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Maternal and perinatal outcomes in twin pregnancies following assisted reproduction: a systematic review and meta-analysis involving 802 462 pregnancies

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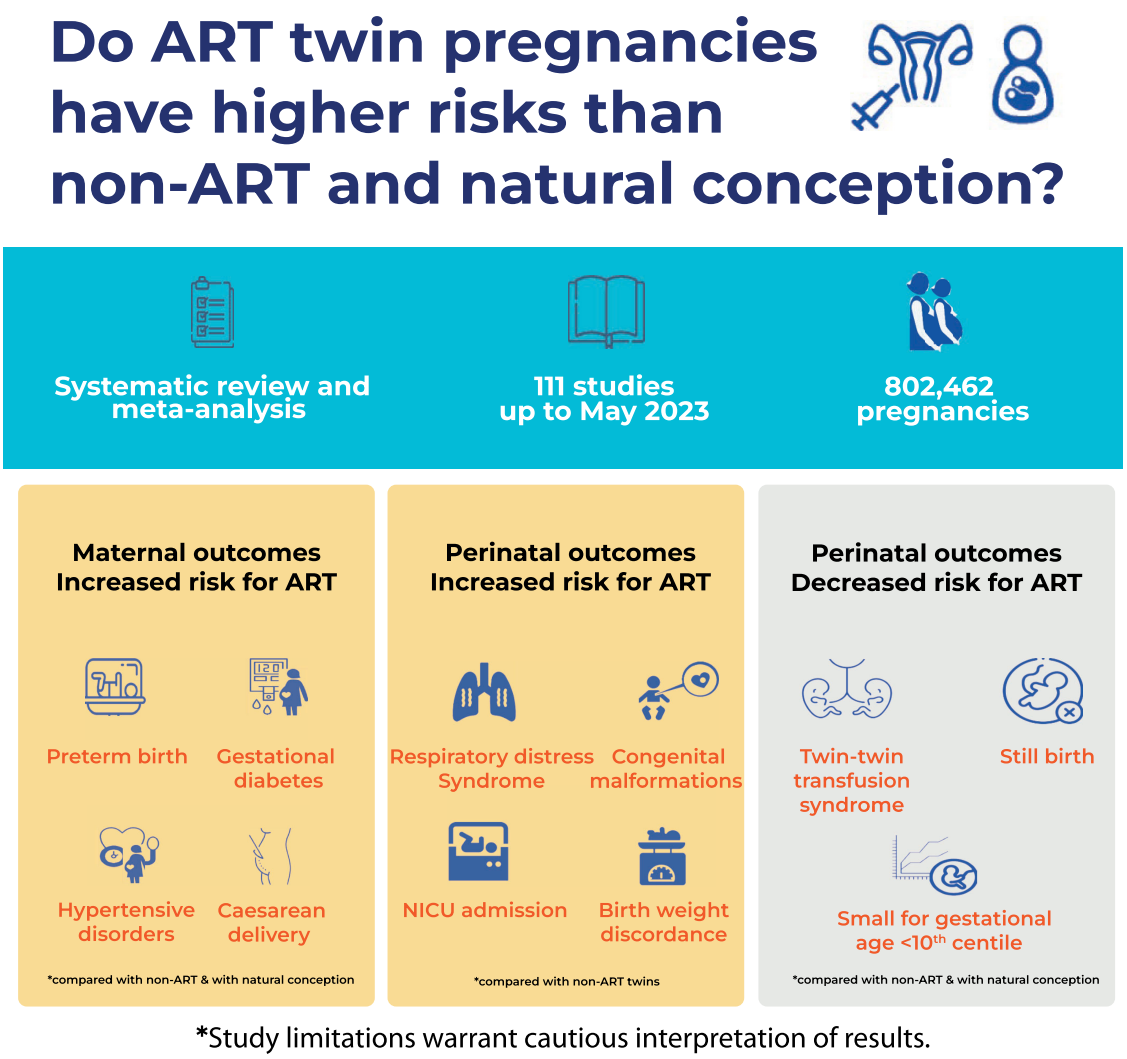
GRAPHICAL ABSTRACT



ART twin pregnancies have higher adverse maternal outcomes; perinatal outcomes vary. NICU: neonatal intensive care unit.

ABSTRACT

BACKGROUND: ART is associated with higher rates of twin pregnancies than singleton pregnancies. Whether twin pregnancies conceived following ART have additional maternal and neonatal complications compared with non-ART twin pregnancies is not known.

OBJECTIVE AND RATIONALE: The objective was to quantify the risk of adverse maternal and perinatal outcomes among twin pregnancies conceived following ART compared with non-ART and natural conception. Existing reviews vary in the reported outcomes, with many studies including triplet pregnancies in the study population. Therefore, we aimed to perform an up-to-date review with an in-depth analysis of maternal and perinatal outcomes limited to twin pregnancies.

SEARCH METHODS: We searched electronic databases MEDLINE and EMBASE from January 1990 to May 2023 without language restrictions. All cohort studies reporting maternal and perinatal outcomes following ART compared with non-ART twin pregnancies and natural conception were included. Case–control studies, case reports, case series, animal studies, and *in vitro* studies were excluded. The Newcastle–Ottawa Scale was used to assess the methodological quality of the studies. Using random-effects meta-analysis, the estimates were pooled and the findings were reported as odds ratios (OR) with 95% CI.

OUTCOMES: We included 111 studies (802 462 pregnancies). Twin pregnancies conceived following ART were at higher risk of preterm birth at <34 weeks (OR 1.33, 95% CI 1.14–1.56, 29 studies, $I^2 = 73\%$), <37 weeks (OR 1.26, 95% CI 1.19–1.33, 70 studies, $I^2 = 76\%$), hypertensive disorders in pregnancy (OR 1.29, 95% CI 1.14–1.46, 59 studies, $I^2 = 87\%$), gestational diabetes mellitus (OR 1.61, 95% CI 1.48–1.75, 51 studies, $I^2 = 65\%$), and caesarean delivery (OR 1.80, 95% CI 1.65–1.97, 70 studies, $I^2 = 89\%$) compared with non-ART twins. The risks for the above maternal outcomes were also increased in the ART group compared with natural conception. Of the perinatal outcomes, ART twins were at significantly increased risk of congenital malformations (OR 1.17, 95% CI 1.05–1.30, 39 studies, $I^2 = 59\%$), birthweight discordance (>25% (OR 1.31, 95% CI 1.05–1.63, 7 studies, $I^2 = 0\%$)), respiratory distress syndrome (OR 1.32, 95% CI 1.09–1.60, 16 studies, $I^2 = 61\%$), and neonatal intensive care unit admission (OR 1.24, 95% CI 1.14–1.35, 32 studies, $I^2 = 87\%$) compared with non-ART twins. When comparing ART with natural conception, the risk of respiratory distress syndrome, intensive care admissions, and birthweight discordance >25% was higher among the ART group. Perinatal complications, such as stillbirth (OR 0.83, 95%

CI 0.70–0.99, 33 studies, $I^2 = 49\%$), small for gestational age <10th centile (OR 0.90, 95% CI 0.85–0.95, 26 studies, $I^2 = 36\%$), and twin–twin transfusion syndrome (OR 0.45, 95% CI 0.25–0.82, 9 studies, $I^2 = 25\%$), were reduced in twin pregnancies conceived with ART versus those without ART. The above perinatal complications were also fewer amongst the ART group than natural conception.

