RISKS OF PRETERM BIRTH AND OTHER COMPLICATIONS IN TWIN PREGNANCIES: SYSTEMATIC REVIEWS AND META-ANALYSES

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STATEMENT OF ORIGINALITY

I, Fathima Shemoon Marleen, confirm that the research included within this thesis is my work or that where it has been carried out in collaboration with or supported by others, that this is duly acknowledged below, and my contribution indicated. Previously published material is also acknowledged below.

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PUBLICATIONS IN PEER-REVIEWED JOURNALS FROM THE THESIS

1) Marleen S, Dias C, Nandasena R, MacGregor R, Allotey J, Aquilina J, Khalil A, Thangaratinam S. Association between chorionicity and preterm birth in twin pregnancies: a systematic review involving 29 864 twin pregnancies. BJOG 2020; DOI: 10.1111/1471-0528.16479.

2) Marleen S, Dias C, MacGregor R, Allotey J, Aquilina J, Khalil A, Thangaratinam S. Biochemical predictors of preterm birth in twin pregnancies: A systematic review involving 6077 twin pregnancies. European Journal of Obstetrics & Gynaecology and Reproductive Biology. 2020 July; 250:130-142.

3) Marleen S, Hettiarachchi J, Dandeniya R, Macgreggor R, Aquilina J, Khalil A, Vogel J, Betrán AP, Thangaratinam S. Maternal clinical predictors of preterm birth in twin pregnancies: A systematic review involving 2,930,958 twin pregnancies. European Journal of Obstetrics & Gynaecology and Reproductive Biology. 2018; 230:159-171.

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Contributions to each paper and chapter are stated in appendix 1.

ABSTRACT

Background

The rates of twin pregnancies have increased globally due to the substantial increase in artificial reproductive techniques (ART) worldwide. Preterm birth is a major contributing factor to twins' significantly higher perinatal morbidity and mortality rates. Early identification of twin pregnancies at high risk of preterm birth is vital to offer targeted care and closer surveillance. This thesis focused on predicting preterm birth in twin pregnancies using maternal clinical characteristics, biochemical markers and chorionicity. In addition, maternal and perinatal outcomes among ART twin pregnancies compared to non-ART and naturally conceived twins were evaluated.

Methods

Systematic reviews and meta-analyses were performed.

Findings

Teenage pregnancies, nulliparity, obesity (BMI >35 kg/m^2), and a previous history of preterm birth was significantly associated with early and late preterm birth in twin gestations among the maternal clinical characteristic studied. Of the various biochemical predictors studied, positive fetal fibronectin was associated with a significantly increased risk of preterm birth. Monochorionicity was significantly associated with an increased risk of preterm birth at all gestations evaluated. ART twins showed significantly higher risks for most maternal complications, including preterm birth, hypertensive disorders in pregnancy, diabetes, antepartum haemorrhage, postpartum haemorrhage, and caesarean section. Perinatal

complications such as congenital malformation, birth weight discordance, neonatal morbidity, and admission to neonatal intensive care units were also significantly higher among ART neonates. ART neonates and their mothers were observed to be at higher risk of adverse outcomes even when adjusted for chorionicity.

Conclusion

Maternal clinical characteristics, biochemical predictors, and monochorionicity are significantly associated with early and late preterm birth in twin pregnancies. ART twin pregnancies demonstrate significantly higher adverse maternal and perinatal outcomes. The risk estimates based on each predictor can be used to identify, counsel and provide targeted care to women with twin pregnancies at high risk of preterm birth. Women seeking fertility treatment should be counselled regarding the increased maternal and neonatal risks associated with ART twin pregnancies.

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DEDICATION

I dedicate this thesis to my parents, siblings, and my husband for their unwavering support and encouragement. A special dedication to my deceased father, who would have been immensely proud of me for completing my research thesis.

I also dedicate this to my two daughters, Aleeza and Liyana for their patience and understanding.

Finally, I would like to dedicate this thesis to all women with twin pregnancies and their babies worldwide who continue to experience adverse outcomes.

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LIST OF ABBREVIATIONS

AFP: Alpha-Fetoprotein

ART: Assisted Reproductive Technology

BMI: Body Mass Index

CDC: Centre for Disease Control

CI: Confidence Interval

CRH: Corticotrophin Releasing Hormone

CRP: C Reactive Protein

fFn: fetal Fibronectin

GIFT: Gamete Intrafallopian Transfer

hCG: human Chorionic Gonadotrophin

HIE: Hypoxic Ischaemic Encephalopathy

ICSI: Intracytoplasmic Sperm Injection

IL-8: Interleukin 8

IUI: Intrauterine Insemination

IVF: In Vitro Fertilization

IVH: Intraventricular Haemorrhage

JLA: James Lind Alliance

MBRRACE-UK: Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries across the UK

MMP-8: Matrix Metalloproteinase 8

MOOSE: Meta-analyses Of Observational Studies in Epidemiology

mshCG: maternal serum human Chorionic Gonadotrophin

NEC: Necrotizing Enterocolitis

NICU: Neonatal Intensive Care Unit

NOS: Newcastle Ottawa Scale

OR: Odds Ratio

PAPP-A: Pregnancy-associated Plasma Protein A

phIGFBP-1: phosphorylated Insulin-like Growth Factor-binding Protein 1

PICO: Population, Intervention, Control, And Outcomes

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PROSPERO: International Prospective Register of Systematic Reviews

PSP: Preterm birth Priority Setting Partnership

RDS: Respiratory Distress Syndrome

SGA: Small for Gestational Age

sFGR: Selective Fetal Growth Restriction

TAPS: Twin Anaemia Polycythaemia Sequence

TRAP: Twin Reversed Arterial Perfusion

TTTS: Twin-to-twin Transfusion Syndrome

UK: United Kingdom

ZIFT: Zygote Intrafallopian Transfer

Chapter 1: Introduction

1.1: Twin pregnancy

Twin pregnancies encompass 2 to 4% of the total births worldwide. [1] Twin pregnancies are classified as dizygotic or monozygotic, with the majority (70%) of twin pregnancies being dizygotic and 30% monozygotic in the UK. [2] Dizygotic twins result from fertilisation of two separate ova with two different sperms, while monozygotic twins result following fertilisation of a single ovum by a single sperm, with the zygote then splitting into two. The pattern of placentation and amnionicity depends on the timing of the division of the zygote. Dichorionic placentation occurs when the division occurs within three days of fertilisation, where each fetus has its own placenta. If the division occurs at 3-9 days, monochorionic placentation occurs where there is sharing of one placenta among the twins. If the cleavage of the zygote occurs after nine days, it results in monoamniotic twinning, where one amniotic sac is shared between the twins, which accounts for <1% of all twin pregnancies. If the cleavage occurs after 12 days, it results in conjoint twins. Figure 1 depicts the process of dizygotic and monozygotic twinning.

The prevalence rates of spontaneous twinning vary across populations. In the 1970s, twinning rates were low in East Asia (less than eight twin pregnancies per 1,000 births), at an intermediate level in Europe and North America (9–16 per 1,000 birth), and high in several African countries with 17 or more per 1,000 births. A widespread steep increase in the incidence of twin pregnancies was observed over three decades, attributed mainly to increasing maternal age at conception and the use of assisted conception. [3, 4, 5] In the United States, the twin birth rate increased from 18.9 per 1000 total births in 1980 to 33.1 per 1,000 total births in 2012. [6] England and Wales reported a similar trend where a 41% increase in twinning was observed

from 1982 to 1997, with 15.6 per 1000 women having had multiple pregnancies in 2013. [7] In France, twin pregnancy rates increased by 80% from the 1970s, with 15.6 twin pregnancies per 1000 women reported in 2011. [8]

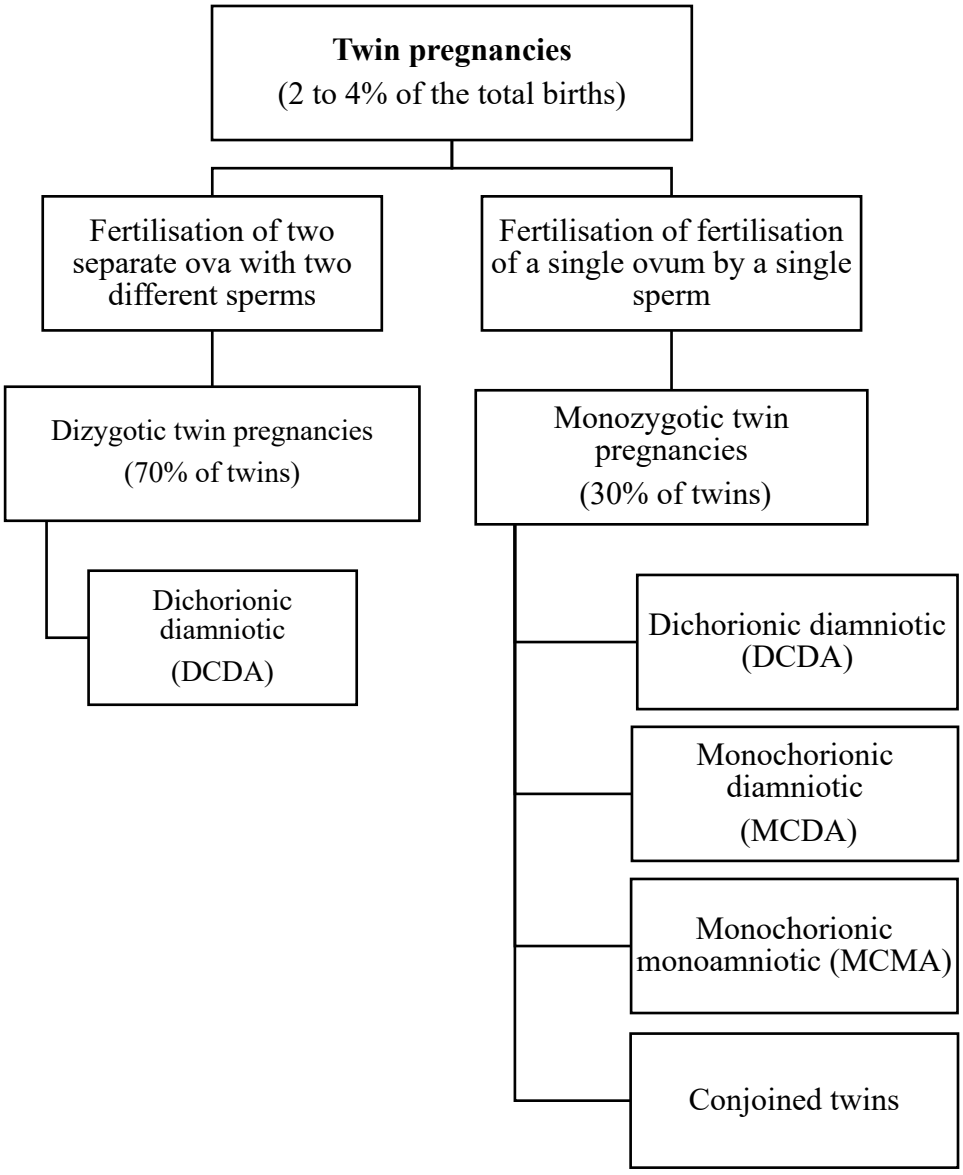


Figure 1: The process of dizygotic and monozygotic twinning

More recently, twin-birth trends have declined in the USA by 4% between 2014 and 2018 and have reached the lowest rate of 32.6 per 1000 total births in over a decade. [9] A similar reduction in twin pregnancies has also been observed in the UK. [10] Although the overall twinning rate remains high, the observed reduction in trends in twinning may have been contributed by the policy of single embryo transfer and advances in IVF techniques. [11]

Twin pregnancies are associated with an increase in both maternal and perinatal morbidity and mortality. [12, 13] Maternal risks include miscarriage, gestational hypertension, pre-eclampsia, gestational diabetes, caesarean delivery, postpartum haemorrhage, blood transfusion, need for intensive care, and a 2.5 times greater risk of maternal mortality. [14, 15, 16, 17] Compared to singletons, the perinatal mortality rate of twins is seven times higher. [18, 19] Infants born from twin gestations have a tenfold increased risk of being admitted to a neonatal unit, while those who survive have a sixfold increased risk of cerebral palsy. [20] The increased perinatal mortality and morbidity are mainly attributed to frequent preterm birth and lower gestational weight. The higher risk of adverse outcomes affects the children born from multiple pregnancies, their families, and the healthcare systems. A report by the National Guideline Alliance on twin pregnancy costing showed that multiple pregnancies are almost three times as expensive compared to singleton pregnancies. [19]

MBRRACE-UK (Mothers and Babies: Reducing Risk through Audits and Confidential Enquiries across the UK) perinatal surveillance report on perinatal deaths for births in the UK from January to December 2017 showed that from 2017 to 2020, the rates of both stillbirths had reduced from 9.03 to 6.99 per 1,000 total births, and neonatal mortality for twins had reduced from 8.01 to 5.45 per 1,000 live births. [21, 22] This observed reduction has been attributed to advances in

antenatal care, invasive procedures for managing complicated monochorionic twin pregnancies and improved neonatal care. Despite these reductions being observed, the increased risk of mortality associated with twins compared to singletons remains double for stillbirths (relative risk (RR) 1.93 in 2017) and over threefold for neonatal deaths (RR 3.53 in 2017). [23]

1.2: Preterm birth in twin pregnancies

Preterm birth is the main contributor to the observed increase in perinatal morbidity and mortality in twin pregnancies. [3] About half of the twins are born before 37 weeks' gestation, and 10% are born before 32 weeks, compared with 1% of singletons. [24] Perinatal data in Scotland estimates that up to 50% of twins deliver before 37 weeks, with around 20% delivering before 34 weeks' gestation. [25] The rate of preterm birth among twins in the United States has risen from 52.3% in 1995 to 62% in 2005, [26] while a similar trend is observed in many other developed countries. [3]

Preterm birth has been identified as the fifth leading cause of disease burden over time, according to the Global Burden of Disease Study 2015. [27] The neonatal mortality rate of twins has been calculated to be 18 per 1,000 live births, a value six to seven times that of singletons. [28] According to the paediatric classification of neonatal death, more than 50% of neonatal deaths among multiple pregnancies are distinctly ascribable to prematurity. [29] Among the surviving children, many short-term and long-term health issues interfere with their quality of life. Common short-term morbidities include infectious and non-infectious ailments, with up to 40% having bronchopulmonary dysplasia, retinopathy of prematurity, neonatal jaundice, hypoxic-ischaemic encephalopathy, and necrotising enterocolitis. [30] A study conducted in France which compared the neurocognitive abilities of 5-year-old children born at 22-32 weeks of gestation

and those of children born at 39-40 weeks of gestation showed that the risk of cognitive and neuromotor disabilities such as cerebral palsy at the age of five is inversely related to the period of gestation. [31] Other long-term morbidities include epilepsy seen in association with cerebral palsy or isolation, cognitive impairment and developmental coordination disorders, including disorders of attention and activity. [30] Prematurely born infants are also at a higher risk of mortality during young adulthood and chronic diseases later in life. [32, 33]

Although the aetiology for preterm birth in multiple pregnancies is not entirely established, the association between the two entities is well-recognized. [34] Similarly to singleton pregnancies, the aetiology of preterm birth among twins is likely to be multifactorial with many possible contributing factors. Potential contributors to the pathophysiology of preterm birth among twin pregnancies include excessive myometrial stretch causing biochemical responses such as increased production of proinflammatory cytokines [35] and increased responses to oxytocin. [36, 37] Other suggested mechanisms for preterm birth among twins include increased levels of a corticotrophin-releasing hormone secreted by the larger placental mass [38] and stimulation of myometrial contractility by higher levels of factors released by the fetal lungs such as surfactant protein A. [34]

Even though the preterm birth rate in twin pregnancies lies around 50%, the contribution of preterm birth to perinatal and infant mortality is out of proportion. [3] The highest mortality rates were observed when preterm birth followed preterm pre-labour rupture of membranes, followed by preterm births occurring after spontaneous labour, and mortality rates were lowest when preterm delivery was medically indicated. [3] Newborns appeared to be the safest when the

healthcare team anticipated and prepared for preterm delivery, despite the delivery being performed due to maternal or fetal complications. [32]

Several studies have evaluated various treatment options to prevent preterm birth in twin pregnancies. A recent multicenter randomised controlled trial of women with twin pregnancies and asymptomatic cervical dilation before 24 weeks of gestation has demonstrated a significant decrease in preterm birth at all evaluated gestational ages using a combination of physical examination–indicated cerclage, indomethacin, and antibiotics. [39] Cerclage in this population was associated with a 50% decrease in early preterm birth at <28 weeks of gestation and a 78% decrease in perinatal mortality. An updated meta-analysis of individual patient data (IPD) of randomised controlled trials comparing vaginal progesterone with placebo or no treatment in women with a twin gestation concluded that administering vaginal progesterone to asymptomatic women with a sonographic short cervix of ≤ 25 mm in the mid-trimester reduced the risk of preterm birth at <30 to <35 gestational weeks, neonatal mortality and some measures of neonatal morbidity. [40]

In women with twin pregnancies, a randomised, placebo-controlled, double-blind trial showed that universal treatment with vaginal progesterone did not reduce the risk of spontaneous preterm birth between 24+0 and 33+6 weeks' gestation. [41] However, post hoc time-to-event analysis suggested that in women with a cervical length of <30 mm, spontaneous preterm birth before 32 weeks' gestation may be reduced by progesterone treatment, while the risk may increase among women with a cervical length of ≥30 mm.

