



HAL
open science

Risk of Autism Spectrum Disorders in children born after assisted conception. A population based follow-up study.

Dorte Hvidtjørn, Jakob Grove, Diana Schendel, Laura Schieve, Claus Sv??rke, Erik Ernst, Poul Thorsen

► **To cite this version:**

Dorte Hvidtjørn, Jakob Grove, Diana Schendel, Laura Schieve, Claus Sv??rke, et al.. Risk of Autism Spectrum Disorders in children born after assisted conception. A population based follow-up study.. Journal of Epidemiology and Community Health, 2010, 65 (6), pp.497. 10.1136/jech.2009.093823 . hal-00591154

HAL Id: hal-00591154

<https://hal.science/hal-00591154v1>

Submitted on 7 May 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Risk of Autism Spectrum Disorders in children born after assisted conception. A population based follow-up study.

Hvidtjørn D, PhD¹, Grove J, associate professor¹, Schendel D, lead of research team², Schieve LA, epidemiologist², Sværke C, statistician¹, Ernst E, professor³, Thorsen P, epidemiologist

¹ Institute of Public Health, at the Department of Epidemiology, University of Aarhus, Paludan-Müllers Vej 17, 8000 Århus C, Denmark.

² Centers for Disease Control and Prevention, National Center on Birth Defects and Developmental Disabilities, 1600 Clifton Rd. Atlanta, GA 30333, USA. ³ Fertility Section, Department of Obstetrics and Gynaecology, Aarhus University Hospital, Skejbygaardsvej, 8200 Aarhus, Denmark.

Corresponding author e-mail: dh@soci.au.dk

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Abstract

Objectives: to assess the risk of Autism Spectrum Disorders (ASD) in children born after assisted conception compared to children born after natural conception (NC). **Design:** population based follow-up study. **Setting:** all children born alive in Denmark 1995-2003. **Participants:** 588,967 children born in Denmark from January 1995 to December 2003. Assisted conception was defined as in vitro fertilization (IVF), with or without intracytoplasmic sperm injection (ICSI) and ovulation induction (OI) with or without subsequent insemination. Children exposed to IVF or OI were identified in the IVF Register and in the Danish Drug Prescription Register. **Main outcome measures:** a diagnosis of Autism Spectrum Disorders in the Danish Psychiatric Central Register. **Results:** 33,139 (5.6%) of all children born in Denmark 1995-2003 resulted from assisted conception and 225 (0.68%) of these exposed children received an ASD diagnosis. Of the 555,828 NC children born in this period 3,394 (0.61%) received an ASD diagnosis. The follow-up time was 4 to 13 years with a median follow-up time of 9 years. In crude analyses children born after assisted conception had an increased risk of getting an ASD diagnosis, crude hazard rate ratio (HRR) 1.25 (95% CI 1.09-1.43). In analyses adjusting for maternal age, educational level, parity, smoking, birth weight and multiplicity the risk disappeared, adjusted HRR 1.13. (95% CI 0.97-1.31). However, subgroup analyses that suggest possible associations in women who received FSH medications indicate the need for further study. **Discussion:** this population based follow-up study demonstrated no risk of ASD in children born after assisted conception.

What is already known on this subject?

- The few studies that have assessed the association between Autism Spectrum Disorders (ASD) and assisted conception present inconsistent results and disclose methodological limitations such as lack of statistical power.
- Associations between ASD and assisted conception might be anticipated due to at least 3 shared risk factors; high parental age, high maternal educational level and hormonal disturbances.
- Furthermore, a higher risk of ASD in children born after assisted conception might also be expected since assisted conception leads to a high rate of preterm delivery (PTD) and low birth weight (LBW) and associations between PTD or LBW and ASD have been reported.

What this study adds

- This population based follow-up study demonstrated no risk of ASD in children born after assisted conception.
- However, we did find an increased risk in a subgroup of women who had IVF treatment and were exposed to FSH

Introduction

The etiology behind infantile autism and autism spectrum disorders (ASD) is multifactorial and largely unknown.¹ Associations between ASD and assisted conception might be anticipated due to at least 3 shared risk factors; high parental age,²⁻⁸ high maternal educational level^{3,9} and hormonal disturbances.¹⁰⁻¹² Furthermore, a higher risk of ASD in children born after assisted conception might also be expected since assisted conception leads to a high rate of preterm delivery (PTD) and low birth weight (LBW)¹³⁻¹⁹ and associations between PTD or LBW and ASD have been reported.^{3;6;20;21} The use of assisted conception has increased over the last 2 decades.^{14;22}

