PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Noda, Aoi; Ishikuro, Mami; Obara, Taku; Murakami, Keiko; Ueno, Fumihiko; Matsuzaki, Fumiko; Onuma, Tomomi; Watanabe, Zen; Shiga, Naomi; Iwama, Noriyuki; Hirotaka, Hamada; Otsuka, Tatsui; Tachibana, Masahito; Tomita, Hiroaki; Saito, Masatoshi; Sugawara, Junichi; Kure, Shigeo; Yaegashi, Nobuo; Kuriyama, Shinichi

VERSION 1 – REVIEW

REVIEWER	Schleussner, Ekkehard Universitätsklinikum Jena, Obstetrics
REVIEW RETURNED	23-Mar-2022

GENERAL COMMENTS	The authors present results from a well-designed large prospective cohort study. They looked for the neurodevelopmental development of children conceived by infertility therapy of their mothers. The results indicate that only a transient effect of developmental delay could be seen at 2 years but not 3.5 y. This early delay are in difference to previous literature did not report any effect of infertility therapy. Within the compared cohort of natural conceived children and children after some kind of infertility treatment exist large differences in regard of risk factors for impaired neurodevelopment of the offspring. Highly significant differences in preterm birth rate, low birth weight, birth defects (does it mean structural anomalies or birth traumata?) as negative predictor as well as higher socio-economic standard and education level as positive predictor were reported in table 1. At least the striking influence of gestational age is only capture roughly with more or less 37 weeks. The more important fact would be very preterm (< 34 weeks) and extremely preterm (< 29 weeks) birth with a need of neonatal intensive care. To interpret the differences to other studies it seems necessary to take in to account the need of NICU therapy as well as preterm birth < 34 weeks for adjusting the results for important risk factors For me the interpretation of the results could be sum up in: the children has more worse starting conditions causing the delay in neurodevelopment, but the better support by parents with higher education and more financial possibilities are able to balance it in further development. This interpretation would be in line with the knowledge about neurodevelopment of extremely preterm infants tracked up to adolescence from Wolke et al. in the Epicure trial.

	The authors mentioned such an explanation shortly in the discussion but not in relation to their own results.

REVIEWER	Mito, Asako Stanford Medicine, Primary Care and Popultion Health
REVIEW RETURNED	25-Mar-2022

GENERAL COMMENTS	The reason for the association between infertility treatment and neurodevelopment of the child is weak as a discussion because it is all speculation, but it seems unavoidable. I thought it would be better to describe the reasons for not adjusting for birth weight when calculating odds ratios.

REVIEWER	Bedoschi, Giuliano Universidade de São Paulo Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, Reproductive Medicine
REVIEW RETURNED	08-Apr-2022

GENERAL COMMENTS	Dear authors, I read the manuscript entitled "Association between infertility treatment and children's neurodevelopment: the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study" with great interest. The study is a prospective cohort that aims to investigate the association between neurodevelopment outcomes in children at 2 and 3.5 years and infertility. The manuscript was written with scientific language and the authors have described all the methods in a detailed manner. The discussion section has deepened the study outcomes and has pointed out some limitations of the study in a proper manner. I have two considerations/suggestions: 1) In Introduction the authors state: "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]" This paragraph should be moved to the discussion. In the introduction, the authors could synthesize these findings exposing the evidence for neurodevelopment disorders and infertility treatments are conflicting. 2) In the Methods, Covariates section: The authors forgot to include one important covariate in the analysis, which is the time to pregnancy. Since subfertility could influence the risk for neurodevelopment disorders (ref), this factor should be taken into account when evaluating these patients. It is not possible to assume a causal relationship between ART treatments steps (embryo culture, ICSI, ovulation induction protocols) and neurodevelopment disorders. This information should be further discussed in the discussion section.

	Ref: Intracytoplasmic sperm injection for male infertility and consequences for offspring Sandro C Esteves et al. Nat Rev Urol. 2018 Sep;15(9):535-562. doi: 10.1038/s41585-018-0051-8. Congratulations on your work! Kind Regards!

VERSION 1 – AUTHOR RESPONSE

Response to Reviewer: 1
Dear Prof. Ekkehard Schleussner, Universitätsklinikum Jena
Thank you for your valuable comment of our manuscript. According to your comment, we revised our manuscript as follows. We also added supplemental tables. We hope that the manuscript has been sufficiently revised.

1. Comments to the Author:
The authors present results from a well-designed large prospective cohort study. They looked for the neurodevelopmental development of children conceived by infertility therapy of their mothers. The results indicate that only a transient effect of developmental delay could be seen at 2 years but not 3.5 y. This early delay are in difference to previous literature did not report any effect of infertility therapy.
Within the compared cohort of natural conceived children and children after some kind of infertility treatment exist large differences in regard of risk factors for impaired neurodevelopment of the offspring. Highly significant differences in preterm birth rate, low birth weight, birth defects (does it mean structural anomalies or birth traumata?) as negative predictor as well as higher socio-economic standard and education level as positive predictor were reported in table 1.
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. We added some sentences in the method section as follows:
Page 8:
“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.”

2. At least the striking influence of gestational age is only capture roughly with more or less 37 weeks. The more important fact would be very preterm (< 34 weeks) and extremely preterm (< 29 weeks) birth with a need of neonatal intensive care.
To interpret the differences to other studies it seems necessary to take in to account the need of NICU therapy as well as preterm birth < 34 weeks for adjusting the results for important risk factors.

