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REVIEW *Reproductive epidemiology*

Are children born after assisted reproductive technology at increased risk of autism spectrum disorders? A systematic review

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STUDY QUESTION: Are children born after assisted reproductive technology (ART) at increased risk of autism spectrum disorders (ASD)?

SUMMARY ANSWER: There is no evidence that ART significantly increases the risk of ASD in the offspring.

WHAT IS KNOWN ALREADY: A few systematic reviews have explored the correlation between assisted conception and ASD with inconclusive results, partly due to the heterogeneity of diagnostic criteria and methodology in the different studies.

STUDY DESIGN, SIZE, DURATION: Systematic review of 7 observational studies (2 cohort and 5 case–control) encompassing 9216 subjects diagnosed with ASD published since 2000.

MATERIALS, SETTING, METHODS: Literature searches were conducted to retrieve observational studies on the risk of ASD in ART population. Databases searched included PubMed, EMBASE and PsycINFO. In order to obtain more consistent results, we only included the studies in which (i) subjects with either infantile autism or ASD could be identified according to international classification systems and (ii) the diagnosis was obtained from hospital records. Seven studies matched the inclusion criteria.

MAIN RESULTS AND THE ROLE OF CHANCE: Four out of seven studies, including the two with the best quality scores, did not show an association between ART and ASD. The two papers supporting an increased risk of autism following ART had the lowest quality scores, due to major methodological limitations. Only one paper showed a protective role of ART.

LIMITATIONS, REASONS FOR CAUTION: In spite of the strict inclusion criteria applied as to the diagnosis of ASD, the papers selected are heterogeneous in many aspects including study design, definitions of ART, data source and analysed confounders.

WIDER IMPLICATIONS OF THE FINDINGS: At present, there is no evidence that ART is significantly associated with ASD and hence that current health policies should be modified. The divergent results of some of the studies suggest that further prospective, large and high-quality studies are still needed.

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Introduction

Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by impaired social interaction and communication, together with restricted and repetitive behaviour (American Psychiatric Association, 2000), due to abnormal brain development beginning early in life (Muratori *et al.*, 2012; Wolff *et al.*, 2012). Although the exact cause of ASD remains unknown, a strong genetic origin has been implicated (Bailey *et al.*, 1995). Nevertheless, recent findings support a crucial role of environmental factors, which are essential in modulating the phenotypical expression of the disorder (Johnson *et al.*, 2007; Kogan *et al.*, 2009). Estimated prevalence of ASD has dramatically

increased in the last decades, reaching values of 1 in 88 children in USA (CDC, 2012). Broadening of diagnostic criteria as well as increased recognition due to higher awareness of symptomatology can account for much of this prevalence increase. However, environmental factors are also suspected of contributing to this rise (Currenti, 2010). The trend in ASD prevalence parallels recent changes in pregnancy and birth factors, such as multiple pregnancies, high maternal age, low birthweight and preterm birth, suggesting a possible link between them (Heron *et al.*, 2010). However, a recent study that investigated through a rigorous mathematical model the role of several prenatal factors [prematurity, birthweight, multiple births, Caesarean delivery, breech presentation and use of assisted reproductive technology (ART)] in the increase of ASD prevalence revealed a minimal contribution of these factors to the ASD increase (Schieve *et al.*, 2011).

Among the environmental factors that have been suggested as potentially associated with ASD, some authors reported the use of ART, including ovulation induction (OI), IVF and ICSI. This relationship could be attributed to at least three shared factors: high parental age, hormonal disturbances, high maternal educational level or social class. However, this last association has been questioned. In fact, although several recent investigations reported an association of ASD diagnoses with higher socioeconomic status (SES) (Mandell *et al.*, 2009; Durkin *et al.*, 2010; Thomas *et al.*, 2012), the majority of studies concluded that results could be biased by differential access to services, as well as differential awareness by parents and providers. Also, a higher risk of ASD in children born after assisted conception might be related to the higher rates of multiplicity, preterm birth and low birthweight deliveries (Bhasin and Schendel, 2007; Koyama *et al.*, 2007; Arpino *et al.*, 2010; Parner *et al.*, 2012).

A few review papers have explored the correlation between assisted conception and ASD with inconclusive results, partly due to the heterogeneity of diagnostic criteria in the different studies (Ludwig *et al.*, 2006; Middelburg *et al.*, 2008; Hvidtjorn *et al.*, 2009b). In the most recent meta-analysis specifically focused on ART, the eight studies selected, as pointed out by the authors, had very broad and heterogeneous diagnostic inclusion criteria (three used infantile autism, three used ASD and two used neurodevelopmental disorder), and differed in the source of the diagnostic information (questionnaires filled out by parents, hospital records, national registers) (Hvidtjorn *et al.*, 2009b).

