0.79	1.40	1.05	0.78	1.41
Communication																	
Natural conception	8,602	411	4.78	ref			ref			452	5.25	ref			ref		
OI/AIH	239	19	7.95	1.72	1.07	2.78	1.77	1.08	2.90	15	6.28	1.21	0.71	2.05	1.30	0.76	2.23
ART	430	19	4.42	0.92	0.58	1.48	0.87	0.53	1.41	22	5.12	0.97	0.63	1.51	1.01	0.64	1.59
Gross motor																	
Spontaneous pregnancy	8,602	452	5.25	ref			ref			336	3.91	ref			ref		
OI/AIH	239	17	7.11	1.38	0.84	2.28	1.14	0.69	1.90	13	5.44	1.42	0.80	2.50	1.26	0.71	2.24
ART	430	40	9.30	1.85	1.32	2.60	1.50	1.05	2.14	21	4.88	1.26	0.80	1.99	1.10	0.69	1.76
Fine motor																	
Natural conception	8,602	363	4.22	ref			ref			479	5.57	ref			ref		
OI/AIH	239	17	7.11	1.74	1.05	2.88	1.51	0.90	2.52	16	6.69	1.22	0.73	2.04	1.19	0.70	2.02
ART	430	30	6.98	1.70	1.16	2.50	1.33	0.89	2.00	26	6.05	1.09	0.73	1.64	1.09	0.71	1.67
Problem solving																	
Natural conception	8,602	326	3.79	ref			ref			475	5.52	ref			ref		
OI/AIH	239	11	4.6	1.23	0.66	2.27	0.98	0.53	1.84	17	7.11	1.31	0.79	2.17	1.34	0.80	2.24
ART	430	25	5.81	1.57	1.03	2.38	1.20	0.77	1.85	22	5.12	0.92	0.60	1.43	0.89	0.57	1.40
Personal-social																	
Natural conception	8,602	417	4.85	ref			ref			372	4.32	ref			ref		
OI/AIH	239	21	8.79	1.89	1.20	2.99	1.55	0.97	2.49	15	6.28	1.48	0.87	2.52	1.35	0.78	2.34
ART	430	35	8.14	1.74	1.21	2.49	1.28	0.88	1.86	22	5.12	1.19	0.77	1.86	1.02	0.65	1.62

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, Assisted reproductive technology: ART.
Multivariable logistic models were adjusted for maternal age, parity, gestational week, childsex, maternal education level, and household income.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1, 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5,6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6,
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	5,6,9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	9
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	9,10
Outcome data	15*	Report numbers of outcome events or summary measures over time	9,10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9,10
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10,11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Association between maternal infertility treatment and child neurodevelopment: findings from the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study in Miyagi and Iwate Prefectures, Japan

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Association between maternal infertility treatment and child neurodevelopment: findings from the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study in Miyagi and Iwate Prefectures, Japan

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Abstract

Objectives: This study aimed to examine the association between infertility treatment and neurodevelopment in children at 2 and 3.5 years of age.

Design: Prospective cohort study.

Setting and participants: The study population consisted of mother-child pairs who participated in the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study in Miyagi and Iwate Prefectures, Japan. Pregnant women were recruited in obstetric clinics or hospitals and their children were followed up by the questionnaire.

Outcome measures: The children's neurodevelopmental outcomes were assessed at 2 and 3.5 years of age using the Ages and Stages Questionnaire, third edition (ASQ-3), which consists of questions on five developmental domains. We performed a multivariate logistic regression analysis of the association between infertility treatment (including ovulation induction [OI], artificial insemination with husband's sperm [AIH], and assisted reproductive technology [ART]) and the clinical range of ASQ-3.

Results: Of 9,655 mother-child pairs, 273 (2.8%) and 487 (5.0%) were conceived through OI/AIH and ART, respectively. The odds of having developmental delays at 2 years of age were

higher in children conceived through OI/AIH (odds ratio [OR], 1.36; 95% confidence interval [95% confidence interval (CI)], 1.00-1.85) and ART (OR, 1.36; 95% CI, 1.07-1.72) than in those conceived naturally. Additionally, OI/AIH and ART were significantly associated with communication (OR, 1.93; 95% CI, 1.25-2.98) and gross motor (OR, 1.50; 95% CI, 1.08-2.09) delays, respectively. There were no statistically significant differences in the odds of having developmental delays at 3.5 years of age in children conceived through OI/AIH (OR, 1.13; 95% CI, 0.79-1.61) and ART (OR, 1.03; 95% CI, 0.78-1.37).

Conclusion: In this study, we found a significant association between infertility treatment and children's neurodevelopment at 2 years of age, whereas no statistically significant differences were found at 3.5 years of age.

Keywords: Ages and Stages Questionnaire, Cohort study, Infertility treatment, Neurodevelopment, Assisted reproductive technology.

Word count: 2488

Strengths and limitations of this study

- The large sample size in this study allowed us to investigate the association between each

type of infertility treatment and neurodevelopmental outcomes.

- We evaluated children's neurodevelopment at two points—2 and 3.5 years of age.
- ASQ-3 is a screening tool (not a diagnostic tool) for developmental delays.
- The number of children born through specific forms of infertility treatment, such as fresh embryo transfer, was insufficient to allow sub-analysis of fresh or frozen embryo transfer.

Introduction

With the rapid progress in infertility treatment, the number of women who use infertility treatment is increasing, and more than 10% of the childbearing population has resorted to assisted reproductive technology (ART) for conception.[1] In recent years, the number of fertility treatments in Japan has also been on the increase. In 2018, 56,000 new-borns were conceived through ART, including in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). This accounts for approximately 6% of all live births in the same year.[2] Because conception using ART involves several processes (such as physical manipulation of gametes and exposure of embryos to hormones and culture media) that differ greatly from those in a natural conception, there is potential for disturbance of normal early developmental processes.[3, 4] Therefore, many studies have investigated the association between ART and neurodevelopmental outcomes. [5–8]

The findings have been almost entirely consistent in showing that singleton children conceived through ART and born at term are no different neurodevelopmentally from those born following natural conception. However, the evidence remains equivocal,[8–11] and another review stated that possible associations between infertility treatment and developmental delay require further assessment in larger studies.[12] The inconsistent results in the previous studies may have been due to the small sample size (fewer than 1000 children),[3] and a longitudinal study with a larger sample size is needed.

This study aims to investigate the association between infertility treatment and children's neurodevelopment at 2 and 3.5 years of age in a Japanese birth cohort.

Methods

Study setting and participants

This study was based on data obtained by the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study (TMM BirThree Cohort Study). The TMM BirThree Cohort Study is a prospective cohort study based in Miyagi and Iwate Prefectures, Japan, and has been published elsewhere.[13, 14] Pregnant women and their family members were contacted in obstetric clinics or hospitals from 2013 to 2017, and 23,406 pregnant women participated in the study. Written informed consent was obtained from all participants. All participants were free to

decline consent to participate in the research and were told that there was no disadvantage or risk involved with their refusal to participate. The TMM BirThree Cohort Study protocol was approved by the Tohoku University Tohoku Medical Megabank Organization's internal review board (2013-1-103-1).