WIDER IMPLICATIONS: ART twin pregnancies are associated with higher maternal complications than non-ART pregnancies and natural conception, with varied perinatal outcomes. Women seeking ART should be counselled about the increased risks of ART twin pregnancies and should be closely monitored in pregnancy for complications. We recommend exercising caution when interpreting the study findings owing to the study's limitations.

Keywords: twins / multiple pregnancy / ART / non-ART / maternal outcomes / neonatal outcomes / offspring outcomes

Introduction

ART significantly contributes to the global increase in multiple pregnancies (Blondel *et al.*, 2002; Adamson *et al.*, 2013). Although trends in multiple births have reduced between 2014 and 2018 (Baxi and Kaushal 2008; Vayssière *et al.*, 2011) owing to single embryo transfer and refinements in IVF techniques, the twin pregnancy rate remains high among women undergoing ART (Khalil 2021). Compared to singleton pregnancies, twin pregnancies are associated with an increase in both maternal and perinatal morbidity and mortality (Obiechina *et al.*, 2011; Chiwanga *et al.*, 2014; Santana *et al.*, 2018), with the risks of adverse outcomes being 4-fold higher for women with twins and 5-fold higher for their offspring than singleton pregnancies (Baxi and Kaushal 2008; Santana *et al.*, 2016). Despite recent reports showing a reduction in stillbirth and neonatal mortality in twins, attributable to advances in antenatal care, invasive procedures for managing complicated monochorionic twin pregnancies, and improved neonatal care, the increased risk for twins compared to singletons remains high (Kilby *et al.*, 2019; Draper *et al.*, 2019, 2021). It is essential to establish if twin pregnancies conceived following ART have additional risks than twin pregnancies conceived naturally. This information is vital in counselling and providing targeted care, including close surveillance and appropriate management.

Existing studies vary in the reported risks of adverse maternal and perinatal outcomes in pregnancies conceived following ART (Dhont *et al.*, 1997; Daniel *et al.*, 2000; Isaksson *et al.*, 2002; Pinborg *et al.*, 2004a,b; Adler-Levy *et al.*, 2007; Boulet *et al.*, 2008; Joy *et al.*, 2008; Weghofer *et al.*, 2009; Suzuki and Miyake 2010; Wen *et al.*, 2010; Hansen *et al.*, 2012; Caserta *et al.*, 2014; Pourali *et al.*, 2016; Hack *et al.*, 2018). Existing systematic reviews and meta-analyses include a small number of studies, provide imprecise estimates, inappropriately include cohorts with triplet pregnancies, and report on a limited number of maternal and offspring outcomes (McDonald *et al.*, 2005; Qin *et al.*, 2015; Qin *et al.*, 2017). Owing to the lack of robust evidence, current guidelines on twin pregnancies provide recommendations for managing all twin pregnancies without considering the mode of conception or the magnitude of the risks.

This systematic review quantifies the risks of maternal and perinatal complications among twin pregnancies conceived by ART compared with non-ART and natural conception to provide a more comprehensive and up-to-date comparison.

Methods

We conducted a systematic review using a prospective protocol complying with standard guidelines on reporting. The PROSPERO ID of the protocol of this study is CRD42020185228.

Search strategy and study selection criteria

We searched the electronic databases Medline and Embase without language restrictions to identify potentially eligible studies on maternal and perinatal outcomes in twin gestations following assisted reproduction, published from January 1990 to May 2023. We used the search terms 'twin pregnancy', and 'multiple pregnancies' and combined them with terms for ART such as 'Intra Cytoplasmic Sperm Injection', 'In Vitro Fertilization', 'Gamete Intra Fallopian Transfer', or 'Zygote Intra Fallopian Transfer'. We additionally included terms for individual maternal and offspring outcomes and combined them with the above terms. The search terms were 'exploded' where applicable. Supplementary File S1 outlines the search strategy. The reference lists of all individual studies and previously published systematic reviews were manually searched to supplement the electronic search.

We followed a two-stage process in study selection. Initially, we screened the citations based on the title and the abstract to select citations suitable for full-text evaluation. The chosen citations' full texts were screened in the second stage, and studies satisfying the inclusion criteria were included. Two reviewers (Shemoon Marleen and R.N.) carried out this process independently, and any disagreements were resolved by consensus after discussion with another reviewer (S.T.).

We included all cohort studies with monochorionic or dichorionic twin pregnancies that evaluated maternal or perinatal outcomes among those conceived following and without ART. All fertility treatments involving *in vitro* manipulation of both oocyte and sperm, such as IVF, ICSI, gamete intrafallopian transfer (GIFT), or zygote intrafallopian transfer, were included in the ART group. All pregnancies conceived naturally or following fertility treatment other than ART, such as ovulation induction and IUI with or without controlled ovarian hyperstimulation, were considered non-ART. The natural conception group included twin pregnancies conceived without fertility treatment.

We excluded case reports, case series, case–control studies, *in vitro* studies, and animal studies. Studies in which the ART group included ovulation induction and IUI with or without controlled ovarian hyperstimulation where data for ART conception could not be extracted separately were also excluded. We excluded data where maternal outcomes were presented as the number of neonates and neonatal outcomes were presented as the number of pregnancies. We accepted the primary study authors' definitions, thresholds, or stratifications for the evaluated outcomes. We accepted any method for the estimation of gestational age.

The maternal outcomes evaluated in the review are preterm birth (PTB) <28 weeks, PTB <32 weeks, PTB <34 weeks, PTB <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, gestational diabetes mellitus (GDM), diabetes in pregnancy, antepartum haemorrhage (APH), placenta previa, placental abruption, postpartum haemorrhage (PPH), and caesarean delivery. The perinatal outcomes that were assessed are stillbirth, neonatal death, perinatal mortality, small for gestational age (SGA) <10th centile, SGA <5th centile, birthweight discordance >25%, twin-to-twin transfusion syndrome (TTTS), any congenital malformation, major congenital malformations,

APGAR <7 at 5 min, neonatal intensive care unit admission, respiratory distress syndrome (RDS), mechanical ventilation, neonatal sepsis, necrotizing enterocolitis (NEC), and neurological complications. Intraventricular haemorrhage, neonatal jaundice, neonatal hypoglycaemia, hypoxic–ischaemic encephalopathy, and umbilical cord pH <7.2 were grouped under 'Other offspring morbidity'.

Study quality assessment and data extraction

Two independent reviewers (S.M. and R.N.) used the Newcastle–Ottawa Scale to assess the methodological quality of the included studies (Wells *et al.*, 2014) The risk of bias in selection, comparability, and outcome assessment of cohorts was evaluated, and stars were allotted for adherence to pre-determined criteria. Studies that scored four stars for selection, two stars for comparability between the cohorts, and three stars for ascertainment of outcome were considered low risk of bias. Studies that scored two or three stars for selection, one for comparability and two for ascertainment of outcomes, were regarded to have a medium risk of bias. Any study that scored one for selection or outcome ascertainment or zero for any of the three domains was considered to have a high risk of bias (Viale *et al.*, 2015).

