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Review Article

Association Between Assisted Reproductive Technology and Cerebral Palsy: A Meta-Analysis

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ABSTRACT

Background: Since 1978 many children are born thanks to assisted reproductive technology (ART). However, the long-term effects of these therapies are still not fully known. Our objective is to evaluate the risk of cerebral palsy (CP) after ART compared with that in those spontaneously conceived (SC) and to examine this risk in single, multiple, and preterm births and the evolution of the risk over the years.
Methods: PubMed, Embase, and Web of Science databases were searched until December 2022. Studies were included if they studied CP cases in children born through ART. 16 studies were finally selected. Quality of studies was assessed using Newcastle Ottawa Scale. Pooled OR was estimated by weighting individual OR/RR by the inverse of their variance. A random-effect model was applied. To assess the causes of heterogeneity, we performed meta-regression analyses.
Results: A significantly high risk of CP was found (OR = 1.27; 95% CI 1.12 to 1.43) in children born through ART compared with those SC. This risk increased in singletons (OR = 1.48; 95% CI 1.23 to 1.79) but disappeared in multiple (OR = 1.05; 95% CI 0.93 to 1.18) and preterm births (OR = 1.09; 95% CI 0.87 to 1.37). We found a higher risk of CP in children born before the year 2000 (OR = 3.40; 95% CI 2.49 to 4.63).
Conclusions: ARTs slightly increase the risk of CP once the effect of multiple gestation is controlled. Further studies are needed to clarify whether the techniques themselves, fertility problems, or associated maternal comorbidities are responsible for this risk.

Introduction

Assisted reproductive technology (ART) is one in which different biomedical techniques are used to facilitate or replace natural fertilization processes. The appearance of these techniques in the 1970s brought new possibilities for conception problems to many couples. Since the first baby was born through *in vitro* fertilization (IVF) in 1978, there has been a rapid increase in the use of these techniques, with more than 8 million children conceived to date worldwide, 2 million in Europe.[1,2] As they have increased, many questions have arisen about the repercussions on the health of the offspring.[3] The investigations carried out have generated

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controversy regarding the development of these children long term.[4] Despite the significant advances that have occurred in the last 40 decades in this field, the effect of ART on the developing human brain is still unclear,[5] as well as the relationship they may have in the development of cerebral palsy (CP).

CP is a group of permanent but not immutable disorders of movement and/or posture and motor function due to nonprogressive interference, injury, or abnormality of the developing/immature brain.[6] The average incidence of CP is estimated to be around 1.5 to 3 per 1000 births, with a decrease in recent years.[7] However, the incidence varies according to the group of patients and depends on several risk factors.[8] These risk factors include prenatal factors, such as multiple gestations or intrauterine growth retardation; perinatal factors, such as prematurity and low birth weight; and postnatal factors. There is evidence that ART is associated with an increased risk of multiple gestations,[9] preterm births, and low birth weight,[10,11] and these have been associated with increased CP rates.[12] The results of the investigations indicate that, contrary to previous beliefs,[13] perinatal pathology is less important in the etiology of CP,[14] estimating that 75% of cases are of prenatal cause.[8] On the other hand, current estimates indicate that up to 30% of cases may have genetic causes.[15] Considering that the procedures employed in ART involve multiple manipulations of gametes and embryos during critical window periods in epigenetic reprogramming, some researchers also question whether ART can induce epigenetic modifications that contribute to neurodevelopmental disorders.[15,16]

The three previously published meta-analyses[17-19] obtained a double overall relative risk of CP after ART approximately. However, none of the included studies were adjusted for the type of pregnancy, so the influence of this factor on that estimation cannot be ruled out. The effect in premature babies shows discrepant results. Table 1 summarizes the main characteristics of these meta-analyses. Several additional observational studies have appeared after the publication of the last meta-analysis,[20-23] which only included articles up to 2019.

The objective of this study is to evaluate the risk of developing CP in children conceived by ART compared with those spontaneously conceived (SC) while controlling for the confounding effect of the type pregnancy and incorporating the new studies conducted since the publication of Wang's meta-analysis. Thus, we will obtain a more valid and reliable measure of the overall effect of these techniques compared with previous meta-analyses. We also evaluated the risk in single and multiple gestations and in preterm infants and the evolution of this risk over time.

Material and Methods

We followed the PRISMA guidelines to report our findings.[24]

Search strategy

Literature search was carried out through Pubmed, Embase, and Web of Science databases. Articles published up to December 2022 were reviewed. The following keywords were used as search terms: (Assisted reproductive techn* OR ART OR ICSI or Intracytoplasmic sperm injection OR IVF OR in vitro fertilization OR ovodonation) AND (cerebral palsy OR brain damage)) AND (offspring OR children OR childhood OR infant OR newborn). In addition, a search for possible articles was carried out, reviewing the references of some of the articles found. Search strategy was carried out independently by two authors (A.C.-I. and J.C.-F.).

Inclusion and exclusion criteria

Studies that met the following criteria were included: studying neurodevelopment and CP cases in children born through ART; study population included children conceived by any ART, without excluding any type; cohort and case-control studies using SC children as a comparison group; and estimated effect calculated in odds ratio (OR), relative risk (RR), or hazard ratio (HR). No language restrictions were applied.

Reviews, systematic reviews, comments, communications to conferences, book chapters or letters to the editor, studies performed with nonhuman samples, and all those articles without information to carry out the analysis were excluded. Applying these criteria and after a complete and exhaustive review, 14 articles were selected2[20,23,25-36]; two more were added through additional searches.[21,22] If more than one paper involved or included the same population, then the most comprehensive and recent study was selected.

Data extraction

A database was created with the relevant information extracted from each article: author, year of publication, country of origin, type of study, type of ART studied, type of pregnancy, type of birth, years of follow-up, RR/OR with its 95% confidence interval (CI), and sample size. The quality of the article was evaluated according to the criteria of the Newcastle Ottawa Scale (NOS).[37] Data extracted from the included studies are shown in Table 2.

TABLE 1.
Characteristics of Previous Meta-Analyses

Author	Year	Number of Included Studies	Provenance of Studies	Design of the Studies Included	Type of Technique Studied	Type of Pregnancy	RR/OR (95% CI)
Wang F[17]	2021	9	Scandinavian countries 5 (Finland 2, Denmark 1, Norway 1, Sweden 1), Australia 3, Israel 1	Case & controls: 2 Cohort: 7	All ART	All Singletons Multiples Preterm	2.17 (1.72-2.74) 1.36 (1.16-1.59) 1.05 (0.86-1.29) 1.53 (0.66-3.56)
Djuwantono T[18]	2020	5	Scandinavian countries 3 (Denmark 2, Sweden 1), Australia 2	Case & control 1 Cohort 4	All ART	All Preterm Birth weight	1.82 (1.41-2.34) 3.01 (2.8-3.24) 2.33 (2.18-2.50)
Hvidtjorn D[19]	2009	9	Scandinavian countries 8 (Finland 1, Sweden 3, Denmark 4), Croatia 1	Cohort: 9	All ART	All Singletons Multiples	2.18 (1.71-2.77) 1.82 (1.31-2.52) 1 (0.65-1.52)