Infertility treatments

We extracted infertility treatment types from maternal medical records, and they included ovulation induction (OI), artificial insemination (AIH), IVF, and ICSI; we refer to IVF and ICSI as ART in this study.

Neurodevelopmental assessments

The third edition of the Ages and Stages Questionnaire (ASQ-3) was used to evaluate child neurodevelopment.[15, 16] ASQ-3 is a comprehensive, reliable screening questionnaire that can be used for children aged 1 and 66 months. The guardians of children aged 2 and 3.5 years filled in the questionnaire. Each questionnaire contains 30 questions divided into five developmental domains: "communication", "fine motor", "gross motor", "problem solving", and "personal-social". Each domain has a set of six items, and each item is given a score of 10, 5, and 0 corresponding to "yes", "sometimes", and "not yet", respectively. The total score ranges

from 0 to 60 for each domain. We defined “developmental delay” in a domain as when the score was greater than two standard deviations (SD) below the mean in that domain.[17] In this study, we used the validated Japanese translation of ASQ-3.[16]

Covariates

Considering previous studies and the characteristics of the population in this study, we included maternal age at delivery, parity, preterm birth (PTB), child sex, birth defects, multiple births, maternal education, and household income as covariates of developmental outcomes.[7, 18] Maternal age, gestational weeks, parity, child sex, birth defect, and multiple birth were obtained from medical records. Birth defects were defined in this study as follow; anencephaly, microcephaly, hydrocephalus, craniotabes, holoprosencephaly, agenesis of the corpus callosum, other head or brain abnormalities, omphalocele, abdominal fissure, epidermolysis bullosa hereditarian, incontinentia pigmenti, myelomeningocele, Down's syndrome, trisomy 18, trisomy 13, achondroplasia, osteogenesis imperfecta, arthrogryposis multiplex congenita, other skeletal or muscle abnormalities, amniotic band syndrome, and other chromosomal abnormality. Data on maternal level of education and household income were obtained using a self-report questionnaire.

Statistical analysis

Characteristics of mothers and children were compared in three groups: natural conception, OI/AIH, and ART. Continuous and categorical variables were described as mean (SD) and frequency or proportion, respectively. Differences in prevalence were analysed using the chi-square test. Logistic regression analyses were performed to determine the associations between infertility treatment and each of the five domains of ASQ-3 at 2 and 3.5 years of age after adjusting for possible confounding factors. Odds ratios (ORs) and confidence intervals (CIs) were calculated. Children who were conceived naturally were used as the reference group in all analyses. For subgroup analyses, participants were classified into five groups as follows: natural conception, OI, AIH, IVF, and ICSI. Furthermore, we compared developmental outcomes among children born following natural conception, fresh embryo transfer (ET), and frozen-thawed ET. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Patient and public involvement

No participants were involved in this study because we used an existing dataset for the analysis.

Results

Characteristics of the participants

We analysed data from 9,655 eligible mother-child pairs who filled in ASQ-3 questionnaires (Figure 1). Of the 9,655 mother-child pairs, 273 (2.8%) were conceived through OI/AIH and 487 (5.0%) through ART. Maternal and child characteristics are shown in Table 1. Mothers who were exposed to infertility treatment were older and had higher levels of educational and household income. Children who were conceived following infertility treatment were likely to have a lower birth weight (LBW) and gestational age.

Infertility treatment and children's neurodevelopment at 2 years of age

In total, 1,437 children (14.9%) had developmental delay at 2 years of age when screened using the ASQ-3. The proportion of children with developmental delays at 2 years of age was 14.3%, 21.3%, and 22.5% for natural conception, OI/AIH, and ART, respectively. In the multivariable models, the odds of having developmental delays at 2 years of age were higher in children conceived through OI/AIH (OR, 1.36; 95% CI, 1.00-1.85) and ART (OR, 1.36; 95% CI, 1.07-1.72) than in those conceived naturally. OI/AIH and ART were significantly associated with communication (OR, 1.93; 95% CI, 1.25-2.98), and gross motor (OR, 1.50; 95% CI, 1.08-2.09) delays, respectively (Table 2).

Infertility treatment and children's neurodevelopment at 3.5 years of age

In total, 1,257 children (13.0%) had developmental delay at 3.5 years of age when screened using the ASQ-3. The proportion of children with developmental delay at 3.5 years of age was 12.9%,14.7%, and 13.8% for natural conception, OI/AIH, and ART, respectively. In the multivariable models, the odds of having developmental delays at 3.5 years of age were higher in children conceived through OI/AIH (OR, 1.13; 95% CI, 0.79-1.61) and ART (OR, 1.03; 95% CI, 0.78-1.37) than in those conceived naturally. Moreover, children conceived through infertility treatment had no statistically significant differences in any domains at 3.5 years of age (Table 2).

Subgroups based on the 5 domains and embryo types were analysed and the results are shown in supplementary tables S1 to S4. Although the number of children conceived through each infertility treatment was less, the results, when compared within the five groups or types of ET, were similar to those within the three groups.

Discussion

We investigated the association between infertility treatment and children's neurodevelopment, among 9,655 mother-child pairs. In multivariable models, a significant association between

infertility treatment and neurodevelopment was observed among the children conceived through infertility treatment, at 2 years of age; no statistically significant differences were found in them at 3.5 years of age.

Children conceived through ART are known to be at risk of LBW, PTB, and birth defects,[19] which are risk factors for neurodevelopmental disorders.[20, 21] However, the data from previous studies among children aged 1–5 years suggest that there are no developmental differences between children conceived through ART and those conceived naturally, after adjusting for confounding variables.[3, 19, 22] Even when children with congenital anomalies or genetic syndromes and multiple births (which are known to affect development) were excluded from the study (n = 9,271), a significant association between infertility treatment and children's neurodevelopment was seen at 2 years of age, while no statistically significant differences were found at 3.5 years of age (Table S5). Furthermore, we took into account very preterm (< 34 weeks) or extremely preterm (< 29 weeks) birth for adjusting the results for important risk factors and the similar result was obtained (Table S6). We also performed analysis adjusted for birth weight and the similar result was obtained (Table S7). A systematic review of neurodevelopmental disorders,[5] neuromotor, cognitive, language, and behavioural outcomes of children born after ART showed that ART had no significant effect on children's neuromotor and cognitive development.[6] A previous study showed no significant difference in the

