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The Association of Infertility Treatment And Autism Spectrum Disorders in Offspring

2025.03.12

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Study question of interest

Infertility

the inability to achieve pregnancy after more than 12 months of regular, unprotected sexual intercourse

Prevalence of Infertility

Taiwan

5–10%

Lee MC, Chien PS, Zhou Y, Yu T. Prevalence and help-seeking for infertility in a population with a low fertility rate. PLoS One. 2024;19(7):e0306572.

Worldwide

18%

World Health Organization. Infertility prevalence estimates, 1990–2021: World Health Organization; 2023.

Study question of interest

Infertility Treatment

Achieve the reproductive goals

- **In Vitro Fertilization (IVF)**
- **Intracytoplasmic Sperm Injection (ICSI)**

Taiwanese government

Action

- **2007**
  Enactment of the Assisted Reproduction Act
- **2015**
  The introduction of the Infertility treatment subsidy program

Proportion of births

via Infertility Treatment

gradually increased with an annual growth rate of 41.2%

Hsu JC, Su Y-C, Tang B-Y, Lu CY. Use of assisted reproductive technologies before and after the Artificial Reproduction Act in Taiwan. PloS one. 2018;13(11):e0206208.
Yu T, Chiu LH, Chen TS. Assisted Reproductive Technology, Multiple Births, and Perinatal Outcomes in Taiwan from 2001 to 2020. J Pediatr. 2024;273:114146.

Study question of interest

Neurodevelopmental Disorder

When the brain or central nervous system encounters obstacles during its growth or development

ASD　ADHD　ID

ASD, Autism Spectrum Disorder
ADHD, Attention Deficit Hyperactivity Disorder
ID, Intellectual Disability

Risk factor for ASD

- Maternal obesity
- Preeclampsia
- Low birth weight

Risk factors for ASD are important influences in the early stages of the life course

The infertility treatment and ASD in offspring is inconclusive, to understand the potential risk is an important aspect of early prevention

Papers

Clinical and Experimental Pediatrics

CEP Vol. 63, No. 9, 368-372, 2020
https://doi.org/10.3345/cep.2020.00073

Original article

Association between assisted reproductive technology and autism spectrum disorders in Iran: a case-control study

2023 IF
3.2
Q1 (21/186)

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Original Investigation | Pediatrics

Infertility and Risk of Autism Spectrum Disorder in Children

2023 IF
10.5
Q1 (12/329)

Paper 1

Clinical and Experimental Pediatrics

CEP Vol. 63, No. 9, 368-372, 2020
https://doi.org/10.3345/cep.2020.00073

Original article

Association between assisted reproductive technology and autism spectrum disorders in Iran: a case-control study

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Dr. Jenabi

- Dr. Jenabi is an Iranian researcher and academic in midwifery, specializing in reproductive health
- She earned her Ph.D. in Reproductive Health from Hamadan University of Medical Sciences
- Since 2017, she has been a faculty member, teaching research methods and menopause courses at MSc and PhD levels

Introduction

One of the most prevalent mental health problem

Autism spectrum disorder (ASD) in adolescence

Adolescent

▼ lead to

Lack of concentration

Academic and social difficulties

strongly ▼ associated

Adult

Mental and Substance use disorders

- Anxiety
- Depression
- Alcohol, drug use disorders
- Suicidal behaviors

Briot, K., Jean, F., et al. (2020). Social anxiety in children and adolescents with autism spectrum disorders contribute to impairments in social communication and social motivation. Frontiers in psychiatry, 11, 710.
Butwicka, A., et al. (2017). Increased risk for substance use-related problems in autism spectrum disorders: a population-based cohort study. Journal of autism and developmental disorders, 47, 80-89.
Ressel, M., et al. (2020). Systematic review of risk and protective factors associated with substance use and abuse in individuals with autism spectrum disorders. Autism, 24(4), 899-918.

Introduction

IVF, In Vitro Fertilization (體外受精)
ICSI, Intracytoplasmic Sperm Injection (顯微授精)

Infertility Treatment (IT)

A category of medical interventions, including IVF and ICSI, among others

Children conceived by IT

Worldwide

2 – 6%

Exposure

IT (+)

Offspring outcomes

ASD

Fountain, 2015
RR 1.5 (1.2 – 1.9)
HR 1.7 (1.5 – 2.1)
Davidovitch, 2018

However, the association between IT and ASD greatly decreased after adjusting for the pregnancy outcomes

HR 1.0 (0.9 – 1.1) Davidovitch, 2018

Silva, S. G. D., et al. (2019). Assisted reproductive technology: prevalence and associated factors in Southern Brazil. Revista de Saúde Pública, 53, 13.
Davidovitch, M., et al. (2018). Infertility treatments during pregnancy and the risk of autism spectrum disorder in the offspring. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 86, 175-179.
Fountain, C., et al. (2015). Association between assisted reproductive technology conception and autism in California, 1997–2007. American Journal of Public Health, 105(5), 963-971.

Introduction

Current Gap

Evidence on the association between infertility treatment (IT) and autism spectrum disorder (ASD) in adolescence remains inconsistent

Aim of the study

This study aims to determine the association between IT and risk of ASD among children through a case-control study

Methods

1 Design 1:2 Case-control study in Hamadan city, Iran

2 Control

- Women who had child without ASD and they had health records at comprehensive health centers in Hamadan city

3 Case

- Women who had child with ASD aged 2–10 years and they were recruited from the Hamadan Autism Community who had medical records
- In their medical record, children with ASD were screened by The Modified Checklist for Autism in Toddlers (M-Chat) and were diagnosed by Autism Diagnostic Interview-Revised (ADI-R)

ADI-R is currently the most adequate and most widely used diagnostic tool for autism study, PMID 20412100

Methods

4 Questionnaire

Eligible women were invited to complete the questionnaire during September 2019 to November 2019

- **Parental age**
- **Mother's occupation**
- **Parity**
- **Preterm birth status**
- **Mode of delivery (Cesarean section vs. Vaginal delivery)**
- **Use of IT**
- **Causes of infertility (ovulation disorders, uterine abnormalities, sperm-related issues, etc.)**

Methods – Statistical Analysis

1 Analysis

- Univariable logistic regression was conducted to estimate crude association between mother and child variables and odds of ASD in child
- Those with P value ≤ 0.2 were considered as potential significant determinants of ASD and were included in multivariable logistic regression