Currently, there are no proven therapies which are very effective in preventing preterm birth among twin pregnancies. Therefore, establishing effective means to correctly and promptly

identify women carrying a twin pregnancy who are at higher risk of preterm birth is vital so that measures can be taken to minimise adverse outcomes to both mother and the newborn. [42]

1.2.1: Predicting preterm birth in twin pregnancies

Due to preterm birth's massive economic, personal and health ramifications, early identification of twin pregnancies at risk of preterm birth using predictive tests is of utmost importance. If women at high risk of preterm delivery could be identified, interventions to delay delivery or minimise the adverse outcomes associated with preterm birth could be initiated. [42] High-risk women asymptomatic for preterm labour could be counselled and offered frequent surveillance. while symptomatic women could be managed with immediate therapeutic options such as tocolysis, antenatal corticosteroids, magnesium sulphate for fetal neuroprotection or in-utero transfer to a tertiary care centre. [43] Identifying twin pregnancies at high risk of preterm birth is particularly important in settings with low resources where high-risk mothers can benefit from counselling and early referral. Predictive tests can also reassure women who are unlikely to deliver early and avoid unnecessary and expensive interventions in mothers at low risk of preterm birth. [43]

In the MBRRACE-UK report published on 14 January 2021, the key findings and recommendations related to preterm birth highlighted the importance of screening and prevention of preterm birth in twin pregnancies. [23] To date, interventions that are effective in predicting preterm birth remain largely uncertain and have been prioritised as the number one uncertainty in preterm birth by the James Lind Alliance (JLA) Preterm birth Priority Setting Partnership (PSP). [44]

Despite the current lack of effective interventions for preventing preterm birth in twin pregnancies, timely referral, administration of steroids and magnesium sulphate have improved neonatal outcomes. However, according to the MBRRACE-UK report, one-third of eligible women remained unidentified until they presented in advanced preterm labour and therefore did not receive these interventions. [23]

Cervical-length assessment at 20–24 weeks is currently the best available screening test that the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) recommends. [45] However, the screening performance of cervical length for preterm birth in twins is lower than that in singletons. [45] A cervical length ≤ 20 mm at 20–24 weeks' gestation has shown to predict preterm birth at < 32 and < 34 weeks' gestation in twin pregnancies with pooled sensitivities of 39% and 29%, specificities of 96% and 97%, positive likelihood ratios of 10.1 and 9.0 and negative likelihood ratios of 0.64 and 0.74, respectively. [42] Thus, there is a need to investigate other predictors to identify twin pregnancies at high risk of preterm birth.

In this thesis, I aim to categorise predictors as maternal clinical characteristics, biochemical markers and chorionicity as predictors of preterm birth in twin pregnancies and explore the existing literature for each predictor to build up more robust recommendations for clinical utility backed by scientific evidence.

1.2.2: Maternal characteristics associated with preterm birth in twin pregnancies

Maternal characteristics identified through history and examination alone could potentially be utilised to predict the likelihood of preterm birth in twin pregnancies. Identifying twin pregnancies at high risk for preterm birth through maternal clinical characteristics has the advantage of not requiring expensive resources and can be done at the first antenatal visit.

Maternal characteristics are advantageous in low-income countries with limited access to expensive resources. Various maternal characteristics, including maternal age, maternal body mass index (BMI), race, history of previous preterm delivery, smoking, parity, gestational diabetes, hypertensive disorders in pregnancy, and maternal anaemia, have been studied as possible indicators of premature birth in twin pregnancies. [46, 47, 48, 49, 50, 51, 52, 53]

- Maternal age

Maternal age plays a vital role in pregnancy outcomes. In recent years there has been a significant increase in the number of pregnancies among the extremes of age. Older mothers have contributed disproportionately to the increase in twin births. From 1990 to 2001, the twin birth rate among women aged 40–44 increased by 50% while increasing by 16% among women of 20–24 years. [54] Due to the advancement of ART and the increase in the number of employed women, many women are getting pregnant at an older age. Therefore, older women undergoing ART are at an increased risk of twin pregnancies.

It is well established that in singleton pregnancies, advanced maternal age (>35 years), independent of confounding factors, is associated with an increase in early and late preterm delivery. [55, 56] However, the evidence for twin pregnancies with advanced maternal age differs. According to Delbaere et al., [57] maternal age of 35 years or over is associated with a lower incidence of preterm birth in twin pregnancies and according to Branum et al., [58] primipara over 40 years of age have a reduced risk of very preterm birth while multipara have the same risk as women of 25–29 years. However, according to Laskov et al., [59] twin pregnancies of maternal age ≥45 years have a statistically significant high risk of early and late preterm birth compared with younger twin mothers.

World Health Statistics from 2017 reported that the average global birth rate among girls between 15 to 19 years is 49 per 1000 girls, accounting for 11% of all global births. 95% of these births occur in low- and middle-income countries. [60] Teen singleton pregnancies have higher risks of preterm birth; therefore, teen twin pregnancies inevitably carry a high risk of preterm birth. Evidence indicates that teen twin pregnancies have a higher risk of very preterm birth than young adult mothers, and the risk of preterm birth increases strongly with decreasing maternal age. [61] Branum et al. showed that very preterm birth occurred in over 20% of twin pregnancies to young teens, regardless of ethnicity. [62]

- Maternal BMI

Over the past 30 years, the prevalence of obesity has increased and has been described as a global epidemic. In 2013, approximately 30% of women were either in the overweight or obese BMI categories. [63] Rates of obesity in pregnancy showed the same increasing trend similar to the global epidemic, [64, 65, 66] which has resulted due to high rates of pre-pregnancy obesity, excess weight gain during the pregnancy [67, 68] and the influence of advanced maternal age. [69] In the UK, the prevalence of obesity in pregnancy has seen an increase, rising from 9–10% in the early 1990s to 16–19% in the 2000s, [70, 71] with similar trends seen in Canada (16% of pregnant women in 2009). [72]

A study by Suzuki et al., [73] on dichorionic twin pregnancies showed that very pre-term delivery was significantly increased among obese women with a BMI ≥ 30 during pre-pregnancy and suggested that maternal obesity was an independent risk factor for early preterm delivery. Another study by Dickey et al., among IVF twin pregnancies, concluded that obese women with BMI >35 kg/m^2 are at an increased risk of preterm birth at <28 and <32 weeks' gestation. [48]

However, a study published by Ram et al., evaluating the relationship between maternal BMI and pregnancy outcomes in twin pregnancies compared with singletons, showed that in twin gestations, underweight was associated with the greatest risk of preterm birth at < 32 weeks while it was not observed among the overweight or obese group. [74]

A considerable disparity is observed among different races regarding obstetric outcomes, including the prevalence of preterm birth. [75] This disparity has been attributed to differences in physical environments, social environments, poverty, maternal stress, quality of accessible health care and different maternal health behaviours such as adequacy of prenatal care, pregnancy weight gain, use of prenatal vitamins, smoking and alcohol use. [76]

- Ethnicity

A study published by Xiong et al., [77] showed that the rates of very early preterm (<28 weeks), early preterm (<32 weeks), and preterm birth (<37 completed weeks) varied across racial and ethnic groups. For singletons, black women were found to have higher odds of very early preterm birth, early preterm birth, and preterm birth. Hispanic women had a significantly lower rate of preterm births than black women and similar or slightly higher rates than white women. Native American women were not at increased risk of any preterm birth, while Asian women had a reduced risk of preterm twin births. In a population-based study of 644,462 Missouri birth records, black women were found to be at higher risk for recurrent preterm birth (aOR = 4.11, 95% CI: 3.78–4.47) compared to white mothers (aOR = 6.4, 95% CI: 3.7–11.0). [78] Although these data strongly indicate the difference in preterm birth among races, limited data exist evaluating the association between maternal race-specific for twin pregnancies.

- History of preterm birth

A previous preterm birth has been identified as a risk factor for subsequent preterm birth. Michaluk et al., in a retrospective cohort study, demonstrated a three-fold increase in the risk of preterm birth in a subsequent twin pregnancy among women with a previous singleton preterm birth. [79] Bloom et al., also found an increase in the risk of preterm birth among twin pregnancies with a previous history of singleton preterm birth. [80] Fox et al., showed that the rate of twin preterm birth before 32 weeks of gestation was 3.5% in women with no history of prior term birth and 26% in women with a prior preterm birth. [81] However, the preterm prediction study conducted by Goldenberg et al., failed to report a significant association between the two entities. [50]

- Maternal smoking

Maternal tobacco smoking has been associated with premature birth for many years. The possible mechanisms include nicotine-induced vasoconstriction, carbon monoxide-induced hypoxia in the fetus, disruption of calcium signals, altered hormone production, disruption of prostaglandin synthesis, and change in responses to oxytocin. However, the contribution of each of these mechanisms is not yet clear. [82] In a study evaluating maternal smoking and gestational age in twin pregnancies by Wisborg et al., it was shown that smoking had a substantial effect on mean gestational age among twin pregnancies where the mean gestational age was five days (95% CI 1-9 days) shorter among smokers compared with non-smokers. [83] Also, the mean gestational age was 261 days (+/-18) among non-smokers, 257 days (+/-23) among women who smoked 1-9 cigarettes per day and 255 days (+/-20) among those who smoked 10+ cigarettes per

day. Therefore, it was also concluded that smoking and preterm birth appeared to have a dose-response relationship.

- Nulliparity

The associations between parity and risk of spontaneous preterm birth have been investigated among singletons and multifetal gestations. A study by Koullali et al., on singleton pregnancies, showed an independent association between nulliparity and spontaneous preterm birth at < 28, < 32 and < 37 weeks. [84] James et al., demonstrated that the gestational age at delivery was significantly increased in parous women carrying a multifetal gestation after controlling for other factors that affect gestational age at birth. [85] Most studies on parity and preterm birth have included multiple pregnancies and not reported outcomes specific to twin gestations.

- Medical disorders in pregnancy

In recent years, chronic medical conditions such as diabetes, hypertension, and cardiac disease have increased significantly among fertile women. [86] Also, the demographic trend of later childbearing has increased pregnancies complicated by chronic diseases. In the United Kingdom, based on a population-based cross-sectional study in 2018, the prevalence of pre-existing multimorbidity (two or more long-term physical or mental health conditions) in pregnant women was estimated to be 44.2%. [87] Maternal chronic diseases are known to increase singleton preterm delivery, and the effect of chronic diseases on the maternal outcome is thought to be similar in singleton and twin pregnancies. Werder et al., showed that women with twin pregnancies and chronic disease delivered significantly earlier than healthy twin mothers (34.1 vs. 34.6 weeks of gestation). [88]

Although individual studies have been conducted evaluating various maternal clinical predictors for preterm birth, their results and conclusions have mostly been inconsistent due to the varying risk estimates provided and small study populations. No systematic reviews exist evaluating the association between various maternal clinical predictors and preterm in twin pregnancies.

1.2.3: Biochemical markers associated with preterm birth in twin pregnancies

Numerous biochemical markers have been assessed for their use in predicting preterm birth. [42] These include markers of extracellular matrix degradation such as fetal fibronectin, hormonal and molecular markers such as pregnancy-associated plasma protein-A (PAPP-A), human chorionic gonadotropin (hCG), estriol, relaxin, α-Fetoprotein (AFP) and cervical phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1), inflammatory and infectious markers such as matrix metalloproteinase-8 (MMP-8), granulocyte elastase, bacterial vaginosis, interleukins (IL) and C-reactive protein (CRP), and markers of fetal stress such as corticotropin-releasing hormone (CRH). [89] Although many of these biochemical markers have been assessed for their use in predicting preterm birth in singleton pregnancies, data for twin gestations is scarce.

One of the most common biochemical markers implicated in preterm birth is fetal fibronectin. It is a glycoprotein secreted by cytotrophoblasts to bind maternal decidua and chorionic membranes together. Finding it in cervicovaginal fluids up to 22 weeks of gestation is considered normal, and its presence in cervicovaginal fluids between 24 to 34 weeks of gestation is considered a risk factor for preterm birth. [90] A study evaluating fetal fibronectin's accuracy in predicting preterm birth in twin gestations found that fetal fibronectin testing was of high negative predictive value in screening women with twin gestations presenting with symptoms of

preterm labour. [91] Another study reported that although the test had a high sensitivity towards preterm birth in twin gestations, the specificity was low. [92]

A meta-analysis conducted in 2010 with 15 studies reported that cervicovaginal fetal fibronectin levels offer minimal to moderate accuracy in predicting preterm birth in multiple pregnancies. Among asymptomatic women, cervicovaginal fetal fibronectin showed pooled sensitivities, specificities, and positive and negative likelihood ratios ranging from 33-39%, 80-94%, 2.0-5.1, and 0.7-0.8 at gestations <32, <34, and <37 weeks' respectively. It also reported that the test was most accurate in women with twin pregnancies and threatened preterm labour within seven days of testing. [93] A retrospective cohort study published in 2014, which included 560 twin gestations, reported similar predictive values for preterm birth at <32 weeks of gestation. [94]

Fetoplacental proteins or hormones such as AFP, PAPP-A, and β-hCG are used for fetal aneuploidy screening. Low levels of maternal serum PAPP-A and elevated levels of maternal serum AFP and β-hCG have been evaluated for predicting preterm birth in twin pregnancies. The overall predictive performance of these markers for preterm birth is poor among the limited studies that have been performed. [95, 96] Elevated maternal relaxin concentrations, a peptide hormone produced by the corpus luteum, have been associated with preterm birth with limited data for twin pregnancies [97, 98] while cervical phIGFBP-1 has shown minimal predictive accuracy for preterm birth. [49, 99]

The concentrations of cytokines in amniotic fluid, cervicovaginal fluid and serum have been studied as potential biomarkers of preterm birth. A cohort study evaluated the ability of interleukin (IL)-1a, IL-6, and IL-8 concentrations in cervicovaginal secretions at 24-34 weeks to predict spontaneous preterm birth in twin gestations. [100] This study showed that IL-8 was

associated with a significant increase in the risk of spontaneous preterm birth at <37 weeks of gestation, although the accuracy of IL-8 in predicting preterm birth at <37 weeks' gestation was low.