neurodevelopmental scores between children conceived through ART and those conceived naturally; however, it showed a decrease in the score units of each scale among children conceived through ART.[23] The reason for the discrepancy in results at 2 years of age between our study and the previous study might be the proportion of children conceived through infertility treatment. Because infertility treatment has become widespread owing to technological advancement, the proportion of children conceived through infertility treatment was higher in our study than in previous studies.[24–26] Therefore, statistical differences might be detectable in our study. In addition, frozen-thawed embryos have been used for most ARTs in Japan.[27] Frozen-thawed ET is associated with a significantly lower incidence of PTB and LBW, which are risk factors for neurodevelopmental disorders.[28] Among children with developmental delay at 2 years of age, the proportion with developmental delay at 3.5 years of age was 47.3%,46.6%, and 38.5% for natural conception, OI/AIH, and ART, respectively. The proportion of children with developmental delay at 3.5 years of age was low, especially in those conceived through ART. Our longitudinal study demonstrated that at 2 and 3.5 years of age, the neurodevelopmental status of children conceived through infertility treatment, especially ART, might eventually catch up with that of those conceived naturally. In Japan, the health check-up for children is done at 1.5 years of age, so the counsel provided by doctors and public health nurses, which is tailored towards individual development, might also help promote the children's

development. A previous study in Japan stated that the developmental state of a child needs to be understood not only based on the characteristics of the child at one point but also on the progress of the child's development from the results of the health check-up at 1.5 years of age. In fact, it has been suggested that it is important to conduct a follow-up health check-up at 3 years of age in relation to the development at the previous age.[29]

Developmental delay in the communication domain was seen at age 2 years in children conceived through OI/AIH but not in those conceived through ART. A previous study showed that compared with children conceived naturally, those conceived through OI had lower verbal ability at 3 years of age, while those conceived through ART had higher verbal ability; children conceived through infertility treatment had higher verbal ability at 5 years of age than those conceived naturally.[30] A previous hospital-based cohort study in Japan showed that children conceived through ART had significantly better language development than those conceived naturally.[7] Although direct comparisons with other studies assessing neurodevelopment in children conceived through infertility treatment are difficult because each researcher uses various assessment tools and control groups, the children conceived through ART might have higher verbal ability or better language development than those conceived through OI/AIH or naturally. Children conceived through infertility treatments generally enjoy advantageous socioeconomic benefits. It is possible that parents who utilise ART to conceive

may invest more into rearing the children, which might have the greatest impact on language skills.[7, 30, 31]

Furthermore, ART was associated with developmental delays in the gross motor domain at 2 years of age. This association was not seen at 3.5 years of age. A hospital-based cohort study in Japan reported no significant difference in gross motor function (evaluated using the Ability for Basic Movement Scale for Children) at 12 months of age between the ART and control groups.[32] In contrast, the Danish National Birth Cohort study showed that infertility treatment, especially ICSI, may be associated with a slight delay in gross motor development at 1.5 years of age.[8] However, another study suggested no differences in motor development at 5 years of age among children conceived through ICSI, IVF, and natural conception.[33] These findings suggest that infertility treatment might be associated with children's development in the early stage, but the neurodevelopment of children conceived through infertility treatment might catch up with that of those conceived naturally.

This study had some limitations. The ASQ-3 is a screening tool (not a diagnostic tool) for developmental delays; however, it is considered to be highly reliable because it has been validated in many countries worldwide and has been used in a variety of studies.[18, 34, 35] Since we focused on children who filled in both questionnaires (at 2 and 3.5 years of age), there was the concern of bias due to the reduced number of participants and the deterioration of

statistical power in the analysis. However, among the children who did not fill in the questionnaire at 3.5 years of age, the percentage of those who had neurodevelopmental delay at 2 years of age was 14.5%,10.8%, and 15.0% for natural conception, OI/AIH, and ART, respectively. Therefore, there was no bias such as more children with neurodevelopmental delay at 2 years of age among children who did not answer the questionnaire at 3.5 years old and the potential applicability of our results to national populations can be discussed. In the subgroup analysis with detailed classification, the number of children conceived through specific forms of infertility treatment, such as fresh ET, was insufficient to compare the ET types. Male infertility or the time to pregnancy should be taken into account when evaluating subfertility patients. [36] However, it was difficult to include these factors because we did not collect them in this study. Research in this field is complex because of the need to collect various data to determine the effects of infertility treatment on offspring outcomes. As this was an observational study, residual confounding might have occurred. However, this study was a large longitudinal birth cohort with detailed information from the participants, and although continued follow-up of children born after infertility treatment is needed, this study helps to increase the understanding of the association between infertility treatment and neurodevelopmental outcomes in Japanese children.

Conclusion

In this study, we found a significant association between infertility treatment and neurodevelopment among children who were conceived through infertility treatments and those conceived naturally, at 2 years of age; however, no statistically significant differences were found at 3.5 years of age.

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Contributors: AN, MI, and T.Obara designed the study. AN, MI, T.Obara, KM, FU, FM, T.Onuma, ZW, NS, NI, HH, MT, MS, JS, S.Kure, NY, and S.Kuriyama contributed to data collection. AN, MI and TO contributed to data analysis and interpretation. AN, MI, T.Obara, KM, FU, FM, T.Onuma, ZW, NS, NI, HH, T. Otsuka, MT, HT, MS, JS, S.Kure, NY, and S.Kuriyama provided advice regarding critically important intellectual content and helped to draft the manuscript.

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Figure Legend

Figure 1. Flow chart of the exclusion criteria in the TMM BirThree Cohort Study

This flow chart describes the exclusion criteria and the number of total participants, excluded participants, and eligible participants. ASQ-3, Ages & Stages Questionnaires, Third Edition.

Table 1. Characteristics of the study population

	Total		Infertility treatment						p
			Natural conception		OI/AIH		ART		
	n=9,655	%	n=8,895	%	n=273	%	n=487	%	
Mother's age, mean (SD), y	32.7	4.7	32.4	4.6	34.3	3.9	37.2	3.8	<0.0001
Pre-pregnancy BMI (kg/m2)									
< 18.5	1,365	14.1	1,279	14.4	36	13.2	50	10.3	0.0707
18.5 to < 25.0	7,204	74.6	6,636	74.6	196	72.1	372	76.3	
≧ 25.0	1,083	11.2	977	11.0	41	15.0	65	13.4	
Educational level									
High school graduate or less	2,711	28.1	2,565	28.8	49	18.0	97	19.9	<0.0001
Junior or vocational college graduate	3,513	36.4	3,215	36.1	100	36.6	198	40.7	
University graduate or above	2,722	28.2	2,457	27.6	102	37.4	163	33.5	
Household income (JPY/year)									
< 4,000,000	3,117	32.3	2,974	33.4	63	23.1	80	16.4	<0.0001
4,000,000 to < 6,000,000	3,063	31.7	2,810	31.6	86	31.5	167	34.3	
≥ 6,000,000	2,992	31.0	2,658	29.9	111	40.7	223	45.8	
Cigarette smoking									
Never	6,194	64.6	5,675	64.3	195	71.4	324	66.7	<0.0001
Stopped before pregnancy	2,291	23.9	2,075	23.5	60	22.0	156	32.1	
Stopped after pregnancy	950	9.9	928	10.5	17	6.2	5	1.0	
Smoked during early pregnancy	152	1.6	150	1.7	1	0.4	1	0.2	
Alcohol consumption									
Drinking at early pregnancy	1,917	20.0	1,766	20.0	57	21.0	94	19.3	0.5426

