JIVFww

Klonoff-Cohen H, Polavarapu M. Assessing the Relationship Between Traditional In Vitro Fertilization and Birth Defects: A Systematic Review and Meta-Analysis. *Journal of IVF-Worldwide*. 2023;1(4). doi:10.46989/001c.91039

Original Research Articles

Assessing the Relationship Between Traditional In Vitro Fertilization and Birth Defects: A Systematic Review and Meta-Analysis

Hillary Klonoff-Cohen[1a], Mounika Polavarapu[2]

[1] Community Health, University of Illinois Urbana-Champaign, 1206 S. Fourth St. Room 3006 Champaign, IL, USA, [2] Population Health, University of Toledo, 2801 W Bancroft St Toledo, OH 43606, USA

Keywords: in vitro fertilization, birth defects, congenital malformations, systematic review, meta-analysis.

https://doi.org/10.46989/001c.91039

Journal of IVF-Worldwide

Vol. 1, Issue 4, 2023

Background

Previously published meta-analyses revealed that IVF combined with intracytoplasmic sperm injection (ICSI) had an increased risk of birth defects in children. ICSI is more invasive, expensive, and time-consuming than IVF, but both result in comparable live birth rates. Currently, despite traditional IVF being used less frequently nationally and internationally than combined IVF/ICSI, it is important to understand the relationship between traditional IVF and birth defects due to a paucity of literature.

Objective

This systematic review and meta-analysis focused on whether traditional IVF techniques increase the risk for “all” birth defects and “major” birth defects in singletons compared to naturally conceived children.

Search Strategy

PubMed and EMBASE databases adhered to PRISMA guidelines.

Selection Criteria

Study selection consisted of original publications in English reporting birth defects for IVF singletons vs. naturally conceived children.

Data Collection and Analysis

Nine selected items from STROBE criteria were employed to rate study quality. Random effect models were used to calculate pooled odds ratios.

Results

From 916 publications, fifteen studies met eligibility criteria. Eight studies were rated as high quality, while the remaining 7 were rated as medium. A higher rate of “all” birth defects (pooled OR= 1.44 (95% CI:1.15-1.80) as well as a higher risk for “major” birth defects (pooled OR= 1.64; 95% CI: 1.24-2.18) were observed among traditional IVF-conceived singletons compared to naturally conceived children.

a Corresponding author: Hillary Klonoff-Cohen, PhD, MS
Saul J. Morse & Anne B. Morgan Professor in Applied Health Sciences
Department of Community Health
University of Illinois Urbana-Champaign
Huff Hall Room 3006
1206 S. Fourth St.
Champaign, IL
Phone: (217) 244-6242
Email: klonoffc@illinois.edu

Conclusions

This is the first systematic review and meta-analysis to date to provide the highest available evidence that IVF is associated with "all" and "major" birth defects among IVF singletons compared to naturally conceived infants. Future large prospective studies should employ standardized reporting and uniform protocols for identifying birth defects with consistent diagnostic criteria for both minor and major birth defects, and comparable durations of follow-up in order to obtain an accurate estimate of birth defects after IVF.

INTRODUCTION

In the US, there has been a dramatic increase in the use of intracytoplasmic sperm injection (ICSI) in combination with *in vitro* fertilization (IVF) over the past 15 years (1996-2012) from a staggering 36% to 76%.[1] Similarly, in Europe, in 1998, the European Society of Human Reproduction and Embryology (ESHRE) reported the percentage of IVF and ICSI at 54% and 46%, respectively, but these percentages dramatically reversed over time until 2020 (last available data), when IVF accounted for 30% and ICSI for 70% of total annual procedures.[2-4] Whether IVF/ICSI should be preferred to traditional IVF remains an open question, particularly with non-male fertility.[5-7] ICSI is more invasive, expensive, and time-consuming than IVF but results in comparable live birth rates.[7] A greater use of ICSI (in cases without male infertility) was propelled by reimbursement for the procedure by ART-mandated states.[8] Overall, the expanded use of ICSI in couples with non-male-factor infertility shows "a gap between clinical practice and evidence".[7] The reported adjusted odds ratio for combined IVF/ICSI and birth defects in 3 meta-analyses from 1.32-1.37.[9-11] The Practice Committee of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology's most recent update in 2020 stated that ICSI has not demonstrated improvement in clinical outcomes in couples with non-male infertility.[12] In light of these considerations, although traditional IVF is used less frequently both nationally and internationally than combined IVF/ICSI, it is imperative to understand the relationship between traditional IVF and birth defects due to a paucity of literature in this specific area.

Several previous published meta-analyses have revealed an increase in birth defects in children who were conceived through assisted reproductive technologies (ART), specifically consisting of combining both IVF and intracytoplasmic sperm injection (ICSI).[9-11,13-15] In contrast, this systematic review and meta-analysis differs from earlier reviews because it focuses solely on IVF-conceived children and the risk of birth defects. Links between birth defects and IVF are poorly understood. Furthermore, recent improvements in IVF protocols in the past decade pose the question of whether the risk of birth defects has decreased over time.

Hence, the purpose of this review is to determine whether children conceived with traditional IVF are at greater risk for birth defects than naturally conceived children based on published studies through June 2023.

METHODS

This systematic review and meta-analysis consisted of secondary data analysis not involving human subject research and, thus, did not require an Institutional Review. We adhered to the preferred reporting items recommended by the "Meta-analysis of Observational Studies in Epidemiology (MOOSE)".[16]

A search on PubMed and EMBASE databases was performed using the keywords *birth defects*, *congenital malformations*, *IVF*, and *in vitro fertilization*. EndNote X9.2 was used to manage retrieved citations. The search strategy is presented as a supplemental file, Appendix S1. The references from the obtained articles were further examined to identify relevant papers.

DESCRIPTION OF EXPOSURE, OUTCOME, AND STUDY POPULATION

In this systematic review and meta-analysis, IVF, the exposure, did not include any other ART procedures, including ICSI, zygote intrafallopian transfer (ZIFT), and gamete intrafallopian transfer (GIFT). Hence, our study population consisted of all IVF-conceived children and all naturally conceived children included in the selected studies. The primary outcome measures were any birth defects or congenital malformations, including major, minor, combined, or unspecified.

ELIGIBILITY CRITERIA AND STUDY SELECTION

Inclusion criteria consisted of 1) original studies published in English, 2) those that reported pediatric birth defects for singletons after IVF treatment, 3) studies containing a naturally-conceived comparison group, and 4) prevalence rates or odds ratios. Exclusion criteria consisted of i) animal studies, ii) genetic studies, iii) reviews, iv) abstracts, case reports, and unpublished studies, v) IVF with oocyte donation, and vi) studies using other ART (e.g., ICSI, GIFT, ZIFT) as a comparison group.

Two investigators independently identified all relevant articles screening for eligibility using inclusion/exclusion criteria. Any disagreements were discussed and resolved among the two reviewers. The only disagreement between investigators pertained to the inclusion of two studies that were subsequently eliminated. Both studies, Olsen and Shevell, defined IVF with other ART procedures, including ICSI, ZIFT, and GIFT, respectively.[17,18]

DATA EXTRACTION

For each included study, data were extracted on author, year, country, study design, source of study sample, description of cases and comparison groups, exposure, predictors, outcome, results, and limitations (**Table 1**). For this systematic review and meta-analysis, the investigators only included relevant information on IVF exposure and birth defects as the outcome.

QUALITY APPRAISAL OF THE EVIDENCE USING STROBE CRITERIA

To assess the quality of every study, STROBE criteria (Strengthening the Reporting of Observational Studies in Epidemiology) were employed by the two investigators. STROBE is an international, collaborative initiative of epidemiologists, methodologists, statisticians, researchers, and journal editors involved in conducting and disseminating observational studies to strengthen the reporting of observational studies in epidemiology.[19]

Nine selected items in the STROBE checklist were used to assess study quality, including: i) study objectives, ii) study design, iii) source of study sample, iv) participants' characteristics, v) sample size, vi) quality of definition of exposure, vii) quality of definition of outcome, viii) results, and ix) study limitations. Quality assessment and final rating are reported in **Table 2**.

