Original Investigation

Autism and Mental Retardation Among Offspring Born After In Vitro Fertilization

Sven Sandin, MSc; Karl-Gösta Nygren, PhD; Anastasia Iliadou, PhD; Christina M. Hultman, PhD; Abraham Reichenberg, PhD

IMPORTANCE Between 1978 and 2010, approximately 5 million infants were born after in vitro fertilization (IVF) treatments. Yet limited information on neurodevelopment after IVF exists, especially after the first year of life.

OBJECTIVE To examine the association between use of any IVF and different IVF procedures and the risk of autistic disorder and mental retardation in the offspring.

DESIGN, SETTING, AND PARTICIPANTS A population-based, prospective cohort study using Swedish national health registers. Offspring born between 1982 and 2007 were followed up for a clinical diagnosis of autistic disorder or mental retardation until December 31, 2009. The exposure of interest was IVF, categorized according to whether intracytoplasmic sperm injection (ICSI) for male infertility was used and whether embryos were fresh or frozen. For ICSI, whether sperm were ejaculated or surgically extracted was also considered.

MAIN OUTCOMES AND MEASURES Relative risks (RRs) for autistic disorder and mental retardation and rates per 100 000 person-years, comparing spontaneously conceived offspring with those born after an IVF procedure and comparing 5 IVF procedures used in Sweden vs IVF without ICSI with fresh embryo transfer, the most common treatment. We also analyzed the subgroup restricted to singletons.

RESULTS Of the more than 2.5 million infants born, 30 959 (1.2%) were conceived by IVF and were followed up for a mean 10 (SD, 6) years. Overall, 103 of 6959 children (1.5%) with autistic disorder and 180 of 15 830 (1.1%) with mental retardation were conceived by IVF. The RR for autistic disorder after any procedure compared with spontaneous conception was 1.14 (95% CI, 0.94-1.39; 19.0 vs 15.6 per 100 000 person-years). The RR for mental retardation was 1.18 (95% CI, 1.01-1.36; 46.3 vs 39.8 per 100 000 person-years). For both outcomes, there was no statistically significant association when restricting analysis to singletons. Compared with IVF without ICSI with fresh embryo transfer, there were statistically significantly increased risks of autistic disorder following ICSI using surgically extracted sperm and fresh embryos (RR, 4.60 [95% CI, 2.14-9.88]; 135.7 vs 29.3 per 100 000 person-years); for mental retardation following ICSI using surgically extracted sperm and fresh embryos (RR, 2.35 [95% CI, 1.01-5.45]; 144.1 vs 60.8 per 100 000 person-years); and following ICSI using ejaculated sperm and fresh embryos (RR, 1.47 [95% CI, 1.03-2.09]; 90.6 vs 60.8 per 100 000 person-years). When restricting the analysis to singletons, the risks of autistic disorder associated with ICSI using surgically extracted sperm were not statistically significant, but the risks associated with ICSI using frozen embryos were significant for mental retardation (with frozen embryos, RR, 2.36 [95% CI, 1.04-5.36], 118.4 vs 50.6 per 100 000 person-years]; with fresh embryos, RR, 1.60 [95% CI, 1.00-2.57], 80.0 vs 50.6 per 100 000 person-years).

CONCLUSIONS AND RELEVANCE Compared with spontaneous conception, IVF treatment overall was not associated with autistic disorder but was associated with a small but statistically significantly increased risk of mental retardation. For specific procedures, IVF with ICSI for paternal infertility was associated with a small increase in the RR for autistic disorder and mental retardation compared with IVF without ICSI. The prevalence of these disorders was low, and the increase in absolute risk associated with IVF was small.

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Author Affiliations: Department of Psychosis Studies, Institute of Psychiatry, King's College London, England (Sandin, Reichenberg); Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden (Sandin, Nygren, Iliadou, Hultman); Department of Psychiatry, Mount Sinai School of Medicine, New York, New York (Reichenberg); Department of Preventive Medicine, Mount Sinai School of Medicine, New York, New York (Reichenberg).

Corresponding Author: Sven Sandin, MSc, Department of Psychosis Studies, Institute of Psychiatry, King's College De Crespigny Park London, SE5 8AF, England, (sven.sandin@kcl.ac.uk) or Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, PO Box 281, SE-171 77 Stockholm, Sweden (sven.sandin@ki.se)

Between 1978 and 2012, approximately 5 million infants worldwide were born from in vitro fertilization (IVF). The original IVF procedure, allowing an egg to be fertilized by sperm in vitro, is usually used in the absence of male-factor infertility. This procedure is used in Sweden in about half of all treatments. Embryos can be transferred immediately after fertilization (fresh) or frozen for later use. The introduction of intracytoplasmic sperm injection (ICSI) in 1992,[1] in which a sperm is injected into an egg, allows treatment for male-factor infertility. For ICSI, sperm can be collected by ejaculation or surgical extraction.

Studies have demonstrated that IVF with or without ICSI is generally safe[2] but can be associated with an increased risk for perinatal complications, including preterm birth.[3] Concern has been raised about ICSI in particular,[4] which bypasses the natural selection of sperm, may physically damage the egg, and may contaminate the cytoplasm of the egg cell with culture media when the sperm is inserted. In vitro fertilization procedures have also been associated with several neurological disorders, including cerebral palsy[5] and the Russell-Silver,[6] Beckwith-Wiedemann, and Angelman syndromes.[6,7] No study has investigated the association between different IVF procedures and neurodevelopment, and few studies have investigated whether IVF treatments are associated with neurodevelopment after the first year of life.[8] Few studies have looked at autistic disorder and mental retardation, 2 of the most severe chronic developmental disorders, affecting 1% to 3% of all children in developed countries.[9(pp362-389)]

This prospective cohort study was designed to analyze the hypotheses that the use of any IVF procedure as well as specific procedures are associated with an increased risk of autistic disorder and mental retardation in the offspring.