Thank you very much for your valuable comment for our manuscript. In accordance with your remark, we took into account very preterm (< 34 weeks) or extremely preterm (< 29 weeks) birth for adjusting the results for important risk factors (Table S6). We added some sentences in the discussion sections as follows:
Page 12:
"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)."

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	2.06	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.

3. For me the interpretation of the results could be sum up in: the children has more worse starting conditions causing the delay in neurodevelopment, but the better support by parents with higher education and more financial possibilities are able to balance it in further development. This interpretation would be in line with the knowledge about neurodevelopment of extremely preterm infants tracked up to adolescence from Wolke et al. in the Epicure trial. The authors mentioned such an explanation shortly in the discussion but not in relation to their own results.
As you mentioned, we could not track up neurodevelopment of infants to adolescence because children participated in this study were born from 2014 to 2017. Continued follow-up of children born after infertility treatment is needed.

Response to Reviewer 2:
Dear Dr. Asako Mito, Stanford Medicine
Thank you for your valuable comment of our manuscript. According to your comment, we revised our manuscript as follows. We hope that the manuscript has been sufficiently revised.

1. Comments to the Author:
The reason for the association between infertility treatment and neurodevelopment of the child is weak as a discussion because it is all speculation, but it seems unavoidable.
As you mentioned, it was unavoidable. Although the reason for the association between infertility treatment and neurodevelopment of the child might be weak, we discussed the association carefully.

2. I thought it would be better to describe the reasons for not adjusting for birth weight when calculating odds ratios.
Thank you for your valuable comment for our manuscript. In this analysis, we excluded birth weight because a previous study excluded demographic variables related to gestational age (i.e. birth weight and postnatal age) to avoid collinearity of predictors. We performed analyses adjusted for birth

weight and the similar result was obtained. We added some sentences in the method section as follows:

Page 12:

"We also performed analysis adjusted for birth weight and the similar result was obtained (Table S7)."

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	
Personalsocial																		
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 weeks, birth weight, child sex, birth defect, multiple birth, maternal education level, and household income.

Response to Reviewer 3:

Dear Dr. Giuliano Bedoschi, Universidade de São Paulo Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto

Thank you for your valuable comment of our manuscript. According to your comment, we revised our manuscript as follows. We hope that the manuscript has been sufficiently revised.

1. Comments to the Author:

Dear authors,

I read the manuscript entitled "Association between infertility treatment and children's neurodevelopment: the Tohoku Medical Megabank Project Birth and Three-Generation Cohort Study" with great interest. The study is a prospective cohort that aims to investigate the association between neurodevelopment outcomes in children at 2 and 3.5 years and infertility.

The manuscript was written with scientific language and the authors have described all the methods in a detailed manner. The discussion section has deepened the study outcomes and has pointed out some limitations of the study in a proper manner.

Thank you very much for your comments on our study.

I have two considerations/suggestions:

2. In Introduction the authors state: "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]" This paragraph should be moved to the discussion. In the introduction, the authors could synthesize these findings exposing the evidence for neurodevelopment disorders and infertility treatments are conflicting.

We appreciate your comment. According to your comment, we moved that paragraph from introduction section to the discussion section as follows:

Page 5:

“Therefore, many studies have investigated the association between ART and neurodevelopmental outcomes. [5-8] 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] ”

Page 12:
“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]”

Page 14:
“A previous hospital-based cohort study in Japan Another study in Japan showed that children conceived through ART had significantly better language development than those conceived naturally.[7]”

Page 14:
“In contrast, the Danish National Birth Cohort study 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]

3. In the Methods, Covariates section: The authors forgot to include one important covariate in the analysis, which is the time to pregnancy. Since subfertility could influence the risk for neurodevelopment disorders (ref), this factor should be taken into account when evaluating these patients. It is not possible to assume a causal relationship between ART treatments steps (embryo culture, ICSI, ovulation induction protocols) and neurodevelopment disorders. This information should be further discussed in the discussion section.

Ref: Intracytoplasmic sperm injection for male infertility and consequences for offspring
Sandro C Esteves et al. Nat Rev Urol. 2018 Sep;15(9):535-562. doi: 10.1038/s41585-018-0051-8.

Thank you for your valuable comment for our manuscript. As you mentioned, the time to pregnancy should be taken into account when evaluating subfertility patients. However, it was difficult to include this factor because we did not have the data. So, we added the sentences in the discussion and reference section as follows:
Page 15:
“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 heps to increase the understanding of the association between infertility treatment and neurodevelopmental outcomes in Japanese children.

Page 23:
"36 Esteves SC, Roque M, Bedoschi G, Haahr T, Humaidan P. Intracytoplasmic sperm injection for male infertility and consequences for offspring. *Nat Rev Urol.* 2018;15(9):535-562."

VERSION 2 – REVIEW

REVIEWER	Schleussner, Ekkehard Universitätsklinikum Jena, Obstetrics
REVIEW RETURNED	15-May-2022

GENERAL COMMENTS	The authors followed carefully the consideration of the reviewer and integrated the revisions into the manuscript. So it seems now ready for publication.

REVIEWER	Bedoschi, Giuliano Universidade de São Paulo Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, Reproductive Medicine
REVIEW RETURNED	27-Apr-2022

GENERAL COMMENTS	Dear authors, Thank you for considering my commentaries and suggestions. All the items were answered in a satisfactory manner. Kind Regards!