Each evaluation criterion was rated as "+" or "-," depending on whether the study adequately did or did not, respectively, meet the specific criteria. When a "-"was assigned, an explanation was provided. The overall study was assigned a low, medium, or high-quality score based on the number of criteria that were rated as "-". Hence, a high-quality study reflected no "-" scores, a medium-quality study reflected 1-4 "-" scores, and a low-quality study reflected 5 or greater.

The overall quality of each study was a reflection of the study authors providing thorough and detailed information in all categories that were directly related to the effects of IVF on birth defects. The paucity of relevant details was possibly a result of authors investigating other primary research questions. Hence, IVF and birth defects results may have been located in a sub-analysis in their publications.

STATISTICAL ANALYSIS

A descriptive analysis of the included studies was performed and presented in Table 1. Meta-analysis was conducted using STATA software, version 17, to calculate the pooled odds ratio for (i) all birth defects and (ii) major birth defects among IVF singletons compared to naturally conceived children. Pooled odds ratios were calculated using unadjusted odds ratios for "all" birth defects. Adjusted odds ratios were not used in this meta-analysis because of the convincing debate in the statistical literature about the appropriateness of combining adjusted odds ratios across studies. It states that if studies adjust for different covariates, then their adjusted odds ratios are not comparable.[20]

We also conducted a subgroup analysis for "major" birth defects. We performed sensitivity analyses to explore sources of heterogeneity and robustness by including one study at a time and recalculating the pooled effect estimates to assess the stability of results.

Heterogeneity was estimated using the I^2 test and interpreted as the percentage of total variation across studies that is due to heterogeneity rather than chance. A value of >50% was indicative of substantial heterogeneity among the included studies.[21] We used a random effect model to account for the expected heterogeneity among the studies due to differences in study populations, methods, and definitions. Egger's test was used as a measure of asymmetry in the funnel plot, which may indicate publication bias or other small-study effects.

RESULTS

Figure 1 depicts the Preferred Recording Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram, mapping the number of studies identified, included, and excluded, and the reasons for exclusion.

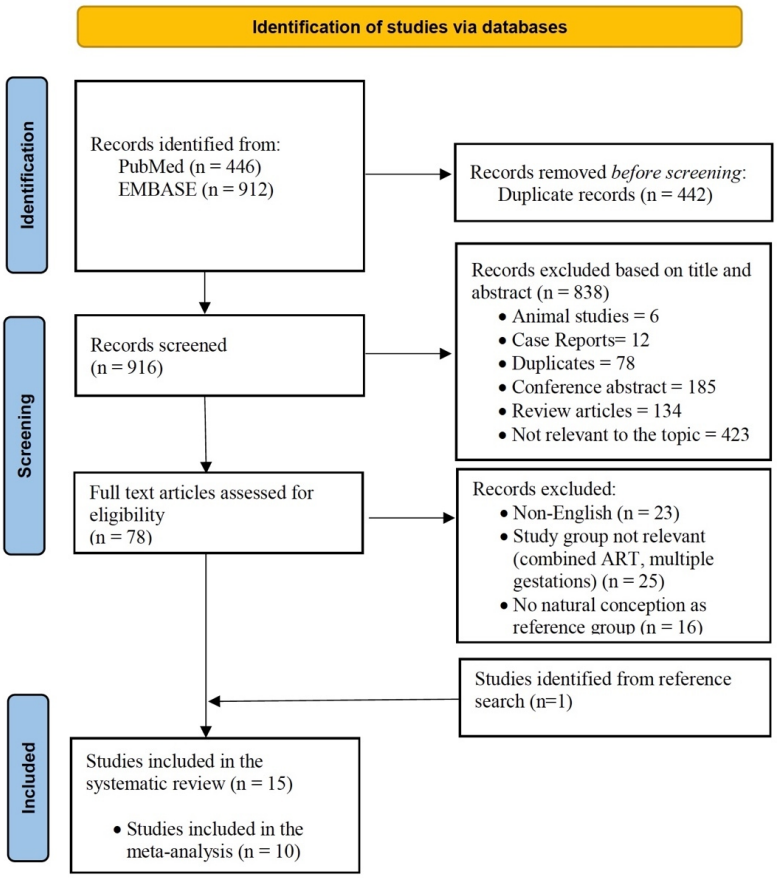
After reviewing 916 articles, 78 full-text articles were reviewed against the eligibility criteria, and 64 were eliminated because they were not published in English (n=23), the study group was not relevant (n=25), and there was no natural conception comparison group (n=16). One relevant article was identified from references (Figure 1).

Ultimately, 15 relevant studies were included in the systematic review and meta-analyses.[22-36]

STUDY CHARACTERISTICS

Of the 15 studies on birth defects and IVF, 8 studies were conducted in Europe[22,23,30-32,34-36] and one each in China,[33] Japan,[27] Canada,[25] and Israel.[26] The remaining three studies were performed in Australia.[24,28,29] A total of 8 studies had a retrospective cohort design,[24-30,36] two were matched case-control studies,[31,35] three were cross-sectional,[22,23,27] and two were prospective cohort studies.[32,33] The majority of studies were retrospective (66.67%), posing a high risk of bias. Most of the studies had sufficient IVF sample sizes ranging from 262[36] to 15,570 patients,[30] except for two studies with low sample sizes of 52 and 140, respectively.[32,35] Information on birth defects was derived from birth or congenital malformations registries,[28-31] hospital discharge registers,[30] a perinatal database/registry/ies,[22,25,27,34] researchers,[33] parents reporting followed by a pediatrician's general examination,[23,32,35] ART centers,[34] and a neonatologist.[36]

Timing of birth defects was diagnosed at birth or detected prior to release from the birth hospital,[24,26,35] during the neonatal and/or perinatal period,[22,27,36] at one year of age,[28] at 4.5 or 5 years of age,[23,32] or not disclosed.[25,29-31,33,34] When assessing confounders, there was heterogeneity among the choice of potential confounders adjusted for in the multivariate analyses (Table 1).



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

For more information, visit: http://www.prisma-statement.org/

Figure 1. PRISMA flowchart indicating study selection process

STUDIES REPORTING SIGNIFICANT ASSOCIATIONS BETWEEN IVF AND BIRTH DEFECTS

Table 1 contains details about STROBE criteria for each of the 15 studies.[22-36] Table 2 summarizes the quality of each study with a rating from low to high. In both Tables 1 and 2, studies are presented by the most recent publication date.

Seven studies reported a statistically significant association between IVF and birth defects (Table 1).[25,28-30,33-35] Results are presented from the newest to oldest studies. Qin reported a six-fold increased risk of congenital malformations in China (adjusted OR= 6.07; 95% CI: 3.14-11.72) while adjusting for a total of 22 potential confounders in 1260 children born with IVF compared to 2,480 naturally conceived children.[33] A retrospective cohort study by Farhi conducted in Israel from 1997-2004 found an increased risk of congenital malformations in 1,680 IVF births compared to 202,935 spontaneously conceived live births (adjusted OR= 1.28; 95% CI: 1.00, 1.63).[26]

In Burgundy, France, between 2000-2009, Sagot and colleagues found an increased risk of congenital malformations (adjusted OR=2.0; 95% CI: 1.3=3.1) in IVF-conceived singletons (n=903) compared to naturally conceived children (n= 4,044).[34]

A slightly increased risk of congenital malformations (OR=1.15; 95% CI 1.07-1.24) was observed among 15,570 infants born after IVF versus 689,157 naturally conceived infants in Sweden during 2001-2007, after adjusting for year of birth, maternal age, parity, smoking, and body mass index.[30]

Data on 3,312 IVF (and 3,634 ICSI singleton pregnancies) were linked to perinatal birth defects occurring between 1991 and 2004 in Victoria, Australia, which were compared to 20,838 outcomes for singleton births.[29] Overall, birth defects were increased after IVF (adjusted OR= 1.33; 95% CI: 1.14-1.55) relative to controls. A specific group, blastogenesis birth defects, were markedly increased among IVF children (adjusted OR= 3.24; 95% CI: 1.79-5.86) compared to the non-ART controls while adjusting for maternal age, year of infant birth, parity, and infant sex.[29]