Former	3,273	34.1	3,023	34.2	93	34.2	157	32.3	
Never	3,871	40.3	3,568	40.4	105	38.6	198	40.7	
Cannot drink because of constitution	536	5.6	482	5.5	17	6.3	37	7.6	
Parity									
Nullipara	4,564	47.3	4,024	45.2	202	74.0	338	69.4	<0.0001
Multipara	5,091	52.7	4,871	54.8	71	26.0	149	30.6	
Child's sex									
Male	4,958	51.4	4,581	51.5	137	50.2	240	49.3	0.5874
Female	4,697	48.7	4,314	48.5	136	49.8	247	50.7	
Gestational age (weeks)									
≥37	9,017	93.4	8,338	93.7	245	89.7	434	89.1	<0.0001
34 to < 37	482	5.0	417	4.7	26	9.5	39	8.0	
29 to < 34	113	1.2	98	1.1	2	0.7	13	2.7	
< 29	33	0.3	32	0.4	0	0.0	1	0.2	
Birth weight (g)									
≥2,500	8,689	90.0	8,037	90.4	229	83.9	423	86.9	0.0004
< 2,500	952	9.9	844	9.5	44	16.1	64	13.1	
Birth defect									
No	9,477	98.3	8,740	98.4	269	98.5	468	96.1	0.0033
Yes	169	1.8	146	1.6	4	1.5	19	3.9	
Multiple birth									
No	9,440	97.8	8,746	98.3	244	89.4	450	92.4	<0.0001
Yes	215	2.2	149	1.7	29	10.6	37	7.6	

ASQ-3（2Y）										
	> Mean -2SD	8,218	85.1	7,626	85.7	215	78.8	377	77.4	<0.0001
	≤ Mean -2SD	1,437	14.9	1,269	14.3	58	21.3	110	22.6	
ASQ-3（3.5Y）										
	> Mean -2SD	8,398	87.0	7,746	87.1	233	85.4	419	86.0	0.5749
	≤ Mean -2SD	1,257	13.0	1,149	12.9	40	14.7	68	14.0	
Father's age, mean (SD), y		n=4,183		n=3,784		n=142		n=257		
		34.5	5.7	34.2	5.6	35.1	4.8	38.8	5.2	<0.0001

Standard deviation: SD, Body mass index: BMI, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH.

Assisted reproductive technology: ART, Ages & Stages Questionnaires, Third Edition: ASQ-3.

Table 2. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,655)

		2 years								3.5 years							
				Crude			Adjusted					Crude			Adjusted		
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI	
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper
Total score																	
Natural conception	8,895	1,269	14.27	ref			ref			1,149	12.92	ref			ref		
OI/AIH	273	58	21.25	1.62	1.21	2.18	1.36	1.00	1.85	40	14.65	1.16	0.82	1.63	1.13	0.79	1.61
ART	487	110	22.59	1.75	1.41	2.19	1.36	1.07	1.72	68	13.96	1.09	0.84	1.42	1.03	0.78	1.37
Communication																	
Natural conception	8,895	445	5.00	ref			ref			482	5.42	ref			ref		
OI/AIH	273	27	9.89	2.09	1.39	3.14	1.93	1.25	2.98	17	6.23	1.16	0.70	1.91	1.13	0.68	1.90
ART	487	27	5.54	1.12	0.75	1.66	0.95	0.62	1.45	29	5.95	1.11	0.75	1.63	1.04	0.69	1.56
Gross motor																	
Natural conception	8,895	483	5.43	ref			ref			367	4.13	ref			ref		
OI/AIH	273	20	7.33	1.38	0.87	2.19	1.15	0.72	1.85	16	5.86	1.45	0.86	2.42	1.26	0.74	2.13
ART	487	49	10.06	1.95	1.43	2.65	1.50	1.08	2.09	25	5.13	1.26	0.83	1.91	1.03	0.67	1.60
Fine motor																	
Natural conception	8,895	389	4.37	ref			ref			513	5.77	ref			ref		
OI/AIH	273	17	6.23	1.45	0.88	2.40	1.28	0.76	2.13	19	6.96	1.22	0.76	1.97	1.20	0.74	1.96
ART	487	34	6.98	1.64	1.14	2.36	1.27	0.86	1.86	31	6.37	1.11	0.76	1.62	1.05	0.71	1.56
Problem solving																	
Natural conception	8,895	354	3.98	ref			ref			505	5.68	ref			ref		
OI/AIH	273	13	4.76	1.21	0.68	2.13	0.90	0.50	1.60	18	6.59	1.17	0.72	1.91	1.20	0.73	1.99

ART	487	32	6.57	1.70	1.17	2.47	1.18	0.79	1.75	27	5.54	0.98	0.66	1.45	0.91	0.60	1.38
Personal-social																	
Natural conception	8,895	449	5.05	ref			ref			400	4.5	ref			ref		
OI/AIH	273	24	8.79	1.81	1.18	2.79	1.46	0.93	2.28	16	5.86	1.32	0.79	2.21	1.20	0.70	2.04
ART	487	42	8.62	1.78	1.28	2.47	1.25	0.88	1.77	28	5.75	1.30	0.87	1.92	1.05	0.69	1.60

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, Assisted reproductive technology: ART

Multivariable logistic models were adjusted for maternal age, parity, gestational week (< 37 weeks), child sex, birth defect, multiple birth, maternal education level, and household income.

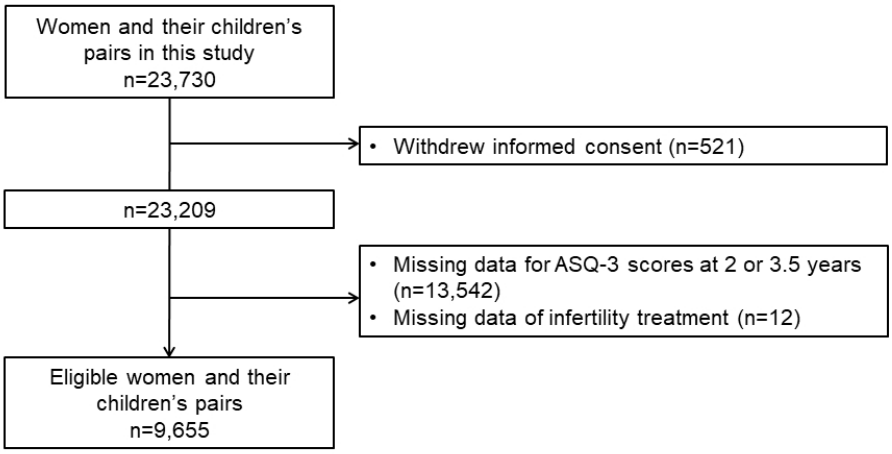

Figure 1. Flow chart of the exclusion criteria in the TMM BirThree Cohort Study

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Table S1. Characteristics of the study population