We identified 8 studies assessing the risk of infantile autism or ASD in children born after assisted conception.²³⁻³⁰ They present inconsistent results and disclose methodological problems such as lack of statistical power²⁶⁻²⁸ or only evaluating a combined group of psychiatric disorders, rather than ASD specifically.^{28;31} Furthermore, 6 studies only assessed one type of assisted conception, in vitro fertilization (IVF). In all, only one study found a significantly higher occurrence of ASD in children born after assisted conception compared to children born after natural conception (NC)²⁶; however, four other studies reported a non-statistically significantly increased risk.^{23;24;28;29} One case-control study reported a protective effect of assisted conception on infantile autism after adjusting for several factors including gestational age.³⁰ None of the studies distinguished between different underlying causes of infertility or between different types of medications used in the treatment. Consequently, the relationship between ASD and assisted conception is still not well understood. To overcome methodological problems in former studies, we assessed the risk of infantile autism and ASD in a large population-based cohort of children born after assisted conception. Assisted conception was defined as IVF, with or without intracytoplasmic sperm injection (ICSI) and ovulation induction (OI) with or without subsequent insemination. We also evaluated underlying causes of infertility and specific medications used in the treatments.

Methods

Study population

This cohort study was based on data from Danish National Registers and linkage between the registers was achieved by use of the unique registration number given to all citizens in Denmark. Our study comprised all children born alive in Denmark from 1 January 1995 to 31 December 2003, identified through the Danish Medical Birth Register (MBR), containing information on all births in Denmark.³²

Exposure

Children exposed to IVF were identified through the IVF Register holding data from all private and public fertility clinics including underlying causes of infertility (tubal, male, ovulation or mixed and unspecified factors).³³ Children exposed to OI were identified through the Danish Drug Prescription Register (DDPR) holding information on all prescription drugs sold at pharmacies in Denmark. The medications used during OI are prescription drugs bought at the pharmacy enabling identification of the women who went through OI in the DDPR. Drugs used in assisted conception were identified by the authors through cross checking with the official Danish Pharmaceutical Classified Catalogue, www.medicin.dk, the US website www.drugs.com, Books of Instruction of Danish fertility clinics from the time period in question and by means of clinical

experience. To identify women who had hormonal treatment in relation to the index pregnancy, we set up a time window for the date of dispatch of 12 weeks before and 4 weeks after last menstrual period (LMP). As the drugs used in OI are also used in IVF, we excluded women from the OI group who were included in the IVF register with the same LMP date.

Outcome

Children diagnosed with an ASD diagnosis (F84.0, F84.1, F84.5, F84.8 and F84.9) or specifically infantile autism (F84.0) through May 8, 2008 were identified via the Danish Psychiatric Central Register (DPCR). The DPCR implements information on all Danish psychiatric in- and out-patient admissions since 1995³⁴ and in Denmark all autism diagnoses are made at public child mental health services, reporting all in- and out-patient discharge diagnoses to the DPCR.

Covariates

Further covariate information was obtained from MBR and Statistics Denmark: birth weight (BW), gestational age (GA), multiplicity, sex, maternal age, educational level, smoking, and parity. Maternal age was divided into 5 year age groups 20-24, 25-29 (referent category (ref.)), 30-34, 35-39, 40+, BW was grouped into 500-1499 g, 1500-2499 g and normal BW: 2500 g or more (ref.): education level was categorized as low (9-10 years of education), intermediate (11-16 years of education) (ref.) and high (17 or more years of education): parity was dichotomized into primipara and multipara (ref.): multiplicity was divided into singletons (ref.) and multiples: and maternal smoking during pregnancy was grouped into non-smoker (ref.), and smoking plus “stopped smoking during pregnancy” combined. As there are no mothers less than 20 years of age in the IVF Register, 10,137 children (1.7%) born from mothers of less than 20 years were excluded.

Multivariate analyses

Cox regression was used to measure the risk of getting an ASD diagnosis in children born after assisted conception combined and in IVF and OI separately. We conducted multivariate analyses including maternal age, parity, smoking, educational level, BW and multiplicity. Due to the skewed sex distribution in ASD, analyses were conducted for all children and stratified by sex.

Sub-analyses

In further analyses we explored if the risk of ASD was associated with a single underlying cause of infertility (tubal, male, ovulation or mixed and unspecified factors), but this information was only available in the IVF Register and only for 9,855 (66%) of the IVF children as many of the IVF children had unspecific or more than one cause. Finally, to disentangle if different hormones used in assisted conception (down-regulating hormones, clomiphene, follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG)) were associated with ASD, we made exploratory analyses with specific hormone exposure. Information about use of specific hormones was available from the DDPR for all OI children but only for 51% of the IVF children. This was due to different dispensing procedures at IVF clinics; some clinics dispense the medication directly to the women while other IVF clinics provide prescriptions for the pharmacy. Only the latter group of women is included in the DDPR.