The aim of this systematic review was to summarize the available published data regarding the association between ART and infantile autism or ASD (i.e. autism, Asperger's disorder or pervasive developmental disorder not otherwise specified). Cohort and case–control studies were included.

Methods

Literature search

Studies were identified by searching multiple literature databases, including Pubmed, EMBASE and PsycINFO. The searches were limited to papers in English and included articles published between January 2000 and February 2013. References were exported into an endnote bibliographic management database and duplicates were removed. The following search strategy was performed: ('intrauterine insemination' OR 'assisted conception' OR 'in vitro fertilization' OR IVF OR 'intracytoplasmic sperm injection' OR ICSI OR 'assisted reproductive technology' OR ART OR 'ovulation induction' OR OI) AND ('infantile autism' OR 'autism spectrum disorder*' OR ASD OR 'asperger' OR 'pervasive development* disorder' OR 'neurological outcome' OR 'neurodevelopmental disorder*').

Study selection

Criteria for inclusion in the study were established prior to the literature search. Inclusion was limited to studies that were either case–control or cohort studies. Reviews were not included in the analysis, but were used to collect original studies. We only selected the studies in which subjects were diagnosed on hospital records with either infantile autism or ASD, according to international classification of diseases (ICD) or diagnostic and statistical manual of mental disorders (DSM).

Validity assessment

Using the Newcastle-Ottawa Scale (NOS) for assessing quality of non-randomized studies in meta-analysis, we assessed the quality and publication bias of included studies (Wells et al.). The NOS was developed using a Delphi process and was subsequently tested on systematic reviews. Different NOS scales exist for cohort and case–control studies. The NOS contains eight items, categorized into three dimensions including selection, comparability, and outcome (cohort studies) or exposure (case–control studies). For each item a series of response options is provided. Differences were resolved by consensus. Publication bias was assessed at the outcome level by visual inspection of funnel plots.

Results

Description of studies

PRISMA Flow Diagram (Liberati *et al.*, 2009; Moher *et al.*, 2009) of the review process is presented in Fig. 1. The search strategy yielded 115 records. Additional records identified through other sources (yielded 12 more records). When duplicates had been removed, the number was 110. All abstracts were independently reviewed by four of the authors (E.C., I.S., S.C. and S.M.) and conflicting judgements were solved by consensus. Eighty papers were excluded during this review based on clear failure to meet the inclusion criteria. Thirty full-text papers were evaluated further and only seven were found to meet the inclusion criteria (Table I).

Of the 23 eliminated papers, six were excluded because the diagnosis of autism was not performed according to standardized criteria (ICD-10 or DSM IV-TR), or was included into broader categories of behavioural disorders (Lidegaard *et al.*, 2005; Klemetti *et al.*, 2006; Knoester *et al.*, 2007; Middelburg *et al.*, 2011; Sanchez-Albisua *et al.*, 2011; Lyall *et al.*, 2012), eight were excluded as they were review papers (Ludwig *et al.*, 2006; Middelburg *et al.*, 2008; Hvidtjorn *et al.*, 2009b; Arpino *et al.*, 2010; Schieve *et al.*, 2011; Eisenberg, 2012; Wiener-Megnazi *et al.*, 2012; Hediger *et al.*, 2013), two as they were commentaries (Bhandari *et al.*, 2011; Szatmari, 2011) and one conference proceedings (Rao, 2008); six papers were excluded because autism was not included in the outcome measures (Agarwal *et al.*, 2005; Sanchez-Albisua *et al.*, 2007; Basatemur and Sutcliffe, 2008; Steel and Sutcliffe, 2009; Pinborg *et al.*, 2010; Kondapalli *et al.*, 2011).

Two independent blinded reviewers (E.C. and S.M.) assessed the quality of the seven selected studies utilizing the NOS scoring system both for case control and cohort studies (Table II). Conflicting judgements were solved by consensus. The average quality of the selected papers was moderately high (mean total score 5, range 2–8).