2 bootstrap

- Bootstrapping using 1,000 bootstrap samples was used to check internal validity of multivariable model and to address the possibility of optimism

Results

Value are presented as number (%)

Boldface indicates a statistically significant difference

ASD, autism spectrum disorder

ART, assisted reproduction technology

CI, confidence interval

OR, odds ratio

Table 1. Univariate logistic regression analysis of predictors of ASD

Variable	ASD		OR (95% CI)	*P* value
	No (n=200)	Yes (n=100)		
Sex				
Girl	94 (47.00)	22 (22.00)	Reference	
Boy	106 (53.00)	78 (78.00)	3.14 (1.82-5.44)	**<0.001**
Type of delivery				
Natural	111 (55.50)	38 (38.00)	Reference	
Cesarean	89 (44.50)	62 (62.00)	2.03 (1.25-3.32)	**0.005**
History of preterm delivery				
No	189 (94.50)	79 (79.00)	Reference	
Yes	11 (5.50)	21 (21.00)	4.57 (2.1-9.92)	**<0.001**
ART				
No	198 (99.00)	92 (92.00)	Reference	
Yes	2 (1.00)	8 (8.00)	8.61 (1.79-41.34)	**0.007**
Maternal age at child birth				
<35 years	180 (90.00)	82 (82.00)	Reference	
≥35 years	20 (10.00)	18 (18.00)	1.97 (0.99-3.93)	**0.049**
Paternal age at child birth				
<35 years	139 (69.50)	68 (68.00)	Reference	
≥35 years	61 (30.50)	32 (32.00)	1.07 (0.64-1.80)	0.79
Maternal education				
Primary school	37 (18.50)	16 (16.00)	Reference	
Guidance school	42 (21.00)	15 (15.00)	0.83 (0.36-1.89)	0.65
Diploma	62 (31.00)	33 (33.00)	1.23 (0.60-2.54)	0.57
Academic	59 (29.50)	36 (36.00)	1.41 (0.69-2.89)	0.35
Paternal education				
Primary school	22 (11.00)	7 (7.00)	Reference	
Guidance school	45 (22.50)	27 (27.00)	1.89 (0.71-5.00)	0.21
Diploma	62 (31.00)	28 (28.00)	1.42 (0.54-3.71)	0.48
Academic	71 (35.50)	38 (38.00)	1.68 (0.66-4.29)	0.28

Results

Table 2. Original and bootstrapped multivariate analyses of mother and neonate variables associated with ASD

Variable	Original model		Bootstrapped model	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Sex				
Girl	Reference		Reference	
Boy	2.66 (1.50–4.72)	**0.001**	2.66 (1.41–5.01)	**0.002**
Type of delivery				
Natural	Reference		Reference	
Cesarean	1.63 (0.96–2.76)	0.07	1.63 (0.94–2.83)	0.08
History of preterm delivery				
No	Reference		Reference	
Yes	4.03 (1.76–9.21)	**0.001**	4.03 (1.72–9.42)	**0.001**
ART				
No	Reference		Reference	
Yes	4.98 (0.91–27.30)	0.065	4.98 (1.06–23.33)	**0.042**
Maternal age at birth (yr)				
<35	Reference		Reference	
≥35	1.72 (0.82–3.64)	0.15	1.72 (0.75–3.93)	0.195

ASD, autism spectrum disorder; OR, odds ratio; CI, confidence interval; ART, assisted reproduction technology.
Boldface indicates a statistically significant difference with $P<0.05$.

Discussion

Main Finding

- The main risk factors were male sex and preterm birth
- The association between IT and ASD was insignificant, supporting previous findings

Limitation

- Lack of unmeasured demographic and parental characteristics
- Majority of participants did not answer the income status
- Family history of ASD in children was not assessed

Discussion

My reflection

- Although the authors mentioned that adverse pregnancy outcomes may affect the association between IT and ASD, the study did not collect comprehensive data on these outcomes. It should be included in further research
- Lack of verity clinical importantly factors, may conduct E-value to calculate the potentially influence of unmeasured confounders
- The CI of odds ratio for IT was wide, as only 3% of children in the study was using IT, suggesting potential instability in the estimate due to the small sample size
- Preterm birth may be a mediator, which allows authors to conduct a mediation analysis

Conclusion

The findings showed that after adjusting for other variables, risk factors for ASD were male sex and history preterm delivery for children with ASD

Therefore, after adjusting for confounder variables, there was not significant association between IT and the risk of ASD among children

Paper 2

JAMA Network Open

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Original Investigation | Pediatrics

Infertility and Risk of Autism Spectrum Disorder in Children

Maria P. Velez, MD, PhD; Natalie Dayan, MD, MSc; Jonas Shellenberger, MSc; Jessica Pudwell, MSc, MPH; Dia Kapoor, MPH; Simone N. Vigod, MD, MSc; Joel G. Ray, MD, MSc

Dr. Velez

- Dr. Velez is a clinician-scientist and associate professor in the Department of Obstetrics and Gynecology at McGill University
- Her research interests include infertility and perinatal outcomes, reproductive health among female cancer patients, and menopause

Introduction

Risk of ASD in children via IT

Initial studies have reported little to no increased risk

Adverse Pregnancy Outcomes in IT

Individuals with subfertility face a higher risk of complications

Uncertain Mediating Effects on ASD Risk

Limited data about the mediating effect of pregnancy outcomes on the association between IT and ASD

Aim of the study

The current study evaluated the association between IT and the risk of ASD, while further modeling the mediating effect of adverse pregnancy outcomes

Methods

1 Design Retrospective cohort study

2 Database Better Outcomes Registry and Network (BORN), Ontario, Canada

3 Inclusion

- Maternal age ranged from 18 to 55 years
- All singleton and multiple live births with a gestational age of at least 24 weeks, from April 2006, to March 2018
- Setting the year 2018 for the last birth permitted all eligible children to be assessed for ASD at a minimum age of 4 years by 2022

Methods

4 Exclusion

- Surrogate pregnancies
- Pregnancies that ended in miscarriage or preterm birth before 24 weeks of gestation
- Infants who died within the first 18 months of life
- Cases with incomplete records

5 Exposure

- Unassisted conception (reference group)
- Subfertility (defined as having a history of an infertility consultation but no infertility treatment)
- OI or IUI
- IVF or ICSI

OI, Ovulation induction (誘導排卵)
IUI, Intrauterine insemination (人工授精)
IVF, In Vitro Fertilization (體外受精)
ICSI, Intracytoplasmic Sperm Injection (顯微授精)

Methods

6 Outcome

- Diagnosis of ASD in the child, starting at age 18 months
- A diagnosis of ASD was based on 2 or more outpatient diagnoses, or 1 or more diagnoses during a hospitalization

PPV 87.4 %

Burke, J. P., et al. (2014). Does a claims diagnosis of autism mean a true case?. Autism, 18(3), 321-330.