An imbalance in the normal vaginal bacteria is known as bacterial vaginosis (BV), where other intravaginal microorganisms replace protective normal vaginal commensals such as Lactobacillus species. [101] BV can cause vaginitis or cervicitis, leading to inflammation of fetal membranes, and causing preterm birth. Studies have been done to assess if bacterial vaginosis is predictive of preterm birth in asymptomatic women with twin gestations. While some studies [102, 103] have reported that the presence of bacterial vaginosis at 24 - 34 weeks of gestation had very low predictive values for spontaneous preterm birth at <32, <35, and <37 weeks of gestation, another study by Ruiz et al., did not find an association between the presence of bacterial vaginosis at 22-34 weeks and preterm birth at <35 weeks of gestation. [104]

Overall, there has been limited data on various biomarkers in predicting preterm birth in twin pregnancies, while meta-analyses studying biochemical predictors of preterm birth in twin pregnancies have been limited to trials conducted up to 2014.

1.2.4: Association between chorionicity and preterm birth in twin pregnancies

Around 30% of twin pregnancies in the UK are monochorionic. The proportion of monochorionic twins among all births varies between 0.76% to 0.96%. One of the main concerns with monochorionic pregnancies is the shared placenta and placental vascular anastomoses that are almost universal and connect the fetal circulations of both twins. Complications specific to inter-twin vascular anastomoses include twin-to-twin transfusion syndrome (TTTS), selective

fetal growth restriction (sFGR), twin anaemia-polycythaemia sequence (TAPS), twin reversed arterial perfusion (TRAP) sequence and single intrauterine death. [105]

TTTS occurs in 15% of monochorionic pregnancies, where the placentas have predominantly unidirectional artery–vein anastomoses leading to a haemodynamic imbalance within the circulations of the twins. [106] Based on the Quintero classification, it has five stages. Selective growth restriction (sGR) is when there is significant intrauterine growth discordance of greater than 20% between the twins. It occurs in up to 10- 15% of monochorionic twins without TTTS and over 50% of monochorionic twins complicated by TTTS. [107] TAPS is associated with fetal anaemia in the donor and polycythaemia in the recipient without significant changes in amniotic fluid. It is an important association in monochorionic pregnancies, occurring in up to 2% of uncomplicated monochorionic diamniotic twins and up to 13% of monochorionic twins post-laser ablation 13%. [108] Approximately 1% of monochorionic twin pregnancies are affected by TRAP sequence where an acardiac twin is being perfused by the anatomically 'normal' pump twin through a large artery–artery anastomosis on the placental surface.

Due to the specific complications associated with monochorionic placentation, monochorionic pregnancies have a higher perinatal risk than dichorionic twin pregnancies. [109, 110, 111, 112, 113] Individual studies have shown that monochorionicity imparts a greater risk of preterm birth, stillbirth, perinatal mortality and neonatal intensive care unit admission compared with dichorionic twins. [111, 114, 115, 116, 117] However, no systematic reviews exist evaluating the relationship between chorionicity and preterm birth in twin pregnancies. Because chorionicity can be determined by prenatal ultrasound scanning, knowing the risk of preterm birth concerning

chorionicity will help estimate the differences, thereby identifying women at high risk of preterm birth and counselling them appropriately.

1.3: Twin pregnancy and assisted reproductive technology

The Centre for Disease Control and Prevention (CDC) defines assisted reproductive techniques (ART) as procedures that involve handling both sperm and oocytes or embryos to establish a pregnancy. [118] It includes in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), zygote intrafallopian transfer (ZIFT) and gamete intrafallopian transfer (GIFT).

Statistics from 74 countries indicate that up to 2.4 million infants were born following ART from 1989 to 2007. [119]. ART is deemed one of the most important contributors to the global increase in multiple pregnancies. [120] The perceived need to stimulate many follicles and transfer multiple embryos to strengthen the chance of pregnancy is the fundamental reason for the higher number of multiple pregnancies observed with ART. [121] In January 2009, the UK Human Fertilisation and Embryology Authority (HFEA) introduced a policy to reduce the chances of multiple pregnancies following IVF treatment. This policy set an overall goal of reducing the national multiple-birth rate following conception by IVF to 10% and set a maximum multiple-birth rate that clinics must not exceed. Although trends in multiple births have reduced between 2014 and 2018, [9, 122] due to single embryo transfer and refinements in IVF techniques, the twin pregnancy rate remains high among women undergoing ART. [12] Majority of twinning following ART is dizygotic (95%); however, ART also increases the risk of monozygotic twinning by 2-4 fold. [123]

Great research interest is seen in comparing neonatal and maternal outcomes in pregnancies conceived by ART and by natural means, as the course of the pregnancy and the infant's health are reasonable measures of the quality of the reproductive techniques. Outcomes such as

pregnancy-related complications, mode of delivery, premature birth, birth weight, admission to neonatal intensive care units, and other neonatal complications have been studied and compared between twins conceived naturally and through assisted reproduction. Some studies report a significantly higher incidence of adverse outcomes in twins conceived by ART, including preterm birth, caesarean delivery, low birth weight, respiratory complications and more extended hospital stay. [121, 124, 125] Women carrying twin gestations following ART have shown to be at a higher risk of gestational diabetes, gestational hypertension, antepartum haemorrhage and discordant growth in foetuses. [124, 125] However, some studies observed that a significantly higher number of naturally conceived twins resulted in low birth weight, lesser gestational age, and admission to neonatal intensive care units. [126]

Attempts to compare the maternal and neonatal outcomes in twin pregnancies following assisted reproductive techniques and natural conception have demonstrated inconsistent results, requiring a review incorporating a more extensive study population, more recent studies and assessing a higher number of maternal and neonatal outcomes. Thereby, more robust evidence could be generated evaluating the risks associated with ART twin pregnancies compared to non-ART and natural conception.

1.4: Aims and objectives

To evaluate, by a systematic review of the literature,

- Maternal clinical predictors of preterm birth in twin pregnancies
- Biochemical predictors of preterm birth in twin pregnancies
- The association between chorionicity and preterm birth in twin pregnancies
- Maternal outcomes in twin pregnancies following assisted reproductive technology

- Perinatal outcomes in twin pregnancies following assisted reproductive technology

Research overview

The specific research questions that I have attempted to answer in this thesis are given below and summarized in a structured format in Table 1.

Maternal clinical predictors of preterm birth in twin pregnancies

- What is the association between maternal clinical characteristics such as maternal age (<20 years vs ≥ 20 years, >35 years vs ≤ 35 years, >40 years vs ≤ 40 years), BMI (>35 vs ≤ 35, >30 vs ≤ 30, <19.8 or 18.5 vs ≥ 19.8), race (black vs non-black, non-white vs white), parity, history of smoking, history of previous preterm birth, maternal medical complications (diabetes in pregnancy, hypertensive disorders in pregnancy, anaemia) and preterm birth (i) before 34 weeks' gestation, and (ii) before 37 weeks' gestation in twin pregnancies?
- What are the risk estimates of the maternal clinical predictors of preterm birth (i) before 34 weeks' gestation, and (ii) before 37 weeks' gestation in twin pregnancies?

Biochemical predictors of preterm birth in twin pregnancies

- What are the biochemical markers associated with a higher risk of preterm birth (i) before 28 weeks, (ii) before 32 weeks, (iii) before 34 weeks, and (iv) before 37 weeks in twin pregnancies?
- What are the risk estimates of the biochemical predictors associated with preterm birth (i) before 28 weeks, (ii) before 32 weeks, (iii) before 34 weeks, and (iv) before 37 weeks in twin pregnancies?

Association between chorionicity and preterm birth in twin pregnancies

- What is the risk of preterm birth at (i) ≤28 weeks, (ii) ≤32 weeks, (iii) ≤34 weeks, (iv) <37 weeks' gestation in monochorionic twin pregnancies compared to dichorionic twin pregnancies?

Maternal outcomes in twin pregnancies following assisted reproductive technology

- Is there a significant difference in maternal outcomes between twin pregnancies conceived following ART and those conceived by non-ART methods?
- Is there a significant difference in maternal outcomes between twin pregnancies conceived following ART and those conceived naturally?
- What are the estimates of maternal risks in twin pregnancies conceived following ART compared to those conceived by non-ART methods and natural conception?

  Perinatal outcomes in twin pregnancies following assisted reproductive technology
- Do perinatal outcomes significantly differ between twin pregnancies conceived following ART compared with those conceived by (i) non-ART methods and (ii) natural conception?
- What are the risk estimates for perinatal outcomes in twin pregnancies conceived following ART compared with those conceived by (i) non-ART methods and (ii) natural conception?

Table 1: Structured questions for each chapter of this thesis

Maternal clinical predictors of preterm birth in twin pregnancies				
Chapter number	**Population**	**Intervention or test**	**Outcome**	**Research design**
3	Twin pregnancies	Maternal clinical predictors: • Maternal age • Maternal body mass index (BMI) • Race • Parity • History of smoking • History of preterm birth in previous pregnancies • Pre-existing or new-onset conditions such as diabetes mellitus, anaemia and hypertensive disorders of pregnancy	Preterm birth at gestations: • <34 weeks • <37 weeks	Systematic review and meta-analysis
Biochemical predictors of preterm birth in twin pregnancies				
Chapter number	**Population**	**Intervention or test**	**Outcome**	**Research design**
4	Twin pregnancies	Biochemical predictors: • Cervicovaginal Fetal fibronectin • Maternal serum PAPP-A • Maternal serum hCG • Maternal serum Relaxin • Maternal serum Alfa fetoprotein • Maternal serum 25 hydroxyvitamin D • Maternal cervical PhIGFBP-1 • Amniotic fluid MMP-8 • Maternal cervical Granulocyte elastase • Maternal cervicovaginal Interleukin-8	Preterm birth at gestations: • <28 weeks • <32 weeks • <34 weeks • <37 weeks	Systematic review and meta-analysis

		• Intrauterine infection (chorioamnionitis, positive amniotic culture) • Bacterial vaginosis		
Association between chorionicity and preterm birth in twin pregnancies				
Chapter number	**Population**	**Intervention or test**	**Outcome**	**Research design**
5	Twin pregnancies	Chorionicity: • Monochorionicity • Dichorionicity	Preterm birth at gestations: • ≤28 weeks • ≤32 weeks • ≤34 weeks • <37 weeks	Systematic review and meta-analysis
Maternal outcomes in twin pregnancies following assisted reproductive technology				
Chapter number	**Population**	**Intervention or test**	**Outcome**	**Research design**
6	Twin pregnancies conceived via spontaneous conception or via assisted reproductive techniques	Assisted reproductive techniques: • In vitro fertilization (IVF) • Intracytoplasmic sperm injection (ICSI) • Fresh or frozen embryo transfer • Gamete intrafallopian transfer (GIFT)	Maternal outcomes: • Preterm birth at gestational ages: <28 weeks, <32 weeks, <34 weeks, <37 weeks • Gestational hypertension • Pre-eclampsia • Hypertensive disorders in pregnancy • Gestational diabetes mellitus • Diabetes in pregnancy • Antepartum haemorrhage • Placenta previa • Placental abruption • Postpartum haemorrhage • Caesarean delivery	Systematic review and meta-analysis

Perinatal outcomes in twin pregnancies following assisted reproductive technology				
Chapter number	**Population**	**Intervention or test**	**Outcome**	**Research design**
7	Twin pregnancies conceived via spontaneous conception or via assisted reproductive techniques	Assisted reproductive techniques: • In vitro fertilization (IVF) • Intracytoplasmic sperm injection (ICSI) • Fresh or frozen embryo transfer • Gamete intrafallopian transfer (GIFT)	Perinatal outcomes: • Stillbirth • Neonatal death • Perinatal mortality • Small for gestational age <10^{th} centile • Small for gestational age <5^{th} centile • Birth weight discordance >25% • Twin-twin transfusion syndrome • Any congenital malformation • Major congenital malformations • APGAR score <7 at 5 min • Admission to the neonatal intensive care unit • Respiratory distress syndrome • Mechanical ventilation • Neonatal sepsis • NEC • Neurological complications • Other offspring morbidity: • IVH, Neonatal jaundice, Neonatal hypoglycaemia, HIE, Umbilical cord pH <7.2	Systematic review and meta-analysis

Chapter 2: Methodology

The evidence obtained by systematic reviews and meta-analysis is considered the highest form in the hierarchy of evidence in medicine and public health. Potential advantages of meta-analyses include the ability to answer questions not posed by individual studies, improved precision, and the opportunity to settle controversies arising from conflicting claims. A well-conducted systematic review contributes to informed decision making in evidence-based medical practice by providing graded recommendations for guidelines and policies. Hence, it is the research method used in this thesis.

Robust systematic reviews identify relevant studies, appraise their quality, and summarise their results using scientific methodology. Using explicit, systematic methods to minimise bias, systematic reviews provide more reliable findings from which conclusions and decisions can be drawn. [127, 128] Meta-analysis is a part of a systematic review and refers to a statistical method for combining the results of several individual studies to produce a summary result. A five steps process was employed when conducting the systematic reviews in this thesis, as depicted in figure 2. [129]

2.1: Framing questions for a review

A structured approach which is unambiguous and specific was used when framing questions, as all aspects of the review flow directly from the original questions. Hence the PICO format with the following question components was utilised; population, intervention, comparison (control) and outcomes. As a systematic review is an analysis of existing studies, it is essential to consider how population characteristics, variations in interventions, outcomes and the differences in study

designs can impact the review results. Thus, review questions in this thesis were formulated a priori, and modifications were allowed only after careful consideration.