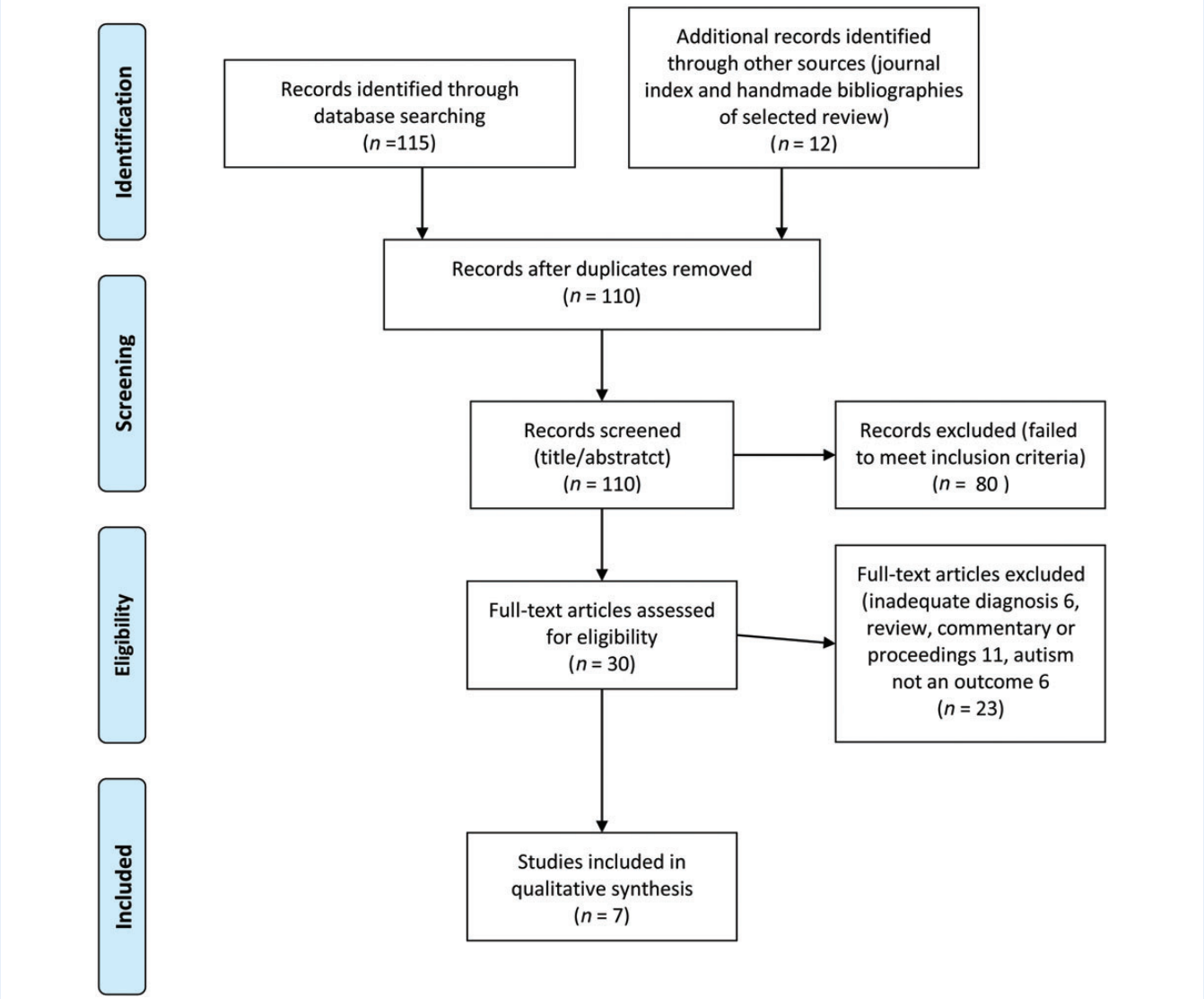

Figure 1 PRISMA four-phase flow diagram of search yield, screening and inclusion steps.

Cohort studies

The quality assessment of the two cohort studies selected is shown in Table II. Both studies were population-based and analysed data extracted from the Danish National Birth Register, which contains information on all births in Denmark. The two studies were conducted in different time periods and overall covered a population born between 1995 and 2003. Although the use of a nationwide register minimizes the risk of a selection bias, generalizability of the findings is limited by the common source used by the two studies which makes them uniform in terms of demographic factors, SES and ethnicity (mainly Caucasian).

ART definition and ascertainment

In both studies, data could be extracted according to the definition of assisted conception as IVF with or without ICSI. Ascertainment of exposure in these studies can be considered secure and inclusive, as children exposed to IVF were identified through the compulsory IVF Register holding data from all private and public fertility clinics in Denmark.

ASD definition and ascertainment

In both studies, children with a diagnosis of ASD were identified via the Danish Psychiatric Central Register, containing information on all Danish psychiatric inpatient and outpatient admissions since 1995 (Munk-Jorgensen and Mortensen, 1997). Diagnosis was based on ICD9 or ICD10 classification systems and all children with ASD were included (codes F84.0, F84.1, F84.5, F84.8 and F84.9). Both cohort studies made no specific reference to the possible presence of subjects lost to follow-up, while Pinborg *et al.* (2004) also used a non-fully adequate follow-up, starting from 2 years of age, when the diagnosis of ASD is not yet stable.

Table I Description of the seven studies included in the review.