Methods

Study Population

A birth cohort of all live births in Sweden from January 1, 1982, to December 31, 2007, was established using data from Swedish national registers, including the Medical Birth Register,[10] Multi-generation Register,[11] Patient Register,[12-14] and IVF Register (eTable 1 in Supplement). Children were followed up until December 31, 2009. The study was approved by the Swedish National Board of Health and Welfare and by the ethics committee at the Karolinska Institutet (Stockholm, Sweden).

Exposure

Information about IVF treatments was obtained from the National Board of Health and Welfare (eTable 1 in Supplement). In vitro fertilization without ICSI is used almost exclusively to treat female infertility, whereas IVF with ICSI is used for male infertility.

We classified mode of conception as spontaneous or IVF. In vitro fertilization was further classified according to use of ICSI; if ICSI was used, it was further classified by the source of sperm, ejaculated or surgically extracted. Treatment with surgically extracted sperm was introduced in 1996. Embryos can either be cultured in vitro for 2 to 3 days (cleavage stage) or for 5 to 6 days (blastocyst). During treatment, several embryos are often produced. The embryos not immediately used can be frozen. In vitro fertilization procedures were also classified by whether the embryo was fresh or frozen. Thus, 6 procedures currently used in Sweden were considered: (1) IVF without ICSI with fresh embryo transfer; (2) IVF without ICSI with frozen embryo transfer; (3) ICSI using ejaculated sperm with fresh embryos; (4) ICSI with ejaculated sperm and frozen embryos; (5) ICSI with surgically extracted sperm and fresh embryos, and (6) ICSI with surgically extracted sperm and frozen embryos.

Outcome

Autistic disorder is characterized by deficits in social interaction and communication as well as restricted, stereotypical, or repetitive behavior. Mental retardation is defined as an IQ lower than 70 plus limitations in adaptive behavior. In Sweden, all infants and preschool children are regularly seen at well-child care clinics and undergo routine medical and developmental screening. At age 4 years, a mandatory developmental assessment (motor, language, cognitive, and social development) is conducted. Children with a suspected developmental disorder are referred for further assessment by a specialized team. Diagnostic information is reported to the Patient Register.

The *International Classification of Diseases* (*ICD*) ninth and tenth revisions were used. We focused on mental retardation and on the narrow diagnosis of infantile and childhood autism (diagnostic codes *ICD*-9 299A or *ICD*-10 F84.0) and do not include other forms of autism spectrum disorders.

Covariates

We considered several factors that might confound or modify the association between IVF treatments and autistic disorder or mental retardation in the child. Parental psychiatric history[15] was classified as present or not-present for each parent separately using any diagnosis at any time before the birth of the child (for *ICD*-codes see eTable 2 in Supplement). We also obtained information on parental age,[16-18] birth year, multiple births, and preterm birth[3] (<week 37). Multiple births and preterm birth may be on a causal pathway to adverse developmental outcome and were therefore examined as effect modifiers.

Statistical Methods

First we examined the association between any IVF procedure and autistic disorder and mental retardation compared with spontaneous conception. This is the most important comparison from a public health perspective. Second, because there may be different risks associated with different procedures or parental factors underlying the choice of procedure, we analyzed the association between 5 of the IVF categories and autistic disorder and mental retardation compared with the most commonly used and least complicated procedure: IVF without ICSI with fresh embryo transfer. As couples undergoing fertility treatment may share common risk factors, the relevant comparison group consists of other couples undergoing IVF

treatment,[19] which controls for reasons for infertility. We also provide the results using spontaneous conception as the reference.

We also combined data to investigate procedures with similar underlying parental factors and to increase power: all ICSI procedures, regardless of sperm source and type of embryo; frozen embryos, regardless of type of IVF procedure; and surgical extraction of sperm, regardless of embryo type. In an exploratory analysis, we also examined whether the embryo was transferred at the cleavage or blastocyst stage; blastocyst transfer data were available from 2002.

For descriptive purposes, we calculated the rate and the percentage of children with autistic disorder and mental retardation and exact 95% confidence intervals.[20]

Using Poisson regression (SAS GLIMMIX version 9.3, SAS Institute Inc), we estimated RRs and 2-sided 95% Wald confidence intervals. We fitted regression models by splitting the follow-up time (child attained age between cohort entry and cohort exit) into 1-year intervals. Poisson regression gives approximately the same parameter estimates and likelihood ratios as Cox proportional hazards regression when the length of follow-up is split into finer intervals but allows for greater flexibility in the modeling.[21] For each child and outcome, we only considered the first event. Each child was followed up from age 1.5 years to death, emigration from Sweden, onset of disease, the age of 28 years, or December 31, 2009, whichever came first. We first fitted crude models including covariates for exposure together with sex, birth year, and attained age, then adjusted models including the potential confounding covariates parental age (paternal: <30, 30-39, 40-49, and ≥50 years; maternal: <25, 25-29, 30-34, and ≥35 years) and paternal and maternal psychiatric history at offspring birth (yes/no). This set of models was fitted for the comparisons of any IVF procedure vs spontaneous conception as well as for the comparison of specific procedures.

To allow the most efficient adjustment of the time variables, attained age and birth year, we fitted natural cubic splines[22,23] allowing for adjustment without assuming a specific functional form such as linear or stepwise.

All statistical tests were performed on the 2-sided 5% level of significance. All RRs are presented together with absolute rates per 100 000 person years adjusted for birth year, sex, and age.