In a Canadian retrospective cohort study, the subgroup analysis consisted of 319 IVF and 43,462 naturally conceived singletons.[25] The prevalence of birth defects was highest among infants born with IVF (3.45%), compared to infants born with ovulation induction (2.35%), intrauter-

Table 1. Summary of Study Characteristics Included in the Systematic Review and Meta-analysis

Author Year Country	Study Design	Sample: Cases	Comparison group	Exposure	Outcome	Results	Limitations
Qin 2016 China	Prospective cohort study	1,260 eligible mothers in IVF group with a history of infertility, >95% from 25-40 years. Parental history of Hepatitis 9.5% Diabetes 1.2% Congenital malformations 1.2%	2,480 fertile group with no history of infertility or infertility treatment Parental history of Hepatitis 3.8% Diabetes 0.2% Congenital malformations 0.2%	IVF	Congenital malformations (under adverse pregnancy outcomes) defined as all major and minor malformations	Congenital malformations (aOR = 6.07; 95% CI: 3.14-11.72) was increased in the IVF group.	No environmental exposures during pregnancy adjusted for in models. Sample size too small to evaluate rare congenital malformation outcomes. There could be multi-collinearity with some of the potential confounders in the model (e.g., smoking condition, active and passive smoking, history of alcohol use).
Farhi 2013 Israel	Retrospective Cohort	6,726 following ART which included 2,518 IVF conceptions. Age of women undergoing IVF: 17-44 years Parental history of other diseases not reported.	208,051 spontaneous conceptions Age of women: 17-44 years. History of other diseases not reported.	All ART, IVF, and ICSI.	Congenital malformations diagnosed at birth or detected prior to release from the hospital.	No significant differences in congenital malformations between IVF singleton group compared to spontaneously conceived singleton infants (adjusted OR= 1.25 (95% CI: 0.88-1.71).	Malformations that might be diagnosed later in life are not included. Sub-fertile women could not be identified as a separate group. Data regarding frozen embryos were not available.
Davies 2012 Australia	Retrospective Cohort	IVF: 1,484 99% between 20-44 years Parental history of diseases not reported.	Spontaneous pregnancies without a history of infertility or ART: 293,314 98% between 22-39 years	Assisted conception including IVF, ICSI, GIFT, IUI, Donor insemination, Ovulation induction, and Clomiphene citrate	Congenital malformations diagnosed at birth or in the neonatal period	Fresh or frozen singleton IVF births had a non-significant risk of birth defects (adjusted OR=1.06, 95% CI: 0.87-1.30).	Birth defects only measured till 28 days.

Author Year Country	Study Design	Sample: Cases	Comparison group	Exposure	Outcome	Results	Limitations
Sagot 2010 France	Retrospective cohort study	1348 ART singletons IVFT: n= 903; IUI: n= 445 Mean maternal age for IVF 31.1 years. Parental history of other diseases not reported.	4044 infants conceived naturally Mean maternal age 30.7 years	IVF, IUI	The risk of major birth defects in IVF or IUI compared with infants conceived naturally	Singletons born after IVFT had a higher prevalence of major congenital malformations, with adjusted odd ratios (aOR) 2.0, 95%CI: 1.3–3.1.	List of predictors is very limited.
Kallen 2010 Sweden	Retrospective Cohort	IVF: 15,570 Parental age not reported. Parental history of diseases not reported.	689,157 naturally conceived infants Parental age not reported.	IVF and ICSI	Congenital malformations coded using ICD-10 since 1997.	Adjusted odds ratio for all congenital malformations aOR= 1.15 (95% CI 1.07-1.24) aOR for severe congenital malformations= 1.25 (95% CI 1.15-1.37)	Multiple testing for various types of malformations with no Bonferroni correction. Crude classification for congenital malformations using ICD-10
Halliday 2010 Australia	Retrospective Cohort	IVF: 3,312 All ART (IVF and ICSI): 6,946 Mean maternal age for IVF mothers= 34.1 years Parental history of diseases not reported.	20,838 naturally conceived singletons Mean maternal age 34 years. Controls frequency matched on maternal age and birth year on a 1:3 ratio.	IVF and ICSI	Congenital malformations: birth defects that have previously been classified as “defects of blastogenesis” classified as neural tube defects, abdominal wall defects, esophageal atresia and anal atresia.	Adjusted odds ratio for all congenital malformations among IVF pregnancies compared to naturally conceived infants= 1.31 (95% CI: 1.10–1.56). AOR for defects of blastogenesis compared to naturally conceived infants= 3.24 (95% CI: 1.79-5.86)	Unable to examine the effects of infertility on birth defects.
El-Chaar 2009 Canada	Retrospective Cohort	1,399 conceived with Assisted Human Reproduction divided into subgroups of	43,462 naturally conceived singletons. Mean maternal age: 29.4 years	Assisted Human Reproduction (AHR): Ovulation induction, Intrauterine	Birth defects, which was not defined.	In the 319 infants conceived by IVF, the prevalence of birth defects was the highest, at 3.45% compared to 1.86% for naturally conceived children.	No information pertaining to classification system for birth defects. Odds ratio for birth defects among IVF or ICSI infants compared

Author Year Country	Study Design	Sample: Cases	Comparison group	Exposure	Outcome	Results	Limitations
		IVF= 319, ovulation induction=298, and IUI=173 Mean maternal age: 35 years.		Insemination, IVF, and ICSI			to non-AHR groups were not presented. Did not adjust for multiple births, use of drugs and alcohol. Smaller sample sizes in the subgroups of AHR types and anomaly types. Potential for underreporting of birth defects in naturally conceived infants.
Fujii 2009 Japan	Cross-sectional study	1,396 singletons after IVF Mean maternal age= 36 years Seventeen pre-pregnancy characteristics were reported. Two were significantly different between cases and controls-. Parental history of blood disorder 1.4%. Infections 1.4%	53,566 singleton births from spontaneous conceptions Mean maternal age- 31 years Parental history of blood disorder 0.9%, infections 2.4%	IVF	Congenital malformations, which was not defined.	Adjusted OR= 1.17 (95% CI: 0.81-1.69) for congenital malformations in newborns after IVF compared to naturally conceived infants	Registry database likely held a biased sample based on high risk pregnancy referrals from larger hospitals. Social factors were not adjusted for in analysis.
Bonduelle 2005 UK, Belgium, Denmark, Sweden, Greece	Cross-sectional	IVF: 437 Mean maternal age- 34 years Any of seven chronic illnesses 12%	Naturally conceived: 538 Matched for age, sex, maternal education, and parental	IVF and ICSI	Major malformations: defined as malformations causing functional impairment or requiring surgical correction.	Adjusted odds ratio= 1.66 (95% CI 0.70–3.95) for major malformations in IVF children compared to naturally conceived children.	High rate of non-participation for IVF, especially in Greece. Potential for survivor bias resulting in underestimation of true relative risk of severe congenital