Author (year)	Study design	Country/birth range	Time of diagnosis	Diagnosis (ICD-DSM)	Source of ASD	Source of cohort/ controls	Type of ART	Source of ART
Pinborg *et al.*, (2004)	Cohort	Denmark 1995–2000	<December 2002	F84.0, F84.5 (ICD 10)	National patients' registry and Danish psychiatric central registry	National medical birth registry and national registry for *in vitro* fertilization	IVF; ICSI	Danish Register for IVF
Hvidtjorn *et al.*, (2011)	Cohort	Denmark 1995-2003	<May 2008	F84.0, F84.1, F84.5, F84.8, F84.9 (ICD 9)	Danish Psychiatric Central Register	Danish Medical Birth Register	IVF; ICSI; OI	IVF register/Danish drug prescription register
Stein *et al.*, (2006)	Case–control	Israel 1970–1998	Not available	Autism (ICD 8; DSM III; IV)	ALUT center of Tel Aviv	Women working at the Chaim Sheba Medical Center	'Infertility requiring medical intervention'	Lewis-Murray scale for rating retrospective obstetric information
Maimburg and Vaeth (2007)	Case–control	Denmark 1990–1999	<February 2001	F84.0 (ICD10) 299.0 (ICD8)	Danish Psychiatric Central Register	Danish Civil Registration System	Hormone therapy or technical treatment	Danish Medical Birth Register/Danish maternity wards
Zachor and Ben Itzchak (2011)	Case–control	Israel 1995–2002	Not available	ASD (DSM IV-TR)	Autism Center within Assaf Harofeh Medical Center, Israel	Large Israel population from infant registry of Rabin Medical Center	IVF; ICSI; (not OI)	Medical files from the infertility and IVF Unit in Rabin Medical Center
Shimada *et al.*, (2012)	Case–control	Japan >1975	April 2006–March 2009	ASD (DSM IV-TR)	University of Tokyo Hospital	General Population of Tokyo	IVF; ICSI	Questionnaires filled by parents
Lehti *et al.*, (2013)	Case–control	Finland 1991–2005	2007	F84 (ICD10)	Finnish Hospital Discharge Register	Finnish Hospital Discharge Register	IVF ± ICSI-FET	Finnish Medical Birth Register

Author (year)	Sample size, ART-exposed (ASD)	Sample size, ART-unexposed (ASD)	Associations, unadjusted	Associations, adjusted	Authors' conclusions
Pinborg *et al.*, (2004)	3393 (3)	10 239 (11)	OR, 0.82 (95% CI, 0.23–2.95)[a]	NA	Individuals from assisted conception have a similar risk of neurological sequelae including ASD, as their naturally conceived peers

Continued

Tablel *Continued*

Author (year)	Study design	Country/birth range	Time of diagnosis	Diagnosis (ICD-DSM)	Source of ASD	Source of cohort/ controls	Type of ART	Source of ART
Hvidtjorn *et al.*, (2011)	33 139 (225)	588 742 (3394)	HRR, 1.25 (95% CI, 1.09–1.43)	HRR, 1.13 (95% CI, 0.97–1.31) Adjusted for maternal age, educational level, parity, smoking, birthweight and multiplicity	In crude analysis children from assisted conception had an increased risk of ASD, which disappeared when adjusting for relevant factors			
Stein *et al.*, (2006)	41 (29)	317 (177)	OR, 1.91 (95% CI, 0.84–3.88)[a]	NA	Individuals from assisted conception have a similar risk of ASD. The presence of nonspecific neonatal factors may account for the elevated neonatal suboptimality found in probands diagnosed with ASD.			
Maimburg and Vaeth (2007)	33 (10)	889 (451)	OR, 0.41 (95% CI, 0.19–0.89)	OR, 0.37 (95% CI, 0.14–0.98) Adjusted for parity, multiplicity, birthweight, gestational age, birth defect, maternal age, and country of origin.	Individuals from assisted conception have a lower risk of developing infantile autism than their matched controls			
Zachor and Ben Itzchak (2011)	1647 (23)	51 718 (262)	OR, 2.78 (95% CI, 1.81–4.27)[a]	NA	Assisted conception appears to be a significant independent risk factor for ASD unassociated with other established risk factors for ASD, including advanced maternal age, prematurity, low birthweight and history of ASD in the family			
Shimada *et al.*, (2012)	2524 (21)	98 061 (446)	OR, 1.84 (95% CI, 1.18–2.85)[a]	NA	The rate of assisted conception in cases of persons with ASD was 1.8 times the frequency expected in the general population			
Lehti *et al.*, (2013)	292 (63)	20 454 (4101)	OR, 1.1 (95% CI, 0.8–1.5)	OR, 0.9 (95% CI, 0.7–1.3) Adjusted for maternal age, social-economical status, gestational age and parity.	Individuals from assisted conception have no increased risk of ASD, as their naturally conceived peers			

ASD, autism spectrum disorders; ART, assisted reproduction technology; CI, confidence interval;DSM, diagnostic and statistical manual of mental disorders; FET, frozen embryo transfer; HRR, hazard risk ratio; ICD, international classification of diseases; NA, not assessed; OI, ovarian induction; OR, odds ratio.
[a]Calculated based on data extracted from the article.