Statistics

The risk of getting an ASD or infantile autism diagnosis was measured by the incidence rate, using the child as the unit of analysis. The incidence rate was defined as the number of children receiving an ASD diagnosis

in a given time frame (1 January 1995 – 8 May 2008) divided by the total number of person-years the children in the cohort were at risk for the diagnosis during that time. We used Cox regression and the incidence rate ratio was estimated by the hazard rate ratio (HRR) with 95% confidence intervals (CI). It was verified that the proportional hazards assumption was met. To account for correlations between siblings in the cohort additional analyses were done employing robust standard errors. To reduce bias that might arise if exposed children at high risk for ASD were more likely to die as infants than unexposed children we conducted additional analyses excluding children less than one year old (i.e. limiting analysis to infant survivors). Stata 8.2 was used for the analyses, StataCorp. 2003. *Stata Statistical Software: Release 8*. College Station, TX: StataCorp LP.

Results

From January 1 1995 to December 31 2003, 588,967 children were born alive in Denmark from 399,598 mothers of 20 years or more. In this period 33,139 (5.6%) children resulted from assisted conception; 14,991 (2.6%) from IVF and 18,148 (3.0%) from OI. Compared to NC mothers, mothers having assisted conception were statistically significantly older ($P < 0.001$); more often primipara ($P < 0.001$); non-smokers ($P < 0.001$); they had higher educational level ($P 0.002$) and delivered more multiples ($P < 0.001$), children with low BW ($P < 0.001$) and PTD ($P < 0.001$) (Table 1). Furthermore, mothers having IVF, as opposed to OI, had the largest difference from mothers who conceived naturally on most of these characteristics. (Table 1)

Table 1. Characteristics of women who used assisted conception and their children. Denmark 1995-2003. Numbers and percentages.

Natural conception compared to the combined assisted conception group and IVF compared to OI

	Natural conception	Assisted conception	P	IVF	OI	P
Maternal age			<0.001			<0.001
20-24	81,371 (14.6)	1,175 (3.6)		206 (1.4)	969 (5.39)	
25-29	211,078 (38.0)	8,423 (25.4)		2,822 (18.8)	5,601 (30.9)	
30-34	186,934 (33.6)	14,185 (42.8)		6,791 (45.3)	7,394 (40.7)	
35-39	66,776 (12.0)	8,174 (24.7)		4,547 (30.3)	3,627 (20.0)	
40+	9,651 (1.8)	1,182 (3.5)		625 (4.2)	557 (3.1)	
Parity			<0.001			<0.001
primipara	228,059 (41.0)	22,171 (66.9)		11,099 (74.0)	11,072 (61.0)	
multipara	327,746 (58.9)	10,968 (33.1)		3,892 (26.0)	7,076 (39.0)	
Educational level (years)			<0.001			0.002
lower (9-10)	111,404 (23.1)	4,641 (16.7)		2,175 (17.5)	2,466 (16.1)	
intermediate (11-16)	331,842 (68.7)	20,187 (72.7)		8,975 (72.3)	11,212 (73.1)	
higher (17+)	39,663 (8.2)	2,930 (10.6)		1,261 (10.2)	1,669 (10.8)	
Smoking			<0.001			<0.001
non-smoker	429,728 (77.7)	26,670 (83.7)		12,293 (82.4)	15,377 (84.8)	
Smoker or stopped in pregnancy	123,512 (22.3)	5,386 (16.3)		2,631 (17.6)	2,755 (15.2)	
Multiplicity			<0.001			<0.001

singletons	541,616 (97.6)	23,483 (70.9)	8,501 (56.7)	14,982 (82.6)
twins	13,376 (2.4)	9,120 (27.5)	6,234 (41.6)	2,886 (15.9)
triplets	215 (0.0)	495 (1.5)	239 (1.6)	256 (1.4)
quadruplets	4 (0.0)	17 (0.1)	5 (0.1)	12 (0.1)
Gestational age (weeks)			<0.001	<0.001
20-27	1,247 (0.2)	397 (1.2)	278 (1.9)	119 (0.7)
28-31	3,275 (0.6)	868 (2.6)	529 (3.6)	339 (1.9)
32-36	26,319 (4.8)	5,177 (15.7)	3,108 (20.8)	2,069 (11.4)
37-41	474,346 (85.9)	24,882 (75.3)	10,613 (71.2)	14,269 (78.6)
42+	46,745 (8.5)	1,728 (5.2)	377 (2.5)	1,351 (7.4)
Birth weight (grams)			<0.001	<0.001
500-1499	3,864 (0.7)	1,116 (3.4)	672 (4.6)	444 (2.5)
1500-2499	19,930 (3.6)	4,680 (14.3)	2,864 (19.4)	1,816 (10.1)
2500+	527,278 (95.7)	29,957 (82.3)	11,214 (76.0)	15,743 (87.4)

Through May 8 2008, 3,619 (0.61%) children from the cohort received an ASD diagnosis; an infantile autism diagnosis was given to 1,385 children (0.24%). For all diagnoses the follow-up time was 4 to 13 years with a median follow-up time of 9 years. Within the time period (1995-2003), 3,479 mothers had one child with an ASD diagnosis, 64 mothers had two children with ASD diagnoses and 4 mothers had 3 children with ASD diagnoses. Mothers of children with ASD differed with statistical significance from mothers of other children according to select characteristics; they were older ($P = 0.002$), more often primipara ($P < 0.001$), smokers ($P < 0.001$), they had higher or lower educational level ($P < 0.001$), more preterm deliveries ($P < 0.001$), and children with low BW ($P < 0.001$) (Table 2).