Author Year Country	Study Design	Sample: Cases	Comparison group	Exposure	Outcome	Results	Limitations
			socioeconomic status. Mean maternal age- 31 years Any of seven chronic illnesses 7%				malformations. Sites of recruitment for naturally conceived children from schools and nurseries may have resulted in underestimation of congenital malformations.
Place 2003 Belgium	Prospective controlled cohort study	52 IVF conceived children Overall mean maternal age for IVF and spontaneously conceived group: 31.9 years Other parental diseases not reported.	59 spontaneous conceived children Matched to cases on basis of mean maternal age.	IVF and ICSI	Combined congenital malformations consisting of both major and minor.	No significant differences in the incidence of combined congenital malformations (p=0.787) among IVF (9.6%), ICSI (10.6%), and spontaneously conceived (13.6%) children. A comparison of rates of major congenital malformations between ICSI, IVF, and spontaneous conception was non-significant.	Small sample size. No odds ratios or potential confounders.
Zadori 2002 Hungary	Retrospective cohort	IVF=262 including 188 neonates from singletons. No maternal age reported. Parental diseases not reported	Naturally conceived (n=262) matched on maternal age, parity, and gravidity. No maternal age reported.	IVF	Congenital malformations were diagnosed by a neonatologist on the basis of physical examination, chest, abdominal or skull X-ray, and ultrasonograph according to ICD criteria	The incidence of major congenital abnormalities (ICD-9) was not significantly higher ($p > 0.05$) among cases (1.90%) than controls (1.15%).	Control group was recruited from a different population. No reported adjusted odds ratios
Anthony 2002 Netherlands	Cross-sectional	IVF-4224 children Mean maternal age= 33.3 years Parental diseases not	Naturally conceived children n=314,605 Mean maternal age= 29.7 years	IVF	Congenital malformations were obtained from 3 national professional perinatal and neonatal	The overall crude odds ratio (OR) for the risk of any malformation for IVF children compared with naturally-conceived children was 1.20 [95% CI: 1.01–1.43]. After correction for differences in maternal age, parity and ethnicity between the IVF and control population the OR=1.03 (95%	A total of 9% of the IVF sample were children conceived by ICSI because no separate coding exists for ICSI in the National Perinatal Database.

Author Year Country	Study Design	Sample: Cases	Comparison group	Exposure	Outcome	Results	Limitations
		reported.	Parental diseases not reported.		registers: the National Perinatal Database for Primary Care, the National Perinatal Database for Secondary Care, and the National Neonatology Database	CI: 0.86–1.23).	
Hansen 2002 Australia	Retrospective cohort	837 infants conceived with IVF. Mean maternal age= 34.1 years. No parental diseases reported.	4,000 naturally conceived infants. Mean maternal age=28.2 years No parental diseases reported.	IVF	Prevalence of major birth defects diagnosed by one year of age	9% of infants conceived with IVF had a major birth defect diagnosed by one year of age compared with 4.2% of naturally conceived infants (P<0.001). For singletons, the aOR for major birth defects in infants by 1 year of age after IVF was 2.2 (95% CI: 1.5-3.2).	
Koudstaal 2000 Netherlands	Matched case-control study	307 IVF children Mean maternal age= 32.8 years. Parental diseases not reported.	307 naturally conceived children selected from the registry. Matched on maternal age, parity, ethnicity, date of parturition, height and weight, smoking at onset of pregnancy, obstetric and medical history, and clinic. Mean	IVF	Congenital malformations	In both groups seven children (2.3%) had congenital malformations. (non-significant, no p-value provided).	No odds ratios reported.

Author Year Country	Study Design	Sample: Cases	Comparison group	Exposure	Outcome	Results	Limitations
			maternal age: 32.7 years				
Verlaenen 1995 Belgium	Case-control	140 singleton pregnancies conceived with IVF. Mean maternal age 31.7 years Other parental diseases not reported.	140 matched controls on parity, height, weight, and age Maternal age 31.6 years	IVF	Minor congenital malformations	Incidence of Minor congenital malformations among IVF children was 5.7% compared to 0% among controls ($p<0.01$). Even though this initially appears to be significant, the high rate of minor abnormalities detected could be a result of IVF infants undergoing an ultrasound scan of the heart and kidney shortly after birth. All defects closed spontaneously and did not appear in a national register. Hence, there were no significant differences between IVF pregnancies and spontaneously conceived ones.	Results were based on a limited number of cases. No odds ratios reported.

Table 2. Study Quality Assessment using Selected STROBE Criteria

	Objectives	Study Design	Source of the study sample	Sample/ participants' characteristics	Sample size	Quality of definition of exposure	Quality of definition of outcomes	Type of results	Study Limitations	Overall quality Rating of the study**
Qin	+	+	+	+	+	+	+	- Possible multicollinearity	- Possible Multicollinearity	High
Farhi	+	+	+	+	+	+	+	+	+	High
Davies	+	+	+	+	+	+	+	+	+	High
Sagot	+	+	+	+	+	+	+	- Only adjusted for pre-existing and gestational diabetes	- Only adjusted for pre-existing and gestational diabetes	Medium
Kallen	+	+	+	+	+	+	+	- Not adjusted for known potential confounders, risk for all malformations were not reported	+	Medium
Halliday	+	+	+	+	+	+	+	+	+	High
El-Chaar	+	+	+	+	+	+	- No information pertaining to classification system for birth defects	- Did not separate IVF from other AHR categories when reported adjusted odds ratios.	- No information on classification of birth defects. Odds ratios for IVF were not presented. Did not adjust for multiple births, use of drugs and alcohol.	Medium
Fujii	+	+	+	+	+	+	+	+		High
Bonduelle	+	+	+	- Quality of records prevented recruitment from	+	+	+	+	+	High

	Objectives	Study Design	Source of the study sample	Sample/ participants' characteristics	Sample size	Quality of definition of exposure	Quality of definition of outcomes	Type of results	Study Limitations	Overall quality Rating of the study**
				several fertility clinics (e.g., Denmark)						
Place	+	+	+	+	-	+	+	- No crude or adjusted odds ratios	- No crude or adjusted odds ratios	Medium
Zadori	+	+	- Study source not disclosed. Controls recruited from a different population.	- No patient characteristic information was provided possibly because it was a short communication.	+	+	+	- No crude or adjusted odds ratios provided	- No crude or adjusted odds ratios provided. No study source and patient characteristics provided.	Medium
Anthony	+	+	- No separate coding for 9% of ICSI cases	+	+	+	+	+	+	High
Hansen	+	+	+	+	+	+	+	+	+	High
Koudstaal	+	+	+	+	+	+	+	- No crude or adjusted odds ratios, only prevalence rates provided with no p-values.	- No crude or adjusted odds ratios, only prevalence rates provided with no p-values.	Medium
Verlaenen	+	+	+	+	+	+	+	- No crude or adjusted odds ratios..	- No crude or adjusted odds ratios..	Medium

ine insemination (2.89%), and spontaneous conception (1.86%).[25]

The prevalence of birth defects diagnosed in Australia within one year of age was significantly higher between 527 singletons born with IVF compared to 3,906 naturally conceived singleton children (adjusted OR= 2.20; 95% CI: 1.50-3.20) after adjusting for maternal age and parity, and sex of infant).[28]

STUDIES REPORTING NON-SIGNIFICANT ASSOCIATIONS BETWEEN IVF AND BIRTH DEFECTS

The remaining 8 studies found no significant effect between IVF and birth defects.[22-24,26,27,31,32,36]

Risks of birth defects diagnosed before a child's fifth birthday were compared in South Australian women who received treatment with ART to women who had spontaneous pregnancies.[24] In a subgroup analysis, the increased risk of birth defects among IVF children (with fresh or frozen embryos n=1,484) compared to those children conceived naturally (n=293,314) was not significant (adjusted OR=1.07; 95% CI: .09-1.26) after adjusting for parental factors.[24]

Fujii and colleagues found no significant differences in congenital malformations between 1,396 singleton Japanese children born after IVF and 53,566 spontaneously conceived singleton children (adjusted OR=1.17; 95% CI: 0.81-1.69).[27] In a European five-nation cohort study, no significant differences in odds of major malformations (assessed at 5 years) were reported for 437 IVF children compared to 538 naturally conceived children (adjusted OR=1.66; 95% CI: 0.70-3.95) after adjusting for social demographic differences.[23]

A Belgian study found no significant differences in the incidence of combined (major and minor) congenital malformations among 52 IVF children (9.6%) compared to 59 spontaneously conceived children (13.6%) (p= .787).[32]

In a retrospective analysis of 12,920 deliveries in Hungary, the incidence of major congenital malformations was not significantly higher (p>.05) among 262 IVF neonates (1.90%) compared to 262 matched naturally conceived neonates (1.15%).[36]

A study based on a Dutch national database between 1995 and 1996 reported that the adjusted odds ratio for the risk of any malformation for IVF children (n= 4,224 compared with naturally conceived children (n= 314,605) was 1.03 (95% CI: 0.86-1.23), after adjusting for maternal age, parity and ethnicity.[22]

No difference in congenital malformations was observed in four Dutch Hospitals among 307 IVF pregnancies compared to 307 naturally conceived pregnancies. (2.3%, 2.3%, respectively, no p-value provided).[31]

Finally, in a case-control study conducted in Belgium, there were eight (5.7%) minor congenital malformations at birth in 140 singleton pregnancies conceived by IVF compared to 140 matched controls (p<0.01).[35] However, all the detected minor malformations spontaneously closed, resulting in this study's conclusion that there were no significant differences between IVF pregnancies and spontaneously conceived ones.