	Total		Infertility treatment										p
			Natural conception		OI		AIH		IVF		ICSI		
	n=9,655	%	n=8,895	%	n=147	%	n=126	%	n=342	%	n=145	%	
Mother's age, mean (SD), y	32.7	4.7	32.4	4.6	33.1	3.7	35.6	3.7	37.1	3.7	37.3	3.9	<0.0001
Pre-pregnancy BMI (kg/m^2)													
< 18.5	1,365	14.1	1,279	14.4	16	10.9	20	15.9	29	8.5	21	14.5	0.0283
18.5 to < 25.0	7,204	74.6	6,636	74.6	104	70.8	92	73.0	261	76.3	111	76.6	
≥ 25.0	1,083	11.2	977	11.0	27	18.4	14	11.1	52	15.2	13	9.0	
Educational level													
High school graduate or less	2,711	28.1	2,565	28.8	28	19.1	21	16.7	74	21.6	23	15.9	<0.0001
Junior college or vocational college graduate	3,513	36.4	3,215	36.1	51	34.7	49	38.9	135	39.5	63	43.5	
University graduate or above	2,722	28.2	2,457	27.6	56	38.1	46	36.5	111	32.5	52	35.9	
Household income (JPY/year)													
< 4,000,000	3,117	32.3	2,974	33.4	42	28.6	21	16.7	64	18.7	16	11.0	<0.0001
4,000,000 to < 6,000,000	3,063	31.7	2,810	31.6	37	25.2	49	38.9	120	35.1	47	32.4	
≥ 6,000,000	2,992	31.0	2,658	29.9	62	42.2	49	38.9	145	42.4	78	53.8	
Cigarette smoking													
Never	6,194	64.6	5,675	64.3	100	68.0	95	75.4	221	64.8	103	71.0	<0.0001
Stoped before pregnancy	2,291	23.9	2,075	23.5	31	21.1	29	23.0	115	33.7	41	28.3	
Stopped after pregnancy	950	9.9	928	10.5	15	10.2	2	1.6	5	1.5	0	0.0	
Smoking at early pregnancy	152	1.6	150	1.7	1	0.7	0	0.0	0	0.0	1	0.7	
Alcohol drinking													
Drinking at early pregnancy	1,917	20.0	1,766	20.0	25	17.0	32	25.6	75	22.0	19	13.1	0.0741
Former	3,273	34.1	3,023	34.2	55	37.4	38	30.4	112	32.8	45	31.0	
Never	3,871	40.3	3,568	40.4	54	36.7	51	40.8	128	37.5	70	48.3	
Cannot drink because of constitution	536	5.6	482	5.5	13	8.8	4	3.2	26	7.6	11	7.6	
Parity													
Nullipara	4,564	47.3	4,024	45.2	100	68.0	102	81.0	242	70.8	96	66.2	<0.0001
Multipara	5,091	52.7	4,871	54.8	47	32.0	24	19.1	100	29.2	49	33.8	
Child sex													
Male	4,958	51.4	4,581	51.5	84	57.1	53	42.1	158	46.2	82	56.6	0.0205
Female	4,697	48.7	4,314	48.5	63	42.9	73	57.9	184	53.8	63	43.5	
Gestational week													
≥37	9,017	93.4	8,338	93.7	130	88.4	115	91.3	302	88.3	132	91.0	<0.0001
34 to < 37	482	5.0	417	4.7	17	11.6	9	7.1	28	8.2	11	7.6	
29 to < 34	113	1.2	98	1.1	0	0.0	2	1.6	11	3.2	2	1.4	
< 29	33	0.3	32	0.4	0	0.0	0	0.0	1	0.3	0	0.0	
Birth weight (g)													
≥2,500	8,689	90.0	8,037	90.4	126	85.7	103	81.8	294	86.0	129	89.0	0.0039
< 2,500	952	9.9	844	9.5	21	14.3	23	18.3	48	14.0	16	11.0	
Birth defect													
No	9,477	98.3	8,740	98.4	145	98.6	124	98.4	330	96.5	138	95.2	0.005
Yes	169	1.8	146	1.6	2	1.4	2	1.6	12	3.5	7	4.8	
Multiple birth													
No	9,440	97.8	8,746	98.3	128	87.1	116	92.1	311	90.9	139	95.9	<0.0001
Yes	215	2.2	149	1.7	19	12.9	10	7.9	31	9.1	6	4.1	
ASQ-3（2Y）													
> Mean -2SD	8,218	85.1	7,626	85.7	119	81.0	96	76.2	265	77.5	112	77.2	<0.0001
≤ Mean-2SD	1,437	14.9	1,269	14.3	28	19.1	30	23.8	77	22.5	33	22.8	
ASQ-3（3.5Y）													
> Mean -2SD	8,398	87.0	7,746	87.1	127	86.4	106	84.1	295	86.3	124	85.5	0.8329
≤ Mean-2SD	1,257	13.0	1,149	12.9	20	13.6	20	15.9	47	13.7	21	14.5	
Father's age, mean (SD), y	n=4,183		n=3,784		n=74		n=68		n=172		n=85		
	34.5	5.7	34.2	5.6	34.3	4.7	35.9	4.7	38.5	5.4	39.6	4.9	<0.0001

Standard deviation: SD, Body mass index: BMI, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, In vitro fertilization: IVF, intracytoplasmic sperm injection: ICSI, Ages & Stages Questionnaires, Third Edition: ASQ-3.

Table S2. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,655)