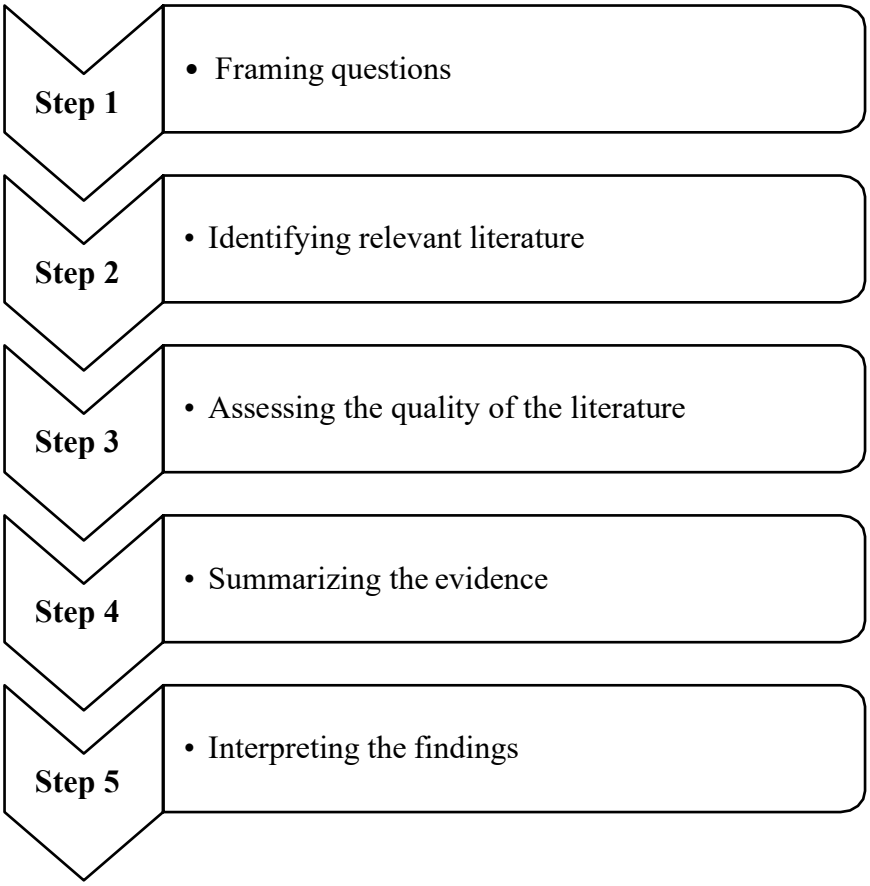


Figure 2: Five-step process in a systematic review

It is good practice to register the review once the protocol has been completed before the commencement of the preliminary search. Prospective enrolment of systematic reviews encourages transparency, helps avoid duplication and reduces the opportunity for reporting bias. The protocols for all the systematic reviews conducted as part of this MD thesis have been registered prospectively in the "The International Prospective Register of Systematic Reviews" (PROSPERO), which is an open-access database of systematic reviews administered by the

Centre of Reviews and Dissemination, University of York, UK. It offers a free online platform for registration. [130]

2.2: Literature search to identify relevant work

A detailed and thorough search of existing literature is crucial for a systematic review and involves utilising multiple resources. Poor searches contribute to imprecision of effect in a review by identifying only a fraction of available studies and leading to wide confidence intervals around summary effects.

The electronic databases in the literature search for the reviews in this thesis included general databases such as Medline, Embase, and Cochrane Library. Search term combinations were designed to capture as many relevant citations as possible. Reference lists from identified studies and relevant reviews were searched, including the latest issues of key journals, conference proceedings and grey literature. The help of the local librarian was sought when required. Citations were retrieved and managed with the reference manager, Endnote. Searches undertaken in the beginning were updated later depending on the length of time taken for the review.

The pre-defined study selection criteria were adhered to in selecting relevant studies. Two independent reviewers conducted the steps of citation screening and study selection to avoid bias, and the full manuscripts considered relevant by any of the two reviewers were retrieved. Disagreements were resolved by consensus or with the involvement of a third reviewer. Only the largest study with the longest follow-up was selected among studies with multiple publications. Language restrictions were not applied in searching or study selection. Reasons for study exclusion were recorded and detailed in a flow chart for transparent reporting. Further details on

the literature search for the systematic reviews conducted are provided in detail in the relevant chapters of this thesis.

2.3: Assessing the quality of studies

Quality assessment should be followed in every step of a systematic review. Study quality is the degree to which a study employs measures to minimize bias and error in its design, conduct and analysis. Studies qualified as relevant are appraised based on the study design, conduct and analysis using quality assessment checklists. There are standardized quality assessment checklists that can be used in systematic reviews depending on the study design and type of review. Two independent reviewers should carry out the quality assessment of the selected studies to eliminate bias.

In this thesis, the primary tool used for the quality assessment of cohort and case-control studies was the Newcastle Ottawa Scale.[131] It introduces a star system based on the three domains; study selection, comparability, and ascertainment of the outcome, where stars are allotted for adherence to pre-specified criteria. A maximum of one star is allotted for each item within the selection and outcome categories, and a maximum of two stars is allotted for comparability. Studies that score four stars for selection, two stars for comparability between the cohorts, and three stars for the ascertainment of outcome are regarded as having a low risk of bias. While studies that have two or three stars for selection, one for comparability and two for outcome ascertainment, have a medium risk of bias. Any study with a score of one for selection or outcome ascertainment or zero for any of the three domains is considered to have a high risk of bias. Table 2 provides the checklist for the Newcastle Ottawa Scale.

Table 2: New Castle Ottawa Scale

SELECTION	**1. Representativeness of the exposure cohort in the community** a. Truly representative □ ***** b. Somewhat representative □ ***** c. Selected group of patients (only certain socio-economic groups/areas) □ d. No description of the derivation of the cohort □
	2. Selection of the non-exposed cohort a. Drawn from the same community as the exposure cohort □ ***** b. Drawn from a different source □ c. No description of the derivation of the non-exposure cohort □
	3. Ascertainment of exposure a. Secure record (e.g., Healthcare record) □ ***** b. Structured interview □ ***** c. Written self-report □ d. Other/no description □
	4. Demonstration that outcome of interest was not present at the start of the study a. Yes □ ***** b. No □
COMPARABILITY	**1. Comparability of the cohorts on the basis of the design or analysis** a. Study controls for age, sex, marital status □ ***** b. Study controls for any additional factors (e.g., Socio-economic status, education) □ *****
OUTCOME	**1. Assessment of outcome** a. Independent blind assessment (stated in the paper/reference to secure records) □ ***** b. Record linkage (ICD codes on databases) □ ***** c. Self-report □

	d. Other/no description □
	2. Was follow up long enough for the outcome to occur a. Yes, if the median duration of follow up ≥ 6 months □ ***** b. No, if the median duration of follow up < 6 months □
	3. Adequacy of follow up of cohorts a. Complete follow up: all subjects accounted for □ ***** b. Subjects lost to follow up unlikely to introduce bias: number lost ≤ 20%, or description of those lost suggesting no difference from those followed up □ ***** c. Follow up rate < 80% and no description of those lost □ d. No statement □

2.4: Summarising the evidence

Data synthesis consists of tabulation of characteristics of studies, quality, and effects. It also uses statistical methods to explore differences between studies and combine their effects. A descriptive summary of the studies' characteristics, design, quality, and effects must be presented, which will help gain a deeper understanding of the evidence and prevent interpretation errors. Tabulation of evidence helps assess the feasibility of statistical analysis and aids in the discovery of differences between studies in their clinical characteristics (clinical heterogeneity) and study design and quality (methodological heterogeneity).

Forest plots allow a graphical display of individual effects. When tabulations only allow an overtly descriptive summary, which is hard to assimilate at a glance, forest plots are utilised. Furthermore, forest plots provide a window for qualitative judgements about the direction, magnitude, precision, and other details on individual effects. This qualitative examination of

observed effects may occasionally lead to conclusions without the necessity of further statistical input.

The qualitative examination is often insufficient, directing the reviewer towards quantitative synthesis. Meta-analysis pools the observed effects of logically comparable studies according to the weight of a measure of importance in each study to produce a weighted average effect. Two models assess the robustness of this summary effect to the variation in statistical methods: random effects and fixed effects statistical model. Compared to fixed effects models, the random-effects model weights smaller studies proportionately higher and where there is heterogeneity, a random-effects model produces wider confidence intervals of the summary effect. The model utilised in this thesis for meta-analysis is the random-effects model. After meta-analysis, the summary effect and the assigned weight of individual studies could be graphically displayed on a forest plot.

The two main threats that affect the validity of a meta-analysis are heterogeneity and small-study effects. [132] Exploring the possible reasons for heterogeneity between studies is essential when conducting a meta-analysis. Heterogeneity may have different sources. There is almost always clinical heterogeneity between patients from different studies, heterogeneity related to study design or other study characteristics, or even statistical heterogeneity.

It is crucial to consider the extent to which the results of studies are consistent. If the confidence intervals for the results of individual studies have poor overlap, statistical heterogeneity is likely. Statistical tests for heterogeneity include I^2 statistics, which focuses on assessing the impact of heterogeneity on the meta-analysis. It describes the percentage of the variability in effect estimates due to heterogeneity rather than chance. I^2 of 0% to 40% can be interpreted as might

not be important, 30% to 60% as moderate heterogeneity, 50% to 90% representing substantial heterogeneity and 75% to 100% suggesting considerable heterogeneity. The importance of the observed value of I^2 depends on the magnitude and direction of effects and the strength of evidence for heterogeneity, such as the P-value from the chi-squared test or a confidence interval for I^2.

Meta-regression is a technique for exploring heterogeneity. It is a linear regression model for examining the influence of study characteristics and quality on the size of the individual effects observed among studies. In the systematic reviews of this thesis, heterogeneity was explored using I^2 statistics and meta-regression. A visual exploration of funnel plot asymmetry to detect small-study effects was performed when more than ten studies were available, the details of which are provided in the relevant chapters.

If substantial heterogeneity prevents utilising an overall meta-analysis, a subgroup meta-analysis can be performed according to clinically relevant subgroups, study designs, and other comparable groups. Re-analysing data under different assumptions to identify the impact of these assumptions on the result can be done using sensitivity analysis. In this thesis, both subgroup analysis and sensitivity analysis have been performed when applicable.

2.5: Interpreting the findings

Interpreting the findings involves critically evaluating the results generated from the preceding steps and drawing plausible conclusions. The validity of the main findings will depend on the strengths and weaknesses of the review. The interpretation of the processed data should be guided with consideration to potential biases, and if there is evidence of bias, it should be reported with the same prominence as the predicted effect.

Funnel plot analysis is a commonly used method to explore biases. It is a scatter plot of individual effects observed among studies against the study size. We have more confidence that publication and related bias are unlikely in a symmetrical funnel plot. However, if the funnel is asymmetrical, it may indicate publication bias or other biases such as location, language, citation, and multiple publication biases. Also, funnel plot asymmetry could result from an overestimation of treatment effects in small studies of low quality. [133] Therefore, funnel plots should also be seen as a means of examining smaller study effects. [134] Several statistical methods are available to investigate funnel plot asymmetry, including the Rank correlation method proposed by Begg and Mazumdar and the linear regression approach introduced by Egger et al. [134] Egger's method was used in this thesis to investigate funnel plot asymmetry. Transparent reporting of a systematic review is ascertained with the guidance provided by the proposed reporting models like PRISMA and MOOSE guidelines. [135] PRISMA, which is the model used in this thesis, focuses on how authors can ensure the transparent and complete reporting of systematic reviews and meta-analyses. [135]

Chapter 3: Maternal characteristics to predict preterm birth in twin pregnancies

3.1: Abstract

Background

Twin pregnancies are at an increased risk of preterm birth. Maternal clinical characteristics have been associated with a higher risk of preterm birth in twins. However, the relationship between the two entities has never been systematically evaluated.

Methods

A systematic review was conducted to assess the risk of both spontaneous and iatrogenic early (<34 weeks) and late (<37 weeks) preterm birth in twin gestations based on maternal clinical characteristics. Electronic databases were searched for relevant literature published from January 1990 to November 2017, without language restrictions. Studies on both dichorionic and monochorionic twin pregnancies were included. The findings were reported as odds ratios (OR) with 95% confidence intervals (CI), and the estimates were pooled using random-effects meta-analysis for various predictor thresholds.

Results

From 12,473 citations, 59 studies (2,930,958 pregnancies) were included in the review. The risks of early preterm birth in twin pregnancies were significantly increased in women with a previous history of preterm delivery (OR 2.67, 95% CI 2.16-3.29, I^2= 0%), teenagers (OR 1.81, 95% CI 1.68-1.95, I^2= 0%), BMI>35 kg/m^2 (OR 1.63, 95% CI 1.30-2.05, I^2= 52%), nulliparity (OR 1.51, 95% CI 1.38-1.65, I^2= 73%), non-white vs. white women (OR 1.31, 95% CI 1.20-1.43, I^2= 0%), black vs. non-black women (OR 1.38, 95% CI 1.07-1.77, I^2= 98%), diabetes (OR 1.73, 95% CI 1.29-2.33, I^2= 0%), and smokers (OR 1.30, 95% CI 1.23-1.37, I^2= 0%). The risks of late preterm

birth were also increased in women with a previous history of preterm delivery (OR 3.08, 95% CI 2.10-4.51, I^2= 73%), teenagers (OR 1.36, 95% CI 1.18-1.57, I^2= 57%), BMI>35 kg/m^2 (OR 1.18, 95% CI 1.02-1.35, I^2= 46%), nulliparity (OR 1.41, 95% CI 1.23-1.62, I^2= 68%), diabetes (OR 1.44, 95% CI 1.05-1.98, I^2= 55%), and hypertension (OR 1.49, CI 1.20-1.86, I^2= 52%).

Conclusion

Maternal clinical characteristics pose an additionally higher risk of early and late preterm birth in twin pregnancies, which should be considered in patient counselling and management.

Publication arising from this chapter

Marleen S, Hettiarachchi J, Dandeniya R, Macgreggor R, Aquilina J, Khalil A, Vogel J, Betrán AP, Thangaratinam S. Maternal clinical predictors of preterm birth in twin pregnancies: A systematic review involving 2,930,958 twin pregnancies. European Journal of Obstetrics & Gynaecology and Reproductive Biology. 2018; 230:159-171.

Poster presentation and resulting peer-reviewed abstract publications

S Marleen, J Hettiarachchi, R Dandeniya, R Macgreggor, J Aquilina, A Khalil, J Vogel, AP Betran, S Thangaratinam, “Maternal Clinical Predictors of Preterm Birth in Twin Pregnancies: A Systematic Review Involving 2,930,958 Twin Pregnancies.” BJOG: an international journal of obstetrics and gynaecology. 126 (2019):64-65 – Top 500 Abstracts (RCOG world congress 2019)

3.2: Introduction

Preterm birth has been identified as the major cause of perinatal morbidity and mortality in developed countries, causing two-thirds of deaths in newborns without congenital malformations. [136] Twins carry a perinatal mortality rate three times that of singletons, with

most deaths accounted to prematurity. [137] Up to 50% of twins are born before completion of 37 weeks, while about one fifth are born before 34 weeks. [138] Preterm twins often require support in managing their short-term and long-term health issues arising from complications, including neurodevelopmental problems. [139]

The aetiology of preterm delivery in twins is most likely multifactorial and different from those of singleton pregnancies. It is essential to identify twin pregnancies at higher risk of preterm birth so that frequency of monitoring and the place of birth can be determined, including administration of antenatal corticosteroids in a timely manner. This is of added value in countries with limited resources for tertiary neonatal care, where resources can be effectively used for twin pregnancies at high risk of preterm birth with early referral and in utero transfer.

Maternal clinical characteristics that pose an added risk of preterm birth can be identified at the first antenatal visit. Various prediction models to predict the likelihood of preterm birth in singletons have been developed, but such data is sparse for twin pregnancies. Individual studies vary in the risk estimates reported, and small study populations often limit them from arriving at solid conclusions. A systematic review has not yet been conducted evaluating the association between maternal clinical predictors and preterm birth in twin pregnancies. Therefore, this systematic review was performed to evaluate the risk of early and late preterm birth in twins for various maternal clinical characteristics.

3.3: Methods

This review was performed using a prospective protocol, and the findings are reported according to the PRISMA guidelines. [140, 141] The PROSPERO ID of this systematic review's protocol is

CRD42015026465.

(http://www.crd.york.ac.uk/PROSPERO/review_print.asp?RecordID=26465&UserID=14183).

Literature search

The electronic databases MEDLINE, CINAHL, LILACS, EMBASE, were searched for relevant studies published from 1 January 1990 to 1 November 2017, without any language restrictions. The search terms used were 'twin pregnancy' and 'multiple pregnancies', combined with terms for outcomes like 'prematurity', 'preterm', 'premature birth' or 'preterm birth'. Additional terms for various clinical predictors were combined with the above terms. Where applicable, we "exploded" the search terms. The reference lists of all primary studies and previously published systematic reviews were manually searched to supplement the electronic search. The search strategy is depicted in Appendix 2.