Supplementary Analyses

To check for confounding potentially present in an observational study such as this and to allow a better understanding of the observed associations, we performed a priori specified analyses.

To confirm that associations were not due to temporal trends, crude models were adjusted for calendar time using splines. To allow the treatment comparisons to be summarized with a single RR, we examined IVF procedure × age interactions, allowing for different RRs at different ages. We calculated RRs separately for male and female offspring.

At the first prenatal visit, mothers are asked about length of involuntary infertility. Additional models included this variable as a linear continuous confounder. Women are also asked about hormone treatment as the only fertility treatment; we compared children of these women with spontaneous conception fitting crude and adjusted models.

We fitted a separate set of models also adjusting for certain genetic diseases and disorders (eTable 2 in Supplement) with known phenotypic and genetic overlap with autistic disorder and mental retardation.[24]

Finally, we repeated all analyses described above restricted to singletons.

For model checking purposes, we fitted supplementary models[25] assuming independence between families and a common correlation within and not requiring the data to follow a particular parametric distribution. We added several analyses post hoc. To complement the analyses of IVF procedures, we calculated RR using spontaneously conceived children as the control group. We analyzed the last 9 birth cohorts as a subgroup. To facilitate interpretation of the mechanism of the associations, we fitted the models separately to the subgroups of preterm and term children. In separate analyses, we calculated and compared RRs for multiple births after IVF and for multiple births after spontaneous conception.

Results

Characteristics of the children are presented in **Table 1**. A total of 2 541 125 children were alive at 1.5 years of age and had complete data on all the covariates; 30 959 (1.2%) were born following an IVF procedure. Of these 18 288 (0.7%) had missing information on parental age or term or preterm status and were not included, 65 with autistic disorder and 204 with mental retardation, including 1 and 2 cases among those born after an IVF procedure. After 1998, 44% of infertility treatments have used ICSI for male reproduction problems and frozen embryos increased from 9% before 1998 to 26% after 2005.

Autistic disorder was diagnosed in 103 of 6959 children (1.5%) and mental retardation in 180 of 15 830 children (1.1%) who were born after an IVF procedure. Cases had a mean follow-up time of 10 years (SD, 6 years), median 14 years (range, 0.1-26.5 years). The rate per 100 000 person-years of autistic disorder was 20.2 and of mental retardation was 46.1 among spontaneously conceived children. The highest rates of autistic disorder (215.0) and mental retardation (161.2) were in children born following ICSI using surgically extracted sperm with fresh embryos (**Table 2**).

Below only adjusted RRs are presented. Crude RRs are presented in **Figure 1**, **Figure 2**, and **Figure 3**. All results, including supplementary analyses, are presented in the eTables in Supplement.

Compared with offspring born following spontaneous conception, those born after any IVF procedure had a statistically significantly increased risk of mental retardation (RR, 1.18 [95% CI, 1.01-1.36]; 46.3 vs 39.8 per 100 000 person-years). The RR for autistic disorder was 1.14 (95% CI, 0.94-1.39; 19.0 vs 15.6 per 100 000 person-years; Figure 1). For both conditions, the risk estimates were slightly lower in singletons and not statistically significant with RRs of 1.01 (95% CI, 0.83-1.24; 38.8 vs 38.5 per 100 000 person-years) for mental retardation and 0.89

Table 1. Distribution of Confounders and Children's Characteristics—All Offspring[a]

Variable	No. (%) of Offspring						
		IVF Without ICSI		ICSI		ICSI, Surgically Extracted Sperm	
	Spontaneously Conceived	Fresh Embryo	Frozen Embryo	Fresh Embryo	Frozen Embryo	Fresh Embryo	Frozen Embryo
Total No. of offspring (% boys)	2 510 166 (51.4)	16 668 (52.9)	2777 (51.1)	9241 (49.7)	1477 (49.6)	628 (49.3)	168 (55.4)
Psychiatric history							
Father	36 405 (1.5)	183 (1.1)	39 (1.4)	136 (1.5)	12 (0.8)	9 (1.4)	8 (4.8)
Mother	46 366 (1.8)	363 (2.2)	51 (1.8)	157 (1.7)	30 (2.0)	12 (1.9)	2 (1.2)
Birth							
Preterm[b]	143 688 (5.7)	3631 (21.8)	435 (15.6)	1562 (16.9)	190 (12.9)	107 (17.1)	17 (10.1)
Multiple	54 673 (2.18)	5285 (31.7)	606 (21.8)	2379 (25.7)	258 (17.5)	164 (26.1)	39 (23.2)
Birth year, median (range)	1994 (1982-2007)	2000 (1982-2007)	2004 (1990-2007)	2002 (1992-2007)	2004 (1988-2007)	2002 (1996-2007)	2004 (1996-2007)
Age, y							
Maternal							
<25	532 141 (21.2)	128 (0.8)	19 (0.7)	183 (2.0)	18 (1.2)	14 (2.2)	3 (1.8)
25-29	889 571 (35.4)	2268 (13.6)	295 (10.6)	1788 (19.3)	234 (15.8)	121 (19.3)	37 (22.0)
30-34	734 554 (29.3)	7254 (43.5)	1113 (40.1)	3970 (43.0)	637 (43.1)	280 (44.6)	73 (43.4)
>34	353 900 (14.1)	7018 (42.1)	1350 (48.6)	3300 (35.7)	588 (39.8)	213 (33.9)	55 (32.7)
Paternal							
<30	969 915 (38.6)	1543 (9.3)	183 (6.6)	943 (10.2)	116 (7.8)	38 (6.0)	14 (8.3)
30-39	1 290 362 (51.4)	11 660 (70.0)	1889 (68.0)	6181 (66.9)	979 (66.3)	365 (58.1)	96 (57.1)
40-49	227 723 (9.1)	3248 (19.5)	647 (23.3)	1892 (20.5)	340 (23.0)	182 (29.0)	45 (26.8)
≥50	22 166 (0.9)	217 (1.3)	58 (2.1)	225 (2.4)	42 (2.8)	43 (6.8)	13 (7.7)
Born after IVF and ICSI							
1983-1987		142 (100.0)	0	0	0	0	0
1988-1992		1588 (93.5)	105 (6.2)	4 (0.2)	2 (0.1)	0	0
1993-1997		4718 (68.5)	572 (8.3)	1412 (20.5)	116 (1.7)	54 (0.8)	14 (0.2)
1998-2002		4577 (48.4)	502 (5.3)	3665 (38.8)	388 (4.1)	286 (3.0)	39 (0.4)
2003-2007		5643 (44.2)	1598 (12.5)	4160 (32.6)	971 (7.6)	288 (2.3)	115 (0.9)
Years of involuntary infertility, median (10th-90th percentiles)	0 (0-0)	3 (0-8)	2 (0-7)	3 (0-7)	2 (0-6)	2 (0-7)	2 (0-6)