STUDIES WITHIN THE SYSTEMATIC REVIEW COMPARING FRESH VS. FROZEN OOCYTES FOR IVF AND ICSI AND THE RISK OF BIRTH DEFECTS

Davies and colleagues evaluated the risk of birth defects among fresh IVF, frozen IVF, fresh ICSI, and frozen ICSI separately compared to spontaneous conceptions.[24] Both fresh and frozen IVF and the risk of birth defects were not significant. In contrast, another study by Halliday and colleagues reported that both fresh (adjusted OR=1.43; 95% CI: 1.23-1.66) and frozen (adjusted OR=1.25; 95% CI: 1.04-1.52) embryo transfers using IVF vs. spontaneous conceptions were significantly associated with birth defects.[29]

Within our systematic review, four studies reported a statistically significant increased risk of birth defects after ICSI compared to spontaneous conception with adjusted odds ratios ranging from 1.40-2.54.[26,28,29,31] Finally, Davies reported that fresh ICSI vs. spontaneous conception was significantly associated with the risk of birth defects (adjusted OR=1.73; 95% CI: 1.35-2.21).[24]

QUALITY OF STUDIES

A total of 8 studies were rated as high,[22-24,26-29,33] while the remaining 7 studies were rated as medium quality.[25,30-32,34-36] The most common reasons for the medium rating were: 1) the absence of diagnostic criteria or a classification system for birth defects, 2) only providing prevalence rates, and 3) the absence of adjusted odds ratios for birth defects among IVF-conceived infants compared to naturally conceived births. (Table 2).

RISK OF ANY BIRTH DEFECTS AND IVF

A random effect model exhibited a statistically significant higher rate of any birth defects among IVF-conceived infants compared to naturally conceived singleton infants with a pooled odds ratio of 1.44 (95%CI: 1.15-1.80) (Figure 2). This model was chosen because the between-study heterogeneity was high (I^2= 74.9%, p<.001). A visual examination suggested some funnel plot asymmetry; however, there was no small-study effect (Egger test, p = 0.14), supporting the absence of publication bias.

RISK OF MAJOR BIRTH DEFECTS AND IVF

Statistically significant heterogeneity was detected in the studies (I^2= 67.6%, p=.005) and hence, a random effect model was used to calculate the pooled odds ratio. The IVF group has a significantly higher risk of major birth defects compared to naturally conceived children with a pooled odds ratio of 1.64 (95% CI: 1.24-2.18) (Figure 3).

SENSITIVITY ANALYSIS

The overall effect for the seven studies remained statistically significant in all sensitivity analyses when removing one study at a time, suggesting the robustness of the meta-analysis results. However, there was variation in heterogeneity based on which study was excluded. Most striking

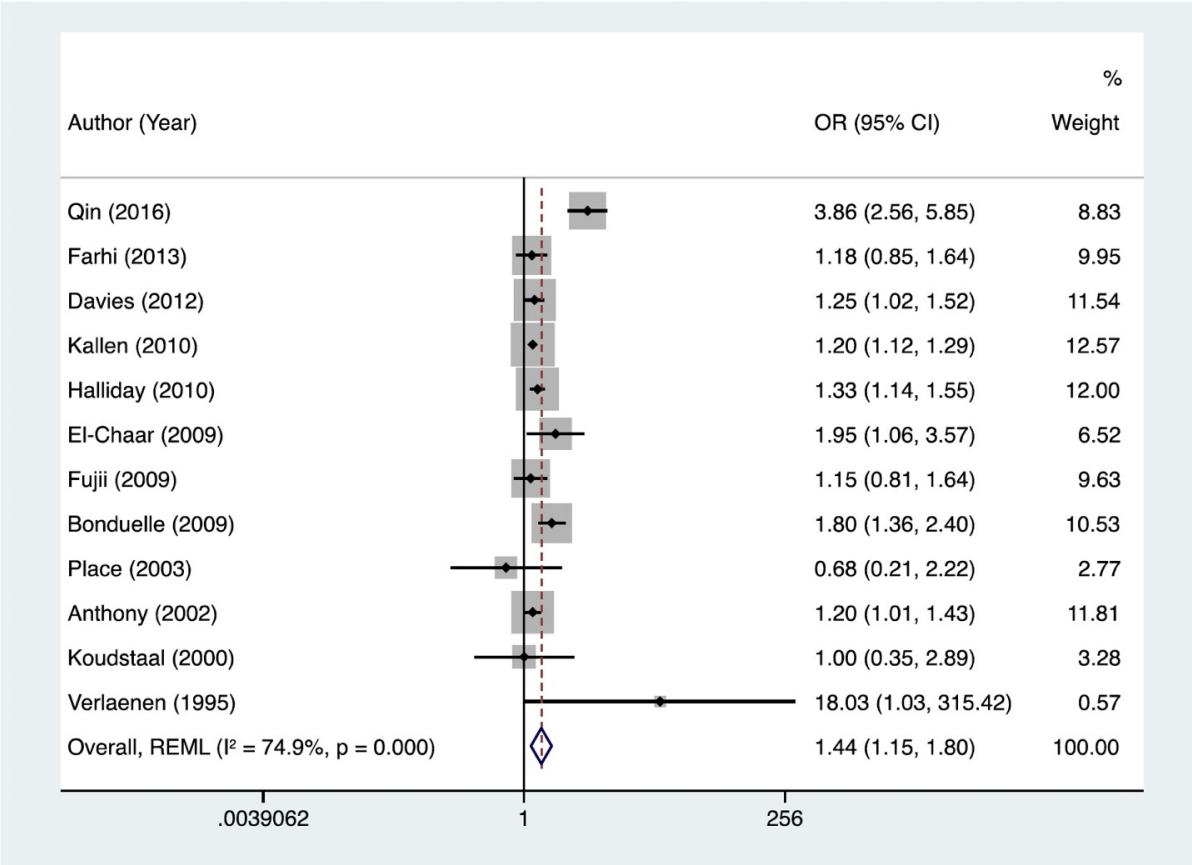


Figure 2. Forest Plot with Pooled Odds Ratios for "any" Birth Defects among IVF-Conceived Children Compared to Naturally-Conceived Children