7 Covariates

- Maternal age, parity, income quintile, rurality, immigration status, smoking, illicit substance use, alcohol use, pre-pregnancy diabetes or chronic hypertension, obesity, history of mental illness, a history maternal ASD, and infant sex

Methods – Statistical Analysis

1 Analysis

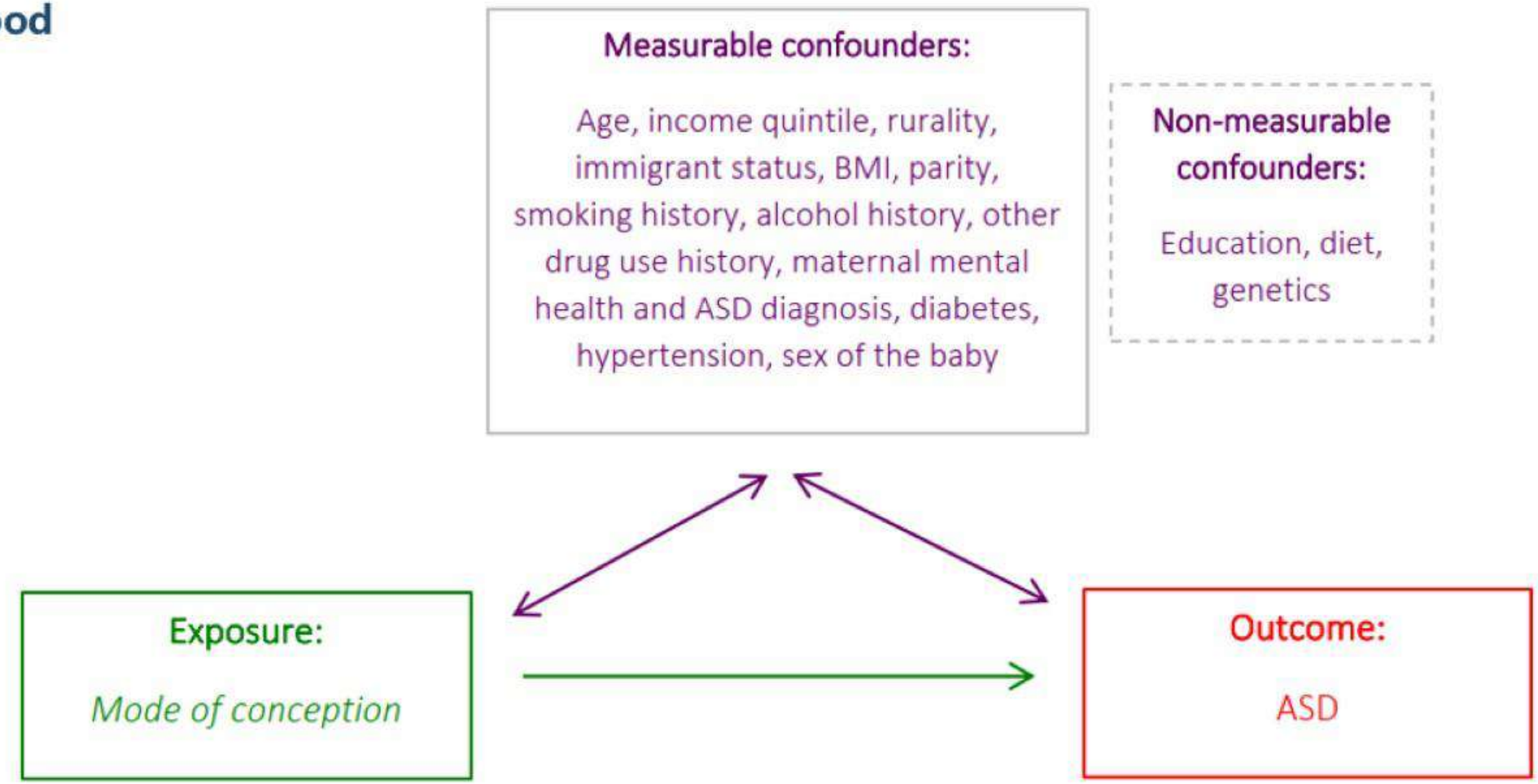
Conventional modeling approach

- Time-to-event analyses were conducted using multivariable Cox regression models to estimate hazard ratios and 95% CIs, with the child’ s age as the underlying time scale, starting at age 18 months (time zero)
- A robust sandwich-type estimator was used to account for the potential of more than 1 birth to the same woman across the study period
- Censoring was at death, lost to follow-up, or arrival at the end of the study period of June 2022

Methods – Statistical Analysis

1 Analysis — Conventional modeling approach

eFigure 1. Conventional Model to Examine the Relation Between Mode of Conception and ASD in childhood



Methods – Statistical Analysis

2 Mediation

Causal mediation analysis based on a counterfactual framework

- **Describe the mediating role of adverse pregnancy outcomes that have been reported to be associated with infertility and fertility treatments**
- **Disentangle the total effect**

  Association between mode of conception and ASD

  - **Natural direct effect**

    The association between each mode of conception and ASD in the absence of the mediator
  - **Natural indirect effect**