		2 years								3.5 years							
				Crude			Adjusted					Crude			Adjusted		
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI	
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper
Total score																	
Natural conception	8,895	1,269	14.27	ref			ref			1,149	12.92	ref			ref		
OI	147	28	19.05	1.41	0.93	2.14	1.22	0.79	1.87	20	13.61	1.06	0.66	1.71	1.01	0.62	1.64
AIH	126	30	23.81	1.88	1.24	2.84	1.53	0.99	2.34	20	15.87	1.27	0.79	2.06	1.29	0.78	2.13
IVF	342	77	22.51	1.75	1.35	2.27	1.34	1.02	1.76	47	13.74	1.07	0.79	1.47	1.02	0.73	1.42
ICSI	145	33	22.76	1.77	1.20	2.62	1.41	0.94	2.11	21	14.48	1.14	0.72	1.82	1.07	0.66	1.74
Communication																	
Natural conception	8,895	445	5	ref			ref			482	5.42	ref			ref		
OI	147	16	10.88	2.32	1.37	3.93	2.12	1.22	3.69	9	6.12	1.14	0.58	2.25	1.05	0.52	2.12
AIH	126	11	8.73	1.82	0.97	3.40	1.71	0.88	3.30	8	6.35	1.18	0.58	2.44	1.23	0.59	2.59
IVF	342	19	5.56	1.12	0.70	1.79	0.94	0.57	1.54	20	5.85	1.08	0.68	1.72	1.02	0.63	1.64
ICSI	145	8	5.52	1.11	0.54	2.28	0.98	0.47	2.05	9	6.21	1.16	0.59	2.28	1.10	0.55	2.20
Gross motor																	
Natural conception	8,895	483	5.43	ref			ref			367	4.13	ref			ref		
OI	147	7	4.76	0.87	0.41	1.87	0.76	0.35	1.64	8	5.44	1.34	0.65	2.75	1.19	0.57	2.48
AIH	126	13	10.32	2.00	1.12	3.58	1.59	0.88	2.89	8	6.35	1.58	0.76	3.25	1.32	0.63	2.77
IVF	342	32	9.36	1.80	1.24	2.62	1.38	0.93	2.05	14	4.09	0.99	0.58	1.71	0.80	0.45	1.40
ICSI	145	17	11.72	2.31	1.38	3.87	1.81	1.07	3.07	11	7.59	1.91	1.02	3.56	1.64	0.87	3.12
Fine motor																	
Natural conception	8,895	389	4.37	ref			ref			513	5.77	ref			ref		
OI	147	7	4.76	1.09	0.51	2.35	1.02	0.47	2.22	10	6.8	1.19	0.62	2.28	1.12	0.58	2.17
AIH	126	10	7.94	1.89	0.98	3.63	1.55	0.80	3.03	9	7.14	1.26	0.63	2.49	1.29	0.64	2.62
IVF	342	27	7.89	1.87	1.25	2.81	1.45	0.95	2.22	18	5.26	0.91	0.56	1.47	0.86	0.52	1.43
ICSI	145	7	4.83	1.11	0.52	2.39	0.87	0.40	1.89	13	8.97	1.61	0.90	2.86	1.49	0.82	2.70
Problem solving																	
Natural conception	8,895	354	3.98	ref			ref			505	5.68	ref			ref		
OI	147	6	4.08	1.03	0.45	2.34	0.80	0.34	1.84	9	6.12	1.08	0.55	2.14	1.12	0.56	2.23
AIH	126	7	5.56	1.42	0.66	3.06	1.00	0.46	2.20	9	7.14	1.28	0.65	2.53	1.31	0.65	2.64
IVF	342	22	6.43	1.66	1.06	2.59	1.13	0.70	1.80	20	5.85	1.03	0.65	1.64	0.98	0.60	1.57
ICSI	145	10	6.9	1.79	0.93	3.43	1.31	0.67	2.55	7	4.83	0.84	0.39	1.81	0.76	0.35	1.66
Personal-social																	
Natural conception	8,895	449	5.05	ref			ref			400	4.5	ref			ref		
OI	147	10	6.8	1.37	0.72	2.63	1.17	0.60	2.27	8	5.44	1.22	0.60	2.51	1.13	0.54	2.35
AIH	126	14	11.11	2.35	1.34	4.13	1.77	0.99	3.19	8	6.35	1.44	0.70	2.97	1.28	0.60	2.70
IVF	342	26	7.6	1.55	1.03	2.33	1.09	0.71	1.68	20	5.85	1.32	0.83	2.10	1.09	0.67	1.78
ICSI	145	16	11.03	2.33	1.38	3.96	1.63	0.94	2.82	8	5.52	1.24	0.60	2.55	0.97	0.46	2.02

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, In vitro fertilization: IVF, intracytoplasmic sperm injection: ICSI.
Multivariable logistic models were adjusted for maternal age, parity, gestational week, child sex, birth defect, multiple birth, maternal education level, and household income.

Table S3. Characteristics of study population for ET

	Total		Natural conception		ART (n=485)						p
					Fresh ET		Frozen-thawed ET		Missing		
	n=9,382	%	n=8,895	%	n=46	%	n=314	%	n=127	%	
Mother's age, mean (SD), y	32.7	4.7	32.4	4.6	37.8	3.6	37.3	3.8	36.8	3.6	<0.0001
Pre-pregnancy BMI (kg/m^2)											
< 18.5	1,329	14.2	1,279	14.4	9	19.6	30	9.6	11	8.7	0.2997
18.5 to < 25.0	7,008	74.7	6,636	74.6	32	69.6	242	77.1	98	77.2	
≥ 25.0	1,042	11.1	977	11.0	5	10.9	42	13.4	18	14.2	
Educational level											
High school graduate or less	2,662	28.4	2,565	28.8	9	19.6	64	20.4	24	18.9	0.0018
Junior college or vocational college graduate	3,413	36.4	3,215	36.1	23	50.0	122	38.9	53	41.7	
University graduate or above	2,620	27.9	2,457	27.6	12	26.1	111	35.4	40	31.5	
Household income (JPY/year)											
< 4,000,000	3,054	32.6	2,974	33.4	6	13.0	53	16.9	21	16.5	<0.0001
4,000,000 to < 6,000,000	2,977	31.7	2,810	31.6	16	34.8	108	34.4	43	33.9	
≥ 6,000,000	2,881	30.7	2,658	29.9	22	47.8	144	45.9	57	44.9	
Cigarette smoking											
Never	5,999	64.4	5,675	64.3	24	52.2	209	66.8	91	71.7	<0.0001
Stoped before pregnancy	2,231	24.0	2,075	23.5	22	47.8	99	31.6	35	27.6	
Stopped after pregnancy	933	10.0	928	10.5	0	0.0	4	1.3	1	0.8	
Smoking at early pregnancy	151	1.6	150	1.7	0	0.0	1	0.3	0	0.0	
Alcohol drinking											
Drinking at early pregnancy	1,860	20.0	1,766	20.0	6	13.0	60	19.2	28	22.1	0.2181
Former	3,180	34.1	3,023	34.2	22	47.8	98	31.3	37	29.1	
Never	3,766	40.4	3,568	40.4	16	34.8	128	40.9	54	42.5	
Cannot drink because of constitution	519	5.6	482	5.5	2	4.4	27	8.6	8	6.3	
Parity											
Nullipara	4,362	46.5	4,024	45.2	35	76.1	212	67.5	91	71.7	<0.0001
Multipara	5,020	53.5	4,871	54.8	11	23.9	102	32.5	36	28.4	
Child sex											
Male	4,821	51.4	4,581	51.5	22	47.8	156	49.7	62	48.8	0.806
Female	4,561	48.6	4,314	48.5	24	52.2	158	50.3	65	51.2	
Gestational week											
≥37	8,772	93.5	8,338	93.7	38	82.6	285	90.8	111	87.4	<0.0001
< 37	610	6.5	557	6.3	8	17.4	29	9.2	16	12.6	
Birth weight (g)											
≥2,500	8,460	90.2	8,037	90.4	41	89.1	277	88.2	105	82.7	0.0814
< 2,500	908	9.7	844	9.5	5	10.9	37	11.8	22	17.3	
Birth defect											
No	9,208	98.2	8,740	98.4	45	97.8	302	96.2	121	95.3	0.0019
Yes	165	1.8	146	1.6	1	2.2	12	3.8	6	4.7	
Multiple birth											
No	9,196	98.0	8,746	98.3	42	91.3	287	91.4	121	95.3	<0.0001
Yes	186	2.0	149	1.7	4	8.7	27	8.6	6	4.7	
ASQ-3（2Y）											
> Mean -2SD	8,003	85.3	7,626	85.7	33	71.7	242	77.1	102	80.3	<0.0001
≤ Mean-2SD	1,379	14.7	1,269	14.3	13	28.3	72	22.9	25	19.7	
ASQ-3（3.5Y）											
> Mean -2SD	8,165	87.0	7,746	87.1	39	84.8	269	85.7	111	87.4	0.8594
≤ Mean-2SD	1,217	13.0	1,149	12.9	7	15.2	45	14.3	16	12.6	
Father's age, mean (SD), y	n=4,041		n=3,784		n=25		n=169		n=63		
	34.5	5.7	34.2	5.6	38.9	4.1	38.9	5.3	38.8	5.4	<0.0001

Standard deviation: SD, Body mass index: BMI, Embryo transfer: ET, Assisted reproductive technology: ART, Ages & Stages Questionnaires, Third Edition: ASQ-3.