Two independent reviewers (S.M. and W.K.) extracted and recorded data on a customized data extraction sheet (Supplementary File S2). Dichotomous data were extracted on 2×2 tables. We emailed the authors for relevant data if the data reported in the original article was insufficient. We included the most recent study if multiple studies were published for the same outcomes from the same cohort of subjects.

Statistical analysis

We calculated the estimates of the individual studies using random-effects meta-analysis and presented the summary estimates as odds ratios (OR) with 95% CI. I^2 statistics were used to gauge the study heterogeneity. We performed a sensitivity analysis by limiting the meta-analysis to dichorionic twins and for spontaneous PTB. An additional sensitivity analysis was conducted excluding the studies with a high risk of bias. Owing to significant advances in reproductive techniques and changes in obstetric care over time, we performed a subgroup analysis comparing outcomes for studies published before 2010 with those published after 2010. A further subgroup analysis between fresh embryo transfer versus frozen embryo transfer cycles was carried out to compare the differences in outcomes. Meta-regression analysis was conducted to adjust the estimated effect on maternal and perinatal outcomes by confounders such as maternal age, parity, and maternal BMI. For outcomes evaluated in >10 studies, we assessed publication bias and the effect of small studies using funnel plots and Egger's tests (Matthias *et al.*, 1997). The Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) approach was used to assess the evidence level of the studies included in the review. Analyses were performed using RevMan (Cochrane, 2014) and Stata 13.0 (StataCorp, 2013).

Results

From 4496 citations, we included 111 studies (802 462 pregnancies). Figure 1 demonstrates the study selection process through the PRISMA flow diagram.

Characteristics of the included studies

Of the 111 studies, 93 were retrospective cohorts, 17 were prospective cohorts, and 1 study was a secondary analysis of a randomized controlled trial. Most studies (84.7%, 94/111) were conducted in high-income countries, while 14 were conducted in upper-middle-income countries. The highest number of studies were carried out in China, followed by the USA and Denmark. Most studies (90%, 100/111) were published after 2000. The sample sizes of the study population ranged from 32 pregnancies (Petersen *et al.*, 1995) to 343 876 pregnancies (Wang *et al.*, 2021).

Forty-eight (43%) of the included studies explicitly reported the exclusion of complicated twin pregnancies such as major foetal anomalies (12 studies), TTTS (5 studies), monochorionic twin pregnancies (25 studies), monoamniotic twin pregnancies (27 studies), chromosomal abnormalities (2 studies), selective foetal reduction (27 studies), and stillbirth (9 studies). Most (97%) studies had included an unselected population of symptomatic and asymptomatic women for preterm labour (108/111).

The ART group included IVF with or without ICSI in most studies (77%, 84/111), GIFT was included in six studies (Bernasko *et al.*, 1997; Ochsenkuhn *et al.*, 2003; Smithers *et al.*, 2003; Kuwata *et al.*, 2004; Hansen *et al.*, 2009; Marino *et al.*, 2014). Four studies stated the use of only frozen embryo transfer cycles (Isaksson *et al.*, 2002; Sagot *et al.*, 2012; Yang *et al.*, 2014; Henningsen *et al.*, 2018), and five studies specified the use of only fresh embryo transfer cycles (Koudstaal *et al.*, 2000; Koivurova *et al.*, 2002; Katalinic *et al.*, 2004; Kuwata *et al.*, 2004; Fedder *et al.*, 2012). Two studies reported data separately for fresh and frozen embryo transfer cycles (Wennerholm *et al.*, 1997; Marino *et al.*, 2014). The rest of the studies did not state the use of either fresh or frozen embryo transfer cycles or both. Five studies reported data after excluding oocyte donation (Malchau *et al.*, 2013; Marino *et al.*, 2014; Bordi *et al.*, 2017; Guilbaud *et al.*, 2017; Algeri *et al.*, 2020). The most commonly reported maternal outcomes were PTB <37 weeks and caesarean delivery (70/111), while any congenital malformation was the most frequently reported perinatal outcome (39/111).

Supplementary Table S1 gives the details of the characteristics of the included studies.

Quality of the included studies

Figure 2 depicts the quality of the included studies. Eighty-one percent of the studies (90/111) were at low risk of bias for study selection, 44% (49/111) were at low risk for comparability, and 97% (108/111) were at low risk for study outcome. Twenty-one studies (19%) had a medium risk of bias for study selection, 23 studies (21%) for comparability, and 3 studies (3%) had a medium risk of bias for outcome assessment. None of the studies showed a high risk of bias for selection and outcome. However, 39 studies (35%) were found to have an increased risk of bias with regard to comparability.

Maternal outcomes in twin pregnancies following assisted reproduction

Women with twin pregnancies conceived by ART were at a significantly higher risk of delivering preterm before 34 weeks (OR 1.33, 95% CI 1.14–1.56, 29 studies) and before 37 weeks (OR 1.26, 95% CI 1.19–1.33, 70 studies) compared to women who conceived via non-ART methods. An increased risk was observed for gestational hypertension (OR 1.35, 95% CI 1.20–1.51, 37 studies), pre-eclampsia (OR 1.35, 95% CI 1.20–1.52, 23 studies), and hypertensive disorders in pregnancy, which included gestational hypertension, pre-eclampsia, and chronic hypertension (OR 1.29, 95% CI 1.14–1.46, 59 studies) among ART twin pregnancies compared with non-ART twin pregnancies. An increased risk was also observed for GDM (OR 1.61, 95% CI 1.48–1.75, 51 studies) and diabetes in pregnancy which comprised both GDM and