Table II Quality assessment by NOS scale of the cohort studies included in the review.

	Item	Pinborg *et al.*, (2004)	Hvidtjorn *et al.*, 2011
Selection		Cross linkage of the National Medical Birth Registry and National Registry for *In Vitro* Fertilization	Cross linkage of National Birth Register, IVF Register and Drug Prescription Register
	1. Representativeness of the exposed cohort (a) Truly representative of the average *ART conceived subjects* in the community (b) Somewhat representative of the average *ART conceived subjects* in the community (c) Selected group of users (e.g. nurses, volunteers) (d) No description of the derivation of the cohort	(a) ★	(a) ★
	2. Selection of the non-exposed cohort (a) Drawn from the same community as the exposed cohort (b) Drawn from a different source (c) No description of the derivation of the non-exposed cohort	Same community as the exposed cohort (a) ★	Same community as the exposed cohort (a) ★
	3. Ascertainment of exposure (a) Secure record (e.g. surgical records) (b) Structured interview (c) Written self-report (d) No description	Registers (a) ★	Registers (a) ★
	4. Demonstration that outcome of interest was not present at start of study (a) Yes (b) No	ASD not present at time of enrolling (birth) (a) ★	ASD not present at time of enrolling (birth) (a) ★
Comparability	1. Comparability of cohorts on the basis of the design or analysis (a) Study controls for *maternal age* (b) Study controls for any additional factors	Maternal age or other confounders are not matched in the design nor adjusted for in the statistical analysis (not provided)	Maternal age and other confounders are adjusted for in the statistical analysis (a,b) ★
Outcome	1. Assessment of outcome (a) Independent blind assessment (b) Record linkage (c) Self-report (d) No description	Record linkage by patient's registry (b) ★	Record linkage by patient's registry (b) ★
	2. Was follow-up long enough for outcomes to occur (a) Yes (3 year minimum follow-up) (b) No	Follow-up shorter than 3 years (b)	Follow-up longer than 5 years (a) ★
	3. Adequacy of follow-up of cohorts (a) Complete follow-up – all subjects accounted for (b) Subjects lost to follow-up unlikely to introduce bias- small number lost 5% (c) Follow-up rate <3% (select and adequate%) and no description of those lost (d) No statement	No statement (d)	No statement (d)
	Total score	5	8

ART, assisted reproduction technology; ASD, autism spectrum disorders; NOS, Newcastle-Ottawa Scale; grey boxes indicate a score of 0.
*indicates a score of 1.

Case–control studies

The quality assessment of the five case–control studies selected is shown in Table III. Although all of the five studies can be technically viewed as case–control studies, in two of them case series have been compared with a more general population group (Zachor and Ben Itzchak, 2011; Shimada *et al.*, 2012), making them closer to case series than true case–control studies. Two of the five papers originated from Scandinavian countries, while the other three explored cohorts were from Israel (Stein *et al.*, 2006; Zachor and Ben Itzchak, 2011) and from Japan (Shimada *et al.*, 2012). This makes the findings potentially more generalizable in terms of geographic distribution than those from the two cohort studies.

Table III Quality assessment of the case–control studies included in the review.

	Item	Stein *et al.*, (2006)	Maimburg and Vaeth (2007)	Zachor and Ben Itzchak (2011)	Shimada *et al.*, (2012)	Lehti *et al.*, (2013)
Selection	1. Is the case definition adequate? (a) Yes, with independent validation (b) Yes, (e.g. record linkage or based on self reports) (c) No description	Cases have been independently evaluated according to ICD8; DSMIII; DSMIV. (a) ★	Cases identified through registers according to ICD10 (b)	Cases independently evaluated according to DSMIV (a) ★	Cases were independently evaluated according to DSMIV (a) ★	Cases identified through record linkage, diagnosed according to ICD 9/10 (b)
	2. Representativeness of the cases (a) Consecutive or obviously representative series of cases (b) Potential for selection biases or not stated	All eligible cases over a defined time/institution. (a) ★	All eligible cases over a defined time/catchment area/institution. (a) ★	All eligible cases over a defined institution. (a) ★	All eligible cases over a defined time/institution. (a) ★	All eligible cases over a defined time/catchment area. (a) ★
	3. Selection of Controls (a) Community controls (b) Hospital controls (c) No description	Controls derived from the same community and would be cases if had outcome (a) ★	Controls derived from the same community and would be cases if had outcome (a) ★	Hospital controls, within same community as cases (b)	Control data abstracted from the general population statistics (not provided)	Controls derived from the same registry and would be cases if had outcome (a) ★
	4. Definition of Controls (a) No history of disease (end-point) (b) No description of source	Controls were checked for having no history of disease (a) ★	No description of specific check on history of disease (b)	No description of specific check on history of disease (b)	No description of specific check on history of disease (b)	Controls were checked for having no history of disease (a) ★
Comparability	1. Comparability of the cases and controls on the basis of the design or analysis (a) Study controls for *maternal age* (b) Study controls for any additional factors	Maternal age or other confounders are not matched in the design nor adjusted for in the statistical analysis (not provided)	Maternal age and other confounders are adjusted for in the statistical analysis (a,b) ★	Maternal age or other confounders are not matched in the design nor adjusted for in the statistical analysis (not provided)	Maternal age or other confounders are not matched in the design nor adjusted for in the statistical analysis (not provided)	Maternal age and other confounders are adjusted for in the statistical analysis (a,b) ★