Abbreviations:
ART = Assisted reproductive technology
CI = Confidence interval
OR = Odds ratio
RR = Relative risk

TABLE 2.
Main Characteristics of the Included Articles

Study	Year	Country	Design	Period of Recruitment	Type of ART	Type of Birth	Subpopulation	Follow-up (years)	RR (95% CI)	N (Study Population)	Proportion of CP in Nonexposed*	NOS
Stromberg[36]	2002	Sweden	RC	Pre	IVF	All	Term	2	3.70 (2.04-6.72)	21,077	1.10	7
						Singleton			2.80 (1.33-5.91)		1.36	
						Multiple			0.90 (0.42-1.91)		5.75	
Lidegaard[32]	2005	Denmark	PC	Pre&aft	IVF	Singleton	Term	14	1.80 (1.18-2.75)	448,401	1.85	6
Klemetti[29]	2006	Finland	RC	Pre	IVF	All	Term	4	2.92 (1.63-5.25)	31,404	1.41	7
						Singleton			1.15 (0.40-3.29)		1.29	
						Multiple			1.52 (0.43-5.39)		N/A	
Hvidtjorn[27]	2010	Denmark	PC	Pre&aft	All ART	All	All	5	0.96 (0.76-1.22)	588,967	1.19	8
						Singleton			1.21 (0.90-1.62)		1.76	
						Multiple			1.04 (0.71-1.53)		2.44	
					IVF	All			2.0 (1.51-2.65)		0.97	
						Singleton			1.21 (0.9-1.62)		1.76	
						Multiple			1.07 (0.69-1.65)		5.44	
					OI	All			1.47 (1.09-1.97)		0.86	
						Singleton			1.21 (0.84-1.74		1.76	
						Multiple			1.0 (0.57-1.74)		5.44	
Kallen[28]	2010	Sweden	RC	Pre&aft	IVF	All	All	N/A	1.81 (1.53-2.14)	2,655,104	2.37	7
							Preterm		1.05 (0.78-1.42)		6.87	
						Singleton	All		1.23 (0.96-1.58)		2.25	
						Multiple			0.99 (0.74-1.33)		6.87	
Klemetti[30]	2010	Finland	RC	Pre	All ART	All	Term	4	2.36 (1.27-4.38)	192,488	1.41	8
						Singleton			2.09 (1.03-4.25)		1.29	
						Multiple			1.23 (0.27-5.58)		N/A	
Pinborg[33]	2010	Denmark	PC	Pre&aft	IVF	Singleton	Term	11	2.44 (1.15-5.20)	16,086	Strom	8
Reid[34]	2010	Australia	CC	Pre&aft	All ART	Singleton	Term	N/A	1.19 (0.63-2.24)	3723	Strom	8
Davies[25]	2012	Australia	RC	Pre&aft	All ART	All	Term	N/A	2.66 (1.79-3.95)	308,974	Strom	7
						Singleton			2.22 (1.35-3.64)		Strom	
						Multiple			1.39 (0.69-2.79)		Strom	
Stoknes[35]	2012	Norway	RC	Pre	All ART	All	All	4	4.90 (2.80-8.59)	176,591	Strom	7
							Preterm		2.50 (1.26-4.95)		Strom	
Abdel-Latif[20]	2013	Australia	PC	Pre&aft	All ART	All	Preterm		1.04 (0.66-1.64)	1473	Strom	8
Goldsmith[26]	2018	Australia	RC	Pre&aft	All ART	All	All	5	2.60 (1.64-4.11)	211,660	Strom	7
						Singleton			2.00 (1.06-3.77)		Strom	
						Multiple			1.00 (0.49-2.05)		Strom	
Levin[31]	2019	Israel	RC	Pre&aft	IVF	All	Term	18	0.92 (0.30-2.87)	241,187	Strom	6
Spangmose[22]	2021	Denmark, Sweden, and Finland	RC	Pre&aft	All ART	All	Term	5	1.56 (1.44-1.69)	4,791,195	Strom	8
						Singleton			1.11 (0.99-1.25)		Strom	
						Multiple			1.05 (0.91-1.22)		Strom	
Roychoudhury[21]	2021	Canada	RC	Pre&aft	All ART	All	Preterm	1.5-2	0.71 (0.42-1.20)	4073	Strom	7
Verhaeghe[23]	2022	France	RC	Pre&aft	All ART	All	Preterm	5	1.00 (0.67-1.49)	3031	Strom	8

Abbreviations:
ART = Assisted reproductive technology
CC = Case-control
CI = Confidence interval
CP = Cerebral palsy
IVF = *In vitro* fertilization
N/A = Not available
NOS = Newcastle Ottawa Scale
OI = Ovulation induction
OR = Odds ratio
PC = Prospective cohort
Pre = Recruitment before 2000
RC = Retrospective cohort
RR = Risk ratio
Pre&aft = Recruitment before and after 2000
* Cases per 1000.

Statistical analysis

Pooled OR were estimated by weighting individual OR by the inverse of their variance. A random-effect model was applied given that the number of studies included in the analysis was lower than 25. Heterogeneity was calculated using the Q and I^2 statistics. The presence of publication bias was explored with graphical (funnel plot) and statistical techniques (Egger test). Separately, we analyzed the studies carried out in preterm births.[20,21,23,26-28,35]

Results are displayed as forest plots showing OR and their 95% CI for each individual study and for the pooled result. We developed stratified analysis according to: (1) the type of pregnancy (classified as singleton and multiple), (2) the period of recruitment (considering recruitment as the selection period of the patients for study; on the one hand, studies with data collected before year 2000 and on the other, those collected before and after year 2000), and (3) the quality of the included studies (assessed with the NOS). To assess the causes of heterogeneity, we performed meta-regression analyses including the year of recruitment, the proportion of effect in the reference group, and the quality of the studies as predictor variables. Finally, a sensitivity analysis was also carried out including only high-quality studies (NOS $\geq$7).[38]

All the statistical analyses were carried out with the package Stata 16/SE (Stata Corporation, College Station, TX, USA).

Results

Study characteristics

Our meta-analysis includes 16 studies: 11 retrospective cohort studies,[21-23,25,26,28-31,35,36] four prospective cohort studies,[20,27,32,33] and one case-control study.[34] A flowchart of the literature search process is shown in Fig 1.