Table 2. Characteristics of children with an ASD diagnosis and their mothers, Denmark 1995-2003. Numbers (percentages)
Children without ASD compared to children with ASD

	no ASD	ASD	P
Maternal age			<0.001
20-24	81,996 (14.0)	550 (15.2)	
25-29	218,242 (37.3)	1,259 (34.8)	
30-34	199,901 (34.2)	1,218 (33.7)	
35-39	74,442 (12.7)	508 (14.0)	
40+	10,749 (1.8)	84 (2.3)	
Parity			<0.001
primipara	248,429 (42.4)	1,801 (49.8)	
multipara	336,896 (57.6)	1,818 (50.2)	
Educational level (years)			<0.001
lower (9-10)	115,139 (22.7)	906 (27.0)	
intermediate (11-16)	349,884 (69.0)	2,145 (63.8)	
higher (17+)	42,283 (8.3)	310 (9.2)	
Smoking			<0.001
non-smoker	454,685 (78.0)	2,713 (75.3)	
smoker or stopped in pregnancy	128,009 (22.0)	889 (24.7)	
Multiplicity			0.028

singletons	561,598 (96.1)	3,501 (96.9)
twins	22,389 (3.8)	107 (3.0)
triplets	703 (0.1)	7 (0.1)
quadruplets	21 (0.0)	0 (0.0)
Gestational age (weeks)		<0.001
20-27	1,624 (0.3)	20 (0.5)
28-31	4,114 (0.7)	29 (0.8)
32-36	31,268 (5.4)	228 (6.4)
37-41	496,232 (85.3)	2,996 (83.5)
42+	48,158 (8.3)	315 (8.8)
Birth weight (grams)		<0.001
500-1499	500 (0.9)	44 (1.2)
1500-2499	24,414 (4.2)	196 (5.5)
2500+	550,889 (94.9)	3,346 (93.3)

In children born after assisted conception the risk of getting an ASD or an infantile autism diagnosis was increased, crude HRR 1.25 (95% CI 1.09-1.43) and crude HRR 1.25 (95% CI 1.01-1.54), respectively. Assessing the risk of an ASD diagnosis divided into specific treatment types IVF or OI, we found a crude HRR of **1.18 (0.95-1.44)** and 1.31 (95% CI 1.10-1.56), respectively. In analyses adjusting for maternal age, educational level, smoking, parity, BW and multiplicity we found a HRR of 1.13 (0.97-1.31) in the combined assisted conception group and higher risk **estimates in OI than in IVF** (Table 3). **In the adjusted analyses only girls born after OI had a statistically significant increased risk of ASD.**

Table 3. Hazard rate ratios and 95% confidence intervals for the association between ASD and assisted conception Crude and adjusted*

	All		Boys		Girls	
	Crude	Adjusted**	Crude	Adjusted**	Crude	Adjusted**
Assisted conception	1.25 (1.09-1.43)	1.13 (0.97-1.31)	1.18 (1.01-1.37)	1.07 (0.91-1.27)	1.55 (1.14-2.10)	1.32 (0.93-1.88)
IVF	1.18 (0.96-1.44)	1.04 (0.83-1.31)	1.15 (0.92-1.44)	1.02 (0.79-1.32)	1.23 (0.75-2.03)	1.02 (0.57-1.81)
OI	1.31 (1.10-1.56)	1.20 (0.99-1.44)	1.20 (0.99-1.47)	1.12 (0.91-1.38)	1.80 (1.24-2.61)	1.56 (1.03-2.36)

* Reference = natural conception

** Adjusted for maternal age, educational level, parity, smoking, BW and multiplicity

Similar results were seen for infantile autism, however with wider confidence intervals (data not shown). The risk of ASD for boys and girls did not differ statistically significant. Restricting analyses to infants who survived their first year showed similar results to the full cohort (data not shown). Dropping variables from the adjusted analyses one at a time showed that the increased risk in the crude analyses seemed to be explained by maternal age and parity but not by smoking, multiplicity, BW and educational level. The HRR in an adjusted analyses not including maternal age was 1.24 (1.06-1.44) and in an adjusted model without parity the HRR was 1.28 (1.10-1.49).