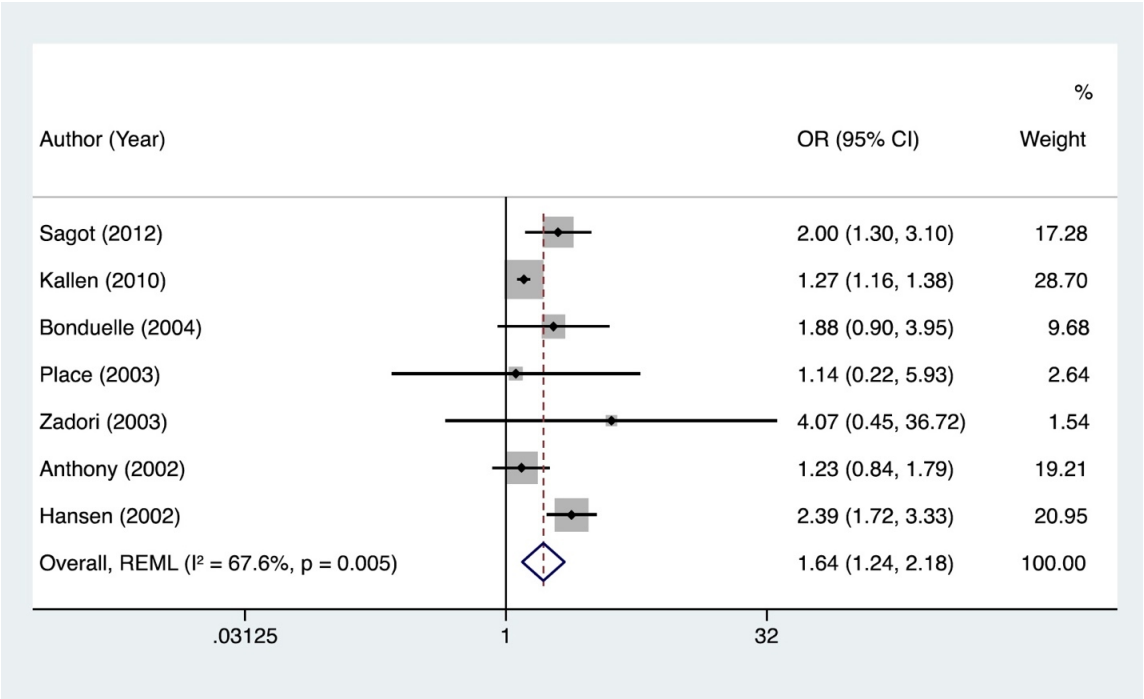


Figure 3. Forest Plot with Pooled Odds Ratios for "major" Birth Defects among IVF-Conceived Children Compared to Naturally-Conceived Children

was when excluding the "Hansen (2002) study,"[28] the overall effect was OR = 1.42 (statistically significant), and the heterogeneity decreased substantially: I^2 = 18.6% (low heterogeneity). In summary, we would conclude that the findings are generally consistent regardless of which individual study is removed, but the degree of heterogeneity among the studies does vary.

DISCUSSION

Our current systematic review and meta-analysis explicitly focused on IVF and all birth defects, consisting of a sample size of 30,171 singletons and 1,623,307 naturally conceived infants. Our study revealed an association between IVF and all birth defects with a pooled OR of 1.44 (95% CI: 1.15-1.80) among singletons. Additionally, the pooled OR for major birth defects was 1.64 (95% CI: 1.24-2.18) in a sub-sample of 21,901 IVF infants and 1,012,497 naturally conceived infants.

EXISTING META-ANALYSES

Six existing meta-analyses in the literature explored the relationship between combined IVF and ICSI technologies (referred to as ART) and birth defects, reporting either unadjusted or adjusted pooled odds ratios.[9-11,13-15] Additionally, single and multiple births were often combined.

Three meta-analyses pooled adjusted odds ratios for birth defects among ART-conceived children (combining IVF/ICSI) ranging from 1.32-1.37.[9-11] Only one study conducted by Zhao in 2020 that included a subgroup analysis, assessed whether traditional IVF procedures increased the risk of birth defects. Their results showed an increased risk of birth defects among IVF children (pooled RR=1.25, 95% CI: 1.12-1.40).[14] In contrast to Zhao's sub-group analysis, our study focused exclusively on IVF singletons and examined both "all" and "major" birth defects.

POSTULATED MECHANISMS

Postulated mechanisms to explain the observed associations between IVF singleton pregnancies and birth defects include: i) advanced age of one or both partners of the infertile couple, ii) factors causing infertility in the mother or father, or prior treatment for infertility, iii) duration of infertility, iv) environmental exposures, v) chronic diseases such as obesity and diabetes, vi) risk behaviors such as alcohol, smoking, recreational drugs, and caffeine, vii) medications used to induce ovulation or to maintain the luteal phase, and viii) the IVF technology procedure itself, such as the culture media composition, the length of time in culture, freezing and thawing of embryos, the altered hormonal environment at the time of implantation, and the manipulation of gametes and embryos.[9,10,15,23,24,28,33,37,38]

It is well-established that subfertility, independent of ART treatment, is associated with poor infant outcomes.[39] Women who struggled to conceive were 21% more likely to give birth to babies with birth defects compared to women who got pregnant without difficulty.[40]

Furthermore, patients diagnosed with infertility and opting for IVF may carry pathogenic genetic variants with variable expressivity, penetrance, and pleiotropic effects.[41] On one hand, these genetic defects lead to sub- or infertility, and on the other hand, genetic errors may affect fetal development and cause birth defects. Alternatively, the parental genome may contain pathogenic genetic variants that predispose to increased mutability in embryogenesis (e.g., pathogenic variants in DNA repair genes). This includes ultra-rapid proliferation of cells (each round preceded by DNA replication) during a short timeframe. Finally, genetic sub/infertility may also increase the risk of DNA errors already in the germline leading to defective genomes in the oocytes or spermatogenic cells. Understanding and determining genetic causes of sub/infertility is extremely important but beyond the scope of our hypothesis for this systematic review and meta-analysis.

With regards to modifiable technical issues of IVF (e.g., medications, culture), there are considerable emotional, monetary, and time costs; hence, it is critical that this technique be optimized, and the risk of birth defects be decreased in the future.

ADVANTAGES AND LIMITATIONS

The major advantage of our study pertained to the exposure, IVF, which was homogenous and did not include any other ART procedures, such as ICSI, ZIFT, and GIFT. When IVF was not the primary exposure, the odds ratios were retrieved from the subgroup analysis of the paper. It should be noted that there was sparse literature investigating the effect of IVF on birth defects. Only 47% of included studies in our meta-analysis utilized IVF solely as the exposure variable.[22,27,28,33] The remaining studies all included other ART procedures (e.g., ICSI, ZIFT) with unadjusted odds ratios obtained from the sub-analyses of IVF and birth defects.

Our study had some potential limitations. First, it only included studies published in English due to a lack of resources which limited its generalizability. Second, the classification of birth defects was different across studies. Some studies included both major and minor malformations,[22,30,32,33] while others only reported major,[23,28,34,36] or minor malformations.[35] Hence, our study separately examined the effects of IVF on "all birth defects" as well as "major birth defects". Additionally, the majority of studies did not provide ICD codes for birth defects/congenital malformations.[22-29,31-35]

Different time frames for diagnosis of birth defects, specifically, at birth and/or hospital release,[26] 28 days,[24] and 1 year[28] were utilized in the studies. Thus, studies diagnosing malformations only at birth may ultimately have led to classification bias. In fact, for the identification of birth defects following IVF, a 1-year follow-up should be the minimum requirement, while 3 years would be the optimal length of follow-up in prospective studies balancing resources with complete ascertainment.[42]

In the future, larger and more homogeneous studies are required to evaluate our hypothesis. Additionally, geneticists/dysmorphologists should make the final birth defects diagnosis rather than pediatricians.

Potential confounders varied from study to study. We took the opportunity to peruse each study to determine whether there were differences (or patterns) with choices of potential confounders between statistically significant and non-significant studies, and two maternal characteristics and one infant characteristic emerged as the most common potential confounders. We found maternal age was included as a potential confounder in 86% of statistically significant studies[25,26,28-30,33] compared to 50% of non-statistically significant studies.[22-24,27] Likewise, parity was adjusted for 71% of statistically significant studies[25,28-30,33] vs. 38% of non-significant studies.[22-24] The sex of the infant was a confounder in 57% of significant[25,26,28,29] and 13% of non-significant studies.[24]

Often, meta-analyses magnify biases and errors by including studies that are methodologically poor or that contain dubious results.[43] Hence, the findings may not be definitive. Nevertheless, a critical review of evidence from meta-analyses for IVF and birth defects is important given their prioritization to inform clinical practice guidelines.[44]

CONCLUSIONS

In conclusion, this current systematic review and meta-analysis (as of June 2023) provide the highest available evidence for reproductive endocrinologists that IVF is associated with both "all" and "major" birth defects among singletons.

FUTURE DIRECTIONS

To better inform physicians and counsel patients, it is imperative to understand epidemiologic causes of birth defects among IVF singletons, including technical aspects of the IVF procedure, parental characteristics, and type(s) and causes of infertility.