Continued

Table III *Continued*

	Item	Stein *et al.*, (2006)	Maimburg and Vaeth (2007)	Zachor and Ben Itzchak (2011)	Shimada *et al.*, (2012)	Lehti *et al.*, (2013)
Exposure	1. Ascertainment of exposure (a) Secure record (e.g. surgical record) (b) Structured interview blind to case/control (c) Interview not blinded to case/control (d) Written self report or medical record only (e) No description	Interview not blinded to case/control status (c)	Secure Records (medical birth records) (a) ★	Interview not blinded to case/control status (c)	Interview or medical records not blinded to case/control status (c/not provided)	Secure record(medical birth register) (a) ★
	2. Same method of ascertainment (a) Yes (b) No	Yes (a) ★	Yes (a) ★	No (Medical record versus general population data) (b)	No (Medical record versus general population data) (b)	Yes (a) ★
	3. Non-response rate (a) Same rate for both groups (b) Non-respondents described (c) Rate different and no designation	Rate different and no designation (c)	Same rate can be assumed (a) ★	Not applicable	Not applicable	Same rate can be assumed (a) ★
	Total score	5	7	2	2	8

DSM, diagnostic and statistical manual of mental disorders; ICD; international classification of diseases; grey boxes indicate a score of 0.
*indicates a score of 1.

ART definition and ascertainment

Definition of ART was heterogeneous in the different studies. Maimburg and Vaeth (2007) included in their analysis all infants conceived both by technical treatment and hormonal therapy, while Zachor and Ben Itzchak (2011) excluded fertility drugs to induce ovulation. Stein *et al.* (2006) used broad inclusion criteria encompassing all cases of 'infertility requiring medical intervention ever'. Three of the five case–control studies used suboptimal means for the ascertainment of ART exposure, namely parental questionnaires or interviews not blinded to case/control status (Stein *et al.*, 2006; Shimada *et al.*, 2012). In the remaining two studies, more reliable methods were used. In Lehti *et al.* (2013) information on ART was retrieved by a national register, the Finnish Medical Birth Register, a nationwide register collecting data on fertilization treatments since October 1990. Maimburg and Vaeth (2007) extracted information from the medical birth records collected from the Danish maternity wards, in which information about fertility status was provided. Moreover, the ascertainment of exposure to ART was not homogeneous between cases and controls in three of the five studies. Zachor and Ben Itzchak (2011) and Shimada *et al.* (2012) did not apply the same method of ascertainment for the control group as they used the general population statistics as control parameter, thus making inapplicable the comparison of the non-respondents between the groups. Stein *et al.* (2006) used the same ascertainment procedure but failed to control for non-response rate between cases and controls.

ASD definition and ascertainment

Although the inclusion criteria of our search were strict as to the definition of ASD, in two of the five papers case ascertainment could not be considered as the result of independent validation, as cases were identified through record linkage registers (the Danish Psychiatric Central Register in Maimburg and Vaeth (2007) and the Finnish Hospital Discharge Register, in Lehti *et al.* (2013)). In all papers the diagnosis was based on clinical evaluation and international coding (ICD or DSM); however, only in one paper diagnosis was supported and confirmed by standardized diagnostic tools such as ADI-R (Lord *et al.*, 1994) and ADOS-G (Lord *et al.*, 2000).