For 9,855 IVF children we had information on a single underlying cause of infertility. The risk of ASD was highest for those children with a maternal infertility diagnosis of ovulation factor; however, estimates are imprecise due to the small sample sizes. (Table 4)

Table 4. Crude hazard rate ratios and 95% confidence intervals for the association between ASD and IVF according to specific underlying cause of infertility. Percentages within categories.

Underlying factor	Numbers exposed	ASD Numbers exposed	Crude HRR
Natural conception	555,828	3,394 (0.61%)	ref.
Mixed or unspecific factors	5,136	39 (0.76%)	1.49 (1.08-2.04)
Tubal factor	4,859	26 (0.54%)	0.86 (0.59-1.27)
Ovulation factor	929	7 (0.75%)	1.63 (0.78-3.42)
Male factor	4,019	23 (0.57%)	1.18 (0.79-1.78)

Information on specific hormonal medications used in assisted conception was available for 25,867 children and in the analyses of the risk of ASD by different hormone exposure the association with ASD was strongest with medications containing FSH; and not clearly evident for other hormonal medications. (Table 5)

Table 5. Hazard rate ratios and 95% confidence intervals for the association between ASD and assisted conception combined and divided into IVF and OI according to specific hormones*

	Assisted conception, all	Assisted conception, boys	Assisted conception, girls	IVF, all	OI, all
Downregulation	0.82 (0.49-1.37)	0.79 (0.45-1.40)	0.93 (0.29-2.92)	0.57 (0.28-1.16)	1.09 (0.48-2.51)
FSH	1.44 (1.16-1.80)	1.32 (1.02-1.70)	1.99 (1.23-3.18)	1.73 (1.10-2.71)	1.29 (0.89-1.89)
hCG	1.28 (1.05-1.55)	1.16 (0.93-1.44)	1.85 (1.23-2.77)	1.37 (0.87-2.15)	1.17 (0.79-1.71)
Clomiphene	1.14 (0.93-1.40)	1.02 (0.80-1.29)	1.75 (1.15-2.65)	1.34 (0.54-3.32)	0.82 (0.53-1.28)

* Reference = natural conception, adjusted for maternal age, education, parity, smoking, BW and multiplicity

** Data from the Danish Drug Prescription Register (DDPR) on specific medications used during IVF treatment available for all OI women and 51% of IVF women

Divided into specific treatment type, the FSH associations were observed only within IVF treatments. The risk was higher for girls than for boys across all hormones and statistically significant for FSH, hCG and clomiphene. We were able to assess FSH use in all OI treatments but only in 51% of the IVF treatments as we could not confirm the specific medications used through the DDPR for the remaining IVF treatments. Women who underwent IVF and had detailed medication data from DDPR were different from those women without DDPR data in that they were older, more often multipara, non-smokers, more highly educated, and more likely to have been treated at a private rather than public clinic. They were also more likely to have ovulation factor infertility, however, even with this increase, it is important to note that while the vast majority of women who underwent IVF and had detailed medication data from DDPR used FSH (85%), only a small minority (9%) had documentation of ovulation factor infertility. We were unable to assess both factors simultaneously because of limited sample size (only 58 cases had data on both single underlying cause of infertility and specific hormone treatment).

Discussion

Main Results

In this large population based cohort study we found no risk of ASD in children born after assisted conception, the adjusted HRR was 1.13 (95% CI 0.97-1.31). The increased risk for ASD observed in unadjusted analyses seemed to be explained by high maternal age and parity but did not seem to be explained by educational level, low BW, smoking or multiplicity. This is the largest population based study to date, including 33,139 children exposed to assisted conception and hence the first study also permitting assessment of different types of assisted conception separately. In the present study the prevalence of ASD was 6 per 1000 children, similar to reports from other studies with ASD prevalence of 4.7, 5.8 and 6-7 per 1000.³⁵⁻³⁷

Sex, maternal age, parity and birth weight

The risk from assisted conception tended to be higher in girls than in boys in all strata. Other studies suggest that the etiologic profile behind ASD in girls and in boys may be different.³⁸ Furthermore, since the a priori risk in boys is so much higher than the risk in girls it is possible that ASD in boys represents either a more heterogeneous mix of autism etiology subtypes or a mix of potential etiologies which could moderate an association of assisted conception with ASD.

The risk of autism or ASD is associated with advanced maternal age in some studies with adjusted analyses²⁻⁵ but not in all.^{6;7;20;21} In our adjusted analysis the risk of ASD from assisted conception was no longer statistically significant when controlling for maternal age (Table 3). Thus the increased risk of ASD in assisted conception seems to be partly explained by high maternal age.

There is also a higher risk of ASD in firstborn children in some studies³⁻⁵ but not in others.^{6;20;21} **A recent meta-analysis find an increased risk in both first born and later born children (>=third).**³⁹ In our analyses the risk of ASD from assisted conception did not remain statistically significant when we adjusted for parity, suggesting that the increased risk of ASD in the crude analyses is also partly explained by a large proportion of primipara mothers having assisted conception.