Abbreviations: ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.

[a] Distribution of confounders and children characteristics for spontaneous conception, across IVF procedures, and for women with hormone treatment as the only fertility treatment.

[b] Preterm indicates birth before week 37.

(95% CI, 0.68-1.17; 14.4 vs 15.0 per 100 000 person-years) for autistic disorder (eTable 3 and eTable 4 in Supplement).

There was a statistically significantly increased risk for autistic disorder after ICSI using surgically extracted sperm with fresh embryos (RR, 4.60 [95% CI, 2.14-9.88]; 135.7 vs 29.3 per 100 000 person-years) compared with those born after IVF without ICSI with fresh embryos (Figure 2). In offspring born preterm, the RR was 9.54 (95% CI, 3.43-26.57; 364.5 vs 38.4 per 100 000 person-years). The increase in risk was not evident in singletons (RR, 0.95 [95% CI, 0.13-7.09]; 21.9 vs 23.9 per 100 000 person-years; eTable 5 in Supplement).

There was an increased risk of mental retardation in offspring born after ICSI using surgically extracted sperm with fresh embryos compared with those born after IVF without ICSI with fresh embryos (RR, 2.35 [95% CI,1.01-5.45]; 144.1 vs 60.8 per 100 000 person-years). In offspring born preterm, the RR was 4.38 (95% CI, 1.53-12.48, 413.9 vs 92.2 per 100 000 person-years). The increase in risk was not evident among singletons (RR, 0.70 [95% CI, 0.10-5.16], 36.1 vs 50.6 per 100 000 person-years).

The RR for mental retardation in offspring born after ICSI using ejaculated sperm was increased with fresh embryos was 1.47 ([95% CI, 1.03-2.09]; 90.6 vs 60.8 per 100 000 person-years) but not frozen. This increase was present also in singletons (RR, 1.60 [95% CI, 1.00-2.57]; 80.0 vs 50.6 per 100 000 person-years). Risk for mental retardation was also statistically significant in singletons after ICSI using ejaculated sperm with frozen embryos (RR, 2.36 [95% CI, 1.04-5.36]; per 100 000 person-years, 118.4 vs 50.6 per 100 000 person-years) but not among all offspring. For other procedures, the RR was not statistically significant (eTable 6 in Supplement).

To further elucidate the effect of specific techniques, we analyzed combined procedures. For autistic disorder, comparing the 2 procedures involving surgically extracted sperm with the 4 procedures involving ejaculated sperm, there was an increase in risk associated with the surgical extraction (RR, 3.29 [95% CI, 1.58-6.87]; 110.1 vs 30.9 per 100 000 person-years). The risk was even higher among preterm births (RR, 8.06 [95% CI, 2.97-21.85]; 319.8 vs 42.3 per 100 000 person-years) but was reduced in magnitude and was no longer statistically

Table 2. Analyses of All Offspring by Autistic Disorder and Mental Retardation

	Autistic Disorder					Mental Retardation				
	No. of Offspring	Person-years[a]	Percent (95% CI)[b]	Rate per 100 000 Person-years (95% CI) Unadjusted	Rate per 100 000 Person-years (95% CI) Adjusted[c]	Cases	Person-years[a]	Percent (95% CI)[b]	Rate per 100 000 Person-years (95% CI) Unadjusted	Rate per 100 000 Person-years (95% CI) Adjusted[c]
Offspring conceived										
Spontaneous conception	6856	33 994 678	0.27 (0.27-0.28)	20.2 (19.7-20.7)	15.6 (15.1-16.1)	15 650	33 947 960	0.62 (0.61-0.64)	46.1 (45.4-46.8)	39.8 (39.0-40.5)
IVF	103	231 118	0.33 (0.27-0.40)	44.6 (36.7-54.1)	19.0 (15.7-23.2)	180	230 710	0.58 (0.50-0.67)	78.0 (67.4-90.3)	46.3 (40.0-53.7)
Children born after specific IVF procedures										
Without ICSI										
Fresh embryo	53	144 207	0.32 (0.24-0.42)	36.8 (28.0-48.2)	29.3 (21.2-40.4)	94	143 924	0.56 (0.46-0.69)	65.3 (53.3-80.0)	60.8 (49.1-75.3)
Frozen embryo	10	17 121	0.36 (0.17-0.66)	58.4 (31.3-109.0)	42.4 (22.1-80.9)	13	17 095	0.47 (0.25-0.80)	76.0 (44.0-131)	69.0 (36.9-119.5)
With ICSI										
Fresh embryo	31	58 262	0.34 (0.23-0.48)	53.2 (37.3-75.8)	34.0 (22.1-52.5)	59	58 177	0.64 (0.49-0.82)	101.4 (78.5-131)	90.6 (68.4-120.1)
Frozen embryo	1	7022	0.07 (0-0.38)	14.2 (2.0-102.3)	9.4 (1.3-67.8)	8	7005	0.54 (0.23-1.06)	114.2 (56.9-229)	103.9 (51.2-210.6)
Fresh embryo, surgically extracted sperm	8	3720	1.27 (0.55-2.49)	215.0 (107.1-431.8)	135.7 (64.6-285.0)	6	3722	0.96 (0.35-2.07)	161.2 (72.1-361)	144.1 (64.1-324.3)
Frozen, embryo surgically extracted sperm	0	787	0 (0-2.17)	NA	NA	0	787	0 (0-2.17)	NA	NA