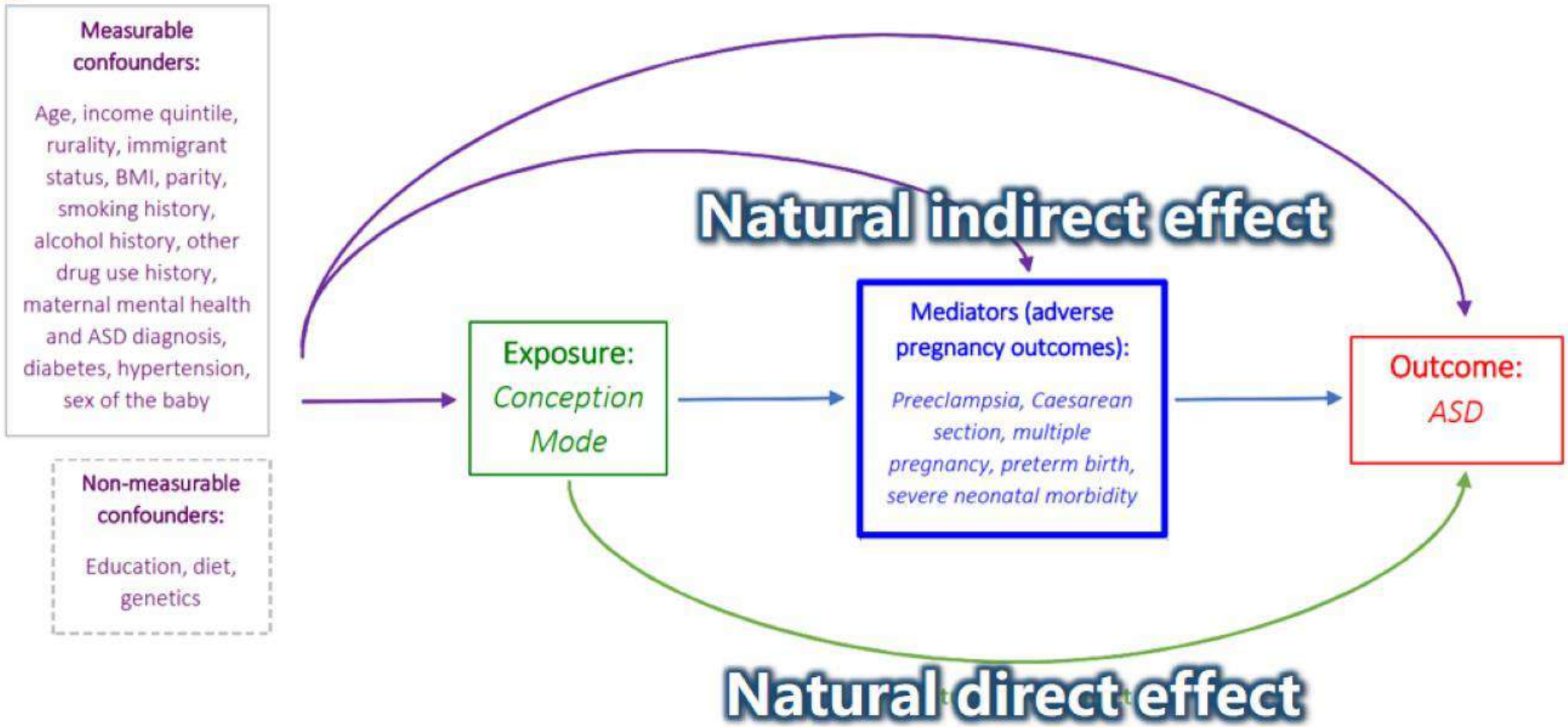
    The association operating through the respective mediators mentioned above

Methods – Statistical Analysis

2 Mediation

Causal mediation analysis based on a counterfactual framework

eFigure 2. Causal Mediation Analysis of the Relation Between Mode of Conception and ASD in children