Table 2 summarizes the main characteristics of the included articles. These studies were published between 2002 and 2022, although data collection dates are longer, from 1986 in some studies[25] to 2016 in others.[21] As shown in Table 2, most of the studies analyzed (11 publications of 16) work with a population from the Nordic countries.[20-23,25-36] Four studies collect data from live newborns in single gestations[31-34]; three of the included studies[20,21,23] study only preterm newborns of less than 29[20,21] and between 24 and 34 gestational weeks (GW),[23] respectively. Finally, another four studies analyze the risk of CP including a subgroup of premature infants.[26-28,35]

Exposure

Nine of the studies included in this meta-analysis examine the combined effect of different ART techniques[20-23,25-27,34,35] and seven analyze the effect of a specific technique: IVF[27-29,31-33,36] or ovulation induction (OI).[27,30,31]

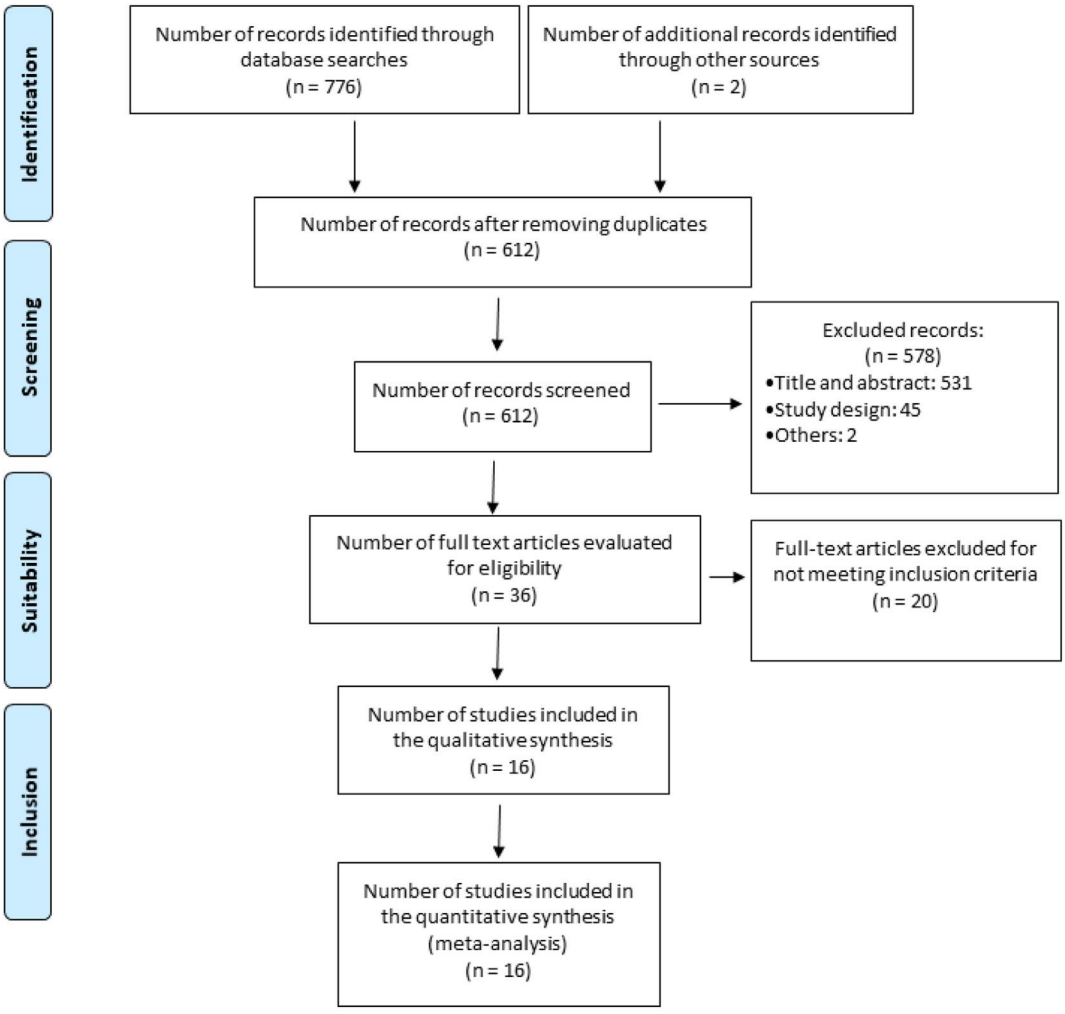

FIGURE 1. Flowchart. The color version of this figure is available in the online edition.

Outcome

13 of the 16 studies[22,25-36] include CP diagnoses from national registries. The diagnostic codes of seven of them follow the International Classification of Diseases (ICD)-10 and ICD-9.[22,28-31,33,36] One of them establishes the diagnoses according to the recommendations of the Surveillance of Cerebral Palsy in Europe[35] and another according to the Birth Paediatric Association,[25] which is a modification of the ICD. Four of them do not refer to the diagnostic criteria followed in their records.[26,27,32,34] Three of the included studies[20,21,23] establish the diagnosis of CP in the selected patients. The studies carried out by Roychoudhury et al.[21] and Verhaeghe et al.[23] follow the diagnostic criteria of the Gross Motor Function System, and the one carried out by Abdel-Latif et al.[20] follows the Griffiths Mental Developmental Scales[39] or Bayley Scales of Infant Development-II4[40] criteria.

Baseline characteristics and potential confounders

Most studies in this meta-analysis found that women undergoing ART had higher maternal age[20-23,25-27,29-31,33] and a greater incidence of pregnancy complications such as hospital treatment during pregnancy,[29,30] diabetes,[20,21,25,31] or urinary tract infections[25,27] compared with those not undergoing ART (natural conceived). More information is collected in Supplementary Table 1. Additionally, studies considering smoking habits[21-23,25,27,28] and socioeconomic factors[21,23,25,27,29,30,34] revealed higher socioeconomic status and fewer mothers smoking during pregnancy in the ART group. To address potential confounding effects related to the mother's condition, most of the studies included maternal age at delivery as an adjustment variable in their analyses. Five studies adjusted for parity.[26,29,30,33,34] However, only two articles[21,31] were adjusted for obstetrics or medical conditions such as diabetes or hypertension. Other variables commonly incorporated for statistical adjustment into the multivariable models were the child's gender,[20-22,25,27,33,34,36] preterm birth,[27,31,34,36] smoking[21-23,25,27,28] or socioeconomic status.[21,23,25,27,29,30,34] Only two of the articles included in the meta-analysis[32,35] did not perform statistical adjustments. A summary of all the confounding factors considered by each article can be found in Supplementary Table 2.

Data analysis

To assess the overall risk of CP we used two different strategies. First, we combined the results of the nine studies that provided an overall analysis of the effect of ART regardless of the type of pregnancy.[22,25-30,35,36] This analysis showed that children conceived by ART had twice the risk of CP than those SC (OR = 2.18, 95% CI 1.68 to 2.82) (Supplementary Figure 1a).

The heterogeneity between the included studies was high (I^2 86.41%, $P < 0.0001$). The funnel plots show asymmetry observing a high risk of publication bias (Egger test: $z = 2.86$ $P = 0.0042$) (Supplementary Figure 1b).

As an alternative analysis, to avoid the confounding effect of multiple gestations, we excluded studies carried out without taking into account the type of pregnancy. With this approach we observed a significant decrease in the strength of association, OR = 1.27 (95% CI 1.12 to 1.43) (Supplementary Figure 2a).

The heterogeneity of the studies included in this case was lower ($I^2 = 37.30\%$, $P = 0.000$). The funnel plots were fairly symmetric (Supplementary Figure 2b), although Egger test cannot rule out a small-study effect (Egger test: $z = 2.87$ $P = 0.004$). Results are shown in Table 3.

To calculate the stratified risk of CP in singletons, results from 12 articles were included,[22,25-34,36] excluding studies on premature population[20,21,23] and those that did not specify the type of pregnancy.[35] A significant risk association was found, OR = 1.48 (95% CI 1.23 to 1.79) (Fig 2).