Parental characteristics such as advancing age as well as past maternal medical history, including diabetes, cytomegalovirus, toxoplasmosis, varicella, rubella, and Streptococcus B are agents that are recognized to potentially cause birth defects in the developing fetus.[45] Maternal psychological history, such as treatment of major depression with paroxetine, may increase teratogenic risk during natural conception[46,47]; hence, studies examining the risks of congenital malformations with first-trimester antidepressant exposure during in vitro fertilization should be explored. Lifestyle habits, including smoking alcohol, and illicit drugs including cannabis, and methamphetamine have been implicated in birth defects after natural conception.[48-55] These risky behaviors could contribute to or confound the relationship between IVF and birth defects.

A greater understanding of the independent roles, particularly of modifiable contributors, as well as the interrelatedness of these factors, may hopefully lead to a decreased risk of birth defects following IVF in the future.

Finally, to obtain an accurate estimate of birth defects after IVF, future large prospective studies should employ standardized reporting and uniform protocols for identifying birth defects (e.g., photos) with consistent diagnostic criteria for both minor and major birth defects, and comparable durations of follow-up.

CONFLICT OF INTEREST STATEMENT

Hillary Klonoff-Cohen: None
Mounika Polavarapu: None

DISCLOSURE STATEMENT

Hillary Klonoff-Cohen: None
Mounika Polavarapu: None

AUTHOR CONTRIBUTIONS

Conceptualization: Hillary Klonoff-Cohen (Lead). Methodology: Hillary Klonoff-Cohen (Equal), Mounika Polavarapu (Equal). Investigation: Hillary Klonoff-Cohen (Equal), Mounika Polavarapu (Equal). Data curation: Hillary Klonoff-Cohen (Equal), Mounika Polavarapu (Equal). Project administration: Hillary Klonoff-Cohen (Lead). Supervision: Hillary Klonoff-Cohen (Lead). Writing – original draft: Hillary Klonoff-Cohen (Equal), Mounika Polavarapu (Equal). Writing – review & editing: Hillary Klonoff-Cohen (Equal), Mounika Polavarapu (Equal). Formal Analysis: Mounika Polavarapu (Equal).

DETAILS OF ETHICAL APPROVAL

This systematic review and meta-analysis consisted of secondary data analysis not involving human subject research and thus did not require an Institutional Review.

FUNDING

None

Submitted: September 18, 2023 CST, Accepted: December 10, 2023 CST

REFERENCES

1. Boulet SL, Mehta A, Kissin DM, Warner L, Kawwass JF, Jamieson DJ. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. *JAMA*. 2015;313(3):255. doi:10.1001/jama.2014.17985

2. European Society of Human Reproduction and Embryology. Publications and Reports. Accessed August 24, 2023. https://www.eshre.eu/Data-collection-and-research/Consortia/EIM/Publications.aspx

3. Wyns C, De Geyter C, Calhaz-Jorge C, et al. O-150 Assisted reproductive technology (ART) in Europe 2019 and development of a strategy of vigilance Preliminary results generated from European registers by the ESHRE EIM consortium. *Human Reproduction*. 2022;37(Supplement_1):deac105.056. doi:10.1093/humrep/deac105.056

4. Smeenk J, Wyns C, De Geyter C, et al. O-153 Assisted Reproductive Technology (ART) in Europe 2020 and development of a strategy of vigilance: Preliminary results generated from European registers by the ESHRE EIM Consortium. *Human Reproduction*. 2023;38(Supplement_1):dead093.014. doi:10.1093/humrep/dead093.186

5. Glenn TL, Kotlyar AM, Seifer DB. The impact of intracytoplasmic sperm injection in non-male factor infertility—a critical review. *JCM*. 2021;10(12):2616. doi:10.3390/jcm10122616

6. Dang VQ, Vuong LN, Luu TM, et al. Intracytoplasmic sperm injection versus conventional in-vitro fertilisation in couples with infertility in whom the male partner has normal total sperm count and motility: an open-label, randomised controlled trial. *Lancet*. 2021;397(10284):1554-1563. doi:10.1016/s0140-6736(21)00535-3

7. Song J, Liao T, Fu K, Xu J. Icsi does not improve live birth rates but yields higher cancellation rates than conventional ivf in unexplained infertility. *Front Med*. 2021;7:614118. doi:10.3389/fmed.2020.614118

8. Zagadailov P, Cho KS, Seifer DB. Differences in ICSI utilization rates among states with insurance mandates for ART coverage. *Reprod Biol Endocrinol*. 2021;19(1):174. doi:10.1186/s12958-021-00856-4

9. Zheng Z, Chen L, Yang T, Yu H, Wang H, Qin J. Multiple pregnancies achieved with IVF/ICSI and risk of specific congenital malformations: a meta-analysis of cohort studies. *Reprod Biomed Online*. 2018;36(4):472-482. doi:10.1016/j.rbmo.2018.01.009

10. Hansen M, Kurinczuk JJ, Milne E, De Klerk N, Bower C. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. *Human Reproduction Update*. 2013;19(4):330-353. doi:10.1093/humupd/dmt006

11. Wen J, Jiang J, Ding C, et al. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. *Fertility and Sterility*. 2012;97(6):1331-1337.e4. doi:10.1016/j.fertnstert.2012.02.053

12. Intracytoplasmic sperm injection (ICSI) for non–male factor indications: a committee opinion. *Fertility and Sterility*. 2020;114(2):239-245. doi:10.1016/j.fertnstert.2020.05.032

13. Chen L, Yang T, Zheng Z, Yu H, Wang H, Qin J. Birth prevalence of congenital malformations in singleton pregnancies resulting from in vitro fertilization/intracytoplasmic sperm injection worldwide: a systematic review and meta-analysis. *Arch Gynecol Obstet*. 2018;297(5):1115-1130. doi:10.1007/s00404-018-4712-x

14. Zhao J, Yan Y, Huang X, Li Y. Do the children born after assisted reproductive technology have an increased risk of birth defects? A systematic review and meta-analysis. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2020;33(2):322-333. doi:10.1080/14767058.2018.1488168

15. Rimm AA, Katayama AC, Katayama KP. A meta-analysis of the impact of IVF and ICSI on major malformations after adjusting for the effect of subfertility. *J Assist Reprod Genet*. 2011;28(8):699-705. doi:10.1007/s10815-011-9583-z

16. Brooke BS, Schwartz TA, Pawlik TM. Moose reporting guidelines for meta-analyses of observational studies. *JAMA Surg*. 2021;156(8):787. doi:10.1001/jamasurg.2021.0522

17. Olson CK, Keppler-Noreuil KM, Romitti PA, et al. In vitro fertilization is associated with an increase in major birth defects. *Fertility and Sterility*. 2005;84(5):1308-1315. doi:10.1016/j.fertnstert.2005.03.086

18. Shevell T, Malone FD, Vidaver J, et al. Assisted reproductive technology and pregnancy outcome. *Obstetrics & Gynecology*. 2005;106(5, Part 1):1039-1045. doi:10.1097/01.aog.0000183593.24583.7c

19. Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *BMJ*. 2007;335(7626). doi:10.1136/bmj.39386.490150.94

20. Chang BH, Hoaglin DC. Meta-analysis of odds ratios: current good practices. *Medical Care*. 2017;55(4):328-335. doi:10.1097/mlr.0000000000000696

21. Deeks JJ, Higgins JP, Altman DG, on behalf of the Cochrane Statistical Methods Group. Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, et al., eds. *Cochrane Handbook for Systematic Reviews of Interventions*. 1st ed. Wiley; 2019:241-284. doi:10.1002/9781119536604.ch10

22. Anthony S, Buitendijk SE, Dorrepaal CA, Lindner K, Braat DDM, den Ouden AL. Congenital malformations in 4224 children conceived after IVF. *Hum Reprod*. 2002;17(8):2089-2095. doi:10.1093/humrep/17.8.2089