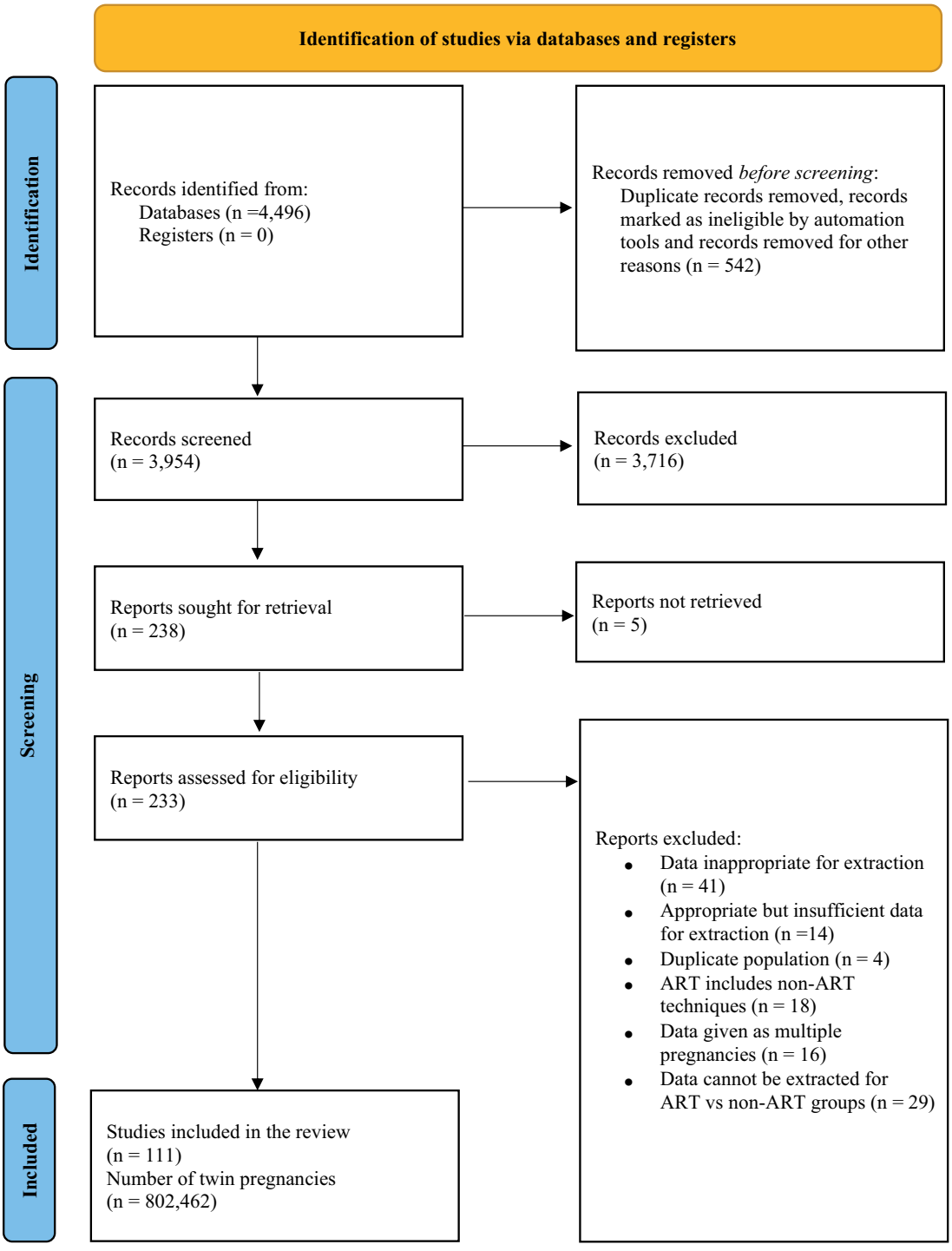


Figure 1. The study selection process in the systematic review of maternal and perinatal outcomes in twin pregnancies following assisted reproduction.

pre-existing diabetes (OR 1.58, 95% CI 1.45–1.73, 52 studies). When considering placental disorders, the risks of APH (OR 1.77, 95% CI 1.26–2.47, 10 studies), placenta previa (OR 1.98, 95% CI 1.73–2.28, 29 studies), and placental abruption (OR 1.10, 95% CI 1.03–1.17, 31 studies) were significantly higher in women who conceived by ART than in those who conceived by non-ART methods. The odds of PPH (OR 1.48, 95% CI 1.31–1.69, 25 studies) and caesarean section (OR 1.80, 95% CI 1.65–1.97, 70 studies) were also significantly higher in ART than in non-ART pregnancies. Table 1 summarizes these results. All of the above outcomes studied, except for pre-eclampsia and placenta previa, were also higher in ART twin pregnancies when compared to naturally conceived twin pregnancies (Table 2). The absolute differences in risk for main maternal outcomes have been included in Supplementary Table S2 (ART versus non-ART) and Supplementary Table S3 (ART versus natural conception).

Subgroup, sensitivity, and meta-regression analysis

When comparing studies published before versus after 2010, a significantly higher association was seen in studies published before 2010 between ART and PTB <28, <34, and <37 weeks when comparing ART with non-ART groups. However, no other

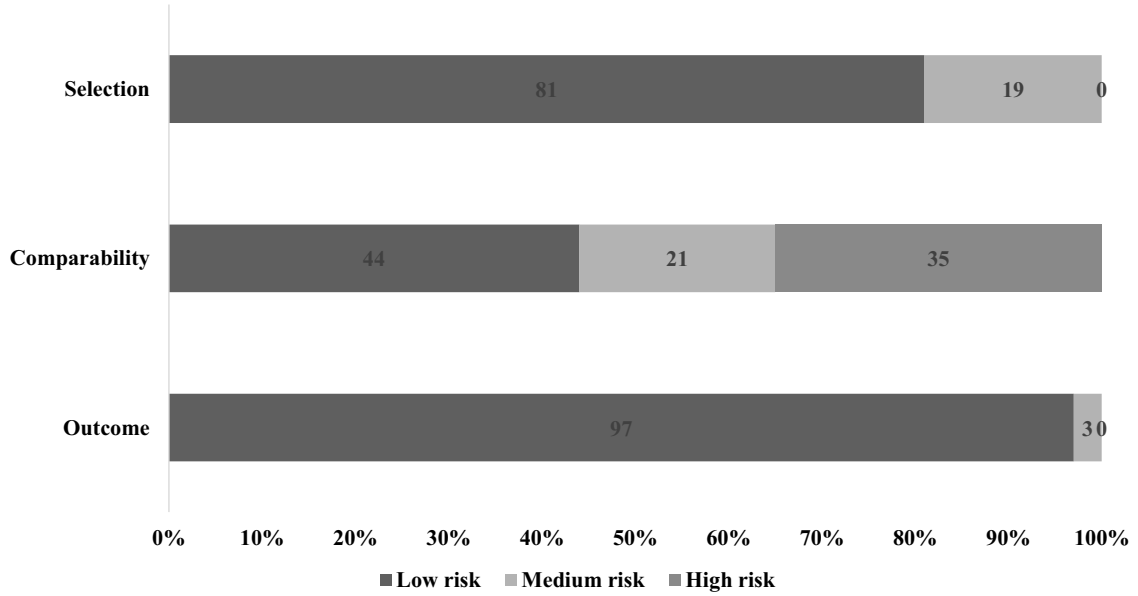


Figure 2. **Quality assessment using the Newcastle–Ottawa Scale in the systematic review of maternal and perinatal outcomes in twin pregnancies following assisted reproduction.**