The strongest association was shown by Strömberg et al.[36] (OR = 2.80 [95% CI 1.33 to 5.91]). Only one of the studies assessed[31] showed a nonstatistically significant protective association between ART and CP in singletons, OR = 0.92 (95% CI 0.30 to 2.87). A moderate heterogeneity was found, $I^2 = 54.03\%$, $P = 0.006$. On the other hand, when evaluating the risk of ART in multiple gestations, we found no association with the development of CP, OR = 1.05 (95% CI 0.93 to 1.18). The eight studies analyzed[22,25-30,36] did not show heterogeneity with $I^2 = 0.0\%$, $P = 0.99$. Only one study[29] obtained an increased risk, not statistically significant, OR = 1.52 (95% CI 0.43 to 5.39). Results are shown in Table 3. Finally, when analyzing the effects of IVF and OI techniques separately, no differences in the strength of the association between IVF and CP were observed (OR 1.29, 95% CI 1.07 to 1.56), and for OI (OR 1.25, 95% CI 0.95 to 1.65), with a test rejecting differences between groups, Q test = 0.02, $P = 0.89$ (Supplementary Figure 3).

CP risk after ART conception according to recruitment period and study quality

We found a progressive decrease in CP risk with evolution over time. For this analysis we grouped the studies according to year of

TABLE 3.
Results From Our Meta-Analysis

Subgroup	Exposition	Number of Studies	Number of Patients	RR (95% CI)	I^2 (%)
Type of birth	All	20	9,337,852	1.27 (1.12-1.43)	37.30
	Singletons	12	9,074,672	1.48 (1.23-1.79)	54.03
	Multiple births	8	263,180	1.05 (0.93-1.18)	0.00
Year of recruitment	Before 2000	6	83,416	1.61 (1.09-2.36)	8.16
	Before & after 2000	14	9,245,935	1.23 (1.09-1.38)	40.19
Quality (singletons)	Moderate (NOS = 6)	2	688,867	1.60 (0.97-2.63)	14.43
	High (NOS ≥7)	10	8,385,805	1.47 (1.20-1.81)	56.40
	All	10	205,493*	1.09 (0.87-1.37)	30.76
Preterm	Singletons	3	12,849*	1.35 (0.70-2.61)	41.15
	Multiple births	2	3363	0.83 (0.37-1.85)	0.00
Extreme preterm		5	11,334	0.99 (0.72-1.37)	36.44

Abbreviations:
ART = Assisted reproductive technology
CI = Confidence interval
CP = Cerebral palsy
NOS = Newcastle Ottawa Scale
RR = Risk ratio
CP risk after ART conception according to type of gestation.
* Data from one study[28] not available.

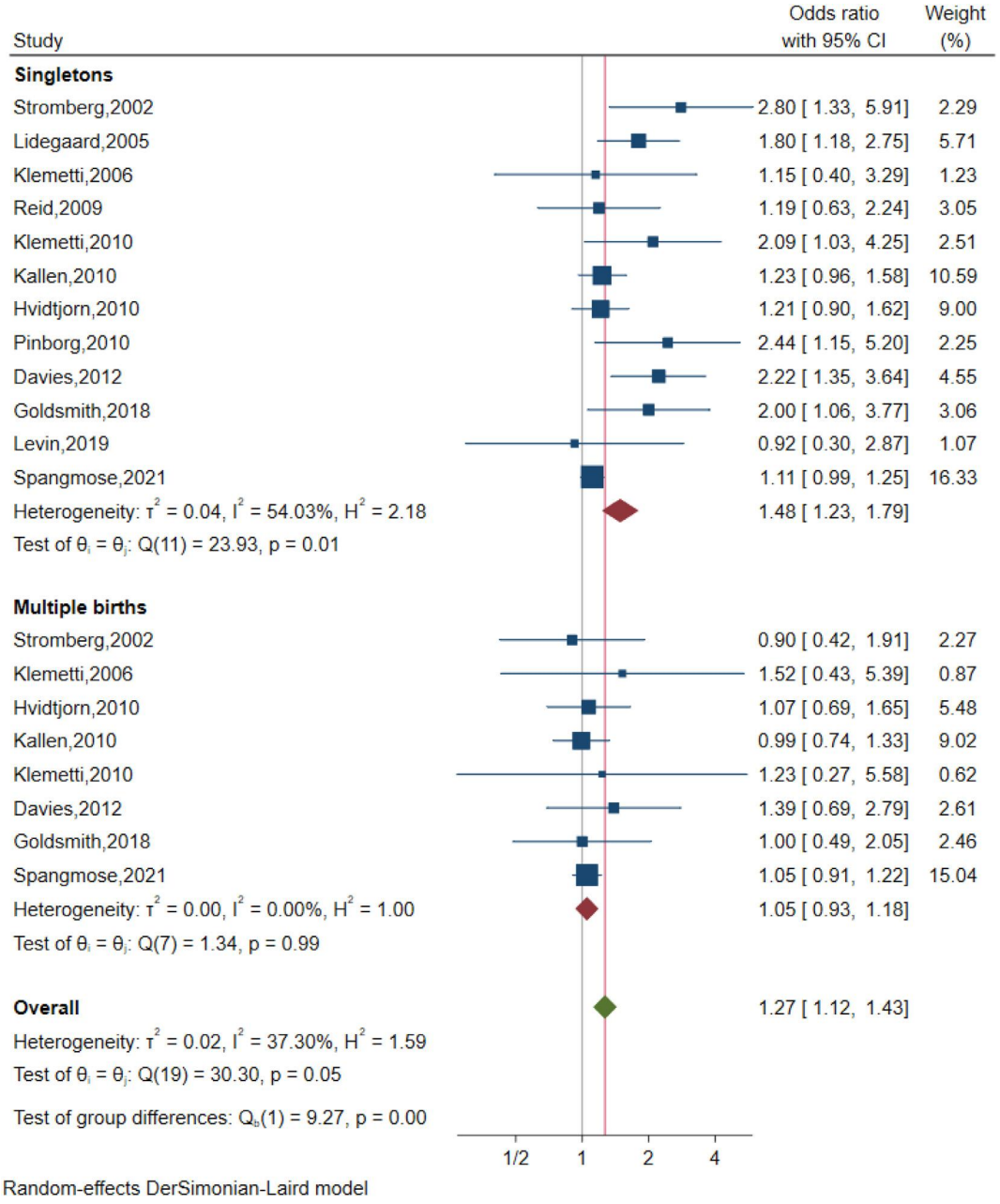

FIGURE 2. Forest plot of the association between assisted reproductive technology and cerebral palsy by type of pregnancy (single or multiple birth). The color version of this figure is available in the online edition.

recruitment: on the one hand, studies with participant recruitment before year 2000 exclusively[29,30,36] and on the other, studies with recruitment of participants for study before and after year 2000, thus overlapping both periods.[22,25-28,31-34] To avoid the confounding effect of the type of birth we performed the analysis selecting only studies in which the type of birth is controlled. Studies conducted on premature population were excluded.[20,21,23] Risk found for studies with recruitment before year 2000 was multiplied by 1.61 (95% CI 1.09 to 2.36), presenting in the combined studies a low heterogeneity, I^2 8.16%, $P = 0.36$. The strongest risk association was provided by Stromberg et al.[36] with an OR 2.80 (95% CI 1.33 to 5.91). On the other hand, when separately analyzing the studies with recruitment in both periods (before and after year 2000), a decrease in the strength of association is noted, OR = 1.23 (95% CI 1.09 to 1.38), with a heterogeneity of $I^2 = 40.19\%$, $P = 0.06$. The highest risk was shown by Pinborg et al.[33] with an OR = 2.44 (95% CI 1.15 to 5.40). Results are shown in Table 3 and Fig 3.