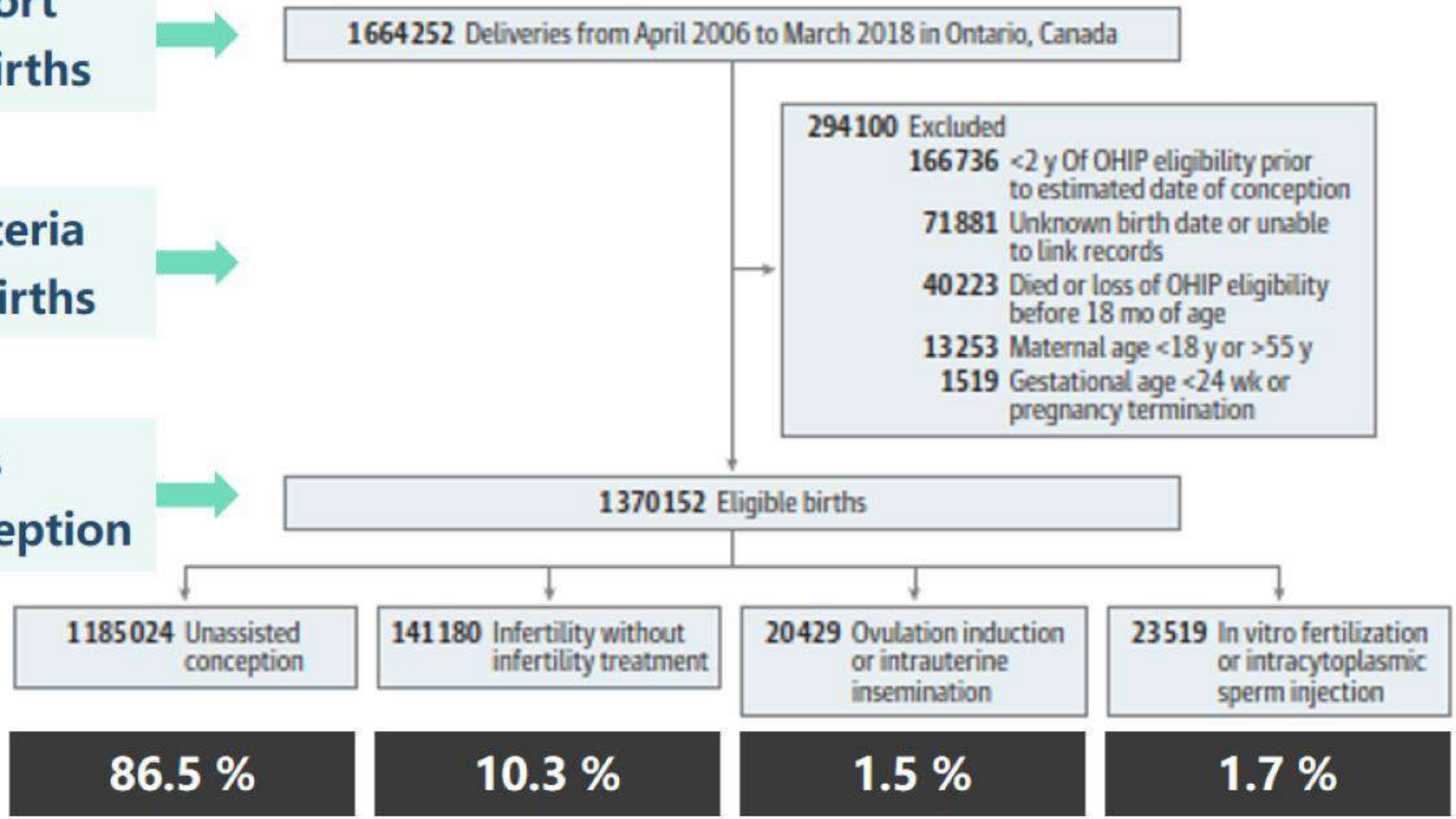
Results

Figure 1. Cohort Creation

At the beginning of cohort recruited 1,664,252 live births

After above exclusion criteria remained 1,370,152 live births

Divided into 4 groups based on the mode of conception



OHIP, Ontario Health Insurance Plan

Results

Table 1. Characteristics of live-born children

Individuals with subfertility or those receiving IT were more likely to be older, nulliparous, reside in a higher-income and urban area, and have higher rates of prepregnancy diabetes and chronic hypertension

Characteristic	Participants, No. (%)			
	Unassisted conception (n = 1 185 024)	Subfertility (n = 141 180)	OI\IUI (n = 20 429)	IVF\ICSI (n = 23 519)
Maternal age				
Mean (SD), y	30.1 (5.2)	33.3 (4.7)	33.1 (4.4)	35.8 (4.9)
<35	943 624 (79.7)	84 739 (60.0)	12 873 (63.0)	9853 (41.9)
35-44	239 884 (20.2)	55 561 (39.4)	7488 (36.7)	12 466 (53.0)
45-55	1516 (0.1)	880 (0.6)	68 (0.3)	1200 (5.1)
Income quintile				
1 (Lowest)	258 610 (21.8)	22 124 (15.7)	2558 (12.5)	2316 (9.9)
2	236 974 (20.0)	24 978 (17.7)	3489 (17.1)	3680 (15.7)
3	245 637 (20.7)	29 906 (21.2)	4403 (21.6)	4945 (21.0)
4	250 125 (21.1)	34 451 (24.4)	5461 (26.7)	6292 (26.8)
5 (Highest)	193 678 (16.3)	29 721 (21.1)	4518 (22.1)	6286 (26.7)
Rural residence	98 755 (8.3)	6260 (4.4)	1216 (6.0)	864 (3.7)
Immigrant to Canada	271 813 (22.9)	42 242 (29.9)	4072 (19.9)	6432 (27.4)
Primiparous	475 996 (40.2)	70 679 (50.1)	12 940 (63.3)	15 912 (67.7)
Body mass index ≥30[a]	142 603 (12.0)	18 592 (13.2)	4212 (20.6)	2803 (11.9)
Smoking	117 049 (9.9)	4538 (3.2)	528 (2.6)	303 (1.3)
Substance use[b]	20 315 (1.7)	557 (0.4)	90 (0.4)	69 (0.3)
Alcohol use	2153 (0.2)	104 (0.1)	13 (0.1)	14 (0.1)
Prepregnancy diabetes	18 392 (1.6)	4193 (3.0)	673 (3.3)	626 (2.7)
Chronic hypertension	27 486 (2.3)	5241 (3.7)	827 (4.1)	896 (3.8)
History of mental illness[c]	306 293 (25.9)	37 132 (26.3)	5088 (24.9)	5628 (23.9)
History of polycystic ovary syndrome	7965 (0.7)	5488 (3.9)	1449 (7.1)	699 (3.0)
History of endometriosis	2625 (0.2)	1787 (1.3)	239 (1.2)	625 (2.7)
Multifetal pregnancy	27 997 (2.4)	8090 (5.7)	3934 (19.3)	7553 (32.1)
Sex of the child				
Male	608 491 (51.3)	72 519 (51.4)	10 489 (51.3)	11 908 (50.6)
Female	576 533 (48.7)	68 661 (48.6)	9940 (48.7)	11 611 (49.4)

OI, Ovulation induction (誘導排卵)
IUI, Intrauterine insemination (人工授精)
IVF, In Vitro Fertilization (體外受精)
ICSI, Intracytoplasmic Sperm Injection (顯微授精)

Results

Table 2. Risk of ASD by Mode of Conception

Starting at age 18 months, children were followed up for a median (IQR) of 8.1 (5.1-11.2) years

A total of 22,409 children (1.6%) with ASD diagnosis, occurring at a mean (SD) age of 3.9 (2.4) years

Mode of conception	No. with ASD/No. at risk	Rate of ASD per 1000 person-years	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[a]
Analysis among all 1 370 152 live-born children (main model)				
Unassisted conception	18 689/1 185 024	1.93	1 [Reference]	1 [Reference]
Subfertility	2858/141 180	2.49	1.29 (1.24-1.34)	1.20 (1.15-1.25)
Ovulation induction or intrauterine insemination	404/20 429	2.72	1.31 (1.18-1.45)	1.21 (1.09-1.34)
In vitro fertilization or intracytoplasmic sperm injection	458/23 519	2.71	1.29 (1.17-1.43)	1.16 (1.04-1.28)

Compared with the unassisted group aHR for ASD

Subfertility
1.20 (1.15–1.25)

OI / IUI
1.21 (1.09–1.34)

IVF / ICSI
1.16 (1.04–1.28)

OI, Ovulation induction (誘導排卵)
IUI, Intrauterine insemination (人工授精)
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ICSI, Intracytoplasmic Sperm Injection (顯微授精)

Results

Table 2. Risk of ASD by Mode of Conception

Mode of conception	No. with ASD/No. at risk	Rate of ASD per 1000 person-years	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[a]
Analysis limited to 185 128 live-born children of individuals with infertility				
Subfertility	2858/141 180	2.49	1 [Reference]	1 [Reference]
Ovulation induction or intrauterine insemination	404/20 429	2.72	1.01 (0.91-1.12)	1.02 (0.92-1.14)
In vitro fertilization or intracytoplasmic sperm injection	458/23 519	2.71	1.00 (0.90-1.11)	0.94 (0.84-1.05)

Compared with the subfertility group aHR for ASD

→ **OI / IUI** 1.02 (0.92–1.14)

→ **IVF / ICSI** 0.94 (0.84–1.05)

Mode of conception	No. with ASD/No. at risk	Rate of ASD per 1000 person-years	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)[a]
Analysis limited to 23 519 live-born children of individuals who underwent in vitro fertilization or intracytoplasmic sperm injection				
In vitro fertilization	408/20 968	2.70	1 [Reference]	1 [Reference]
Intracytoplasmic sperm injection	50/2551	2.77	1.01 (0.75-1.37)	1.05 (0.77-1.42)