Table 1. Pooled odds ratios for maternal outcomes comparing ART versus non-ART in the systematic review of maternal and perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No. studies	ART		Non-ART		*P*-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
PTB <28 weeks	23	939	26 616	6777	185 308	0.41	1.11 [0.87, 1.43]	65
PTB <32 weeks	47	2611	28 352	6252	71 791	0.10	1.11 [1.00, 1.24]	65
PTB <34 weeks	29	1673	7610	3474	17 319	0.02	1.33 [1.14, 1.56]	73
PTB <37 weeks	70	53 037	122 028	138 507	351 694	<0.00001	1.26 [1.19, 1.33]	76
Gestational hypertension	37	10 848	123 390	34 090	456 813	<0.00001	1.35 [1.20, 1.51]	88
Pre-eclampsia	23	12 024	94 898	30 004	270 283	<0.00001	1.35 [1.20, 1.52]	37
Hypertensive disorders in pregnancy	59	8629	40 905	31 251	207 072	<0.0001	1.29 [1.14, 1.46]	87
GDM	51	22 342	123 705	46 487	457 374	<0.00001	1.61 [1.48, 1.75]	65
Diabetes in pregnancy	52	23 550	123 042	51 163	456 924	<0.00001	1.58 [1.45, 1.73]	68
APH	10	321	6885	532	17 234	0.0009	1.77 [1.26, 2.47]	59
Placenta previa	29	3983	104 403	5925	293 314	<0.00001	1.98 [1.73, 2.28]	14
Placental abruption	31	1432	100 465	3625	280 231	<0.004	1.10 [1.03, 1.17]	0
PPH	25	11 762	97 126	22 304	269 907	<0.00001	1.48 [1.31, 1.69]	54
Caesarean delivery	70	100 482	122 468	253 854	341 793	<0.00001	1.80 [1.65, 1.97]	89

P-value <0.05 is considered statistically significant.
APH, antepartum haemorrhage; GDM, gestational diabetes mellitus; OR, odds ratios; PPH, postpartum haemorrhage; PTB, preterm birth.

differences between the subgroups were observed for the remaining maternal outcomes comparing studies published before 2010 versus those after 2010. In the ART versus natural conception comparison, a significant difference was observed only for the association between ART and PTB before 28 weeks and 37 weeks (Supplementary Tables S4 and S5). A further subgroup analysis was performed comparing fresh versus frozen embryo transfer cycles; no significant differences were observed for any maternal outcomes between ART versus non-ART pregnancies and ART versus naturally-conceived pregnancies (Supplementary Tables S6 and S7).

Sensitivity analysis excluding monochorionic twin pregnancies showed an increased risk of PTB <37 weeks, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes in pregnancy, APH, placenta previa, PPH, and caesarean delivery in mothers who conceived by ART when compared with non-ART twin pregnancies (Supplementary Table S8). Compared with natural conception, ART mothers were at a higher risk for the same outcomes except for APH and PPH, and additionally for placental abruption, with monochorionicity excluded (Supplementary Table S9). Limiting the analysis to studies that reported spontaneous PTBs showed a higher risk of spontaneous preterm delivery at <34 weeks in ART pregnancies when compared to non-ART conception and natural conception (Supplementary Tables S10 and S11). Sensitivity analysis performed after excluding studies with a high risk of bias showed that ART twin pregnancies were at a higher risk of PTB <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes in pregnancy, placenta previa, placental abruption, PPH, and caesarean delivery when compared with non-ART twins. The risks were also increased for the same outcomes when compared with natural conception except for placental abruption (Supplementary Tables S12 and S13).

The effect of ART on hypertensive disorders of pregnancy, GDM, PTB <34 weeks, and PTB <37 weeks was independent of maternal age. However, the effect of ART on caesarean section

Table 2. Pooled odds ratios for maternal outcomes comparing ART versus natural in the systematic review of maternal and perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No. studies	ART		Natural		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
PTB <28 weeks	18	787	20 706	6052	159 693	0.42	1.16 [0.81, 1.65]	68
PTB <32 weeks	38	1504	16 027	2878	28 161	0.1	1.14 [0.98, 1.32]	67
PTB <34 weeks	21	1139	5818	1970	10 404	0.02	1.24 [1.04, 1.49]	66
PTB <37 weeks	52	10 411	18 185	17 817	33 496	<0.00001	1.33 [1.21, 1.47]	73
Gestational hypertension	30	6409	32 962	21 827	178 421	0.0002	1.37 [1.16, 1.62]	88
Pre-eclampsia	19	757	7381	821	8618	0.13	1.35 [1.14, 1.61]	27
Hypertensive disorders in pregnancy	27	5763	26 497	24 057	166 740	0.0002	1.44 [1.18, 1.75]	87
GDM	46	4296	33 898	13 802	180 053	0.0002	1.67 [1.50, 1.85]	49
Diabetes in pregnancy	45	4096	32 460	15 007	177 646	<0.00001	1.60 [1.44, 1.77]	39
APH	7	140	3946	102	6778	<0.00001	2.55 [1.86, 3.50]	5
Placenta previa	23	410	11 461	205	15 498	0.06	2.00 [1.54, 2.59]	34
Placental abruption	27	276	12 688	314	19 188	0.0007	1.36 [1.14, 1.62]	0
PPH	23	1398	10 144	1359	10 984	<0.00001	1.46 [1.24, 1.71]	44
Caesarean delivery	56	15 120	21 303	22 167	38 518	<0.00001	1.99 [1.76, 2.25]	84

P-value <0.05 is considered statistically significant.
APH, antepartum haemorrhage; GDM, gestational diabetes mellitus; OR, odds ratios; PPH, postpartum haemorrhage; PTB, preterm birth.

Table 3. Pooled odds ratios for perinatal outcomes comparing ART versus non-ART in the systematic review of maternal and perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No. studies	ART		Non-ART		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
Stillbirth	33	568	52 375	2431	152 985	0.04	0.83 [0.70, 0.99]	49
Neonatal death	30	663	44 267	1950	120 616	0.55	1.06 [0.88, 1.28]	55
Perinatal mortality	21	904	36 886	2668	89 099	0.46	0.92 [0.74, 1.15]	75
SGA <10th centile	26	6998	37 971	16 356	81 784	0.0002	0.90 [0.85, 0.95]	36
SGA <5th centile	4	4696	30 714	20 919	132 777	0.30	0.88 [0.69, 1.12]	70
Birth weight discordance >25%	7	157	1862	348	6020	0.01	1.31 [1.05, 1.63]	0
TTTS	9	19	1209	224	4178	0.009	0.45 [0.25, 0.82]	25
Any congenital malformation	39	3804	69 145	11 304	218 152	0.004	1.17 [1.05, 1.30]	59
Major congenital malformations	8	1818	33 319	6226	145 385	0.06	1.26 [0.99, 1.61]	69
APGAR <7 at 5 min	29	2841	57 208	19 788	369 617	0.55	1.06 [0.88, 1.27]	82
NICU admission	32	29 751	65 787	159 255	390 601	<0.00001	1.24 [1.14, 1.35]	87
RDS	16	915	8386	889	9435	0.0008	1.32 [1.09, 1.60]	61
Mechanical ventilation	8	1399	14 483	4053	35 093	0.21	1.17 [0.91, 1.50]	72
Neonatal sepsis	11	149	3838	215	4838	0.47	1.12 [0.82, 1.53]	31
NEC	7	69	4996	54	4826	0.22	1.39 [0.82, 2.35]	32
Neurological complications	2	42	1104	43	1780	0.03	1.61 [1.04, 2.48]	0

P-value <0.05 is considered statistically significant.
NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; OR, odds ratios; RDS, respiratory distress syndrome; SGA, small for gestational age; TTTS, twin-to-twin transfusion syndrome.

was significantly associated with maternal age ($P = 0.022$). The effect of ART on hypertensive disorders of pregnancy and PTB <34 weeks was independent of nulliparity. However, the effect of ART on GDM, PTB <37 weeks, and caesarean section was significantly higher when the proportion of nulliparous women in the ART group was higher. None of the evaluated maternal outcomes was significantly associated with the maternal BMI (Supplementary Fig. S1).