Findings

Of the seven studies included in this systematic review, only two reported evidence of an increased risk of ASD in children born after assisted conception (Zachor and Ben Itzchak, 2011; Shimada *et al.*, 2012). Interestingly, these are the two studies with the lowest quality scores at the NOS, mainly due to the fact that ASD subjects were compared with general population statistics thus making the ascertainment of both exposure and outcome different between cases and controls (strong selection bias). In contrast, one paper reported a protective effect of assisted conception on infantile autism after adjusting for several confounding factors (Maimburg and Vaeth, 2007). The remaining four papers, however, did not confirm this result, as they found no evidence of a different risk of ASD in children born after ART. The two cohort studies, which analysed partially overlapping nationwide populations, either found no difference (Pinborg *et al.*, 2004) or found an increased risk of ASD after assisted conception, which did not hold true after adjusting for main confounders (i.e. maternal age, educational level, parity, smoking, birthweight and multiplicity) (Hvidtjorn *et al.*, 2011). Stein *et al.* (2006) used a loose definition of assisted reproduction including all cases of infertility requiring medical intervention, and found a higher risk of developing ASD, which was however not statistically significant. Finally, Lehti *et al.* (2013), the most recent and the highest-ranked paper of this review, found no increased risk of ASD in children born after ART.

Discussion

This systematic review included studies assessing the risk of ASD in children born after assisted conception and revealed no evidence of a significant association between ASD and ART. Only one study showed a protective role of assisted conception, which did not change after adjusting for potential confounders (i.e. maternal age, multiplicity, parity and prematurity/birthweight). To explain their findings, the authors focused on the potential advantages related to pregnancies following assisted conception, including the closer contact with the health system or better promotion of good health behaviour before and during early pregnancy, for example, folic acid intake. No other studies however found supporting evidence since their first observation. None of the other studies of this review in which the analysis was adjusted for potential confounders a significant association between ART and ASD was found, and the only two studies suggesting an increased risk of ASD in children born after assisted conception had major methodological limitations.

The quality of the studies, as assessed by NOS, ranged between very low (score of 2) and very good (score of 8). The two papers with the highest quality scores (Hvidtjorn *et al.*, 2011; Lehti *et al.*, 2013) support the absence of any significant association between exposure and outcome. It is of interest that the only two papers showing a significant association between ART and ASD (Zachor and Ben Itzchak, 2011; Shimada *et al.*, 2012) obtained the lowest quality scores (score = 2) as their design was suboptimal in all domains explored (i.e. selection, comparability and exposure). A good quality score was obtained by the only paper favouring a protective role of ART (Maimburg and Vaeth, 2007); however, it has to be noted that it had one of the lowest sample sizes and number of events among the reviewed studies.

In spite of the strict inclusion criteria applied in relation to the diagnosis of ASD, the papers selected are heterogeneous in many aspects including definition of ART, analysed confounders and diagnostic procedure.

While there is no consensus on the definition of assisted reproduction technology, this is generally considered to include all fertility treatments in which both eggs and sperm are handled, thus excluding procedures limited to medical treatment to the woman (CDC, 2012). Half of the papers analysed in this review have not followed this definition. Stein *et al.* (2006) have used broad inclusion criteria encompassing all cases of 'infertility requiring medical intervention ever' as determined by structured questionnaires for retrospective obstetric information. Maimburg and Vaeth (2007) gathered information about fertility from birth records including in their analysis all infants conceived both by technical treatment and hormonal therapy. Similarly, Hvidtjorn *et al.* (2011) include both IVF that can be broadly assimilated to current definition of ART, and OI, with or without subsequent insemination. Although we were unable to extract separate data (IVF versus OI) for our analysis from this latter study, it is of interest that the authors calculated separate risk ratios finding no association between ASD and IVF.

In addition to the variability in the definition of ART, papers did not use the same criteria for the ascertainment of exposure. In most cases

structured interviews were used, while in some others the information was gathered from medical registers. Although both approaches are considered reliable methods of ascertainment (Hvidtjorn *et al*., 2009a; Barradas *et al*., 2012), getting full scores within the NOS quality scale, it has to be underlined that some authors have questioned the reliability of birth records, which can potentially result in underestimation and biased ascertainment (Zhang *et al*., 2010). Another aspect potentially contributing to the heterogeneity among studies is the year of birth, which ranged between 1970 and 2006, thus potentially reflecting the application of different techniques of assisted conception. It has to be noted however that the interpretation of the findings does not change even when limiting the analysis to those papers that only include subjects born after 1995.

All selected papers, with the exception of Stein *et al*. (2006), take into account possible confounders. Maimburg and Vaeth (2007), Hvidtjorn *et al*. (2011) and Lehti *et al*. (2013) consider covariates such as maternal age, gestational age, birthweight and multiplicity, using adjusted values for well-known risk factors for autism and assisted conception in analysis regarding measures of effect. Some authors dealt with the potential bias of prematurity and low birthweight, which are common in both ART and ASD, by considering multiplicity within the selection criteria, either excluding multiple births from the analysis (Zachor and Ben Itzchak, 2011) or just focusing on twin pregnancies (Pinborg *et al*., 2004). In two of the seven papers, singletons and twins were analysed separately but no differences were detected, with the exception of a significant association between singletons and Asperger syndrome (Lehti *et al*., 2013).