Study selection

The identified citations were first screened by their title and abstract to select potentially relevant studies. The selected citations were then subjected to full-text evaluation. Studies that fulfilled the inclusion criteria were included in the review. Two independent reviewers (SM and RD) undertook study selection, and any disagreements were resolved by consensus after discussion with a third reviewer (ST). Studies on dichorionic or monochorionic twin pregnancies which assessed the risk of preterm birth posed by maternal clinical predictors were included in the review.

At the time of registering the review with PROSPERO, we aimed to assess the following predictors: maternal age, maternal height, maternal weight, maternal weight gain during

pregnancy, maternal BMI, ethnicity, parity, maternal education, occupational status, alcohol intake, caffeine intake, maternal smoking, marital status, previous history of preterm birth, previous miscarriages, gestational age of mother's birth, method of conception, multifetal reduction, activity during pregnancy, prenatal care, maternal comorbidities (pre-eclampsia, chronic hypertension, diabetes mellitus, maternal pregestational or gestational DM, asthma, depression, thyroid disease, heart disease, GI disorders, renal disorders), maternal anaemia, uterine contractions, and vaginal bleeding.

However, due to the extensive list of maternal predictors mentioned in the PROSPERO registration, only the predictors considered to be of high importance were included in this review. Additionally, certain predictors could not be assessed due to insufficient data, and this thesis analysed data on ART as an independent review.

The maternal clinical predictors assessed were: age, body mass index (BMI), race, parity, history of smoking, history of preterm birth in previous pregnancies, pre-existing or new-onset conditions such as diabetes in pregnancy (gestational diabetes, pre-existing diabetes, or both), anaemia and hypertensive disorders of pregnancy (gestational hypertension, pre-eclampsia or pre-existing hypertension). Studies that assessed spontaneous or iatrogenic, early (<34 weeks) or late (<37 weeks) preterm birth were included. During the PROSPERO registration, we had aimed to analyse preterm birth at <37 weeks, <32 weeks and <28 weeks cut-offs. However, as studies varied vastly in their reporting of preterm birth, we decided to stratify data on preterm birth as <37 weeks and <34 weeks. Studies that reported only on assisted reproduction as a predictor was excluded in this review as it was assessed in an independent review included in this thesis. In vitro studies, animal studies, case reports and case series were also excluded. The

primary authors' definitions, stratifications or thresholds for the various factors evaluated were accepted. A minimal sample size was not considered. Assessment of gestational age by any method was accepted.

Study quality assessment and data extraction

The Newcastle Ottawa Scale (NOS) was used by two independent reviewers (SM and RD) to assess the methodological quality of the studies. [142] The risk of bias in the selection, comparability and outcome assessment of cohorts was evaluated, and stars were allocated for adherence to the pre-specified criteria [143] as elaborated in the methodology chapter of this thesis.

Data extraction was carried out in duplicate by two reviewers (SM and RD), and the data was recorded in a customized data extraction form. Dichotomous data were extracted to 2×2 tables. If the potentially acceptable articles had insufficient data, we contacted the authors by email for the required information. Only the most recent study was included if several articles had been published from the same cohorts for the same outcomes.

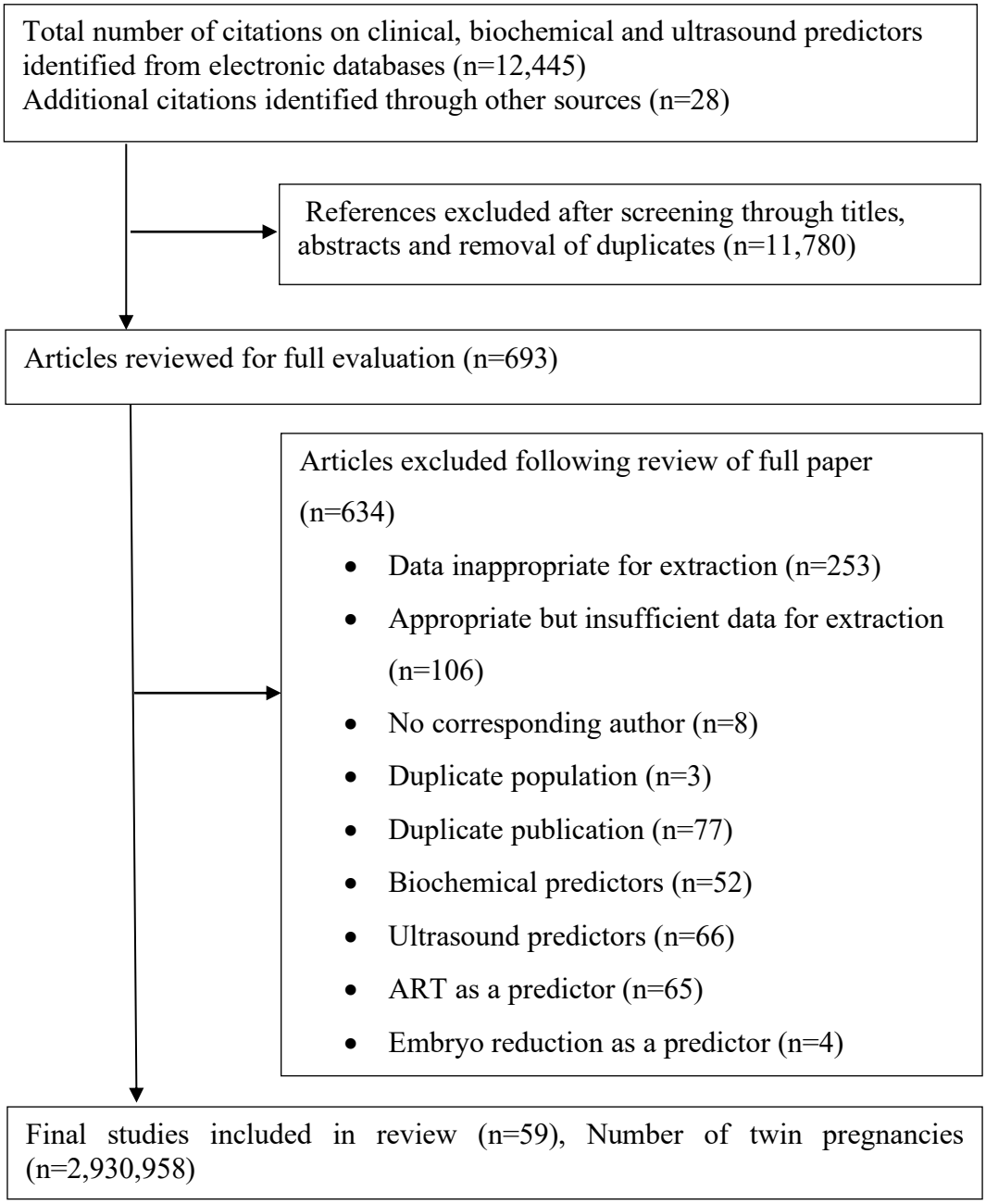
Statistical analysis

The estimates of the individual studies were pooled with random-effects meta-analysis. The summary estimates were reported as odds ratios (OR) with 95% confidence intervals (CI) for variously reported thresholds of the predictors. I^2 statistics were used to evaluate the heterogeneity of the studies. Sensitivity analysis was performed, limiting the meta-analysis to twin pregnancies with spontaneous preterm birth. Funnel plot asymmetry was evaluated to detect potential small-study effects where more than ten studies were available. [144] The software RevMan was used to carry out all analyses. [145]

3.4: Results

Out of 12,473 citations, 59 studies (2,930,958 pregnancies) were included in our analysis. The study selection process is depicted in detail in Figure 3.

Figure 3: Study selection process in the systematic review of maternal clinical predictors of preterm birth in twin pregnancies



3.4.1: Characteristics of the included studies

Out of the 59 studies, 15 were prospective cohorts [83, 100, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158], 40 were retrospective cohorts [47, 48, 51, 59, 61, 62, 77, 79, 81, 95, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188], three were cohorts nested within randomised trials [189, 190, 191], and one was a case-control study. [73] 17 studies provided data from registries. [47, 48, 62, 77, 159, 160, 166, 168, 169, 171, 175, 176, 178, 180, 186, 187, 188] Fifty seven studies were conducted in high-income countries (USA 35, Israel 4, Canada 3, Italy 3, UK 3, Sweden 2, Denmark 2, Japan 2, Belgium 1, Korea 1, Brazil 1). Two studies were conducted in middle-income countries (Iran, South Africa). 88% (52/59) of the studies were published after 2000. The sample sizes ranged from 20 [158] to 779,387. [160]

49% (29/59) of the studies explicitly reported exclusion of complicated twin pregnancies such as those with major fetal anomalies (21 studies) [79, 81, 95, 147, 150, 151, 154, 156, 160, 161, 162, 167, 170, 173, 176, 177, 180, 182, 183, 184, 185], twin to twin transfusion syndrome (10 studies), [79, 81, 147, 151, 161, 162, 167, 172, 173, 174] and stillbirth (16 studies). [48, 51, 61, 79, 95, 147, 151, 153, 161, 167, 169, 170, 177, 184, 186, 188] Monochorionic twin pregnancies were excluded from four studies [73, 164, 172, 179] and eight studies excluded monoamniotic twin pregnancies. [81, 151, 161, 162, 167, 173, 174, 183] Five studies excluded chromosomal abnormalities [79, 160, 161, 173, 180] and three excluded selective fetal reduction. [95, 161, 189] Only one study reported the exclusion of twin pregnancies with selective intrauterine growth restriction. [153]

Parity was reported as a maternal clinical predictor in 28 studies (47%). [47, 51, 73, 81, 83, 95, 100, 146, 147, 149, 150, 151, 152, 153, 155, 157, 158, 162, 163, 165, 170, 173, 174, 180, 181, 182, 185, 191] 85% of studies (50/59) were not

selective about their study populations and included women both symptomatic and asymptomatic for preterm labour. [47, 48, 51, 59, 61, 62, 77, 79, 81, 83, 95, 100, 148, 150, 152-157, 159, 160-163, 165-189] Treatment or prevention of preterm labour was assessed in seven studies. [100, 156, 157, 158, 163, 173, 181] Three-quarters of studies reported data on early preterm birth (<34 weeks) (44/59, 75%), [47, 48, 51, 59, 61, 62, 73, 77, 81, 83, 95, 146-148, 150, 151, 153-155, 157, 159, 160, 162, 164-168, 171-174, 177, 178, 180-182, 183-187, 189, 190, 191] and a similar proportion reported on late (<37 weeks) preterm birth (45/59, 76%). [48, 51, 59, 61, 77, 79, 81, 83, 100, 146, 149, 150, 152, 153, 154, 156, 158, 159, 160, 161, 162, 163, 164, 166, 167, 168, 169, 170, 172, 175, 176, 177, 178, 179, 180, 182-191] Spontaneous preterm birth was evaluated by 32% of the studies (19/59). [100, 146-149, 151, 153-155, 163, 165, 170, 173, 181, 188-190, 192, 193] The description of characteristics of the included studies is provided in Appendix 3.

3.4.2: Quality of the included studies

Over 75% of studies (46/59) [47, 51, 59, 61, 62, 79, 81, 95, 100, 146, 147, 149, 150, 151, 153-156, 158, 160-163, 167, 168, 169-171, 173-185, 187-191] were low risk of bias for study selection, 41% of studies (24/59) were low risk for comparability of cohorts [48, 51, 61, 83, 95, 100, 146-149, 160-163, 167-169, 171, 174, 180, 183, 184, 186, 188] and 93% of studies (55/59) were low risk for outcome assessment. [47, 48, 51, 59, 61, 62, 77, 79, 81, 83, 95, 100, 146-163, 166-189, 191] 22% of studies (13/59) were medium risk of bias for study selection, [48, 73, 77, 83, 148, 152, 157, 159, 164-166, 172, 186] 29% of studies (17/59) were medium risk of bias for comparability of cohorts, [47, 62, 73, 77, 79, 81, 151, 153-156, 158, 159, 173, 181, 187, 191] and 5% of studies (3/59) were medium risk of bias for outcome. [73, 164, 165] No studies were at high risk of bias for study selection. A third of studies (18/59) were high risk of bias for comparability of cohorts [59, 81, 150, 152, 157, 164-166, 170, 172, 175-179, 182, 185, 189, 190] while one study was considered to have a high risk of bias for outcome assessment. [190] The quality of the included studies is given in Appendix 4.

3.4.3. Maternal clinical predictors of preterm birth in twin pregnancies

The pooled OR for early and late preterm birth for the clinical predictors evaluated are given in Table 3. The forest plots are included in Appendix 5.

Maternal demographic characteristics

Fifteen studies (386,421 pregnancies) reported age as a predictor of preterm birth in twin pregnancies. [47, 59, 61, 62, 73, 152, 154, 159, 164-166, 172, 178, 187, 188] When the age was less than 20 years, the odds of both early (OR 1.81, 95% CI 1.68–1.95, I^2 = 0%) and late preterm birth (OR 1.36, 95% CI 1.18– 1.57, I^2 = 57%) were significantly increased. In women over 35 years of age, the odds of early but not late preterm birth were significantly lowered (OR 0.89, 95% CI 0.82-0.96, I^2 = 37%). The risk of early or late preterm birth was not significantly associated with age over 40 years.

10 studies (44,052 pregnancies) evaluated the association between maternal BMI and preterm birth in twins. [48, 73, 154, 156, 167, 168, 183, 186, 188, 190] A BMI of >35 kg/m^2 placed the woman at a higher risk for both early (OR 1.63, 95% CI 1.30–2.05, I^2 = 52%) and late (OR 1.18, 95% CI 1.02–1.35, I^2 = 46%) preterm birth. BMI values >30 kg/m^2 and <19.8 or 18.5 kg/m^2 were not significantly associated with early or late preterm birth.

Table 3: Pooled odds ratios (OR) for early and late preterm birth (PTB) in the systematic review of maternal clinical predictors of preterm birth in twin pregnancies.