Perinatal outcomes in twin pregnancies following assisted reproduction

Twins conceived by ART were at a significantly higher risk of congenital malformation (OR 1.17, 95% CI 1.05–1.30, 39 studies), birthweight discordance (OR 1.31, 95% CI 1.05–1.63, 7 studies), neonatal intensive care admission (OR 1.24, 95% CI 1.14–1.35, 32 studies), neonatal RDS (OR 1.32, 95% CI 1.09–1.60, 16 studies), and neurological complications (OR 1.61, 95% CI 1.04–2.48, 2 studies) when compared to twins conceived by non-ART methods. We observed a reduction in the risk of stillbirth (OR of 0.83, 95% CI 0.70–0.99, 33 studies), SGA <10th centile (OR 0.90, 95% CI 0.85–0.95, 26 studies), and twin–twin transfusion syndrome (OR 0.45, 95% CI 0.25–0.82, 9 studies) in twins conceived by ART when compared to non-ART twins. ART twins did not show any significant increase in odds compared to non-ART for outcomes such as neonatal death, perinatal mortality, SGA <5th centile, major congenital malformation, APGAR <7 at 5 min, mechanical ventilation, neonatal sepsis, and NEC. Table 3 summarizes the results. The increased or decreased risk in ART twins for the above outcomes followed a similar trend compared with naturally conceived twins, except for congenital malformation, where no significant difference was observed (Table 4). The absolute risk differences for main perinatal outcomes when comparing ART twins with non-ART twins and ART twins with naturally conceived twins are shown in Supplementary Tables S14 and S15, respectively.

Intraventricular haemorrhage, neonatal jaundice, neonatal hypoglycaemia, hypoxic–ischaemic encephalopathy (HIE), and

Table 4. Pooled odds ratios for perinatal outcomes comparing ART versus natural in the systematic review of maternal and perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No. studies	ART		Natural		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
Stillbirth	25	316	34 752	1167	90 577	0.01	0.78 [0.65, 0.95]	29
Neonatal death	24	377	27 719	974	63 868	0.47	1.09 [0.87, 1.37]	48
Perinatal mortality	17	615	25 474	1365	49 272	0.66	0.94 [0.71, 1.24]	76
SGA <10th centile	25	6985	37 899	15 257	76 055	<0.0001	0.90 [0.85, 0.94]	28
SGA <5th centile	4	4696	30 714	20 919	132 777	0.30	0.88 [0.69, 1.12]	70
Birth weight discordance >25%	7	157	1862	322	5542	0.01	1.32 [1.06, 1.64]	0
TTTS	7	6	893	120	2946	0.02	0.35 [0.14, 0.87]	28
Any congenital malformation	34	1054	28 326	1726	44 960	0.18	1.12 [0.95, 1.34]	63
Major congenital malformations	4	173	2310	334	6781	0.62	1.22 [0.56, 2.68]	86
APGAR <7 at 5 min	28	2672	52 628	18 365	336 607	0.71	1.04 [0.85, 1.28]	83
NICU admission	29	26 656	60 077	140 499	352 363	<0.0001	1.22 [1.11, 1.34]	87
RDS	14	901	8244	857	8987	0.01	1.30 [1.05, 1.59]	66
Mechanical ventilation	8	1399	14 483	3478	30 185	0.27	1.15 [0.90, 1.47]	71
Neonatal sepsis	9	145	3696	204	4390	0.56	1.11 [0.78, 1.58]	44
NEC	7	69	4996	52	4658	0.24	1.38 [0.81, 2.36]	33
Neurological complications	2	42	1104	34	1708	0.004	1.95 [1.23, 3.09]	0

P-value <0.05 is considered statistically significant.
NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; OR, odds ratios; RDS, respiratory distress syndrome; SGA, small for gestational age; TTTS, twin-to-twin transfusion syndrome.

umbilical cord pH <7.2 were grouped as other offspring morbidities (Supplementary Tables S16 and S17). We observed a significant reduction in odds for HIE when comparing ART to non-ART neonates (OR 0.43, 95% CI 0.20–0.92, 1 study). However, none of the other evaluated outcomes showed a significant difference in odds when ART twins were compared with non-ART or naturally conceived twins.

Subgroup, sensitivity, and meta-regression analysis

A significant increase in risk for SGA <5th centile and NEC was observed among ART twins when compared with non-ART twins in studies published before 2010 versus studies published after 2010. The risk of NEC was also higher among ART twins in studies published before 2010 than among naturally conceived twins. Other perinatal outcomes did not differ between the subgroups in studies published before 2010 and after 2010 when comparing ART twins with non-ART twins and natural conception (Supplementary Tables S18, S19, S20, and S21). No significant difference was observed in the subgroup analysis comparing fresh versus frozen embryo transfers for ART versus non-ART twins. Significantly higher odds of major congenital malformations for frozen embryo transfer cycles were seen when comparing ART with naturally conceived twins. However, only one study was available for comparison (Supplementary Tables S22 and S23).

The sensitivity analysis excluding monochorionicity showed higher odds of birthweight discordance, neonatal intensive care (NICU) admission, and RDS among ART neonates compared with non-ART twins. The trend in risk was similar for the same outcomes except for NICU admission when compared with naturally conceived neonates (Supplementary Tables S24 and S25). As for other offspring morbidities, significantly increased odds were observed only for neonatal jaundice among ART twins than in non-ART conception (Supplementary Tables S26 and S27). When the meta-analysis was performed after excluding studies with a high risk of bias, significantly higher odds for NICU admission and a lower risk of APGAR <7 at 5 min were observed among ART twins compared to non-ART twins. The risk reduction for the latter outcome was observed when ART twins were compared with naturally conceived twins as well (Supplementary Tables S28 and S29).

Meta-regression showed that maternal age, nulliparity, and maternal BMI were not significantly associated with the effect of ART on stillbirth, NICU admission, SGA <10th centile, and congenital malformations (Supplementary Fig. S2).

Small study effects

Funnel plot asymmetry was assessed for outcomes with at least 10 studies. There was evidence of small studies effect (Egger test of asymmetry) for pre-eclampsia ($P = 0.010$), GDM ($P = 0.016$), and diabetes in pregnancy ($P = 0.029$) in the comparisons with non-ART twins, and for PTB before 37 weeks ($P = 0.001$), and caesarean section ($P = 0.029$) when compared to natural conception (Supplementary Fig. S3).