Besides, when evaluating the influence of the quality of the included studies, we observe that when combining the results of high-quality studies (NOS ≥7) a clearly lower strength of association between ART and CP in singletons is obtained than the one observed when combining studies with moderate quality (NOS = 6), OR = 1.47, 95% CI (1.20 to 1.81) if NOS ≥7 vs OR = 1.60, 95% CI (0.97 to 2.63) if NOS = 6 (Supplementary Figure 4).

CP risk after ART conception and preterm birth

When the analysis is performed selecting exclusively studies with premature population no increased risk of CP after ART was observed, OR = 1.09 (95% CI 0.87 to 1.37).[20,21,23,26,28,35] No

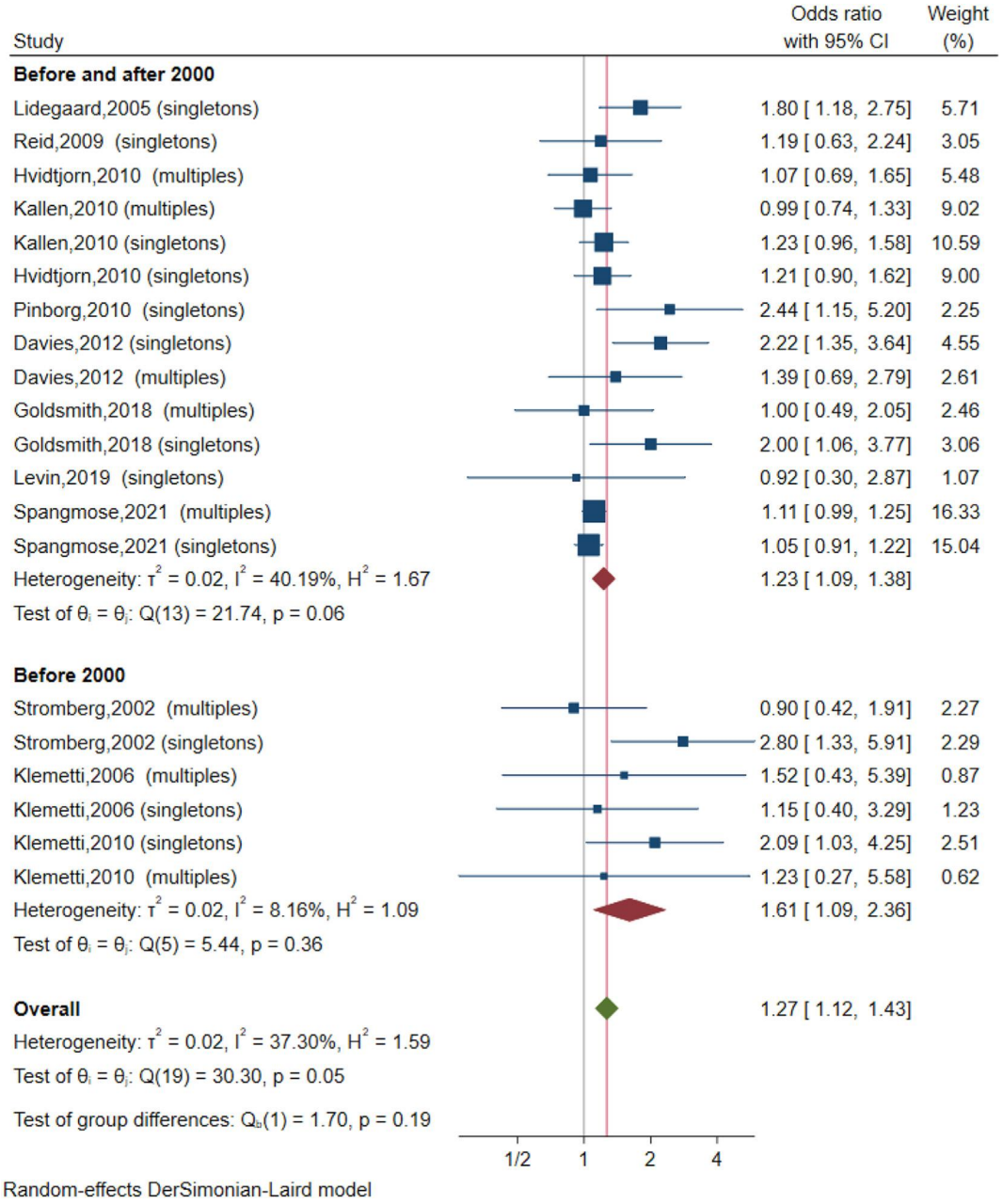

FIGURE 3. Forest plot of the association between assisted reproductive technology and cerebral palsy by period of recruitment. The color version of this figure is available in the online edition.

significant heterogeneity was observed between the included studies, I^2 30.76%, $P = 0.16$, and risk of publication bias, Egger test: $z = 0.91$ $P = 0.36$ (Fig 4).

To identify the effect in very preterm infants, we repeated the analysis, including only studies carried out in preterm infants born at less than 32 GW.[20,21,23,26] We also found no risk modification in this subpopulation, OR = 0.99 (95% CI 0.72 to 1.37). No heterogeneity (I^2: 0%) or risk of publication bias was observed in this subgroup (Egger test z: 0.81, P: 0.417).

In both the global and the stratified analysis, the most important risk association was shown by Goldsmith et al.,[26] OR = 2.70 (95% CI 1.03 to 7.09) (Supplementary Figure 5).

We carried out an analysis by type of gestation; four studies with premature population were included, regardless of the gestational age at birth.[20,21,23,35] A nonsignificant higher risk was found in premature singleton, OR = 1.35 (95% CI 0.70 to 2.61). No risk of publication bias was observed (Egger z test = 0.91, P: 0.361). We found no association for preterm twins after conception with ART, with an estimated OR = 0.83 (95% CI 0.37 to 1.85). This latter analysis was performed using the estimated risks in the different subpopulations of premature twins provided by Goldsmith et al.[26] (Supplementary Figure 6). Results are shown in Table 3.