In assisted conception more children are born with low BW and in general the risk of ASD increases with decreasing BW in most studies,^{3;6;20;21} however not in one smaller study by Glasson using mean BW.⁵ In our study low BW did not seem to explain the increased risk of ASD in assisted conception. This is in contrast to studies of cerebral palsy where the increased risk of cerebral palsy in children born after IVF is partly explained by multiplicity and PTD, factors leading to a high proportion of low BW children^{31;40}.

Other studies

Our results contradict previous studies finding a higher occurrence of ASD in children born after assisted conception.^{23;24;26;28;29} One case-control study showed a protective association between a general notation of assisted conception on the birth record and infantile autism³⁰ The possibility of biased exposure ascertainment cannot be ruled out in the latter case-control study.

Type of assisted conception

There was a tendency of higher risk estimates in the OI group than in the IVF group, but the differences in risk between the two types of treatment were not statistically significant. However, this trend was consistent in both boys and girls with ASD and might be explained by competing risk factors in the IVF group, as this group is at higher risk for ASD a priori due to higher maternal age, more multiple pregnancies and more

primipara mothers (Table 1). However, the risk of ASD was not statistically significant in the adjusted analyses in either type of assisted conception.

Hormonal exposure

In adjusted analyses an increased risk of ASD from FSH exposure was seen, HRR 1.44 (95% CI 1.16-1.80), divided into sex the risk was statistically significant in both boys and girls, but divided into specific treatments the risk was only statistically significant in IVF. Hormonal disorders could link assisted conception with ASD as some underlying causes of infertility are hormone related and hormonal medications are used as part of infertility treatments. Further, various theories for associations between abnormal levels of sex hormones and ASD have been presented based on reports of elevated levels of fetal testosterone found in children with ASD,¹⁰ women with ASD were found to have statistically significantly more Polycystic Ovary Syndrome, delayed puberty and irregular menstrual cycles in adulthood than controls¹¹ and children with ASD were found to have statistically significantly lower levels of serum follicle stimulating hormone and statistically significantly higher levels of serum total testosterone relative to normal reference ranges.¹² Our findings concerning the risk of ASD from specific hormone exposure in assisted conception should be interpreted with caution. In treatment regimes in assisted conception hormones are used in various combinations and hormone exposure groups are thus overlapping; we did not have data to assess specific medication dosages or protocols; we were able to assess FSH use in all OI treatments but only in 51% of the IVF treatments as we could not confirm the specific medications used through the DDPR for the remaining IVF treatments we could not assess medication use in the context of the underlying fertility condition being treated. Accordingly, it is unclear whether it is factors linked to use of FSH (underlying cause of infertility or other conditions), a teratogenic effect from FSH medication per se, the combined effect of exogenous FSH on the endogenous hormonal profile or some perhaps some yet unmeasured factor, underlying the association between FSH medications and ASD. The risk of ASD in different hormone regimes would best be assessed in a randomized controlled trial but this possibility is ruled out by the infrequency of ASD. Consequently, observational studies like this are the only alternative and our findings indicate the need for further, more specific investigations of possible associations between exposure to exogenous FSH and ASD.

Strengths and limitations of the study

Our sources of data were Danish national health registers. These registers are maintained for administrative or monitoring purposes and will therefore not always include specific details desirable for research. For an exposure to IVF we used the IVF Register and according to the Danish legislation, it is mandatory for all public and private fertility clinics in Denmark to report all initiated IVF and ICSI treatments, successful or not, to the IVF Register and the register is therefore considered close to complete.³³ For an exposure to OI we used the DDPR with data on all dispatched prescriptions for hormones used in OI. The main purpose of the DDPR is entirely administrative, that is to ensure that the prescription is not used twice and therefore it is expected that all dispatches are reported to the DDPR. However, it was not possible to establish the date the woman actually administered the medicine. Hormones used in OI can be prescribed for the next 3 cycles and consequently, we had to select a time-window of prescriptions within 3 months before LMP to define the use of OI in the index pregnancy. Therefore the hormones could have been administered 1 or 2 months earlier than the index pregnancy. Additionally we cannot be sure that the woman ever administered the

medicine she purchased and therefore the findings regarding OI have to be interpreted with caution. However, we have no reason to believe such exposure misclassification would be related to ASD; thus, to the extent that we erroneously included some NC children in the OI category, our results might actually be attenuated. The quality of autism diagnoses found in the DPCR has been validated by experts ⁴¹; after evaluating close to 500 medical records of children registered with infantile autism in the DPCR, 94% of the children met the criteria for a correct diagnosis.