Discussion

Main findings

Twin pregnancies conceived following ART have significantly higher adverse maternal and perinatal outcomes than non-ART twin pregnancies. Women with twin pregnancies conceived by ART are more likely to deliver preterm, develop medical complications including hypertensive disorders and GDM, develop placental disorders, and deliver by caesarean section. Their newborns are at an increased risk of congenital malformation, birthweight discordance, and neonatal morbidity requiring admission to neonatal intensive care units. Twins born by ART are less likely to be stillborn, be SGA, and have twin–twin transfusion syndrome. The findings were broadly similar when comparing ART pregnancies with naturally conceived pregnancies. The association between ART and all the perinatal and maternal outcomes except for preterm delivery <28, <34, and <37 weeks has stayed consistent over time, despite advances in ART.

Strengths and limitations

Our systematic review is a comprehensive analysis of maternal and perinatal outcomes in twin pregnancies following assisted reproduction, involving the largest number of twin pregnancies to date. We used a prospective protocol with a thorough literature search without language restrictions, thereby increasing our chances of capturing all relevant citations. We explored the

association between ART and maternal and perinatal outcomes important for clinical practice. We performed a comprehensive study quality assessment. We assessed maternal and offspring outcomes among ART twins compared to non-ART twin pregnancies and ART with naturally conceived twins to quantify the effects specifically caused by ART on twin pregnancies.

The meta-regression analysis findings, which were carried out to adjust for effects on specific studied outcomes by confounders such as maternal age, parity, and maternal BMI, did not generally deviate from the findings of the main analysis. Meta-regression analysis showed that the effect of ART on caesarean section increased with increasing difference in average maternal age between ART and non-ART and when the proportion of nulliparous women increased in the ART group compared to the non-ART group, which is in keeping with what is already known. Such observations validate the meta-analysis findings of our review.

In addition to maternal age, parity, and BMI that have been evaluated, other factors such as causes of infertility, uterine malformations, and previous uterine surgery among the ART group may have contributed to study heterogeneity (Lin, 2004; Hosseinirad *et al.*, 2021). These could not be assessed owing to limited data. Such heterogeneity could confound the study conclusions. Although none of the evaluated maternal and perinatal outcomes were significantly associated with maternal BMI, it is worth noting that we used aggregate data from the studies included in the review and aggregated data on BMI of the groups compared (ART and non-ART). It is well known that using aggregate data is not a powerful enough analysis to detect the confounding effect, and it is very likely that some ecological bias could explain this lack of association. Given the scarce information provided in the studies for covariates, a multiple meta-regression model for the statistically significant outcomes, including maternal age, BMI, and parity, was not performed as it is under a high risk of over-fitting. In all instances, the number of studies available for the meta-regression model was less than or equal to 10 studies.

This review is limited by the heterogeneity in the study population, exposure (including the type of ART), comparison, and outcomes. The inclusion criteria varied among different studies. Some studies excluded monochorionic twins, monoamniotic twin pregnancies, multifoetal pregnancy reduction, foetal anatomic or chromosomal anomalies, twin–twin transfusion syndrome, and those conceived following ovulation induction or IUI. We used the primary study authors' definitions for the outcomes studied, which may have varied across different studies. In the non-ART group, there was heterogeneity among studies; some included those conceived naturally and those conceived following ovulation induction or IUI. Many studies did not provide information regarding donor eggs, which has likely influenced the outcomes. Ideally, a sensitivity analysis excluding the oocyte donation should be conducted to verify this association; however, we could not perform the analysis owing to the lack of data. Part of the heterogeneity in our study could also be explained by the small study effects for some of the outcomes, which may have introduced some bias. Also, there was limited data on fresh and frozen embryo transfer cycles. Although it was shown that frozen embryo transfer cycles carry a higher risk of congenital malformations compared to fresh embryo transfer, it was based on one study and the results should be interpreted with caution. Additionally, as monochorionicity is associated with poorer perinatal outcomes than dichorionic twins, in our study we performed a sensitivity analysis assessing the outcomes comparing ART versus non-ART after excluding this cohort of twins.

Our cut-off for selecting the year 2010 for the subgroup analysis by year was arbitrary as there was no clearly defined time when ART had significantly improved, and individual studies did not report on the specific period when these techniques changed. As the year of publication of the study is likely to be different from the year in which it was conducted, a meta-regression based on the year of publication may not truly represent the effect of time on ART and outcomes. Therefore, a subgroup analysis comparing two broad time periods was performed. Additionally, as a double subgroup analysis according to both the year of publication and type of embryo transfer (fresh versus frozen) has been performed, the results of this subgroup analysis should be interpreted with caution because of limited statistical power.

Crucially, the results obtained from the sensitivity analysis excluding studies with high risk of bias were highly consistent with those from the primary analysis with regard to maternal outcomes. The only discrepancy was in PTB <34 weeks, where the significant association observed in the primary analysis was no longer evident. However, the findings in the sensitivity analysis for perinatal outcomes differed from those of the primary analysis. The significant association of ART with stillbirth, SGA <10th centile, congenital malformations, and RDS shown in the primary analysis was not observable in the sensitivity analysis. As the estimates were still in the same direction as the primary analysis, the lack of statistical significance could be explained by the lower number of studies included in the sensitivity analysis. The only salient discrepancy is the significant reduction in risk for APGAR <7 at 5 min among ART twins compared to both non-ART and natural conception shown in the sensitivity analysis.

The evidence presented in this review is primarily based on observational studies, which are initially considered 'low level' according to GRADE guidelines. We carefully examined the included studies but did not find circumstances warranting an upward adjustment of the evidence level. There were no large effect sizes, complete consideration of confounding factors, or dose-response relationships observed. Consequently, the certainty of our findings is low or very low, particularly when concerns about bias or statistical heterogeneity arise. As a result, the quality of evidence for these critical outcomes is expected to be graded as low or very low, resulting in recommendations with limited strength. Additionally, most of the studies were conducted in high-income countries, warranting additional research before applying these findings to low-income settings.

Comparison with previous studies

Previous systematic reviews have reported increased risk for maternal outcomes such as pregnancy-induced hypertension GDM, PTB, and caesarean section among ART twin pregnancies (McDonald *et al.*, 2005; Rossi and D'Addario 2011; Palomba *et al.*, 2016). The risks of perinatal outcomes, such as low birthweight, NICU admission, and perinatal death, were also higher among ART twins than among non-ART twins (McDonald *et al.*, 2005; Rossi and D'Addario 2011; Palomba *et al.*, 2016). Although, broadly, the findings of our review are in keeping with previously published reviews, most previous reviews had not adjusted the results for confounders such as maternal age and parity. Our review did not impose any language restrictions on the primary studies, which allowed a larger study population to be included. The list of maternal and perinatal outcomes assessed in our review was more extensive than in any of the above reviews.