Meta-regression analysis

Finally, to assess the potential causes of heterogeneity in the developed analyses, a meta-regression was performed, including as predictors the proportion of the effect in the unexposed group, the year of recruitment, and the quality of the articles included. Globally, the three factors explained 64.3% of the variability observed;

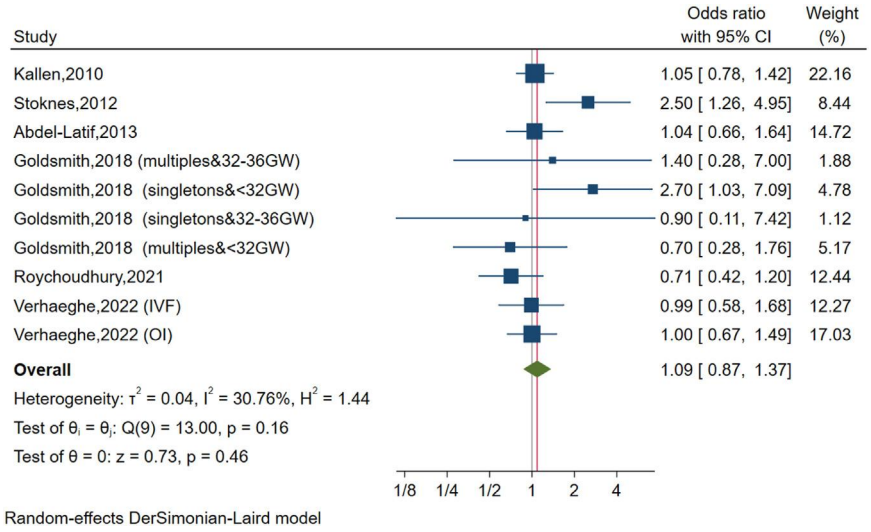

FIGURE 4. Forest plot of the association between assisted reproductive technology and cerebral palsy in preterm birth conceived with assisted reproductive technology. The color version of this figure is available in the online edition.

only the proportion of CP in SC children presented a significant negative association with the OR. Results are shown in Table 4.

Discussion

According to our results, children conceived with ART have a slightly increased risk of developing CP compared with SC children. However, the effect size of this association is comparatively smaller than that obtained in previous meta-analyses.[17-19] One possible explanation for this disparity is that the majority of published articles analyzing the relationship between ART and CP did not adequately control for the confounding effect of pregnancy type. When jointly analyzing the studies carried out without taking into account the type of pregnancy, the risk is doubled. However, this association is reduced to 27% by including in the analysis only studies that separately evaluate the effect of ART in single or multiple gestations.

In singletons conceived through ART, we observed a 48% increased risk of CP. However, we did not find an increased risk in children born from multiple gestations. These results confirm those provided by the three meta-analyses previously published on this matter.[17-19] Nowadays, the impact of ART on singletons remains uncertain. Some studies suggest an increased risk of perinatal adverse effects, including CP,[2,32,36] whereas others report no association.[29,31] The underlying etiology for the increased risk of CP is not well-understood but is likely multifactorial in origin. Possible explanations include subfertility or infertility that leads couples to resort to ART, the "vanishing embryo syndrome," and the ART procedures themselves.[17,19]

Infertility is a frequent health problem and is considered a possible factor involved in the increased risk of CP. Numerous studies have investigated its repercussion at different levels. Some authors[2,29,33,34,41-44] consider that subfertility itself contributes significantly to the risk of adverse effects, including those of the central nervous system, observed in children conceived after ART. Increased risk has been noted even in infertile women who achieved pregnancy without treatment.[29,34,43] However, other studies neither find a significant influence of infertility on the association between ART and CP[12,44] nor have they been able to determine whether the increased risk of adverse perinatal outcomes is due to ART or to the inherent infertility of couples undergoing these treatments.[45,46] Another possible cause related to the increased risk of CP in singletons is embryonic and fetal death (or vanishing embryo syndrome).[47] The incidence of this phenomenon after ART is estimated to be around 10% to 30%,[10] or even higher.[48] Although the literature suggests that the surviving twin is at increased risk of negative health outcomes, including increased risk of CP,[49,50] there is controversy. A few authors[26,34] did not observe any cases of vanishing embryo syndrome among the children with CP who were studied or of CP when eliminating this possible phenomenon by single embryo transfer (SET). It has also been pointed out that the survivor's prognosis would be fundamentally determined by the moment in which this phenomenon occurs, when later a greater risk of perinatal adverse effects and higher rates of neurological sequelae were found.[48,50] Although the vanishing embryo

TABLE 4.
Meta-Regression Analysis

Possible Causes of Heterogeneity	Coeff. (95% CI)	τ^2	Adj. R^2 (%)	I^2
Model		0.011	40.08	21.82%
High quality (NOS ≥7)	−0.18 (−0.65 to 0.28)			
Period of recruitment before 2000	−0.22 (−0.19 to 0.62)			
Proportion of CP in nonexposed	−40.88 (−77.6 to −4.13)			

Abbreviations:
Adj. = Adjusted
CI = Confidence interval
Coeff. = Coefficient
CP = Cerebral palsy
NOS = Newcastle Ottawa Scale

syndrome is not the sole explanation for the higher risk of CP in singletons,[32,41,45] several studies recommend SET to minimize the incidence and neonatal morbidity.[10,11,17,22,28,29,41,50-52]

Multiple gestations are often recognized as a significant risk factor in the occurrence of CP after ART procedures1[17,53]; some researchers consider that these techniques do not increase the risk of CP that multiple gestations themselves already have. Instead, these authors highlight the influence of various factors that impact the neurodevelopment of twins, including those conceived through IVF, such as prematurity,[11] fetal growth restrictions, and zygosity.[5,54] Our findings provide support for the latter argument by demonstrating no elevated risk of CP in cases of multiple gestation. Nevertheless, literature shows discrepancies[9,10,55,56] on the risk of ART versus spontaneous twins. It is important to highlight, however, that studies often do not take zygosity into account, despite the fact that monozygosity is associated with an increased risk of CP.[9,57,58]

In our meta-analysis, when separately analyzing studies carried out in premature infants, no association between ART and CP was observed. This result reinforces the hypothesis that ART does not increase the risk of CP that preterm births already have. As we mentioned, prematurity is a potential confounding factor in the relationship between ART and CP; it is one of the known causes of long-term neurological disability[59,60] and is also associated with ART.[61] In line with our findings, a recent review emphasizes the role of prematurity as a confounding factor when evaluating the impact of ART on the development of CP.[62] In our analysis, we did not find a risk modification in preterm infants even when stratifying according to the type of pregnancy (single or multiple). Just one study[26] showed a high and significant risk of CP in ART singletons born at less than 32 GW. Stoknes et al.[35] observed that the risk of CP increased when more combined risk factors were present. The authors concluded that CP was the result of a sequence of events among preterm infants, whereas in term infants, individual vulnerability played a more important role.

Another relevant finding in our study has been the progressive decrease in the risk of CP after ART over time. Around the year 2000, a growing body of research and the implementation of reproductive policies in various countries started endorsing the use of SET. This approach aimed to effectively reduce the occurrence of multiple gestations and its inherent risks associated.[63,64] Studies that recruited participants before the year 2000[29,30,36] show a stronger association compared with studies involving children recruited both before and after that period.[22,25-28,31,32,34,50] It is important to highlight that in future investigations involving only participants recruited after the year 2000, the risk could potentially be even lower. Our results are consistent with other research[22,28,65] showing a trend toward a progressive decrease in the risk of CP over time, probably related to the use of SET. In addition, use of SET has been facilitated by improvements in culture media and in morphologic selection of embryos, which have made it possible to obtain embryos of higher quality and better prognosis and thus achieve a successful pregnancy.[66] However, these are probably not the only influential factors since we also have to consider the evolution toward better knowledge and use of ART, the improvement in health care, and the development of new diagnostic tools.[67]

Our meta-analysis is, to date, the one that includes the largest number of studies[20-23] and adds data on populations not included in previous publications of this type.[17,18,27] This fact has allowed us to separately analyze the effect of ART in subpopulations such as preterm infants, included only in one previous meta-analysis,[17] thus controlling the confounding effect of this variable. We also analyzed the evolution of the risk of CP in children conceived after ART over time according to the year of recruitment of the patients, data not provided to date in any other meta-analysis. We have also controlled by type of pregnancy, which has allowed us to improve the validity of our estimations. Last, our meta-analysis is the first published study to use meta-regression to assess the causes of heterogeneity.