As a number of children were followed for less than 6 years some children with ASD may not have been diagnosed before the end of follow up. Also, since the number and proportion of assisted conceptions increased over the study period, a disproportionate number of children born after assisted conception had a shorter follow up time than NC children. To deal with these aspects of the data we used Cox regression models in the statistical analysis which account for differences in follow-up time.

Conclusion

In conclusion, we found no increased risk of ASD in children born after assisted conception overall. However, in sub analyses we did find an increased risk in a subgroup of women who had OI treatment and were exposed to FSH. Additional studies with more detailed information on underlying causes of infertility and use of specific hormonal medications may help to pinpoint the underlying mechanism of this increased risk.

Competing interests: all authors declare that the answer to the questions on your competing interest form are all No and therefore have nothing to declare.

Contributions: DH, PT and JG were responsible for conception and design. DH, JG and CS conducted the statistical analyses and the interpretation of data. DH wrote the first draft of the manuscript. All authors provided critical input at all stages and critically reviewed and contributed to the final draft. PT and DH are guarantors of the study.

Funding: the study was funded as a co-financed PhD project by The Danish Agency for Science, Technology and Innovation, University of Aarhus and The Elsass Foundation. Further funding was applied by Sofiefonden, The Health Insurance Foundation, The Augustinus Foundation, Julie von Müllens Foundation, Direktør Jacob Madsen & Hustru Olga Madsens Fond and Aase and Ejnar Danielsen Foundation.

Ethical approval: approval for this study was obtained from the Danish Data Protection Agency.

Access statement: all authors had full access to the data.

Exclusive licence: the Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in BMJ editions and any other BMJ PGL products.

Reference List

- (1) Baron IS. Autism Spectrum Disorder: Complex, Controversial, and Confounding. *Neuropsychol Rev* 2008.
- (2) Durkin MS, Maenner MJ, Newschaffer CJ, Lee LC, Cunniff CM, Daniels JL et al. Advanced parental age and the risk of autism spectrum disorder. *Am J Epidemiol* 2008; 168(11):1268-1276.
- (3) Croen LA, Grether JK, Selvin S. Descriptive epidemiology of autism in a California population: who is at risk? *J Autism Dev Disord* 2002; 32(3):217-224.
- (4) Durkin MS, Maenner MJ, Newschaffer CJ, Lee LC, Cunniff CM, Daniels JL et al. Advanced parental age and the risk of autism spectrum disorder. *Am J Epidemiol* 2008; 168(11):1268-1276.
- (5) Glasson EJ, Bower C, Petterson B, de KN, Chaney G, Hallmayer JF. Perinatal factors and the development of autism: a population study. *Arch Gen Psychiatry* 2004; 61(6):618-627.
- (6) Larsson HJ, Eaton WW, Madsen KM, Vestergaard M, Olesen AV, Agerbo E et al. Risk factors for autism: perinatal factors, parental psychiatric history, and socioeconomic status. *Am J Epidemiol* 2005; 161(10):916-925.
- (7) Lauritsen MB, Pedersen CB, Mortensen PB. Effects of familial risk factors and place of birth on the risk of autism: a nationwide register-based study. *J Child Psychol Psychiatry* 2005; 46(9):963-971.
- (8) Reichenberg A, Gross R, Weiser M, Bresnahan M, Silverman J, Harlap S et al. Advancing paternal age and autism. *Arch Gen Psychiatry* 2006; 63(9):1026-1032.
- (9) Bhasin TK, Schendel D. Sociodemographic risk factors for autism in a US metropolitan area. *J Autism Dev Disord* 2007; 37(4):667-677.
- (10) Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G. Fetal testosterone and autistic traits. *Br J Psychol* 2008.
- (11) Ingudomnukul E, Baron-Cohen S, Wheelwright S, Knickmeyer R. Elevated rates of testosterone-related disorders in women with autism spectrum conditions. *Horm Behav* 2007; 51(5):597-604.
- (12) Geier DA, Geier MR. A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. *Horm Res* 2006; 66(4):182-188.
- (13) Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004; 328(7434):261.