Abbreviations: ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization.
[a]Person-years represents the total number of years all the offspring were followed up.
[b]Percentage of children is calculated as number of children observed with a disease diagnosis divided by the number of offspring born who reached age 1.5 years. Exact confidence intervals were calculated on crude proportion autistic disorder and mental retardation without adjusting for possible confounding.
[c]Adjusted for age, birth year, and sex.

significant when restricted to singletons (RR, 0.73 [95% CI, 0.10-5.30]; 18.3 vs 24.3 per 100 000 person-years; eTable 7 in Supplement).

For mental retardation, comparing the 4 different ICSI procedures with the 2 procedures without ICSI, the RR was 1.51 (95% CI, 1.10-2.09; 93.5 vs 61.8 per 100 000 person-years). The RR was similar in singletons (RR, 1.50 [95 CI, 0.98-2.29]; 80.2 vs 54.8 per 100 000 person-years) and among preterm births (RR, 1.73 [95% CI, 1.05-2.86]; 166.7 vs 96.0 per 100 000 person-years). The risk increase comparing procedures using surgical extraction vs ejaculated sperm was present only for mental retardation among preterm births (RR, 3.31 [95% CI, 1.18-9.31]; 356.7 vs 109.4 per 100 000 person-years; eTable 8 in Supplement).

Comparing IVF procedures using blastocyst transfer with those using cleavage-stage transfer and comparing procedures using frozen embryos with those using fresh embryos, the risks of autistic disorder and mental retardation were not statistically significant.

Supplementary Analyses

The RRs for specific IVF procedures using spontaneously conceived offspring as the reference group were almost identical to the RRs using IVF without ICSI fresh embryo as the reference group (eTable 10 in Supplement). The RRs did not change when adjusting for calendar year. When restricting to birth cohorts after 1998, the overall adjusted results remained stable and statistical significance remained except the risk for mental retardation following ICSI using surgically extracted sperm with fresh embryos, which dropped in precision (RR, 2.08 [95% CI, 0.74-5.89]; 119.3 vs 61.2 per 100 000 person-years).

There were no major differences in risk of autistic disorder and mental retardation by age. The estimated RRs were similar in male and female offspring.

There was no increase in risk associated with years of infertility. Adjustment for this variable did not change the estimated associations with IVF or ICSI. The RR comparing hormone stimulation as the only treatment vs spontaneous conception was not statistically significantly different.

There were a total of 366 cases with known genetic diseases in the cohort, only 3 born following IVF (all following IVF with fresh embryos). Adjusting for presence of such conditions did not change the RR estimates or CIs.

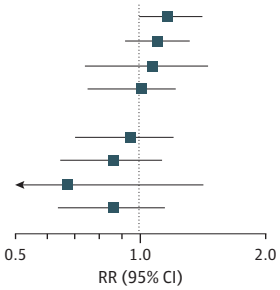
For spontaneously conceived children, multiple births contributed 2% of the person-years compared with 38% for IVF without ICSI with fresh embryos and 18% to 31% for other procedures. Among children with a diagnosis of autistic disorder or mental retardation, 3% were multiple births compared with 2% among children with no diagnosis at end of follow-up.

Among spontaneously conceived children, the RR for autistic disorder in multiple births compared with singletons was 1.15 ([95% CI, 0.99-1.34]; 15.9 vs 13.8 per 100 000 person-years). Among children born after any IVF procedure, the RR was 1.88 (95% CI, 1.28-2.77; 46.0 vs 24.9 per 100 000 person-

Figure 1. Comparison of Offspring Born After Any In Vitro Fertilization vs Being Spontaneously Conceived (Reference)—All Offspring

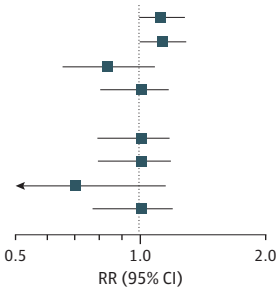
A Relative risk of autistic disorder

	No. of Cases		No. of Offspring		RR (95% CI)
	IVF	Spontaneous	IVF	Spontaneous	
All offspring					
Crude	103	6856	30959	2510166	1.22 (1.01-1.49)
Adjusted	103	6856	30959	2510166	1.14 (0.94-1.39)
Preterm birth	35	665	5942	143688	1.10 (0.78-1.54)
Term birth	68	6191	25017	2366478	1.00 (0.79-1.28)
Singletons					
Crude	54	6683	22 228	2455493	0.96 (0.74-1.26)
Adjusted	54	6683	22 228	2455493	0.89 (0.68-1.17)
Preterm birth	7	596	1844	120702	0.71 (0.34-1.50)
Term birth	47	6087	20 384	2334791	0.89 (0.67-1.19)