Compared with the IVF group aHR for ASD

→ **ICSI group** 1.05 (0.77–1.42)

OI, Ovulation induction (誘導排卵)
IUI, Intrauterine insemination (人工授精)
IVF, In Vitro Fertilization (體外受精)
ICSI, Intracytoplasmic Sperm Injection (顯微授精)

Results

Table 3. Mediation Analysis of the Effect of Selected Adverse Pregnancy Outcomes

Adverse pregnancy outcome mediator assessed and mode of conception[b]	Adjusted hazard ratio (95% CI)[a]			Proportion mediated (%)
	Total effect	Natural direct effect	Natural indirect effect	
Preeclampsia				
Subfertility	1.19 (1.16-1.23)	1.19 (1.17-1.22)	1.00 (0.98-1.02)	1.2
Ovulation induction or intrauterine insemination	1.20 (1.14-1.27)	1.20 (1.14-1.26)	1.01 (0.99-1.03)	4.0
In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.22)	1.14 (1.09-1.20)	1.01 (0.99-1.03)	8.7

Proportion mediated by preeclampsia was less than 10% and not statistically significant

Results

Table 3. Mediation Analysis of the Effect of Selected Adverse Pregnancy Outcomes

Following OI or IUI

the proportion mediated
by cesarean birth was 11%,
by multifetal pregnancy was 36%,
by preterm birth was 26%,
and by severe neonatal morbidity
was 14%

OI, Ovulation induction (誘導排卵)
IUI, Intrauterine insemination (人工授精)
IVF, In Vitro Fertilization (體外受精)
ICSI, Intracytoplasmic Sperm Injection (顯微授精)

Adverse pregnancy outcome mediator assessed and mode of conception[b]	Adjusted hazard ratio (95% CI)[a]			
	Total effect	Natural direct effect	Natural indirect effect	Proportion mediated (%)
Cesarean birth				
Subfertility	1.20 (1.16-1.23)	1.18 (1.16-1.21)	1.01 (0.99-1.03)	7.4
Ovulation induction or intrauterine insemination	1.21 (1.14-1.27)	1.19 (1.13-1.25)	1.02 (1.00-1.04)	10.6
In vitro fertilization or intracytoplasmic sperm injection	1.14 (1.09-1.20)	1.10 (1.05-1.16)	1.04 (1.02-1.06)	28.9[c]
Planned cesarean birth[d]				
Subfertility	1.18 (1.14-1.21)	1.16 (1.14-1.19)	1.01 (0.99-1.03)	7.1
Ovulation induction or intrauterine insemination	1.18 (1.11-1.26)	1.16 (1.09-1.23)	1.02 (1.00-1.04)	12.0
In vitro fertilization or intracytoplasmic sperm injection	1.12 (1.05-1.20)	1.08 (1.02-1.15)	1.04 (1.02-1.06)	34.7[c]
Unplanned Caesarian birth[e]				
Subfertility	1.19 (1.16-1.23)	1.19 (1.16-1.21)	1.01 (0.99-1.03)	4.2
Ovulation induction or intrauterine insemination	1.21 (1.14-1.29)	1.20 (1.13-1.27)	1.01 (0.99-1.03)	5.8
In vitro fertilization or intracytoplasmic sperm injection	1.12 (1.05-1.19)	1.09 (1.03-1.15)	1.02 (1.00-1.05)	22.7[c]
Multiple pregnancy				
Subfertility	1.17 (1.13-1.21)	1.15 (1.12-1.18)	1.01 (0.99-1.03)	8.5
Ovulation induction or intrauterine insemination	1.20 (1.13-1.27)	1.13 (1.07-1.19)	1.06 (1.04-1.09)	35.8[c]
In vitro fertilization or intracytoplasmic sperm injection	1.14 (1.08-1.21)	1.03 (0.98-1.09)	1.11 (1.08-1.14)	78.3[c]
Preterm birth <37 wk				
Subfertility	1.19 (1.16-1.23)	1.17 (1.15-1.20)	1.02 (1.00-1.03)	9.2
Ovulation induction or intrauterine insemination	1.19 (1.13-1.26)	1.14 (1.09-1.20)	1.04 (1.02-1.06)	25.6[c]
In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.23)	1.08 (1.03-1.14)	1.07 (1.05-1.10)	49.8[c]
Severe neonatal morbidity				
Subfertility	1.20 (1.16-1.23)	1.19 (1.16-1.21)	1.01 (0.99-1.03)	5.1
Ovulation induction or intrauterine insemination	1.20 (1.14-1.27)	1.17 (1.11-1.23)	1.02 (1.00-1.04)	13.9[c]
In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.22)	1.12 (1.07-1.18)	1.04 (1.02-1.06)	25.0[c]

Results

Table 3. Mediation Analysis of the Effect of Selected Adverse Pregnancy Outcomes

After IVF or ICSI

mediation by cesarean birth was 29%, by multifetal pregnancy was 78%, by preterm birth was 50%, and by severe neonatal morbidity was 25%

OI, Ovulation induction (誘導排卵)
IUI, Intrauterine insemination (人工授精)
IVF, In Vitro Fertilization (體外受精)
ICSI, Intracytoplasmic Sperm Injection (顯微授精)