- (14) Nyboe AA, Goossens V, Gianaroli L, Felberbaum R, de MJ, Nygren KG. Assisted reproductive technology in Europe, 2003. Results generated from European registers by ESHRE. *Hum Reprod* 2007.
- (15) Kallen B, Olausson PO, Nygren KG. Neonatal outcome in pregnancies from ovarian stimulation. *Obstet Gynecol* 2002; 100(3):414-419.
- (16) Wang JX, Norman RJ, Kristiansson P. The effect of various infertility treatments on the risk of preterm birth. *Hum Reprod* 2002; 17(4):945-949.
- (17) Olivennes F, Rufat P, Andre B, Pourade A, Quiros MC, Frydman R. The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. *Hum Reprod* 1993; 8(8):1297-1300.
- (18) Gaudoin M, Dobbie R, Finlayson A, Chalmers J, Cameron IT, Fleming R. Ovulation induction/intrauterine insemination in infertile couples is associated with low-birth-weight infants. *Am J Obstet Gynecol* 2003; 188(3):611-616.
- (19) Ombelet W, Martens G, De SP, Gerris J, Bosmans E, Ruysinck G et al. Perinatal outcome of 12,021 singleton and 3108 twin births after non-IVF-assisted reproduction: a cohort study. *Hum Reprod* 2006; 21(4):1025-1032.
- (20) Eaton WW, Mortensen PB, Thomsen PH, Frydenberg M. Obstetric complications and risk for severe psychopathology in childhood. *J Autism Dev Disord* 2001; 31(3):279-285.
- (21) Hultman CM, Sparen P, Cnattingius S. Perinatal risk factors for infantile autism. *Epidemiology* 2002; 13(4):417-423.
- (22) Wright VC, Chang J, Jeng G, Macaluso M. Assisted reproductive technology surveillance--United States, 2003. *MMWR Surveill Summ* 2006; 55(4):1-22.
- (23) Lidegaard O, Pinborg A, Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. *Hum Reprod* 2005; 20(4):950-954.
- (24) Ericson A, Nygren KG, Olausson PO, Kallen B. Hospital care utilization of infants born after IVF. *Hum Reprod* 2002; 17(4):929-932.
- (25) Stromberg B, Dahlquist G, Ericson A, Finnstrom O, Koster M, Stjernqvist K. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet* 2002; 359(9305):461-465.
- (26) Pinborg A, Loft A, Schmidt L, Andersen AN. Morbidity in a Danish national cohort of 472 IVF/ICSI twins, 1132 non-IVF/ICSI twins and 634 IVF/ICSI singletons: health-related and social implications for the children and their families. *Hum Reprod* 2003; 18(6):1234-1243.
- (27) Pinborg A, Loft A, Schmidt L, Greisen G, Rasmussen S, Andersen AN. Neurological sequelae in twins born after assisted conception: controlled national cohort study. *BMJ* 2004; 329(7461):311.

- (28) Klemetti R, Sevón T, Gissler M, Hemminki E. Health of children born as a result of in vitro fertilization. *Pediatrics* 2006; 118(5):1819-1827.
- (29) Stein D, Weizman A, Ring A, Barak Y. Obstetric complications in individuals diagnosed with autism and in healthy controls. *Compr Psychiatry* 2006; 47(1):69-75.
- (30) Maimburg RD, Vaeth M. Do children born after assisted conception have less risk of developing infantile autism? *Hum Reprod* 2007; 22(7):1841-1843.
- (31) Hvidtjorn D, Schieve L, Schendel D, Jacobsson B, Svaerke C, Thorsen P. Cerebral Palsy, Autism Spectrum Disorders, and Developmental Delay in Children Born After Assisted Conception: A Systematic Review and Meta-analysis. *Arch Pediatr Adolesc Med* 2009; 163(1):72-83.
- (32) Knudsen LB, Olsen J. The Danish Medical Birth Registry. *Dan Med Bull* 1998; 45(3):320-323.
- (33) Andersen AN, Westergaard HB, Olsen J. The Danish in vitro fertilisation (IVF) register. *Dan Med Bull* 1999; 46(4):357-360.
- (34) Munk-Jorgensen P, Mortensen PB. The Danish Psychiatric Central Register. *Dan Med Bull* 1997; 44(1):82-84.
- (35) Tebruegge M, Nandini V, Ritchie J. Does routine child health surveillance contribute to the early detection of children with pervasive developmental disorders? An epidemiological study in Kent, U.K. *BMC Pediatr* 2004; 4:4.
- (36) Chakrabarti S, Fombonne E. Pervasive developmental disorders in preschool children: confirmation of high prevalence. *Am J Psychiatry* 2005; 162(6):1133-1141.
- (37) Centers of Disease Control and Prevention. MMWR. Prevalence of Autism Spectrum Disorders. Surveillance summaries. MMWR 56 (No. SS-1). 2-9-2007. Surveillance Summaries.

Ref Type: Report

- (38) Schendel D, Bhasin TK. Birth weight and gestational age characteristics of children with autism, including a comparison with other developmental disabilities. *Pediatrics* 2008; 121(6):1155-1164.
- (39) Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. *Br J Psychiatry* 2009; 195(1):7-14.
- (40) Hvidtjorn D, Grove J, Schendel DE, Vaeth M, Ernst E, Nielsen LF et al. Cerebral palsy among children born after in vitro fertilization: the role of preterm delivery--a population-based, cohort study. *Pediatrics* 2006; 118(2):475-482.
- (41) Lauritsen MB JMMKLSTSGJSDTP. Quality of Childhood Autism in the Danish Psychiatric Central Register Findings From a Cohort Sample Born 1990-1999. *Journal of Autism and Developmental Disorders*, 2009. In press 2009.