B Relative risk of mental retardation

	No. of Cases		No. of Offspring		RR (95% CI)
	IVF	Spontaneous	IVF	Spontaneous	
All offspring					
Crude	180	15647	30959	2510166	1.16 (1.00-1.35)
Adjusted	180	15647	30959	2510166	1.18 (1.01-1.36)
Preterm birth	70	2127	5942	143688	0.87 (0.69-1.11)
Term birth	110	13520	25017	236478	1.01 (0.84-1.22)
Singletons					
Crude	101	15178	22 228	2455494	1.01 (0.83-1.23)
Adjusted	101	15178	22 228	2455494	1.01 (0.83-1.24)
Preterm birth	17	1875	1844	120702	0.74 (0.46-1.20)
Term birth	84	13303	20 384	2334791	1.01 (0.81-1.25)



All models were adjusted for sex, attained age, birth year, paternal age categorically, maternal age categorically, maternal psychiatric history at offspring birth (yes or no), paternal psychiatric history at offspring birth (yes or no), except for "crude," which was adjusted for sex, attained age, and birth year. Term and preterm births are subgroups of the data. IVF indicates in vitro fertilization; RR, relative risk.

years), which was statistically significantly higher than among spontaneous conceived children (P = .021; eTable 11 in Supplement).

For mental retardation, the comparable RR was 1.49 (95% CI,1.11-2.00; rates per 100 000 person-years, 91.4 vs 60.0) among multiple births following IVF treatment and 1.42 (95% CI, 1.29-1.56; rates per 100 000 person-years, 51.1 vs 36.4) among spontaneously conceived multiples (eTable 11 in Supplement).

Discussion

Studies on long-term neurodevelopment of children born following IVF treatment, especially after the first year of life, are limited. Studies investigating the association between IVF and autism[26-28] or mental retardation[5,19,29-33] show mixed results. A case-control study showed IVF to be a risk factor for autistic disorder,[34] whereas 2 other studies did not.[26,27] Increased risk of developmental delay was reported in twins born following IVF[5] and in singletons following ICSI,[35] whereas a similar study did not find any difference.[36]

To the best of our knowledge, this is the largest study examining the relationship between specific IVF procedures and autistic disorder and mental retardation, examining the full range of IVF procedures. Although the data did not show an association between any IVF procedure and autistic disorder, compared with spontaneous conception, there was a small, statistically significant increase in the risk for mental retardation. When restricted to singletons, the risk for mental retardation was no longer statistically significant. However, the results demonstrated an association between autistic disorder and mental retardation and specific IVF procedures with ICSI related to paternal origin of infertility compared with IVF without ICSI.

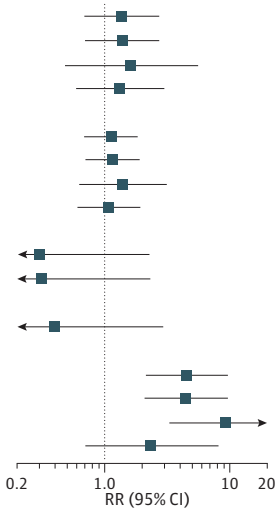
The absolute differences in rates were small, fewer than 7 per 100 000 person-years for mental retardation comparing any IVF procedure with spontaneous conception. The highest rate difference occurred with ICSI using surgically extracted sperm and fresh embryo transfer, compared with IVF without ICSI with fresh embryos (178.2 per 100 000 person-years for autistic disorder).

Our investigation of specific procedures was done in the subset of the population who all shared some degree of fertility problems. Although this is the correct comparison for evaluating the effect of IVF beyond the general effects of subfertility, the question of how generalizable the data are can be raised.[19] For this reason, we also presented these results using children born following spontaneous conception as the comparison group (eTable 10 in Supplement).

Figure 2. Comparison of Offspring Born After Specific IVF Procedures vs IVF Without ICSI, Fresh Embryo (Reference)—All Offspring

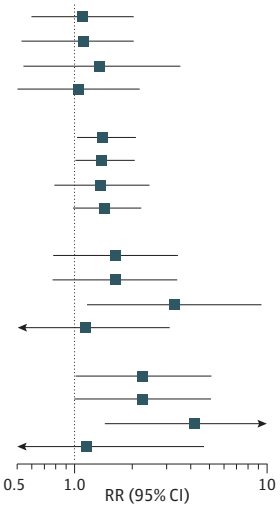
A Relative risk of autistic disorder by IVF procedures

	No. of Cases		No. of Offspring		
	Specific Procedure	Reference Procedure	Specific Procedure	Reference Procedure	RR (95% CI)
IVF without ICSI, frozen					
Crude	10	53	2777	16668	1.44 (0.73-2.85)
Adjusted	10	53	2777	16668	1.46 (0.74-2.89)
Preterm birth	3	17	453	3626	1.69 (0.49-5.79)
Term birth	7	36	2342	13042	1.39 (0.62-3.14)
ICSI, fresh					
Crude	31	53	9241	16668	1.16 (0.73-1.85)
Adjusted	31	53	9241	16668	1.20 (0.75-1.91)
Preterm birth	10	17	1562	3626	1.47 (0.66-3.26)
Term birth	21	36	7679	13042	1.11 (0.64-1.93)
ICSI, frozen					
Crude	1	53	1477	16668	0.32 (0.04-2.34)
Adjusted	1	53	1477	16668	0.33 (0.05-2.40)
Preterm birth					
Term birth	1	36	1287	13042	0.42 (0.06-3.09)
ICSI, fresh surgery					
Crude	8	53	628	16668	4.64 (2.17-9.92)
Adjusted	8	53	628	16668	4.60 (2.14-9.88)
Preterm birth	5	17	107	3626	9.54 (3.43-26.6)
Term birth	3	36	521	13042	2.42 (0.74-7.97)