Our study has some limitations. First, it was impossible to separate the effect of the technique itself from that of other confounding factors, such as zygosity in multiple gestations or underlying subfertility. This limitation is due to the heterogeneity in the variables collected in the few existing studies. Second, it has not been possible to investigate in depth the effect of each type of technique separately. Most of the articles do not specify the type of technique, considering several within the term ART, or compare the effect of one with another instead of comparing with the SC population. Third, none of the studies included in the analysis provided data on the relationship between ART and CP stratified by maternal age or medical conditions. Therefore, it was not possible to incorporate this information into the meta-analysis. Finally, generalizing the findings of the included studies, primarily from Nordic countries, requires caution due to differing socioeconomic conditions.

In conclusion, ART slightly increases the risk of developing CP once the effect of multiple gestations is controlled. Also, a slight decrease in this risk is observed over time, which may be related to the increasing use of single transfer of embryos. Based on these results, clinicians could provide couples with information regarding the small increase in CP and reinforce the use of single transfer of embryos technique. Additionally, advancements in ART are leading to safer pregnancies, which may also contribute to the reduction of costs of these treatments, both at individual and public levels. Further research is needed to identify whether the risk of CP after ART is caused by the techniques used themselves, underlying fertility issues, or other maternal health conditions.

Declaration of competing interest

The authors have no conflicts of interest to disclose.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pediatrneurol.2023.12.019.

References

1. De Geyter C, Calhaz-Jorge C, Kupka MS, et al. ART in Europe, 2015: results generated from European registries by ESHRE. Hum Reprod Open. 2022;2020:1–20.
2. Graham ME, Jelin A, Hoon AH, Wilms Floet AM, Levey E, Graham EM. Assisted reproductive technology: short- and long-term outcomes. Dev Med Child Neurol. 2023;65:38–49.
3. Supramaniam P, Mittal M. Assisted reproductive technology: where did the journey begin, and where are we today – a 40-year history. World J Gynecol Womens Health. 2018;1:10–12.
4. Bay B, Mortensen EL, Kesmodel US. Assisted reproduction and child neurodevelopmental outcomes: a systematic review. Fertil Steril. 2013;100:844–853.
5. Middelburg KJ, Heineman MJ, Bos AF, Hadders-Algra M. Neuromotor, cognitive, language and behavioural outcome in children born following IVF or ICSI-a systematic review. Hum Reprod Update. 2008;14:219–231.
6. Bax M, Goldstein M, Rosenbaum P, et al. Proposed definition and classification of cerebral palsy, April 2005. Dev Med Child Neurol. 2005;47:571–576.
7. Sellier E, Platt MJ, Andersen GL, et al. Decreasing prevalence in cerebral palsy: a multi-site European population-based study, 1980 to 2003. Dev Med Child Neurol. 2016;58:85–92.
8. Sadowska M, Sarecka-Hujar B, Kopyta I. Cerebral palsy: current opinions on definition, epidemiology, risk factors, classification and treatment options. Neuropsychiatr Dis Treat. 2020;16:1505–1518.
9. Qin J-B, Sheng X-Q, Wu D, et al. Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. Arch Gynecol Obstet. 2017;295:285–301.