Table S4. Odds ratios (95% CIs) from logistic regression models for ET and children's neurodevelopment at the age of 2 and 3.5 years (n=485)

	2 years									3.5 years								
				Crude			Adjusted					Crude			Adjusted				
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI			
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper		
Total score																			
Natural conception	8,895	1,269	14.27	ref			ref			1,149	12.92	ref			ref				
Fresh ET	46	13	28.26	2.37	1.24	4.51	1.71	0.88	3.33	7	15.22	1.21	0.54	2.71	1.12	0.49	2.58		
Frozen-thawed ET	314	72	22.93	1.79	1.37	2.34	1.39	1.05	1.85	45	14.33	1.13	0.82	1.56	1.07	0.76	1.50		
Communication																			
Natural conception	8,895	445	5	ref			ref			482	5.42	ref			ref				
Fresh ET	46	4	8.7	1.81	0.65	5.07	1.43	0.49	4.19	1	2.17	0.39	0.05	2.82	0.33	0.04	2.42		
Frozen-thawed ET	314	16	5.1	1.02	0.61	1.70	0.88	0.52	1.50	23	7.32	1.38	0.89	2.13	1.28	0.81	2.02		
Gross motor																			
Natural conception	8,895	483	5.43	ref			ref			367	4.13	ref			ref				
Fresh ET	46	5	10.87	2.12	0.84	5.40	1.51	0.59	3.90	2	4.35	1.06	0.26	4.37	0.84	0.20	3.53		
Frozen-thawed ET	314	34	10.83	2.12	1.47	3.06	1.65	1.12	2.43	21	6.69	1.67	1.06	2.63	1.37	0.85	2.21		
Fine motor																			
Natural conception	8,895	389	4.37	ref			ref			513	5.77	ref			ref				
Fresh ET	46	3	6.52	1.53	0.47	4.94	1.06	0.32	3.47	3	6.52	1.14	0.35	3.69	1.11	0.34	3.67		
Frozen-thawed ET	314	21	6.69	1.57	1.00	2.47	1.21	0.75	1.94	19	6.05	1.05	0.66	1.69	1.01	0.62	1.65		
Problem solving																			
Natural conception	8,895	354	3.98	ref			ref			505	5.68	ref			ref				
Fresh ET	46	3	6.52	1.68	0.52	5.45	1.07	0.32	3.56	1	2.17	0.37	0.05	2.68	0.34	0.05	2.49		
Frozen-thawed ET	314	23	7.32	1.91	1.23	2.95	1.33	0.84	2.11	18	5.73	1.01	0.62	1.64	0.95	0.57	1.56		
Personal-social																			
Natural conception	8,895	449	5.05	ref			ref			400	4.5	ref			ref				
Fresh ET	46	3	6.52	1.31	0.41	4.25	0.82	0.25	2.74	2	4.35	0.97	0.23	4.00	0.75	0.18	3.19		
Frozen-thawed ET	314	36	11.46	2.44	1.70	3.49	1.76	1.20	2.58	17	5.41	1.22	0.74	2.00	0.99	0.59	1.66		

Confidence interval: CI, odds ratio: OR, Embryo transfer: ET.

Multivariable logistic models were adjusted for maternal age, parity, gestational week, child sex, birth defect, multiple birth, maternal education level, and household income.

Table S5. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,271)

	2 years									3.5 years								
				Crude			Adjusted					Crude			Adjusted				
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI			
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper	
Total score																		
Natural conception	8,602	1,200	13.95	ref			ref			1,092	12.69	ref			ref			
OI/AIH	239	48	20.08	1.55	1.12	2.14	1.36	0.98	1.89	35	14.64	1.18	0.82	1.70	1.20	0.83	1.75	
ART	430	90	20.93	1.63	1.28	2.08	1.33	1.03	1.71	57	13.26	1.05	0.79	1.40	1.05	0.78	1.41	
Communication																		
Natural conception	8,602	411	4.78	ref			ref			452	5.25	ref			ref			
OI/AIH	239	19	7.95	1.72	1.07	2.78	1.77	1.08	2.90	15	6.28	1.21	0.71	2.05	1.30	0.76	2.23	
ART	430	19	4.42	0.92	0.58	1.48	0.87	0.53	1.41	22	5.12	0.97	0.63	1.51	1.01	0.64	1.59	
Gross motor																		
Spontaneous pregnancy	8,602	452	5.25	ref			ref			336	3.91	ref			ref			
OI/AIH	239	17	7.11	1.38	0.84	2.28	1.14	0.69	1.90	13	5.44	1.42	0.80	2.50	1.26	0.71	2.24	
ART	430	40	9.30	1.85	1.32	2.60	1.50	1.05	2.14	21	4.88	1.26	0.80	1.99	1.10	0.69	1.76	
Fine motor																		
Natural conception	8,602	363	4.22	ref			ref			479	5.57	ref			ref			
OI/AIH	239	17	7.11	1.74	1.05	2.88	1.51	0.90	2.52	16	6.69	1.22	0.73	2.04	1.19	0.70	2.02	
ART	430	30	6.98	1.70	1.16	2.50	1.33	0.89	2.00	26	6.05	1.09	0.73	1.64	1.09	0.71	1.67	
Problem solving																		
Natural conception	8,602	326	3.79	ref			ref			475	5.52	ref			ref			
OI/AIH	239	11	4.6	1.23	0.66	2.27	0.98	0.53	1.84	17	7.11	1.31	0.79	2.17	1.34	0.80	2.24	
ART	430	25	5.81	1.57	1.03	2.38	1.20	0.77	1.85	22	5.12	0.92	0.60	1.43	0.89	0.57	1.40	
Personal-social																		
Natural conception	8,602	417	4.85	ref			ref			372	4.32	ref			ref			
OI/AIH	239	21	8.79	1.89	1.20	2.99	1.55	0.97	2.49	15	6.28	1.48	0.87	2.52	1.35	0.78	2.34	
ART	430	35	8.14	1.74	1.21	2.49	1.28	0.88	1.86	22	5.12	1.19	0.77	1.86	1.02	0.65	1.62	

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, Assisted reproductive technology: ART.
Multivariable logistic models were adjusted for maternal age, parity, gestational week, childsex, maternal education level, and household income.