B Relative risk of mental retardation by IVF procedures

	No. of Cases		No. of Offspring		
	Specific Procedure	Reference Procedure	Specific Procedure	Reference Procedure	RR (95% CI)
IVF without ICSI, frozen					
Crude	13	94	2777	16668	1.14 (0.63-2.04)
Adjusted	13	94	2777	16668	1.16 (0.54-2.07)
Preterm birth	5	38	453	3626	1.44 (0.56-3.66)
Term birth	8	56	2342	13042	1.07 (0.51-2.25)
ICSI, fresh					
Crude	59	94	9241	16668	1.49 (1.05-2.11)
Adjusted	59	94	9241	16668	1.47 (1.03-2.09)
Preterm birth	19	38	1562	3626	1.46 (0.83-2.59)
Term birth	40	56	7679	13042	1.51 (0.99-2.31)
ICSI, frozen					
Crude	8	94	1477	16668	1.71 (0.82-3.58)
Adjusted	8	94	1477	16668	1.70 (0.81-3.56)
Preterm birth	4	38	190	3626	3.47 (1.22-9.90)
Term birth	4	56	1287	13042	1.19 (0.43-3.30)
ICSI, fresh surgery					
Crude	6	94	628	16668	2.37 (1.03-5.48)
Adjusted	6	94	628	16668	2.35 (1.01-5.45)
Preterm birth	4	38	107	3626	4.38 (1.53-12.5)
Term birth	2	56	521	13042	1.21 (0.29-4.99)



In vitro fertilization (IVF) without intracytoplasmic sperm injection (ICSI), fresh embryo transfer is the reference category. All models were adjusted for sex, attained age, birth year, paternal age categorically, maternal age categorically, maternal psychiatric history at offspring birth (yes or no), paternal psychiatric history at offspring birth (yes or no), except for "crude," which was are adjusted for confounding sex, attained age, and birth year. Term and preterm births are subgroups of the data. Groups in the figures without error bars do not have sufficient data to allow an estimation of the confidence intervals. For the same reason, ICSI, frozen embryo, and surgically extracted sperm are not shown. RR indicates relative risk.

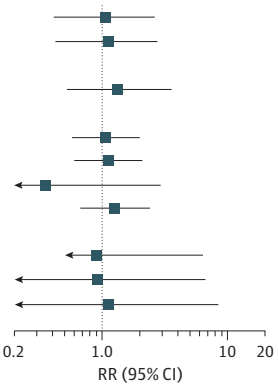
Mental retardation was associated with ICSI with fresh embryos. This association was robust and not due to multiple births, premature birth, or parental characteristics. Mental retardation was also associated with ICSI with frozen embryos among children born prematurely (multiples or singletons) and with IVF without ICSI with frozen embryos among preterm singleton infants.

Autistic disorder and mental retardation were also associated with ICSI using surgically extracted sperm. The increase in risk was present in the analysis including all offspring and was stronger in preterm births. Although the complete resolution of the risk in singletons can be explained by the reduction in statistical power, it also suggests that the risk was, at least in part, mediated through multiple embryo transfer or preterm birth. In this context, the formal statistical analysis comparing multiples and singletons showed a higher rate of autistic disorder among multiples, although multiple birth may not be a risk factor for autistic disorder gener-

Figure 3. Comparison of Offspring Born After Specific IVF Without ICSI, Fresh Embryo (Reference)—Singletons

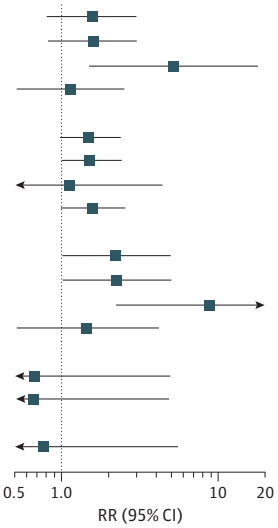
A Relative risk of autistic disorder by IVF procedures

	No. of Cases		No. of Offspring		
	Specific Procedure	Reference Procedure	Specific Procedure	Reference Procedure	RR (95% CI)
IVF without ICSI, frozen					
Crude	5	30	2171	11383	1.09 (0.42-2.82)
Adjusted	5	30	2171	11383	1.14 (0.44-2.95)
Preterm birth					
Term birth	5	24	2019	10336	1.41 (0.53-3.71)
ICSI, fresh					
Crude	18	30	6862	11383	1.09 (0.60-2.00)
Adjusted	18	30	6862	11383	1.17 (0.63-2.15)
Preterm birth	1	6	519	1047	0.37 (0.04-3.11)
Term birth	17	24	6343	10336	1.35 (0.71-2.57)
ICSI, fresh surgery					
Crude	1	30	464	11383	0.92 (0.12-6.79)
Adjusted	1	30	464	11383	0.95 (0.13-7.09)
Term birth	1	24	435	10336	1.19 (0.16-8.89)



B Relative risk of mental retardation by IVF procedures

	No. of Cases		No. of Offspring		
	Specific Procedure	Reference Procedure	Specific Procedure	Reference Procedure	RR (95% CI)
IVF without ICSI, frozen					
Crude	11	48	2171	11383	1.65 (0.85-3.21)
Adjusted	11	48	2171	11383	1.67 (0.86-3.24)
Preterm birth	4	7	152	1047	5.47 (1.58-18.9)
Term birth	7	41	2019	10336	1.20 (0.53-2.70)
ICSI, fresh					
Crude	34	48	6852	11383	1.58 (0.99-2.53)
Adjusted	34	48	6852	11383	1.60 (1.00-2.57)
Preterm birth	3	7	519	1047	1.18 (0.30-4.65)
Term birth	31	41	6343	10336	1.66 (1.01-2.73)
ICSI, frozen					
Crude	7	48	1219	11383	2.34 (1.03-5.31)
Adjusted	7	48	1219	11383	2.36 (1.04-5.36)
Preterm birth	3	7	87	1047	9.26 (2.35-36.6)
Term birth	4	41	1132	10336	1.52 (0.53-4.33)
ICSI, fresh surgery					
Crude	1	48	464	11383	0.71 (0.10-5.22)
Adjusted	1	48	464	11383	0.70 (0.10-5.16)
Preterm birth	0	7		1047	
Term birth	1	41	435	10336	0.81 (0.11-5.96)