Shimada *et al*. (2012) reported significantly higher paternal and maternal age in relation to ASD; however, they did not put this finding in relation to ART. Overall, the heterogeneity of the studies in relation to the role of the different confounders, and in particular prematurity and multiplicity, does not allow for a clear evaluation of their contribution to the definition of ART-related risk in ASD.

Although we limited our selection to studies in which ASD was identified according to international classification systems, some differences in the diagnostic procedure were present among the studies. Zachor and Ben Itzchak (2011) were the only authors to systematically apply gold standard tools for ASD diagnosis, such as the Autism Diagnostic Interview-Revised (ADI-R (Lord *et al*., 1994)), the Autism Diagnostic Observation Schedule-Generic (ADOS-G (Lord *et al*., 2000)) and ADOS Severity Scale (Gotham *et al*., 2009) assessed by trained and reliability-tested experts. Lehti *et al*. (2013) used clinical diagnoses based on the ICD; however, their general validity was reassessed in a validation study using the ADI-R, demonstrating that 96% of the cases with registry diagnoses of childhood autism fulfil the ADI-R diagnostic criteria (Lampi *et al*., 2010). Although the ADOS-G and the ADI-R are two internationally recognized and widely used diagnostic instruments for ASD, it has to be noted that their validity is questionable for disorders other than infantile autism. For example, the ADOS-G has had lower specificity and sometimes sensitivity for distinctions involving children with Pervasive developmental disorder not otherwise specified and Asperger's Disorder (Gotham *et al*., 2007). Moreover, the ADOS-G tends to underdiagnose children with higher verbal and nonverbal skills (Chawarska *et al*., 2007). In the remaining papers, less information is provided as to the diagnostic procedure; the ASD diagnosis is made according to different classification systems, such as DSM-IV-TR (Shimada *et al*., 2012) and ICD 8, ICD 10, DSM III or DSM III-R in the less recent papers (Pinborg *et al*., 2004; Stein *et al*., 2006; Hvidtjorn *et al*., 2011). It is of note that, at variance with the other analysed studies, Maimburg and Vaeth (2007) only included patients who satisfied strict criteria of infantile autism according to ICD 8 and ICD 10, namely patients belonging to the most severe end of the spectrum and thus not reflecting the large heterogeneity of ASD. Conversely, Stein *et al*. (2006) only included cases with idiopathic autism, in which all children with a diagnosis of secondary autism related to genetic or metabolic causes were excluded. Generally speaking, the use of different inclusion criteria for ASD might hinder the possibility to reliably compare results of different studies. It is of interest however that the only paper that reported a clear-cut protective effect of ART is also the only one limiting the inclusion criteria to infantile autism (Maimburg and Vaeth, 2007).

In summary, our systematic review suggests that ART does not represent a risk factor for ASD; however, the divergent results of some of the studies suggest that further prospective, large and high quality studies are still needed. The main methodological limitation that has led to this outcome is the heterogeneity of the selected studies, particularly in terms of study design (cohort versus case control), ART data recruitment strategy (registers, medical records, parental interviews), ASD clinical assessment (standardized tests versus clinical evaluation) and assessment of confounders. It is of interest however that the papers with the highest value in this review based on their methodological quality reached similar conclusions, supporting the absence of significant associations between ART and ASD.

In future studies it will be interesting to determine the risk of association between ART and ASD separately for each subtype of intervention, and to explore if children with ASD born with assisted reproduction show a different clinical phenotype if compared with ASD children born without ART, in terms of gender, severity of ASD symptoms, IQ level and psychiatric comorbidity. In particular, the identification of environmental exposure that represents a possible risk factor for the development of ASD could potentially be important for the surveillance of vulnerable subjects and, ultimately, to accelerate the diagnostic process. In wider terms, further research on larger, well-characterized samples of children born after ART, followed longitudinally, may allow for the identification of subgroups of subjects with different developmental profiles, and could ultimately contribute to a better understanding of the aetiological underpinnings of ASD phenotypes.

Authors' roles

E.C. was responsible for defining the research question and aims and designing the strategy used to perform the literature search. She also participated to study selection and papers' quality assessment. She assisted in the interpretation of the results and was the major contributor to manuscript writing. S.M. was responsible for the statistical analysis and performed the actual data analysis. She also participated to study selection and papers' quality assessment and assisted in the interpretation of the results and contributed to manuscript writing. S.C. participated to study selection and assisted in the interpretation of results and contributed to manuscript writing. I.S. contributed to database search, paper selection and qualitative assessment of selected papers. A.G. assisted in defining the research question and writing the manuscript. He was primarily responsible for the interpretation of the findings.

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Conflict of interest

The authors have no competing interests to declare.

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