Predictor	Outcome PTB	No. of studies	Experiment		Control		OR (95% CI)	P-value	I^2 (%)
			No. of PTB	No. of women	No. of PTB	No. of women			
Age (years)									
<20 vs >20	Early (<34)	5	3656	14836	24312	148645	1.72(1.60-1.86)	0.00001	39
	Late (<37)	5	7362	12296	43227	81231	1.36(1.18-1.57)	0.0001	57
Age (years)									
>35 vs <35	Early (<34)	8	5571	50625	21328	169966	0.89(0.82-0.96)	0.003	37
	Late (<37)	6	9430	16008	18691	29561	0.77(0.35-1.70)	0.52	98
Age (years)									
>40 vs <40	Early (<34)	4	887	7935	25712	209259	0.89(0.71-1.12)	0.33	74
	Late (<37)	3	1294	2183	24389	39353	1.28(0.73-2.22)	0.39	82
BMI (kg/m^2)									
>30 vs <30	Early (<34)	7	944	7025	3629	33108	1.15(0.88-1.52)	0.31	85
	Late (<37)	8	4006	7777	19548	35691	0.83(0.54-1.26)	0.41	98
BMI (kg/m^2)									
>35 vs <35	Early (<34)	2	217	1784	2229	27838	1.63(1.30-2.05)	0.0001	52
	Late (<37)	4	1180	2147	15890	31086	1.18(1.02-1.35)	0.02	46
BMI (kg/m^2)									
<19.8 or 18.5 vs	Early (<34)	2	85	363	1753	8530	1.22(0.95-1.56)	0.12	0
>19.8 or 18.5	Late (<37)	3	345	550	6552	10672	1.15(0.96-1.37)	0.13	0
Race									
Black vs Non-black	Early (<34)	7	27358	106756	64374	431816	1.38(1.07-1.77)	0.01	98
	Late (<37)	5	119946	251032	470417	1125379	1.17(0.89-1.55)	0.26	100
Race									
Non-white vs White	Early (<34)	4	772	5903	2131	20731	1.31(1.20-1.43)	0.00001	0
	Late (<37)	4	3649	5902	12787	20520	0.98(0.92-1.04)	0.54	0
Parity									
Nulliparity vs Multiparity	Early (<34)	21	30002	198536	32018	306894	1.51(1.38-1.65)	0.00001	73
	Late (<37)	17	59803	131222	72707	195395	1.41(1.23-1.62)	0.00001	68
Smoking									
Smoking vs Non-smoking	Early (<34)	12	1774	9768	10375	71231	1.30(1.23-1.37)	0.00001	0
	Late (<37)	9	6968	10166	48468	70398	1.16(0.92-1.46)	0.21	65
Previous preterm birth									
	Early (<34)	13	159	516	820	6050	2.67(2.16-3.29)	0.00001	0
	Late (<37)	13	553	906	3577	9841	3.08(2.10-4.51)	0.00001	73
Hypertensive disorders in pregnancy									
	Early (<34)	3	36	296	218	1683	0.91(0.63-1.33)	0.64	0
	Late (<37)	4	14929	23207	142378	257900	1.49(1.20-1.86)	0.0003	52
Diabetes in pregnancy									
	Early (<34)	3	70	280	440	2994	1.73(1.29-2.33)	0.0003	0
	Late (<37)	3	9143	15004	228773	409252	1.44(1.05-1.98)	0.03	55
Anaemia in pregnancy									
	Early (<34)	1	520	2893	13382	77602	1.05(0.95-1.16)	0.31	NA
	Late (<37)	1	1653	2893	43225	77602	1.06(0.98-1.14)	0.13	NA

gestations and maternal race. [51, 62, 77, 79, 146, 149, 154, 160, 169, 170, 177, 181, 188] A significantly higher number of early preterm births was observed in non-white women in comparison to white women (OR 1.31, 95% CI 1.20–1.43, I^2 = 0%), and in black women compared to non-black women (OR 1.38, 95% CI 1.07–1.77, I^2 = 98%). However, there was no significant association between maternal race and late preterm delivery for non-white women vs white women and black women vs non-black women.

The relationship between preterm birth in twin pregnancies and the mother's smoking status was assessed in 15 studies (83,955 pregnancies). [51, 73, 79, 83, 100, 146, 148, 150, 154, 165, 171, 173, 181, 188, 194] Women who smoked were observed to have a significantly higher risk of early preterm birth when compared to women who did not smoke (OR 1.30, 95% CI 1.23–1.37, I^2 = 0%). A significant effect was not observed for late preterm birth.

Pregnancy characteristics

Twenty-eight studies (508,021 pregnancies) evaluated the association between nulliparity and preterm delivery in twin gestations. [47, 51, 73, 81, 83, 95, 100, 146, 147, 149, 150-153, 155, 157, 158, 162, 163, 165, 170, 173, 174, 180-182, 185, 191] Nulliparity placed the women at a higher risk for both early (OR 1.51, 95% CI 1.38–1.65, I^2 = 73%) and late (OR 1.41, 95% CI 1.23– 1.62, I^2 = 68%) preterm birth, in comparison to multiparous women. A previous preterm birth was evaluated as a predictor of preterm birth in nineteen studies (9,924 pregnancies). [79, 81, 83, 95, 100, 146, 147, 151, 154, 155, 163, 165, 173, 179, 181, 184, 188, 189, 191] Women with a history of a previous preterm delivery were identified to have a higher risk for both early (OR 2.67, 95% CI 2.16– 3.29, I^2 = 0%) and late (OR 3.08, 95% CI 2.10–4.51, I^2 = 73%) preterm birth in their subsequent twin pregnancies.

Medical disorders in pregnancy

The relationship between preterm birth and maternal diabetes was assessed in five studies (425,918 pregnancies). [51, 73, 79, 161, 176] Women with diabetes in pregnancy were found to have a significantly higher risk of early preterm birth (OR 1.73, 95% CI 1.29–2.33, I^2 = 0%) and late preterm birth (OR 1.77, 95% CI 1.05–1.98, I^2 = 55%) when compared with women without diabetes. Hypertensive disorders were assessed in 5 studies involving 281,376 pregnancies. [51, 73, 79, 150, 175] A significantly higher risk of late preterm birth was identified in women with hypertensive disorders in pregnancy (OR 1.49, 95% CI 1.20-1.86, I^2= 52%). No significant association was found for early preterm birth. One study involving 80,495 pregnancies assessed maternal anaemia as a possible predictor of preterm birth in twin pregnancies. [166] No significant association was found between maternal anaemia and early or late preterm birth.

3.4.4: Sensitivity analysis

Sensitivity analysis was performed on the data for spontaneous preterm birth. The risk of spontaneous preterm birth was significantly elevated in women with a history of previous preterm delivery, for both early (OR 2.83, 95% CI 2.00–4.00, I^2 = 0%) and late (OR 2.77, 95% CI 1.66- 4.63, I^2 = 85%) preterm birth. On the contrary, one study showed that women over 40 years of age had a significantly lower risk of early (OR 0.14, 95% CI 0.03 – 0.58) and late (OR 0.53, 95% CI 0.31– 0.90) spontaneous preterm birth. [172] A similar reduction in spontaneous late preterm birth was observed in one study among black women, compared to non-black women (OR 0.48, 95% CI 0.24 – 0.98). [102] A significant association was not observed for maternal age

>35 years, BMI, non-white vs white race, nulliparity and maternal smoking. Pooled ORs for spontaneous early and late preterm birth are given in Table 4.

Table 4: Pooled odds ratios (OR) for spontaneous early and late preterm birth (PTB) in the systematic review of maternal clinical predictors of preterm birth in twin pregnancies.

Predictor	Outcome SPTB	No. of studies	Experiment		Control		OR (95% CI)	P-value	I^2 (%)
			No. of SPTB	No. of women	No. of SPTB	No. of women			
Age (years)									
>35 vs <35	Early (<34)	1	10	192	24	232	0.48(0.22-1.02)	0.06	NA
	Late (<37)	0							
Age (years)									
>40 vs <40	Early (<34)	1	2	165	22	265	0.14(0.03-0.58)	0.007	NA
	Late (<37)	1	22	165	60	265	0.53(0.31-0.90)	0.02	NA
BMI (kg/m^2)									
>30 vs <30	Early (<34)	1	1	29	16	252	0.53(0.07-4.12)	0.54	NA
	Late (<37)	2	61	207	183	714	0.93(0.38-2.31)	0.88	63
BMI (kg/m^2)									
<19.8 or 18.5 vs	Early (<34)	1	3	18	10	129	2.38(0.59-9.63)	0.22	NA
>19.8 or 18.5	Late (<37)	1	10	18	70	129	1.05(0.39-2.84)	0.92	NA
Race									
Black vs Non-black	Early (<34)	2	11	154	36	417	0.69(0.30-1.60)	0.38	0
	Late (<37)	1	47	97	33	50	0.48(0.24-0.98)	0.04	NA
Race									
Non-white vs	Early (<34)	3	13	426	44	163	0.19(0.02-2.28)	0.19	91
White	Late (<37)	3	59	178	142	199	0.74(0.44-1.25)	0.26	NA
Parity									
Nulliparity vs	Early (<34)	7	175	1493	135	1506	1.30(0.92-1.85)	0.14	32
Multiparity	Late (<37)	7	631	1291	437	1193	1.58(1.00-2.52)	0.05	76
Smoking									
Smoking vs	Early (<34)	6	19	133	138	1582	1.53(0.87-2.70)	0.14	0
Non-smoking	Late (<37)	3	29	59	116	254	0.89(0.36-2.16)	0.26	32
Previous preterm birth									
	Early (<34)	9	57	220	261	2702	2.83(2.00-4.00)	0.00001	0
	Late (<37)	8	390	788	2136	7828	2.77(1.66-4.63)	0.00001	85

3.4.5: Small study effects

Where more than ten studies were present, we investigated for small study effects for women's parity, smoking (for early preterm birth) and history of previous preterm delivery. Funnel plot asymmetry was not observed for any of the above maternal clinical characteristics (Appendix 6).

3.5: Discussion

Summary of main findings

This review provided quantitative estimates of the relationship between maternal clinical characteristics and preterm birth in twin gestations. Apart from the inherent baseline risk of premature birth in twins, young age (<20 years), obesity (BMI >35 kg/m^2), nulliparity, and multiparity with a history of preterm delivery impose an additional risk of both early and late preterm birth. Being of a non-white race, black race, having diabetes in pregnancy, and smoking was found to increase the likelihood of early preterm birth. Having a history of previous preterm birth appeared to be the most robust clinical predictor of both early and late preterm birth in twin pregnancies, and this observation was also demonstrated for spontaneous preterm birth.

Comparison with existing studies/reviews

The results yielded in this analysis show that most clinical predictors significant to singleton pregnancies are applicable for twin pregnancies. However, one place where it differs is where age greater than 35 years came as protective for preterm birth in twins, while it is known to be a risk factor for premature delivery in singletons. Older women being multiparous in the study population may have contributed to this finding. Also, maternal anaemia did not significantly affect preterm birth among twin pregnancies, but it is an identified risk factor in singletons. This

may be because only one study assessed the relationship between the two entities in twin pregnancies. A systematic review performed by Kazemier et al. [195] in 2014 showed a 57% (CI 51.9%– 61.9%) absolute risk of recurrence of spontaneous preterm birth before 37 weeks in a subsequent twin pregnancy following a previous singleton preterm delivery. This observation is on par with the results in our review, where the risk of both early and late preterm delivery among twins was shown to increase with a previous preterm birth.

Strengths and limitations

This is the first systematic review evaluating the relationship between maternal clinical characteristics and preterm birth in twin gestations. The detailed literature search without any language restrictions increased the probability of capturing all studies relevant to the research topic. A prospective protocol was followed for this review, and the sources of heterogeneity were explored. The quality of the studies and the effect of study quality on the results were assessed in detail. The large sample size in this meta-analysis provided high precision for significant clinical predictors.

Limitations of our study include estimation of the gestational age, which was based on the last menstrual period in many studies. Only a few studies have been published using ultrasound-based assessment to estimate gestational age. [196] Studies differed in their definition of the lower limit for preterm birth, while some studies did not mention a lower limit. Low-income countries were represented less in the data as only a few studies were available. There were also inconsistencies regarding the exclusion criteria. Some studies excluded varying complications such as monochorionic pregnancies, chromosomal and structural anomalies in the fetus, cases of

selective fetal reduction, stillbirth, twin to twin transfusion syndrome, and selective intrauterine growth restriction, which may have interfered with the outcome assessed.

The selection of women among studies varied; some had women asymptomatic for preterm labour, some had those who were symptomatic, and others included both groups. Many studies did not report on the interventions performed for preterm labour, such as tocolysis, pessary, progesterone, and cerclage. Treatment for preterm labour in some groups may have caused an underestimation of the absolute risk of preterm birth in that group.

Even though more studies were identified than those included in this analysis, some had to be excluded because various cut-offs for gestational age and delivery were used. It was not possible to pool such data, and therefore not all the existing data could be assessed for each predictor. Most studies reported data on spontaneous and iatrogenic delivery grouped as one entity. Additionally, there was considerable heterogeneity among the studies regarding the total study population, with some studies including registry data with significantly larger cohorts. Only a few studies indicated data specifically for spontaneous preterm birth, which led to a reduced precision in the sensitivity analysis for spontaneous preterm birth. Most studies included both monochorionic and dichorionic twins or excluded complicated monochorionic twins with insufficient data to perform a sensitivity analysis on dichorionic twins. IPD data would have estimated predictive factors more reliably than summary statistics.

Clinical and research implications

All guidelines have repeatedly emphasized the need for robust evidence in predicting preterm delivery in twins. Cervical length assessment and fetal fibronectin have been the focus in most attempts to establish predictors of preterm birth among twin pregnancies. Current evidence for

predicting preterm birth based on existing systematic reviews and IPD meta-analysis supports the use of cervical length screening at 18-24 weeks for asymptomatic twin pregnancies. [197, 198, 199] The IPD meta-analysis published by Kindinger et al [199] in 2015 found that when the cervical length was <30 mm at 18 weeks of gestation, it was most predictive of birth at ≤28 weeks. Prediction of later spontaneous preterm birth (28-34 weeks) improved with cervical length measurements taken at ≥22weeks gestation. However, from the limited evidence available, cervical length screening has shown a poor predictive value for preterm birth in twins in symptomatic women. [197]

Fetal fibronectin shows limited accuracy in predicting preterm birth in asymptomatic twin pregnancies and is not currently recommended by NICE to predict preterm birth in twin pregnancies. In a meta-analysis published in 2010, [80] the use of fetal fibronectin was evaluated in predicting preterm birth in asymptomatic twins, and it showed that it gives better negative prediction rates (6% risk of birth before 34 weeks with a negative test) than positive prediction rates (33% risk of birth before 34 weeks with a positive test). Nonetheless, not every obstetric unit has access to fetal fibronectin testing and ultrasonography, especially in countries with limitations resource-wise.

3.6: Conclusion

This review emphasizes the importance of maternal characteristics identified by history and examination alone in recognizing women at a higher risk of preterm delivery of twins. The information on maternal clinical characteristics is made available to the clinician at the booking visit via the obstetric history without requiring expensive resources. The risk estimates based on each maternal clinical predictor can be utilized to counsel women carrying a twin pregnancy

regarding their risk of premature delivery, even at the very early stages. High-risk women can then be offered appropriate care, including early referral, closer monitoring, and available interventions to prevent preterm birth.

Chapter 4: Biochemical Predictors of Preterm Birth in Twin Pregnancies

4.1: Abstract

Background

Biochemical markers are not routinely used in clinical settings to predict preterm birth in twins. This systematic review aimed to assess the risk of spontaneous and iatrogenic preterm birth among twin pregnancies using biochemical markers.

Methods

Studies that assessed the association between biochemical markers and preterm birth in twins, published from January 1990 to June 2019, were searched in electronic databases without any language restrictions. The findings were reported as odds ratio (OR) with a 95% confidence interval (CI). We pooled the risk estimates with random-effects meta-analysis for different predictor thresholds.

Results

Thirty-three studies (6,077 pregnancies) were included in the review out of 12,623 citations. A positive fetal fibronectin (fFN) test in women either symptomatic or asymptomatic for preterm labour was associated with an increased risk of birth before 28 weeks (OR 12.06, 95 % CI 4.90-29.70, I^2 = 0%), before 32 weeks (OR 10.03, 95 % CI 6.11-16.47, I^2 = 0%), before 34 weeks (OR 6.26, 95 % CI 3.85-10.17, I^2 = 30 %), before 37 weeks (OR 5.34, 95 % CI 3.68-7.76, I^2 = 15 %), and within 14 days of testing (OR 13.95, 95 % CI 4.33-44.98, I^2 = 0%). An increased risk for preterm birth was also observed among women asymptomatic for preterm labour with a positive fFN test at <32 weeks (OR 10.54, 95 % CI 5.66-19.64, I^2 = 19 %), <34 weeks (OR 8.07, 95 % CI 5.28-12.33, I^2 = 0%), and <37 weeks' gestation (OR 6.21, 95 % CI 4.34-8.87, I^2 = 0%).