Adverse pregnancy outcome mediator assessed and mode of conception[b]	Adjusted hazard ratio (95% CI)[a] Total effect	Natural direct effect	Natural indirect effect	Proportion mediated (%)
Cesarean birth				
Subfertility	1.20 (1.16-1.23)	1.18 (1.16-1.21)	1.01 (0.99-1.03)	7.4
Ovulation induction or intrauterine insemination	1.21 (1.14-1.27)	1.19 (1.13-1.25)	1.02 (1.00-1.04)	10.6
In vitro fertilization or intracytoplasmic sperm injection	1.14 (1.09-1.20)	1.10 (1.05-1.16)	1.04 (1.02-1.06)	28.9[c]
Planned cesarean birth[d]				
Subfertility	1.18 (1.14-1.21)	1.16 (1.14-1.19)	1.01 (0.99-1.03)	7.1
Ovulation induction or intrauterine insemination	1.18 (1.11-1.26)	1.16 (1.09-1.23)	1.02 (1.00-1.04)	12.0
In vitro fertilization or intracytoplasmic sperm injection	1.12 (1.05-1.20)	1.08 (1.02-1.15)	1.04 (1.02-1.06)	34.7[c]
Unplanned Caesarian birth[e]				
Subfertility	1.19 (1.16-1.23)	1.19 (1.16-1.21)	1.01 (0.99-1.03)	4.2
Ovulation induction or intrauterine insemination	1.21 (1.14-1.29)	1.20 (1.13-1.27)	1.01 (0.99-1.03)	5.8
In vitro fertilization or intracytoplasmic sperm injection	1.12 (1.05-1.19)	1.09 (1.03-1.15)	1.02 (1.00-1.05)	22.7[c]
Multiple pregnancy				
Subfertility	1.17 (1.13-1.21)	1.15 (1.12-1.18)	1.01 (0.99-1.03)	8.5
Ovulation induction or intrauterine insemination	1.20 (1.13-1.27)	1.13 (1.07-1.19)	1.06 (1.04-1.09)	35.8[c]
In vitro fertilization or intracytoplasmic sperm injection	1.14 (1.08-1.21)	1.03 (0.98-1.09)	1.11 (1.08-1.14)	78.3[c]
Preterm birth <37 wk				
Subfertility	1.19 (1.16-1.23)	1.17 (1.15-1.20)	1.02 (1.00-1.03)	9.2
Ovulation induction or intrauterine insemination	1.19 (1.13-1.26)	1.14 (1.09-1.20)	1.04 (1.02-1.06)	25.6[c]
In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.23)	1.08 (1.03-1.14)	1.07 (1.05-1.10)	49.8[c]
Severe neonatal morbidity				
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In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.22)	1.12 (1.07-1.18)	1.04 (1.02-1.06)	25.0[c]

Discussion

Strengths

- Large population-based cohort, comprising validated datasets representing more than 99% of live births in Ontario
- Comprehensive collection of covariates, including socioeconomic factors, medical history, and perinatal complications
- Causal mediation analysis, quantifying the mediating effects of adverse pregnancy outcomes

Limitation

- Underestimation of ASD diagnosis may have occurred using current datasets
- Parents with subfertility may be more likely to seek ASD evaluation
- Early pregnancy mediators (multiple pregnancy) may influence later mediators (cesarean delivery), making it impossible to quantify the combined mediation effect

Conclusion

There was a slightly higher risk of ASD in those born to an individual with infertility independent of IT, which appeared partly mediated by certain adverse pregnancy outcomes

Efforts to decrease multiple pregnancy following OI or IUI and IVF should continue to be reinforced

Variables	Paper 1	Paper 2
Study question	Association between IT and ASD in children	Association between IT and ASD in children, and whether factors mediate the association
Study design	Case-control study	Retrospective cohort study
Study setting	City of Hamadan, Iran	Ontario, Canada
Population	Case: mothers of children with ASD Control: mothers of children without ASD	Mothers of children born at ≥24 weeks of gestation
Exposure and measures	Use of IT (IVF, OI, or IUI) Collected by questionnaires	Infertility without IT Infertility with OI or IUI Infertility with IVF or ICSI Recorded in the BORN dataset
Outcome and measures	Child diagnosis of ASD at age 2–10, based on ADI-R confirmation from the local autism center	Child diagnosis of ASD at age 1.5–16, based on ICD codes
Inclusion period	Study enrollment conducted from September to November 2019	Births in Ontario from 2006 to 2018, with follow-up starting at 18 months of age and continuing until June 2022
Statistical analysis	Logistic regression with an effect size "OR" Bootstrapping using 1,000 bootstrap samples	Cox proportional models, with an effect size "HR" Causal mediation analysis

Variables	Paper 1	Paper 2
Selection Bias	Moderate, case and control enrollment relied on volunteers, and some non-participation occurred	Low, only deaths, out-of-province migration, or loss of health insurance end follow-up, and these events are unlikely to differ between exposure groups
Information Bias	Moderate, reliance on mothers' self-reported, may introduce a recall bias; certain variables like income status had low response rates	Moderate, administrative data may have misclassification of exposure or diagnosis; and parents with infertility may be more careful
Confounding	High, lacked full data on adverse pregnancy outcomes, family history, and income status	Low, study collected relevant variables following previous research; and performed mediation analyses to further account for indirect effects
Advantages	Case-control design examining the association in a relatively quick approach Provides initial local data in Iran	Nationally representative cohort Large sample size Control for multiple confounders Mediation analysis offers a refined view
Disadvantages	Relatively small sample Heavy reliance on self-report Did not analyze detailed IT subtypes	Potentially risk of detection bias and misclassification bias

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Original article

Association between assisted reproductive technology and autism spectrum disorders in Iran: a case-control study

Ensiyeh Jenabi, PhD[1], Mahdieh Seyedi, MSc[1], Ronak Hamzehei, MSc[2], Saeid Bashirian, PhD[3], Mohammad Rezaei, PhD[1], Katayoon Razjouyan, MD[4], Salman Khazaei, PhD[5]

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D2 Chih-Wei Tseng

Comment 1: Total only 10 ART in these 300 ASD It's underpowered to say ART "NOT" a/w ASD Will Firth Logistic Regression help?

Table 1. Univariate logistic regression analysis of predictors of ASD

Variable	ASD		OR (95% CI)	*P* value
	No (n=200)	Yes (n=100)		
Sex				
Girl	94 (47.00)	22 (22.00)	Reference	
Boy	106 (53.00)	78 (78.00)	3.14 (1.82–5.44)	**<0.001**
Type of delivery				
Natural	111 (55.50)	38 (38.00)	Reference	
Cesarean	89 (44.50)	62 (62.00)	2.03 (1.25–3.32)	**0.005**
History of preterm delivery				
No	189 (94.50)	79 (79.00)	Reference	
Yes	11 (5.50)	21 (21.00)	4.57 (2.1–9.92)	**<0.001**
ART				
No	198 (99.00)	92 (92.00)	Reference	
Yes	2 (1.00)	8 (8.00)	8.61 (1.79–41.34)	**0.007**

Comment
My references

Statistics in Medicine

Research Article

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Firth's logistic regression with rare events: accurate effect estimates and predictions?