In vitro fertilization (IVF) without intracytoplasmic sperm injection (ICSI), fresh embryo transfer is the reference category. All models were adjusted for sex, attained age, birth year, paternal age categorically, maternal age categorically, maternal psychiatric history at offspring birth (yes or no), paternal psychiatric history at offspring birth (yes or no), except for "crude," which was adjusted for sex, attained age, and birth year. Term and preterm births are subgroups of the data. Groups in the figures without error bars do not have sufficient data to allow an estimation of the confidence intervals. For the same reason ICSI, frozen embryos, and surgically extracted sperm are not shown. RR indicates relative risk.

ally. An indirect cause for this might be the use of multiple embryo transfer in more severe cases of infertility or a direct effect of parental infertility factors.

We examined several alternative explanations for the results. First, hormone stimulation is part of IVF treatment. It has been suggested that use of hormones, not IVF treatment, is associated with increased risk of autistic disorder.[37] We compared the risk of autistic disorder and mental retardation in children born to mothers reporting hormone treatment who had no IVF procedure. The risk for autistic disorder and mental retardation were not increased compared with the control population with RR point estimates lower than 1.

Second, any risk associated with an IVF procedure could be due to advancing parental age or other parental characteristics. Adjusting for paternal and maternal age and for parental psychiatric history did not attenuate the risk associated with the IVF procedures.

Third, since 1981 the single-embryo transfers have increased from 10% to 70% of all treatments while the rate of premature births dropped from 40% to 10%. However, our results were not restricted to the earlier years of treatment, and we adjusted for birth year. Also, the RR remained unchanged in the sensitivity analyses restricted to birth after 1998.

Although we did not have information on the number of treatment cycles, there was no association with years of infertility. This association may however be complicated with different causes acting differently, eg, if couples with paternal infertility tend to apply for IVF earlier.

A possible mechanism linking IVF and neurodevelopmental disorders is epigenetic modifications.[38,39] Epigenetic processes have been associated with Rett[40] and Angelman syndromes,[41] disorders characterized by autistic-like features in some patients. Experiments in mice have suggested that some of the steps involved in IVF might be related to epigenetic defects.[42,43] Mammal embryos cultured in vitro are also susceptible to imprinting control.[43] The risk of epigenetic changes may be modified the longer an embryo spends in culture. Although blastocyst transfer is rare and also involves sperm selection, it offers an indirect test of this hypothesis. We did not find any change in risk with blastocyst transfer.

The strengths of this study include the large, prospective, population-based sample and a health system with equal access. We included more detailed IVF treatment information with longer follow-up and control for confounding than previously done. Closest in comparison is a cohort study of autistic spectrum disorder from 2011[27] that also included detailed control for confounding but only 9 years of follow-up, a sample size one-fourth of ours, and no results on specific procedures. The detailed information allowed direct comparison of specific IVF procedures with IVF with fresh embryo transfer, allowing adjustment for shared confounding by causes of infertility and treatment.

The study has several limitations. We could not examine whether multiple birth was associated with zygosity. We only had information on live births and cannot rule out confounding by miscarriage.

We did not have information on parental educational achievement or socioeconomic status. In Sweden IVF treatment is free for childless women for up to 3 treatment cycles. Additional cycles are not expensive compared with many other countries, but there are still many couples in Sweden that cannot afford treatment beyond the 3 free-of-charge attempts. Any potential bias is likely to be small. Information about the number of embryos transferred was only available from 2003. Therefore, this effect could not be reliably examined.

We did not adjust for multiple comparisons. The overall study objective of testing for an association between IVF and ICSI and autistic disorder or mental retardation is built from a composite hypothesis involving 10 statistical tests, of which 4 had unadjusted *P* values below the .05 limit. After adjusting for multiplicity using Holm's procedure,[44] only 1 was statistically significant.

Finally, some outcomes were based on small numbers, some estimates have wide confidence intervals, and many others have lower confidence limits close to 1. Future studies in different populations are needed to further examine these issues.

Conclusions

In Sweden, compared with spontaneous conception, any IVF treatment was not associated with autistic disorder but was associated with a small but statistically significantly increased risk of mental retardation. Regarding specific procedures, the use of IVF with ICSI for paternal infertility was associated with a small increase in the RR for autistic disorder and mental retardation compared with IVF without ICSI. The prevalence of these disorders was low, and the increase in absolute risk associated with IVF was small. These associations should be assessed in other populations.

Our results should be applicable to most countries where IVF and ICSI are used. There are no major differences in equipment or laboratory work across countries but there may be some differences in choice of procedure. For instance, in several countries (like the United States), ICSI is often used when the sperm sample is normal because of a presumed (but unproven) higher efficiency. Blastocyst transfer is infrequently used in Sweden but is more common in the United States.

ARTICLE INFORMATION

Author Contributions: Mr Sandin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Hultman, Reichenberg, Sandin.
Acquisition of data: Hultman, Sandin.
Analysis and interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Iiadou, Reichenberg, Sandin.
Obtained funding: Hultman.
Study supervision: Nygren, Hultman, Reichenberg.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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