Significantly increased odds of birth before 37 weeks were observed in women with raised maternal serum human Chorionic Gonadotrophin (mshCG) levels (OR 1.51, 95 % CI 1.07-2.13, I^2 = 0%), Interleukin 8 (IL-8) levels (OR 3.13, 95 % CI 1.18-8.34, I^2=NA), and in women with a positive test for phosphorylated Insulin-like Growth Factor Binding Protein 1 (phIGFBP-1) (OR 4.23, 95 % CI 1.97-9.09, I^2 = 0%). An increased risk of delivery before 34 weeks was seen in women with serum Alpha-Fetoprotein (AFP) >3.5 MoM (OR 2.35, 95 % CI 1.12-4.96, I^2=NA), while an increase in the risk of birth before 32 weeks was observed among women with 25 Hydroxy Vitamin D levels below 75 nmol/l (OR 3.01, 95 % CI 1.26-7.19, I^2=NA). Delivery within seven days of testing was significantly higher in women with a positive test for Matrix Metallo Protein-8 (MMP-8) (OR 10.59, 95 % CI 3.70–30.29, I^2=NA).

Conclusion

Fetal fibronectin showed a statistically significant association with preterm birth among women with twin pregnancies either asymptomatic or symptomatic for preterm birth and in asymptomatic women. Several other biomarkers have also demonstrated a positive correlation with preterm birth among twin pregnancies. We recommend further studies to assess their role.

Publication arising from this chapter

Marleen S, Dias C, Macgreggor R, Allotey J, Aquilina J, Khalil A, Thangaratinam S. Biochemical predictors of preterm birth in twin pregnancies: A systematic review involving 6077 twin pregnancies. European journal of obstetrics, gynecology, and reproductive biology. 2020; 250: 130-142.

Poster presentation and resulting peer-reviewed abstract publication

Marleen S, Dias C, MacGregor R, Allotey J, Aquilina J, Khalil A, Thangaratinam S. Biochemical predictors of preterm birth in twin pregnancies. (2021), Category – Fetal Medicine. BJOG: Int J Obstet Gy, 128: 45-55. DOI: 10.1111/1471-0528.5_16715 - Top 500 Abstracts (RCOG virtual world congress 2021)

4.2: Introduction

The perinatal mortality rate of twins is three times that of singletons, and most cases are attributed to prematurity. [137] If reliable predictors of preterm delivery in twin gestations could be established, neonatal morbidity could be minimized through closer monitoring, early transfer to a tertiary care facility, and well-timed administration of corticosteroids. Therefore, more attention should be given to recognizing women at a higher risk of preterm birth so that interventions could be focused on where the benefit will be maximal.

Different biochemical markers have been evaluated in predicting preterm birth among singleton pregnancies, but data on such markers for twins is scarce. Routine clinical practice does not utilize any biomarkers to predict preterm delivery in twins. Even though many studies have been conducted on fetal fibronectin (fFN), other molecular, hormonal, infectious and inflammatory markers, including maternal serum beta-human Chorionic Gonadotrophin (mshCG), Relaxin, Pregnancy Associated Plasma Protein A (PAPP-A), 25-Hydroxy Vitamin D, maternal serum Alpha Fetoprotein (AFP), Insulin-like Growth Factor Binding Protein-1 (IGFBP-1), Matrix Metallo Protein-8 (MMP-8), Interleukin-8 (IL-8), Granulocyte Elastase, presence of bacterial vaginosis and intrauterine infection have not received as much focus.

Systematic reviews on twin pregnancies performed so far include studies on fFN published only up to 2010 and those on other biomarkers published up to 2014. [42, 200, 201] This systematic review was undertaken to incorporate more recent studies and biochemical markers to evaluate the risk of premature delivery in twins based on different biochemical predictors.

4.3: Methods

This systematic review was performed using a prospective protocol, and reporting was done according to PRISMA guidelines. [140, 141] The PROSPERO ID of this review is CRD42019141397. (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019141397).

Literature search

The electronic databases MEDLINE, CINAHL, EMBASE, and LILACS were searched for citations on preterm birth in twins published from 1 January 1990 to June 2019, without any language restrictions. The search terms 'twin pregnancy' and 'multiple pregnancies' were combined with terms for outcomes such as 'prematurity', 'preterm', 'premature birth' and 'preterm birth'. Search terms used for biochemical predictors were 'fetal Fibronectin', 'PAPP-A', 'Maternal serum beta hCG', 'Relaxin', '25-Hydroxy Vitamin D', 'Maternal serum Alpha fetoprotein', 'IGFBP-1', 'Granulocyte Elastase', 'MMP-8', 'IL-8', 'Intrauterine infection' and 'bacterial vaginosis'. The search terms for the biochemical predictors were combined with the primary search terms mentioned above. The terms were "exploded" where applicable. Appendix 7 provides the search strategy in detail. The reference lists of the primary studies and previously published systematic reviews were manually searched to locate more potentially eligible studies.

Study selection

Study selection was done in two stages. First, titles and abstracts of all citations were perused to identify those which appeared relevant for the review. The full texts of the selected citations were then obtained and evaluated, and the studies which fulfilled the inclusion criteria were included. Two independent reviewers (SM and CD) conducted study selection, and consensus settled disagreements after discussion with a third reviewer (ST). Studies on monochorionic and dichorionic twin pregnancies evaluating the association between biochemical markers and preterm birth were included in the review.

Studies that reported data on birth <28 weeks, <32 weeks, <34 weeks, <37 weeks and birth occurring within seven days or fourteen days of testing were considered eligible. Spontaneous preterm birth was evaluated, but when it was not separately reported, data for preterm birth as a collective was included. Case reports, case series, animal studies and in vitro studies were excluded. The minimal sample size was not considered. The primary authors' definitions, thresholds or stratifications for the factors evaluated were accepted. Assessment of gestational age by all methods was accepted.

Study quality assessment and data extraction

The Newcastle Ottawa Scale (NOS) [142] was used by two independent reviewers (SM and CD) to assess the methodological quality of the studies. Pre-specified criteria were used to assess the risk of bias regarding selection, comparability and outcome assessment of cohorts, and stars were allocated to each study. [143] Further details on study quality assessment are provided in this thesis's methodology chapter.

Data extraction was carried out in duplicate by two reviewers (SM and CD), and the data was recorded in a customized data extraction form. If the potentially acceptable articles had insufficient data, we contacted the authors by email for the required information. Only the most recent study was included in our review if several articles had been published from the same cohorts for the same outcomes.

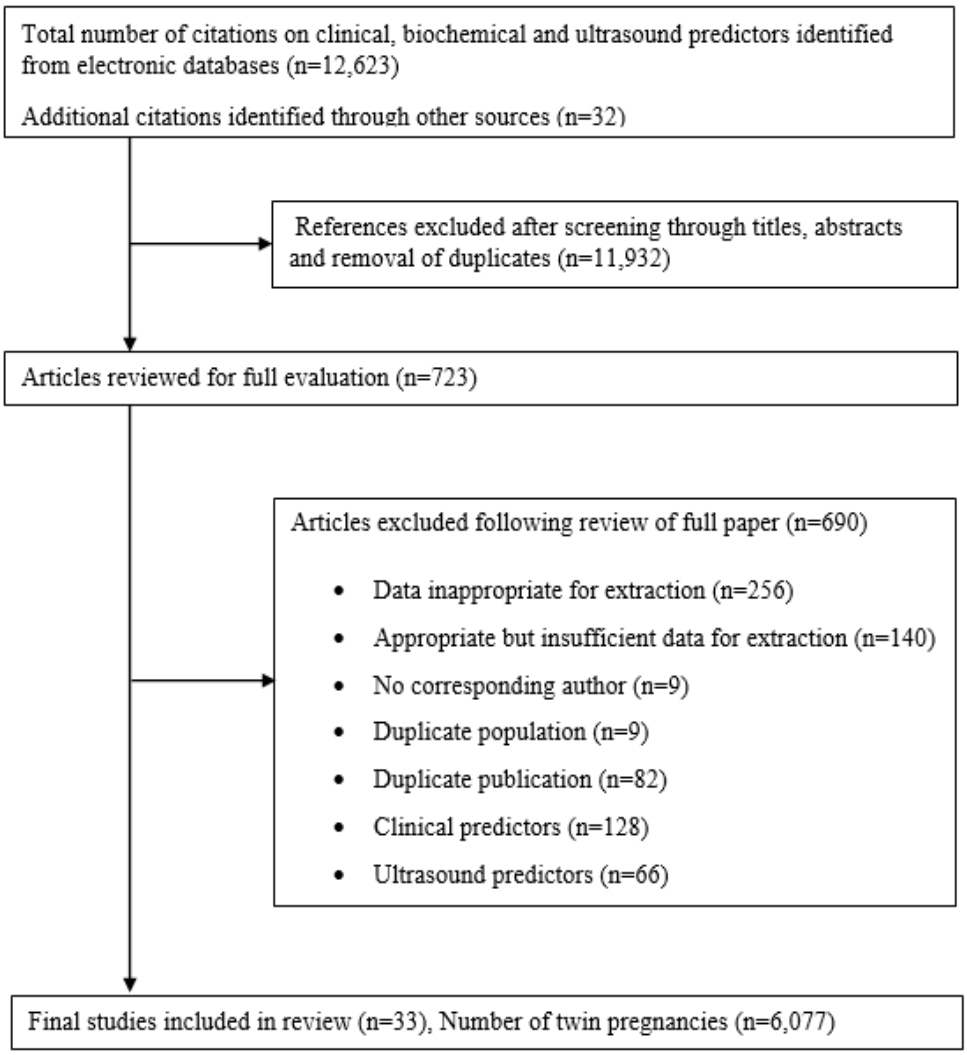
Statistical analysis

The estimates of the individual studies were pooled with random-effects meta-analysis. The summary estimates were reported as odds ratios (OR) with 95% confidence intervals (CI) for varyingly reported thresholds of the predictors. I^2 statistics were used to evaluate the heterogeneity of the studies. Sensitivity analysis was performed with data on twin pregnancies ending with spontaneous preterm birth. Funnel plot asymmetry was evaluated using Egger's method to detect potential small-study effects where more than ten studies were available. [144] The software RevMan [202] and Stata 13.0 [203] were used to carry out all analyses.

4.4: Results

Thirty-three studies, involving 6,077 pregnancies, were included out of 12,623 citations. The study selection process is given in detail in Figure 4.

Figure 4: Study selection process in the systematic review of biochemical predictors of preterm birth in twin pregnancies



4.4.1: Characteristics of the included studies

Of the 33 studies included in the systematic review, 20 were prospective cohort studies, [97, 98, 100, 102-104, 149, 155, 204-215] 10 were retrospective cohort studies, [91, 95, 99, 216-222] 2 were cohorts within randomized trials, [223, 224] and one was a case-control study. [96] The setting was a high-income

country in thirty-one studies (USA 18, Korea 3, Sweden 2, France 2, UK 2, Italy 2, Israel 2, Poland 1, Japan 1), while two studies were based in upper-middle-income (Brazil and Turkey). Twenty-two studies (67%) were published after the year 2000. The population sizes ranged from 7 [212] to 1,589 pregnancies. [96]

The following is a break-down of exclusion criteria in different studies: major congenital anomalies (11 studies), [95, 96, 98, 102, 204, 205, 209, 217, 220-222] twin to twin transfusion syndrome (6 studies), [100, 207, 209, 220-222] monoamniotic twin pregnancies (5 studies), [204, 207, 220-222] monochorionic twin pregnancies (1 study), [224] selective fetal reduction (3 studies), [95, 98, 219] chromosomal anomalies (2 studies), [96, 217] and selective intrauterine growth restriction (1 study). [204]

48% of the studies (16/33) reported data on fetal Fibronectin, [91, 102-104, 149, 204, 205, 211-215, 220-222, 224] making it the most assessed biomarker. Five out of the twelve biochemical markers studied were measured during the second trimester (PAPP-A, mshCG, 25-Hydroxy Vitamin D, AFP, and IGFBP-1), and the rest were measured during either the second or the third trimester (Relaxin, fFN, MMP-8, Granulocyte Elastase, IL-8, intrauterine infection, and bacterial vaginosis).

Thirteen studies out of 33 (40%) included women both symptomatic and asymptomatic for preterm labour in their populations, while ten studies (30%) only included asymptomatic women. Nine studies reported data on treatment or prophylaxis of preterm labour. Twenty studies (61%) considered only spontaneous preterm birth as their outcome, while the rest included both iatrogenic and spontaneous preterm delivery. The characteristics of the included studies are described in detail in Appendix 8.

4.4.2: Quality of the included studies

Twenty-eight studies (85%) [95, 96, 98, 99, 100, 102, 103, 149, 155, 204, 205, 207, 208, 209, 210, 212, 213, 214, 215, 217, 218, 219, 221, 222, 223, 224, 225, 226] recorded a low risk of bias in study selection, while four studies (12%) [91, 97, 104, 220] were of medium risk and one study was of high risk. [211] 24% of the studies (8/33) [95, 98, 100, 103, 149, 213, 221, 223] indicated a low risk for comparability. Twenty-five studies (76%) [91, 96, 97, 99, 102, 104, 155, 204, 205, 207, 208, 209, 210, 211, 212, 214, 215, 217, 218, 219, 220, 222, 224, 225, 226] were of medium risk and none recorded a high risk of bias. In outcome assessment, approximately 79% (26/33) [95, 96, 98, 99, 100, 102, 149, 155, 204, 205, 207, 208, 210, 212, 213, 215, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226] of the studies were of low risk, while 15% studies (5/33) [91, 103, 104, 209, 214] had a medium risk and 6% (2/33) [97, 211] a high risk of bias. Appendix 9 shows the quality of the included studies.

4.4.3: Biochemical predictors of preterm delivery in twin pregnancies

- *Fetal Fibronectin*

Sixteen studies (2,347 pregnancies) assessed fetal fibronectin as a biomarker to predict preterm delivery among twins. [91, 102, 103, 104, 149, 204, 205, 211, 212, 213, 214, 215, 220, 221, 222, 224] A positive fetal fibronectin (fFN) test in women either symptomatic or asymptomatic for preterm labour was found to indicate an increased risk of preterm birth before 28 weeks (OR 12.06, 95% CI 4.90-29.70, $I^2 = 0\%$), before 32 weeks (OR 10.03, 95% CI 6.11-16.47, $I^2 = 0\%$), before 34 weeks (OR 6.26, 95% CI 3.85-10.17, $I^2 = 30\ \%$), before 37 weeks (OR 5.34, 95% CI 3.68-7.76, $I^2 = 15\ \%$), and within 14 days of testing (OR 13.95, 95% CI 4.33-44.98, $I^2 = 0\%$). Most studies considered an fFN level ≥50 ng/ml as a positive test. However, the measurement was done at varying gestational ages, which included 28 weeks, 24-26 weeks, 22-32 weeks, 24-36 weeks, 22-28 weeks, 24-34 weeks, 24-34+6 weeks, 22-25+6 weeks and before 34 weeks.

- *Hormones and other molecular biomarkers*

Two studies (174 pregnancies) [218, 219] reported the association between PAPP-A and preterm delivery in twins. PAPP-A levels <10th or <25th centile were not associated with a higher risk of birth before 32 weeks (OR 2.95, 95% CI 0.69-12.60, I^2= NA), before 34 weeks (OR 3.27, 95% CI 0.87-12.35, I^2=NA), or before 37 weeks (OR 1.28, 95% CI 0.42-3.86, I^2=NA).