Existing individual studies have demonstrated varied findings on outcomes in twin pregnancies following ART. Many studies have shown that naturally conceived twins and those conceived

following ART have no significant difference in obstetric or neonatal outcomes (Isaksson et al., 2002; Liang et al., 2002; Huang et al., 2006; Eskandar 2007; Boulet et al., 2008; Vasario et al., 2010; Yang et al., 2011; Szymusik et al., 2012; Andrijasevic et al., 2014; Kim et al., 2015; Bensdorp et al., 2016; Bordi et al., 2017; Barda et al., 2017; Deltombe-Bodart et al., 2017; Henningsen et al., 2018; Chen et al., 2019). In contrast, other studies have shown that twin pregnancies following ART carry worse pregnancy outcomes (Moise et al., 1998; Daniel et al., 2000; Koudstaal et al., 2000; Koivurova et al., 2002; Kuwata et al., 2004; Katalinic et al., 2004; Manoura et al., 2004; Olson et al., 2005; Adler-Levy et al., 2007; Hansen et al., 2009; Kallen et al., 2010; Sagot et al., 2012; Malchau et al., 2013; Michaluk et al., 2013; Yang et al., 2014; Simoes et al., 2015; Gocmen et al., 2015; Ombelet et al., 2016; Zhu et al., 2016; Wang et al., 2018; Feng et al., 2018; Lei et al., 2019; Couck et al., 2020; Algeri et al., 2020). A study has also demonstrated higher birthweight, fewer congenital anomalies, and a lower risk of NICU admission among ART twins than naturally conceived twins (Joy et al., 2008).

In previous systematic reviews on singleton pregnancies conceived by ART versus non-ART, the risk of PTB, SGA foetus, caesarean delivery, perinatal mortality, and NICU admission was shown to be significantly higher among ART singletons (Helmerhorst et al., 2004; McDonald et al., 2005; Pandey et al., 2012). Outcomes like APH, premature rupture of membranes, GDM, and hypertensive disorders of pregnancy were also higher among mothers who conceived singleton pregnancies following ART (Pandey et al., 2012). The trends in perinatal and maternal outcomes following ART are generally similar between singletons and twins.

The reason for the observed higher maternal, and offspring risks among ART twin pregnancies warrants further research. Women undergoing ART tend to be older than those who conceive naturally, increasing their risks of medical complications during pregnancy. Underlying medical problems in ART mothers may also place their neonates at an increased risk for complications. Higher anxiety levels among subfertile women and the obstetricians' tendency to avoid vaginal delivery and its related complications in what is deemed a 'precious' pregnancy may contribute to adverse outcomes, such as higher rates of PTB and caesarean delivery, among ART twins, contributing to increased perinatal morbidity. The higher rate of PTB among ART twins was not observed after 2010, reflecting a possibly improved confidence among clinicians in managing ART twin pregnancies and a subsequent change in practice.

Although ART twin pregnancies were associated with higher risks for many of the neonatal complications evaluated, the risk of stillbirth, SGA <10th centile, and TTTS showed a statistically significant reduction among ART twins. Plausible reasons for this reduction in stillbirth rates (OR of 0.83, 95% CI 0.70–0.99, 33 studies) among ART twins compared to non-ART twins include closer foetal surveillance offered for ART pregnancies and the observed increased late PTB rates. Additionally, the reduction in SGA <10th centile (OR 0.90, 95% CI 0.85–0.95, 26 studies) among ART twins could be attributed to higher preterm deliveries, which may have resulted in fewer cases of late-onset foetal growth restriction. Published individual studies vary in the rates of monochorionicity among ART twins compared with non-ART twins (Miura and Niikawa, 2005; Ben-Ami et al., 2016; Bordi et al., 2017; Couck et al., 2020). The reduction in the odds of TTTS among ART twins in our review (OR 0.45, 95% CI 0.25–0.82, 9 studies) may have been related to the fewer number of monochorionic diamniotic twin pregnancies among the ART group, although only one study provided data comparing monochorionicity rates. Differences in the study designs and populations could also explain the reduced neonatal risks observed in the ART group.

Relevance to clinical practice and research recommendations

As the number of twin pregnancies following ART is high globally, it is paramount to accurately quantify maternal and offspring risks involved with ART for counselling and proper management. Current guidelines on twin pregnancies do not categorize those conceived by ART as a higher-risk group than non-ART twins (National Institute for Health and Care Excellence, 2019). The higher maternal and offspring risks observed in our review emphasize the need for ART twin pregnancies to be recognized in guidelines as a separate group so that appropriate counselling and closer surveillance can be offered in addition to what is currently being offered. With the recent trends of single embryo transfer and the impact it has on reducing the prevalence of twin pregnancies and the consequent decrease in complications (Chambers et al., 2016; Kushnir et al., 2017; Adamson and Norman 2020), women can make an informed choice of single embryo transfer and evade the higher maternal and neonatal risks that ART twin pregnancies entail. However, the technique of single embryo transfer needs improvement, as it still tends to be associated with lower live birth rates compared to conventional ART methods (Kushnir et al., 2017).

We recommend further research, with more refined inclusion and exclusion criteria, identifying which aspect of ART is responsible for this observed increase in risk and how it can be minimized. Definitions of various maternal and perinatal outcomes need to be standardized for uniformity across studies. More data on risk factors, such as race and ethnicity, are required for a better understanding of their effect on twin pregnancies conceived by ART. A systematic review conducted by Zaat et al. (2021) comparing fresh versus frozen embryo transfer cycles concluded that the risk of maternal hypertensive disorders of pregnancy, a large-for-gestational-age baby, and a higher birthweight of the children born may be increased among frozen embryo transfer cycles (Zaat et al., 2021). Subgroup analysis of fresh versus frozen embryo transfer with a larger cohort will likely provide additional information regarding the differences in outcomes based on the ART technique. We also recommend individual participant data meta-analysis to obtain more robust information on specific subgroups. As women who undergo ART are at an increased risk of complications in addition to the increase in risk inherently associated with twin pregnancies, they should be followed up to assess the long-term effects of ART on their health.

Conclusion

Twin pregnancies conceived following ART have significantly higher adverse maternal and offspring outcomes than non-ART twin pregnancies, including GDM, hypertensive disorders in pregnancy, PTB, caesarean delivery, NICU admission, and congenital malformation. Therefore, ART twin pregnancies should be recognized and managed as a higher-risk group. Additionally, women seeking assisted reproduction should be counselled regarding the increased risks of ART twin pregnancies. However, the limitations of the study findings warrant a cautious interpretation.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Data availability

The data underlying this article are available in the article and its online supplementary material.

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Authors' roles

S.Ma. was fully involved in the conception, planning, literature search, selection of studies, data extraction, data analysis, and writing up the review. W.K. was involved in the literature search, study selection, data extraction, analysis, and writing up of the review. R.N. was involved in the literature search, study selection, data extraction, analysis, and writing up of the review. S. Mo. was involved with the literature search and writing up the review. J.A. was involved with writing up the review and analysis of small-study effects. S.F.-G. was involved with the grading of evidence. Andrea Gaetano was involved with the meta-regression analysis. G.R.-C. was involved with the meta-regression analysis. J.A. was involved with writing up the review and supervision. A. K. was involved with the writing up of the review and supervision. P.B. was involved with writing up the review and supervision. J.Z. was involved with the meta-regression analysis. S.T. was involved in the conception, planning, data extraction, data analysis, writing up, and overall supervision.

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Conflict of interest

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