10. Broughton DE, Jungheim ES. Multiple gestations and assisted reproductive technology. In: Avery's Diseases of the Newborn, Vol. 2011. 10th ed. Seattle, WA: Elsevier; 2018:70–77.
11. Blickstein I. Cerebral palsy in multifoetal pregnancies. Dev Med Child Neurol. 2002;44:352–355.
12. Hvidtjørn D, Grove J, Schendel DE, et al. Cerebral palsy among children born after in vitro fertilization: the role of preterm delivery—a population-based, cohort study. Pediatrics. 2006;118:475–482.
13. Nielsen LF, Schendel D, Grove J, et al. Asphyxia-related risk factors and their timing in spastic cerebral palsy. BJOG. 2008;115:1518–1528.
14. Verdú A. Parálisis Cerebral Infantil. In: Manual de Neurología infantil. 2ª. Spain: Médica Panamericana; 2014:287–298.
15. Fahey MC, Maclennan AH, Kretzschmar D, Gecz J, Kruer MC. The genetic basis of cerebral palsy. Dev Med Child Neurol. 2017;59:462–469.
16. La Rovere M, Franzago M, Stuppia L. Epigenetics and neurological disorders in ART. Int J Mol Sci. 2019;20:4169.
17. Wang FF, Yu T, Chen XL, Luo R, Mu DZ. Cerebral palsy in children born after assisted reproductive technology: a meta-analysis. World J Pediatr. 2021;17:364–374.
18. Djuwantono T, Aviani JK, Permadi W, Achmad TH, Halim D, Review AS. Risk of neurodevelopmental disorders in children born from different ART treatments: a systematic review and meta-analysis. J Neurodev Disord. 2020;12:1–12.
19. Hvidtjørn D, Schieve L, Schendel D, Jacobsson B, Sværke C, Thorsen P. Cerebral palsy, autism spectrum disorders, and developmental delay in children born after assisted conception. Arch Pediatr Adolesc Med. 2009;163:72.
20. Abdel-Latif ME, Bajuk B, Ward M, Oei JL, Badawi N. Neurodevelopmental outcomes of extremely premature infants conceived after assisted conception: a population based cohort study. Arch Dis Child Fetal Neonatal Ed. 2013;98:F205–F211.
21. Roychoudhury S, Lodha A, Synnes A, et al. Neurodevelopmental outcomes of preterm infants conceived by assisted reproductive technology. Am J Obstet Gynecol. 2021;225:276.e1–276.e9.
22. Spangmose AL, Christensen LH, Henningsen AA, et al. Cerebral palsy in ART children has declined substantially over time: a Nordic study from the CoNARTaS group. Hum Reprod. 2021;36:2358–2370.
23. Verhaeghe C, Marchand-Martin L, Kaminski M, et al. Neurodevelopment at 5 years of age for preterm-born children according to mode of conception: a cohort study. Am J Obstet Gynecol. 2022;227:606.e1–606.e21.
24. Kamioka H. Preferred reporting items for systematic review and meta-analysis protocols (prisma-p) 2015 statement. Jpn Pharmacol Ther. 2019;47:1177–1185.
25. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. N Engl J Med. 2012;366:1803–1813.
26. Goldsmith S, Mcintyre S, Badawi N, Hansen M. Cerebral palsy after assisted reproductive technology: a cohort study. Dev Med Child Neurol. 2018;60:73–80.
27. Hvidtjorn D, Grove J, Schendel D, et al. Multiplicity and early gestational age contribute to an increased risk of cerebral palsy from assisted conception: a population-based cohort study. Hum Reprod. 2010;25:2115–2123.
28. Källén AJ, Finnström O, Lindam AP, Nilsson EME, Nygren KG, Otterblad Olausson PM. Cerebral palsy in children born after in vitro fertilization. Is the risk decreasing? Eur J Paediatr Neurol. 2010;14:526–530.
29. Klemetti R, Sevóna T, Gissler M, et al. Health of children born as a result of in vitro fertilization. Pediatrics. 2006;118:1819–1827.
30. Klemetti R, Sevón T, Gissler M, et al. Health of children born after ovulation induction. Fertil Steril. 2010;93:1157–1168.
31. Levin S, Sheiner E, Wainstock T, et al. Infertility treatments and long-term neurologic morbidity of the offspring. Am J Perinatol. 2019;36:949–954.
32. Lidegaard Ø, Pinborg A, Andersen AN. Imprinting diseases and IVF: Danish National IVF cohort study. Hum Reprod. 2005;20:950–954.
33. Pinborg A, Loft A, Aaris Henningsen A-K, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995–2006. Fertil Steril. 2010;94:1320–1327.
34. Reid SM, Jaques AM, Susanto C, Breheny S, Reddihough DS, Halliday JANE. Cerebral palsy and assisted reproductive technologies: a case-control study. Dev Med Child Neurol. 2010;52:e161–e166.
35. Stoknes M, Andersen GL, Elkamil AI, et al. The effects of multiple pre- and perinatal risk factors on the occurrence of cerebral palsy. A Norwegian register based study. Eur J Paediatr Neurol. 2012;16:56–63.
36. Strömberg B, Dahlquist G, Ericson A, Finnström O, Köster M, Stjernqvist K. Neurological sequelae in children born after in-vitro fertilisation: a population-based study. Lancet. 2002;359:461–465.
37. Wells G, Shea B, O'Connell D, et al. Newcastle Ottawa scale. Coding Manual for Case-Control Studies 2013. Available at: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed July 3, 2023.
38. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol. 2010;25:603–605.
39. Pino MC, Donne IL, Vagnetti R, Tiberti S, Valenti M, Mazza M. Using the Griffiths Mental Development Scales to evaluate a developmental profile of children with autism spectrum disorder and their symptomatologic severity. Child Psychiatry Hum Dev. 2022. https://doi.org/10.1007/s10578-022-01390-z. Online ahead of print.
40. Gauthier SM, Bauer CR, Messinger DS, Closius JM. The Bayley Scales of infant development. II: where to start? J Dev Behav Pediatr. 1999;20:75–79.
41. Pinborg A, Wennerholm UB, Romundstad LB, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update. 2013;19:87–104.
42. Romundstad LB, Romundstad PR, Sunde A, et al. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. Lancet. 2008;372:737–743.
43. Zhu JL, Basso O, Obel C, Bille C, Olsen J. Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. BMJ. 2006;333:679.
44. Zhu JL, Hvidtjørn D, Basso O, et al. Parental infertility and cerebral palsy in children. Hum Reprod. 2010;25:3142–3145.
45. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Hum Reprod Update. 2012;18:485–503.
46. Rumbold AR, Sevoyan A, Oswald TK, Fernandez RC, Davies MJ, Moore VM. Impact of male factor infertility on offspring health and development. Fertil Steril. 2019;111:1047–1053.
47. Pharoah POD, Cooke RWI. A hypothesis for the aetiology of spastic cerebral palsy - the vanishing twin. Dev Med Child Neurol. 2008;39:292–296.
48. Li Y-X, Sun T, Lv M, et al. Is vanishing twin syndrome associated with adverse obstetric outcomes of ART singletons? A systematic review and meta-analysis. J Assist Reprod Genet. 2020;37:2783–2796.
49. Hvidtjorn D, Grove J, Schendel D, et al. Short communication: `Vanishing embryo syndrome' in IVF/ICSI. Human Reproduction. 2005;20:2550–2551.
50. Pinborg A, Lidegaard Ø, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. Hum Reprod. 2005;20:2821–2829.
51. Thurin A, Hausken J, Hillensjo?? T, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. Obstet Gynecol Surv. 2005;60:376–377.
52. Bergh C, Kamath MS, Wang R, Lensen S. Strategies to reduce multiple pregnancies during medically assisted reproduction. Fertil Steril. 2020;114:673–679.
53. Bonellie SR, Currie D, Chalmers J. Comparison of risk factors for cerebral palsy in twins and singletons. Dev Med Child Neurol. 2005;47:587–591.
54. Briana DD, Malamitsi-Puchner A. Twins and neurodevelopmental outcomes: the effect of IVF, fetal growth restriction, and preterm birth. J Matern Fetal Neonatal Med. 2019;32:2256–2261.
55. Pinborg A, Loft A, Schmidt L, Greisen G, Rasmussen S, Andersen AN. Neurological sequelae in twins born after assisted conception: controlled national cohort study. BMJ. 2004;329:311.
56. Minakami H, Sayama M, Honma Y, et al. Lower risks of adverse outcome in twins conceived by artificial reproductive techniques compared with spontaneously conceived twins. Hum Reprod. 1998;13:2005–2008.
57. Hack KEA, Derks JB, Elias SG, et al. Increased perinatal mortality and morbidity in monochorionic versus dichorionic twin pregnancies: clinical implications of a large Dutch cohort study. BJOG. 2007;115:58–67.
58. Hack KEA, Koopman-Esseboom C, Derks JB, et al. Long-term neurodevelopmental outcome of monochorionic and matched dichorionic twins. PLoS One. 2009;4:e6815.
59. Himpens E, Van den Broeck C, Oostra A, Calders P, Vanhaesebrouck P. Prevalence, type, distribution, and severity of cerebral palsy in relation to gestational age: a meta-analytic review. Dev Med Child Neurol. 2008;50:334–340.
60. Fuchs F, Senat M-V. Multiple gestations and preterm birth. Semin Fetal Neonatal Med. 2016;21:113–120.
61. Wang R, Shi Q, Jia B, et al. Association of preterm singleton birth with fertility treatment in the US. JAMA Netw Open. 2022;5:e2147782.
62. Gullo G, Scaglione M, Cucinella G, et al. Impact of assisted reproduction techniques on the neuro-psycho-motor outcome of newborns: a critical appraisal. J Obstet Gynaecol. 2022;42:2583–2587.
63. Van Landuyt L, Verheyen G, Tournaye H, Camus M, Devroey P, Van Steirteghem A. New Belgian embryo transfer policy leads to sharp decrease in multiple pregnancy rate. Reprod Biomed Online. 2006;13:765–771.
64. Vilska S, Tiitinen A, Hydén-Granskog C, Hovatta O. Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. Hum Reprod. 1999;14:2392–2395.
65. Finnstrom O, Kallen B, Lindam A, Nilsson E, Nygren K-G, Olausson PO. Maternal and child outcome after in vitro fertilization - a review of 25 years of population-based data from Sweden. Acta Obstet Gynecol Scand. 2011;90:494–500.
66. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Reprint of: Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril. 2019;112:e81–e84.
67. Sermon K, Van Steirteghem A, Liebaers I. Preimplantation genetic diagnosis. Lancet. 2004;363:1633–1641.