Table S6. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,655)

		2 years								3.5 years							
				Crude			Adjusted					Crude			Adjusted		
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI	
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper
Total score																	
Natural conception	8,895	1,270	14.27	ref			ref			1,150	12.92	ref			ref		
OI/AIH	273	58	21.32	1.55	1.12	2.14	1.38	1.01	1.87	40	14.71	1.18	0.82	1.70	1.15	0.81	1.64
ART	487	109	22.47	1.63	1.28	2.08	1.37	1.08	1.73	67	13.81	1.05	0.79	1.40	1.04	0.78	1.37
Communication																	
Natural conception	8,895	446	5.01	ref			ref			483	5.43	ref			ref		
OI/AIH	273	27	9.93	1.72	1.07	2.78	1.99	1.29	3.08	17	6.25	1.21	0.71	2.05	1.16	0.69	1.94
ART	487	26	5.36	0.92	0.58	1.48	0.96	0.63	1.47	28	5.77	0.97	0.63	1.51	1.05	0.70	1.57
Gross motor																	
Spontaneous pregnancy	8,895	484	5.44	ref			ref			367	4.12	ref			ref		
OI/AIH	273	20	7.35	1.38	0.84	2.28	1.18	0.73	1.89	16	5.88	1.42	0.80	2.50	1.32	0.78	2.24
ART	487	48	9.90	1.85	1.32	2.60	1.53	1.10	2.13	25	5.15	1.26	0.80	1.99	1.04	0.67	1.62
Fine motor																	
Natural conception	8,895	389	4.37	ref			ref			514	5.78	ref			ref		
OI/AIH	273	17	6.25	1.74	1.05	2.88	1.34	0.80	2.23	19	6.99	1.22	0.73	2.04	1.24	0.76	2.02
ART	487	34	7.01	1.70	1.16	2.50	1.29	0.88	1.90	30	6.19	1.09	0.73	1.64	1.05	0.71	1.57
Problem solving																	
Natural conception	8,895	354	3.98	ref			ref			506	5.69	ref			ref		
OI/AIH	273	13	4.78	1.23	0.66	2.27	0.93	0.52	1.67	18	6.62	1.31	0.79	2.17	1.24	0.75	2.05
ART	487	32	6.60	1.57	1.03	2.38	1.19	0.80	1.77	26	5.36	0.92	0.60	1.43	0.91	0.60	1.37
Personal-social																	
Natural conception	8,895	449	5.05	ref			ref			401	4.51	ref			ref		
OI/AIH	273	24	8.82	1.89	1.20	2.99	1.52	0.98	2.38	16	5.88	1.48	0.87	2.52	1.23	0.72	2.09
ART	487	42	8.66	1.74	1.21	2.49	1.27	0.89	1.80	27	5.57	1.19	0.77	1.86	1.06	0.70	1.60

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, Assisted reproductive technology: ART.

Multivariable logistic models were adjusted for maternal age, parity, gestational week (< 29, 29–34, 34–37, ≥ 37 weeks), child sex, birth defect, multiple birth, maternal education level, and household income.

Table S7. Odds ratios (95% CIs) from logistic regression models for infertility treatment and children's neurodevelopment at the age of 2 and 3.5 years (n=9,655)

		2 years								3.5 years							
				Crude			Adjusted					Crude			Adjusted		
				OR	95% CI		OR	95% CI				OR	95% CI		OR	95% CI	
	N	n	%		Lower	Upper		Lower	Upper	n	%		Lower	Upper		Lower	Upper
Total score																	
Natural conception	8,895	1,270	14.27	ref			ref			1,150	12.92	ref			ref		
OI/AIH	273	58	21.32	1.55	1.12	2.14	1.35	0.99	1.84	40	14.71	1.18	0.82	1.70	1.12	0.79	1.60
ART	487	109	22.47	1.63	1.28	2.08	1.37	1.08	1.73	67	13.81	1.05	0.79	1.40	1.04	0.79	1.38
Communication																	
Natural conception	8,895	446	5.01	ref			ref			483	5.43	ref			ref		
OI/AIH	273	27	9.93	1.72	1.07	2.78	1.93	1.25	2.97	17	6.25	1.21	0.71	2.05	1.12	0.67	1.88
ART	487	26	5.36	0.92	0.58	1.48	0.96	0.63	1.46	28	5.77	0.97	0.63	1.51	1.06	0.70	1.59
Gross motor																	
Spontaneous pregnancy	8,895	484	5.44	ref			ref			367	4.12	ref			ref		
OI/AIH	273	20	7.35	1.38	0.84	2.28	1.14	0.71	1.84	16	5.88	1.42	0.80	2.50	1.24	0.73	2.11
ART	487	48	9.90	1.85	1.32	2.60	1.52	1.09	2.11	25	5.15	1.26	0.80	1.99	1.05	0.68	1.62
Fine motor																	
Natural conception	8,895	389	4.37	ref			ref			514	5.78	ref			ref		
OI/AIH	273	17	6.25	1.74	1.05	2.88	1.27	0.76	2.12	19	6.99	1.22	0.73	2.04	1.19	0.73	1.94
ART	487	34	7.01	1.70	1.16	2.50	1.28	0.88	1.89	30	6.19	1.09	0.73	1.64	1.06	0.71	1.57
Problem solving																	
Natural conception	8,895	354	3.98	ref			ref			506	5.69	ref			ref		
OI/AIH	273	13	4.78	1.23	0.66	2.27	0.89	0.50	1.59	18	6.62	1.31	0.79	2.17	1.19	0.72	1.97
ART	487	32	6.60	1.57	1.03	2.38	1.19	0.80	1.77	26	5.36	0.92	0.60	1.43	0.92	0.60	1.39
Personal-social																	
Natural conception	8,895	449	5.05	ref			ref			401	4.51	ref			ref		
OI/AIH	273	24	8.82	1.89	1.20	2.99	1.45	0.93	2.27	16	5.88	1.48	0.87	2.52	1.19	0.70	2.02
ART	487	42	8.66	1.74	1.21	2.49	1.25	0.88	1.78	27	5.57	1.19	0.77	1.86	1.07	0.70	1.62

Confidence interval: CI, odds ratio: OR, Ovulation induction: OI, Artificial Insemination with husband's semen: AIH, Assisted reproductive technology: ART.

Multivariable logistic models were adjusted for maternal age, parity, gestational week, birth weight, child sex, birth defect, multiple birth, maternal education level, and household income.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1, 2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5,6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6,
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 7
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	5,6,9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	9
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	9,10
Outcome data	15*	Report numbers of outcome events or summary measures over time	9,10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	9,10
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
Discussion			
Key results	18	Summarise key results with reference to study objectives	10,11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.