Rainer Puhr,[a] Georg Heinze,[b] Mariana Nold,[c] Lara Lusa[d] and Angelika Geroldinger[b*†]

PMID 28295456

BRIEF RESEARCH COMMUNICATION

Firth's penalized logistic regression: A superior approach for analysis of data from India's National Mental Health Survey, 2016

Satish Suhas[1], Narayana Manjunatha[1], Channaveerachari Naveen Kumar[1], Vivek Benegal[1], Girish N. Rao[2], Mathew Varghese[1], Gopalkrishna Gururaj[3]

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PMID 38298875

Conventional Logistic Regression

- **Rare exposure** may introduce biased maximum likelihood estimation (MLE)
- MLE overfits the estimate point due to insufficient data, leading to an **overestimation of the OR**

Firth Logistic Regression

- It uses Penalized Likelihood Estimation (PLE), adding a penalty term to MLE
- Ensure finite estimates even in the presence of complete separation (avoiding OR = ∞)

Comment 2: Rich men afford ART and variables for SES listed here is only education? What would recommend the authors to add on?

Maternal education				
Primary school	37 (18.50)	16 (16.00)	Reference	
Guidance school	42 (21.00)	15 (15.00)	0.83 (0.36–1.89)	0.65
Diploma	62 (31.00)	33 (33.00)	1.23 (0.60–2.54)	0.57
Academic	59 (29.50)	36 (36.00)	1.41 (0.69–2.89)	0.35
Paternal education				
Primary school	22 (11.00)	7 (7.00)	Reference	
Guidance school	45 (22.50)	27 (27.00)	1.89 (0.71–5.00)	0.21
Diploma	62 (31.00)	28 (28.00)	1.42 (0.54–3.71)	0.48
Academic	71 (35.50)	38 (38.00)	1.68 (0.66–4.29)	0.28

Comment

I agree with your comment

SES status is a potential confounder, and most studies of this type adjust for SES using variables such as education, income, family structure, and residential area

Although the questionnaire included a question on income, the participants did not provide responses

As a result, this study only adjusted for education, which is a limitation

Comments on Paper 2 of Jheng Yan

Ya-Ling Hsieh (T88121031)
2nd Year PhD Student

Comment 1-Statistical Analysis

ASD has a strong genetic component, and if either parent has ASD, the risk of ASD in their child may be significantly higher. This study only considered the mother's ASD history but did not account for the father's age or family genetic history (such as ASD or psychiatric disorders), which may lead to an underestimation of the genetic influence.

Should the study further adjust for paternal ASD risk factors to better identify the true source of ASD risk?

Comment

I agree with your comment

Paternal age or a family history of ASD was associated with the risk of offspring ASD

Hultman, C. M., et al. (2011). Advancing paternal age and risk of autism: new evidence from a population-based study and a meta-analysis of epidemiological studies. Molecular psychiatry, 16(12), 1203-1212.
Croen, L. A., Najjar, D. V., Fireman, B., & Grether, J. K. (2007). Maternal and paternal age and risk of autism spectrum disorders. Archives of pediatrics & adolescent medicine, 161(4), 334-340.

The authors acknowledged this as a limitation due to the lack of data in the dataset

Comment 2-Results

Table 3. Mediation Analysis of the Effect of Selected Adverse Pregnancy Outcomes on the Association Between Mode of Conception and Autism Spectrum Disorder

Adverse pregnancy outcome mediator assessed and mode of conception[b]	Adjusted hazard ratio (95% CI)[a]			
	Total effect	Natural direct effect	Natural indirect effect	Proportion mediated (%)
Preeclampsia				
Subfertility	1.19 (1.16-1.23)	1.19 (1.17-1.22)	1.00 (0.98-1.02)	1.2
Ovulation induction or intrauterine insemination	1.20 (1.14-1.27)	1.20 (1.14-1.26)	1.01 (0.99-1.03)	4.0
In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.22)	1.14 (1.09-1.20)	1.01 (0.99-1.03)	8.7
Cesarean birth				
Subfertility	1.20 (1.16-1.23)	1.18 (1.16-1.21)	1.01 (0.99-1.03)	7.4
Ovulation induction or intrauterine insemination	1.21 (1.14-1.27)	1.19 (1.13-1.25)	1.02 (1.00-1.04)	10.6
In vitro fertilization or intracytoplasmic sperm injection	1.14 (1.09-1.20)	1.10 (1.05-1.16)	1.04 (1.02-1.06)	28.9[c]
Planned cesarean birth[d]				
Subfertility	1.18 (1.14-1.21)	1.16 (1.14-1.19)	1.01 (0.99-1.03)	7.1
Ovulation induction or intrauterine insemination	1.18 (1.11-1.26)	1.16 (1.09-1.23)	1.02 (1.00-1.04)	12.0
In vitro fertilization or intracytoplasmic sperm injection	1.12 (1.05-1.20)	1.08 (1.02-1.15)	1.04 (1.02-1.06)	34.7[c]
Unplanned Caesarian birth[e]				
Subfertility	1.19 (1.16-1.23)	1.19 (1.16-1.21)	1.01 (0.99-1.03)	4.2
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In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.23)	1.08 (1.03-1.14)	1.07 (1.05-1.10)	49.8[c]
Severe neonatal morbidity				
Subfertility	1.20 (1.16-1.23)	1.19 (1.16-1.21)	1.01 (0.99-1.03)	5.1
Ovulation induction or intrauterine insemination	1.20 (1.14-1.27)	1.17 (1.11-1.23)	1.02 (1.00-1.04)	13.9[c]
In vitro fertilization or intracytoplasmic sperm injection	1.16 (1.10-1.22)	1.12 (1.07-1.18)	1.04 (1.02-1.06)	25.0[c]

This study shows that adverse pregnancy outcomes (e.g., cesarean birth, multiple pregnancy, preterm birth) are linked to ASD risk, especially in children conceived via IVF/ICSI.

Among these, **multiple pregnancy has the strongest impact**, mediating **78.3% of ASD risk**.

When **multiple pregnancy is accounted for**, the **indirect effect of IVF/ICSI on ASD risk remains significant** (**aHR = 1.11, 95% CI: 1.08-1.14**).

Does this suggest that IVF itself is not the main risk factor for ASD, but rather that multiple pregnancies caused by IVF are the key driver?

Comment

Yes, the study suggests that IVF or ICSI itself may not be the primary risk factor for ASD; rather, multiple pregnancies resulting from IVF or ICSI appear to be the key driver

IVF, In Vitro Fertilization (體外受精)
ICSI, Intracytoplasmic Sperm Injection (顯微授精)