23. Bonduelle M, Wennerholm UB, Loft A, et al. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Human Reproduction*. 2005;20(2):413-419. doi:10.1093/humrep/deh592

24. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. *N Engl J Med*. 2012;366(19):1803-1813. doi:10.1056/nejmoa1008095

25. El-Chaar D, Yang Q, Gao J, et al. Risk of birth defects increased in pregnancies conceived by assisted human reproduction. *Fertility and Sterility*. 2009;92(5):1557-1561. doi:10.1016/j.fertnstert.2008.08.080

26. Farhi A, Reichman B, Boyko V, et al. Congenital malformations in infants conceived following assisted reproductive technology in comparison with spontaneously conceived infants. *J Matern Fetal Neonatal Med*. 2013;26(12):1171-1179. doi:10.3109/14767058.2013.776535

27. Fujii M, Matsuoka R, Bergel E, van der Poel S, Okai T. Perinatal risk in singleton pregnancies after in vitro fertilization. *Fertil Steril*. 2010;94(6):2113-2117. doi:10.1016/j.fertnstert.2009.12.031

28. Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med*. 2002;346(10):725-730. doi:10.1056/nejmoa010035

29. Halliday JL, Ukoumunne OC, Baker HWG, et al. Increased risk of blastogenesis birth defects, arising in the first 4 weeks of pregnancy, after assisted reproductive technologies. *Human Reproduction*. 2010;25(1):59-65. doi:10.1093/humrep/dep364

30. Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Otterblad PO. Congenital malformations in infants born after in vitro fertilization in Sweden. *Birth Defects Res A Clin Mol Teratol*. 2010;88(3):137-143. doi:10.1002/bdra.20645

31. Koudstaal J, Braat DD, Bruinse HW, Naaktgeboren N, Vermeiden JP, Visser GH. Obstetric outcome of singleton pregnancies after IVF: a matched control study in four Dutch university hospitals. *Hum Reprod*. 2000;15(8):1819-1825. doi:10.1093/humrep/15.8.1819

32. Place I, Englert Y. A prospective longitudinal study of the physical, psychomotor, and intellectual development of singleton children up to 5 years who were conceived by intracytoplasmic sperm injection compared with children conceived spontaneously and by in vitro fertilization. *Fertil Steril*. 2003;80(6):1388-1397. doi:10.1016/j.fertnstert.2003.06.004

33. Qin J, Sheng X, Wu D, et al. Adverse obstetric outcomes associated with in vitro fertilization in singleton pregnancies. *Reprod Sci*. 2017;24(4):595-608. doi:10.1177/1933719116667229

34. Sagot P, Bechoua S, Ferdynus C, et al. Similarly increased congenital anomaly rates after intrauterine insemination and IVF technologies: a retrospective cohort study. *Hum Reprod*. 2012;27(3):902-909. doi:10.1093/humrep/der443

35. Verlaenen H, Cammu H, Derde M, Amy J. Singleton pregnancy after in vitro fertilization: Expectations and outcome. *Obstetrics & Gynecology*. 1995;86(6):906-910. doi:10.1016/0029-7844(95)00322-i

36. Zádori J, Kozinszky Z, Orvos H, Katona M, Kaáli SG, Pál A. The incidence of major birth defects following in vitro fertilization. *J Assist Reprod Genet*. 2003;20(3):131-132. doi:10.1023/a:1022682908307

37. Fedder J, Loft A, Parner ET, Rasmussen S, Pinborg A. Neonatal outcome and congenital malformations in children born after ICSI with testicular or epididymal sperm: a controlled national cohort study. *Human Reproduction*. 2013;28(1):230-240. doi:10.1093/humrep/des377

38. Sánchez Soler MJ, López-González V, Ballesta-Martínez MJ, et al. Risk of mayor and minor birth defects in children conceived by assisted reproductive technology (Ivf/icsi): A prospective controlled cohort study. *An Pediatr (Engl Ed)*. 2021;95(6):448-458. doi:10.1016/j.anpede.2021.06.009

39. Luke B, Brown MB, Wantman E, et al. Risk of prematurity and infant morbidity and mortality by maternal fertility status and plurality. *J Assist Reprod Genet*. 2019;36(1):121-138. doi:10.1007/s10815-018-1333-z

40. Hwang SS, Dukhovny D, Gopal D, et al. Health of infants after art-treated, subfertile, and fertile deliveries. *Pediatrics*. 2018;142(2):e20174069. doi:10.1542/peds.2017-4069

41. Laan M, Kasak L, Punab M. Translational aspects of novel findings in genetics of male infertility—status quo 2021. *British Medical Bulletin*. 2021;140(1):5-22. doi:10.1093/bmb/ldab025

42. Liu CL, Li P, Cai GF, et al. Optimal follow-up duration for assessment of birth defects after in vitro fertilization–embryo transfer: a multicenter 5-year cohort study in china. *Front Endocrinol*. 2022;13:817397. doi:10.3389/fendo.2022.817397

43. Andrade C. Understanding the basics of meta-analysis and how to read a forest plot: as simple as it gets. *J Clin Psychiatry*. 2020;81(5). doi:10.4088/jcp.20f13698

44. Lunny C, Ramasubbu C, Gerrish S, et al. Impact and use of reviews and 'overviews of reviews' to inform clinical practice guideline recommendations: protocol for a methods study. *BMJ Open*. 2020;10(1):e031442. doi:10.1136/bmjopen-2019-031442

45. Harmful maternal illnesses - birth defect prevention. Birth Defect Research for Children. Accessed September 17, 2023. https://birthdefects.org/healthy-baby/maternal-illness/

46. Bar-Oz B, Einarson T, Einarson A, et al. Paroxetine and congenital malformations: Meta-Analysis and consideration of potential confounding factors. *Clinical Therapeutics*. 2007;29(5):918-926. doi:10.1016/j.clinthera.2007.05.003

47. Budenholzer B. Paroxetine use should be avoided during pregnancy. *Am Fam Physician*. 2012;85(8):747-748.

48. Lee LJ, Lupo PJ. Maternal smoking during pregnancy and the risk of congenital heart defects in offspring: a systematic review and metaanalysis. *Pediatr Cardiol*. 2013;34(2):398-407. doi:10.1007/s00246-012-0470-x

49. Zhao L, Chen L, Yang T, et al. Parental smoking and the risk of congenital heart defects in offspring: An updated meta-analysis of observational studies. *Eur J Prev Cardiolog*. 2020;27(12):1284-1293. doi:10.1177/2047487319831367

50. Fetal alcohol syndrome (FAS) (For parents) - nemours kidshealth. Accessed September 17, 2023. https://kidshealth.org/en/parents/fas.html

51. Grant KS, Conover E, Chambers CD. Update on the developmental consequences of cannabis use during pregnancy and lactation. *Birth Defects Research*. 2020;112(15):1126-1138. doi:10.1002/bdr2.1766

52. Van Gelder MMHJ, Donders ART, Devine O, Roeleveld N, Reefhuis J, National Birth Defects Prevention Study. Using bayesian models to assess the effects of under-reporting of cannabis use on the association with birth defects, national birth defects prevention study, 1997-2005: assessing effects of exposure misclassification. *Paediatr Perinat Epidemiol*. 2014;28(5):424-433. doi:10.1111/ppe.12140

53. Viteri O, Soto E, Bahado-Singh R, Christensen C, Chauhan S, Sibai B. Fetal anomalies and long-term effects associated with substance abuse in pregnancy: a literature review. *Amer J Perinatol*. 2014;32(05):405-416. doi:10.1055/s-0034-1393932

54. Smith LM, LaGasse LL, Derauf C, et al. The infant development, environment, and lifestyle study: effects of prenatal methamphetamine exposure, polydrug exposure, and poverty on intrauterine growth. *Pediatrics*. 2006;118(3):1149-1156. doi:10.1542/peds.2005-2564

55. Wouldes T, LaGasse L, Sheridan J, Lester B. Maternal methamphetamine use during pregnancy and child outcome: what do we know? *N Z Med J*. 2004;117(1206):U1180.