The relationship between maternal serum beta-hCG and premature birth in twins was evaluated in four studies (2042 pregnancies), [96, 217-219] and there was no significant correlation with birth before 32 weeks (OR 1.19, 95% CI 0.54-2.63, I^2 = 0%) or with birth before 34 weeks (OR 1.25, 95% CI 0.30-5.22, I^2=NA). Nevertheless, the odds of preterm birth occurring before 37 weeks was significantly elevated (OR 1.51, 95% CI 1.07-2.13, I^2 = 0%).

Two studies (216 pregnancies) [97, 98] assessed relaxin against preterm birth in twins. In cases where the maternal serum relaxin level exceeded the 90th centile or 2SD, the risk of preterm birth was not significantly increased at <32 weeks (OR 1.13, 95% CI 0.24-5.36, I^2=NA) or at <37 weeks (OR 3.77, 95% CI 0.79-18.05, I^2 = 27 %). One study with 267 pregnancies [95] in the population reported that maternal serum AFP of >3.5 MoM was associated with 2.35 higher odds of delivery occurring before 34 weeks in twin pregnancies (OR 2.35, 95% CI 1.12-4.96, I^2=NA). Another study (211 pregnancies) [223] demonstrated that a 25-Hydroxy Vitamin D level lower than 75 nmol/l was associated with a significantly higher risk of preterm birth at <32 weeks (OR 3.01, 95% CI 1.26-7.19, I^2=NA) and at <37 weeks (OR 2.59, 95% CI 1.35-4.95, I^2=NA).

Three studies (388 pregnancies) [49, 99, 207] evaluated the relationship between phIGFBP-1 and preterm delivery in twins. The risk of preterm birth before 37 weeks was significantly increased in women with a positive phIGFBP-1 test (OR 4.23, 95% CI 1.97-9.09, I^2 = 0%), but a similar

correlation was not observed for birth before 28 weeks (OR 3.04, 95% CI 0.36-25.47, I^2 = 0%), before 32 weeks (OR 1.71, 95% CI 0.50-5.85, I^2 = 0%) or before 34 weeks (OR 1.59, 95% CI 0.68-3.73, I^2 = 15%).

- *Infectious and inflammatory markers*

One study (88 pregnancies) [210] evaluated the association between preterm delivery in twins and MMP-8. A positive MMP-8 test indicated a significantly higher risk of delivery within seven days of testing (OR 10.59, 95% CI 3.70–30.29, I^2=NA). Another study involving 54 pregnancies failed to demonstrate a significant relationship between a positive granulocyte elastase test and birth before 34 weeks (OR 0.61, 95% CI 0.16-2.32, I^2=NA). [209]

When the relationship between preterm birth and intrauterine infection was assessed in four studies, [100, 208, 225, 226] it was found that there was no significant increase in odds of preterm delivery before 37 weeks in women with intrauterine infection (OR 1.17, 95% CI 0.54-2.54, I^2 = 0%). IL-8 and preterm birth in twins was evaluated in one study (101 pregnancies) [100], and the odds of delivery occurring before 37 weeks was significantly increased when IL-8 level exceeded 1.75 ng/g of mucus (OR 3.13, 95% CI 1.18-8.34, I^2=NA).

Two studies incorporating 248 pregnancies assessed the association between bacterial vaginosis and premature delivery among twins. [100, 102] The odds ratio was not significantly raised for birth before 32 weeks (OR 0.17, 95% CI 0.01-2.91, I^2=NA) or before 37 weeks (OR 0.93, 95% CI 0.27-3.20, I^2 = 60 %). The pooled OR for preterm birth for each factor studied are shown in Table 5. The forest plots of the pooled OR are given in Appendix 10.

Table 5: Pooled odds ratios (OR) for preterm birth (PTB) in the systematic review of biochemical predictors of preterm birth in twin pregnancies.

Predictor	Outcome PTB	No. of studies	Experiment		Control		OR (95% CI)	P value	I^2 (%)
			No. of PTB	No. of women	No. of PTB	No. of women			
Cervicovaginal factors									
Fetal Fibronectin[δ]									
	<28 weeks	2	14	92	9	674	12.06 (4.90-29.70)	<0.00001	0
	<32 weeks	6	44	130	56	1097	10.03 (6.11-16.47)	<0.00001	0
	<34 weeks	8	90	202	126	1061	6.26 (3.85-10.17)	<0.00001	30
	<37 weeks	14	221	302	641	1915	5.34 (3.68-7.76)	<0.00001	15
	Delivery within 14 days of testing	3	14	40	7	130	13.95 (4.33-44.98)	<0.0001	0
phIGFBP-1[˜]									
	<28 weeks	2	2	73	4	275	3.04 (0.36-25.47)	0.31	0
	<32 weeks	2	5	73	13	275	1.71 (0.50-5.85)	0.39	0
	<34 weeks	3	13	75	35	313	1.59 (0.68-3.73)	0.28	15
	<37 weeks	2	47	58	65	133	4.23 (1.97-9.09)	0.0002	0
Granulocyte elastase[ˇ]									
	<34 weeks	1	4	23	8	31	0.61 (0.16-2.32)	0.46	NA
IL-8[-]									
	<37 weeks	1	26	58	7	34	3.13 (1.18-8.34)	0.02	NA
Maternal serum factors									
PAPP-A[a]									
	<32 weeks	1	4	17	5	53	2.95 (0.69-12.60)	0.14	NA
	<34 weeks	1	5	10	22	94	3.27 (0.87-12.35)	0.08	NA
	<37 weeks	1	10	17	28	53	1.28 (0.42-3.86)	0.67	NA
hCG[e]									
	<32 weeks	2	9	62	36	287	1.19 (0.54-2.63)	0.66	0
	<34 weeks	1	3	10	24	94	1.25 (0.30-5.22)	0.76	NA
	<37 weeks	3	93	155	819	1783	1.51 (1.07-2.13)	0.02	0
Relaxin[g]									
	<32 weeks	1	2	17	18	171	1.13 (0.24-5.36)	0.87	NA
	<37 weeks	2	15	20	91	196	3.77 (0.79-18.05)	0.10	27
Alpha feto protein[ˆ]									
	<34 weeks	1	13	42	36	225	2.35 (1.12-4.96)	0.02	NA
25 Hydroxy vitamin D[γ]									
	<32 weeks	1	16	85	9	126	3.01 (1.26-7.19)	0.01	NA
	<37 weeks	1	29	85	21	126	2.59 (1.35-4.95)	0.004	NA
Amniotic fluid markers									
MMP-8''									
Delivery within 7 days of testing		1	27	33	17	57	10.59 (3.70-30.29)	<0.0001	NA
Maternal infection									
Intrauterine infection[´]									
	<37 weeks	4	39	48	368	517	1.17 (0.54-2.54)	0.68	0
Bacterial vaginosis[fi]									
	<32 weeks	1	0	24	13	123	0.17 (0.01-2.91)	0.22	NA
	<37 weeks	2	15	34	101	214	0.93 (0.27-3.20)	0.91	60

ð Cervicovaginal fetal Fibronectin ≥50ng/ml measured at any time during pregnancy or at 28 weeks or at 24-26 weeks or at 22-32 weeks or at 24-36 weeks or at 22-28 weeks or at 24-34 weeks or before 34 weeks or at 24- 34+6/7 weeks or at 22-25+6 weeks.

ª Maternal serum PAPP-A <10th centile or <25th centile at 11-14 weeks.

º Maternal serum hCG >75th centile or >90th centile or >2.5 MoM or >5 MoM at 11-14 weeks or at 15-20 weeks or at 16-18 weeks.

ß Maternal serum relaxin >90th centile or >2 SD at 24 weeks or at ≤32 weeks.

ˆ Maternal serum AFP >3.5 MoM at 15-20 weeks.

ª Maternal serum 25 Hydroxy vitamin D <75nmol/l at 24-28 weeks.

˜ Maternal cervical phIGFBP-1 two blue lines on the dipstick or >10 µg/l at 26 weeks or at the time of anomaly scan or between 20-24 weeks.

˝ Amniotic fluid MMP-8 measured by a commercially available point of care test at ≤34 weeks.

ˇ Maternal cervical Granulocyte elastase >1.6 µg/ml at 22-29 weeks.

´ Maternal Intrauterine infection including chorioamnionitis and positive amniotic fluid culture. Chorioamnionitis defined as maternal fever >38°C and either uterine tenderness or unexplained fetal tachycardia or histologically when there was lymphocytic infiltration into the chorionic trophoblast layer and/or chorioamniotic connective tissue. Intra-amniotic infection defined by Gram stain examination and/or possible amniotic fluid culture for aerobic, anaerobic or mycoplasma species at 20+0/7 to 34+6/7 weeks or on admission with preterm labour.

¯ Maternal cervicovaginal IL-8 >1.75ng/g mucus at 28 weeks.

fi Maternal Bacterial vaginosis defined by presence of clue cells in any concentration between 24-34 weeks during cervicovaginal sampling or by a pH >4.5 in addition to a Gram stain score of 7 to 10 according to the criteria of Nugent et al to grade the presence of bacterial morphotypes at 28 weeks.

4.4.4: Sensitivity analysis

Separate sensitivity analyses were performed for fFN in women asymptomatic and symptomatic for preterm labour. In women asymptomatic for preterm labour, the odds of preterm birth before 32 weeks (OR 10.54, 95% CI 5.66-19.64, I^2 = 19 %), before 34 weeks (OR 8.07, 95% CI 5.28-12.33, I^2 = 0%) and before 37 weeks (OR 6.21, 95% CI 4.34-8.87, I^2 = 0%) were significantly elevated. Among women symptomatic for preterm birth, the risk of preterm birth before 32 weeks (OR 28.47, 95% CI 1.32-612.60, I^2=NA), birth before 34 weeks (OR 3.07, 95% CI 1.44-6.57, I^2 = 0%) and delivery within fourteen days of testing (OR 13.95, 95% CI 4.33-44.98, I^2 =

0%) was observed to be significantly higher. There was no significant association with delivery before 37 weeks' gestation (OR 1.80, 95% CI 0.53-6.10, I^2 = 0%). Details of the sensitivity analysis are shown in Table 6.

Table 6: Pooled odds ratios (OR) among symptomatic and asymptomatic women for fetal fibronectin in predicting preterm birth (PTB) in twin pregnancies.

Predictor	Outcome PTB	No. of studies	Experiment		Control		OR (95% CI)	P value	I^2 (%)
			No. of PTB	No. of women	No. of PTB	No. of women			
Asymptomatic women									
	<32 weeks	4	38	118	39	922	10.54 (5.66-19.64)	<0.00001	19
	<34 weeks	5	66	152	97	940	8.07 (5.28-12.33)	<0.00001	0
	<37 weeks	8	170	240	491	1636	6.21 (4.34-8.87)	<0.00001	0
Symptomatic women									
	<32 weeks	1	3	3	7	37	28.47 (1.32-612.60)	0.03	NA
	<34 weeks	3	24	50	29	121	3.07 (1.44-6.57)	0.004	0
	<37 weeks	3	28	32	62	90	1.80 (0.53-6.10)	0.35	0
	Delivery within 14 days	3	14	40	7	130	13.95 (4.33-44.98)	<0.0001	0

4.4.5: Small study effects

Where more than ten studies were present for the analysis, we investigated for small study effects on the analysis of fFN against preterm birth before 37 weeks. There was no evidence of small study effects observed (Appendix 11).

4.5: Discussion

Summary of the main findings

The relationship between preterm birth among twin pregnancies and different biochemical markers has been quantified through pooled risk estimates in the current systematic review. A positive fFN test indicated a significantly higher risk of preterm birth before 28 weeks, 32 weeks,

34 weeks, 37 weeks', and within 14 days of testing in women either symptomatic or asymptomatic for preterm labour. A positive fFN test was also associated with an increased risk of preterm birth at <32 weeks, <34 weeks and <37 weeks in women asymptomatic for preterm labour. Among symptomatic women, a positive test indicated an increased risk of preterm delivery before 32 weeks, 34 weeks, and delivery within 14 days of testing.

A higher risk of twin preterm birth at <37 weeks was associated with other biomarkers such as elevated phIGFBP-1, mshCG, IL-8 and lowered levels of 25 Hydroxy Vitamin D. A risk of preterm delivery at <34 weeks was observed with elevated levels of AFP in the maternal serum, while a positive MMP-8 test correlated with a significantly higher risk of delivery within seven days of testing.

Comparison with existing studies/reviews

The association between cervicovaginal fFN and spontaneous preterm birth of twins in women either symptomatic or asymptomatic for preterm labour was shown to be limited in predicting premature delivery by a meta-analysis conducted in 2010. [(200)] A meta-analysis by the same author published in 2014 [(42)] reported on the same data on fFN as the previous review but with additional data reported on other biomarkers. The conclusion was that women at low risk of giving birth within seven of testing could be reliably identified through a negative fFN test.

The most recent meta-analysis performed on the topic in question was published in 2018, [(201)] with only three studies published between 2005 and 2015, and the conclusion was that the precision of fFN in predicting preterm birth in twin pregnancies was indecisive.
It should be emphasized that previous reviews reported data in the form of sensitivity, specificity, negative predictive values, and positive predictive values, while we reported our

findings using odds ratios, as cervicovaginal fFN and other biomarkers serve as predictive tests. Compared to previous systematic reviews, our review includes more studies exploring the role of fFN and novel biomarkers. We were able to show that a positive test on fFN and several other biomarkers was associated with significantly increased odds of preterm delivery in women with twin gestations.

Strengths and limitations

The current systematic review provides a thorough and updated assessment of the relationship between different biochemical markers and premature delivery in twins. Even though the association has been studied in previous reviews, [42, 200, 201] studies on fFN included in them were published only until 2010, while studies on other biomarkers were published up to 2014. The current review includes seven additional studies done on fFN, and data on Granulocyte Elastase, MMP-8 and intrauterine infection, none of which was included in the previous analyses.

Preceding reviews mainly focused on the accuracy of the tests, but we reported the results in the form of odds ratios so that positive correlations between preterm birth in twins and different biomarkers could be demonstrated. A comprehensive literature search, carried out without language restrictions, ensured that all studies relevant to our research question were captured. Our review also followed a prospective protocol, and the sources of heterogeneity were investigated. The methodological quality of the studies was evaluated in detail, along with how the results were affected by study quality.

The dissimilarity in the inclusion and exclusion criteria of the included studies can be considered a limitation of our review. Some studies reported data on women either asymptomatic or symptomatic for preterm labour, while other studies included women of both groups. Similarly,

some studies reported spontaneous preterm birth, while the rest included spontaneous and iatrogenic preterm birth. Studies differed in the exclusion criteria they had followed; different studies excluded cases of monoamniotic pregnancies, monochorionic pregnancies, chromosomal anomalies, major congenital anomalies, selective fetal reduction, selective intrauterine growth restriction and twin to twin transfusion syndrome.

A vast proportion of the studies were based in high-income countries, with only two studies in upper-middle-income countries. There were no studies from low-income countries, probably due to the high cost of biochemical tests such as fFN. Only a few studies included data on measures to treat or prevent preterm labour. There is a possibility that the number of preterm birth were underestimated because of such interventions. Some studies could not be included in our review as they had followed cut-offs for preterm birth different from most studies. We categorized the outcomes studied in our review to obtain the maximum yield.

Clinical and research implications

Our systematic review indicates a significant relationship between preterm delivery among women with twin pregnancies, both symptomatic and asymptomatic for preterm labour, and tests for fFN and other biochemical predictors. Fetal fibronectin is a bedside test that can be performed with ease and speed, helping screen for women at a high risk of preterm birth. The high-risk women can be appropriately managed with early referral and timely administration of antenatal corticosteroids.

Other biomarkers, including phIGFBP-1, 25 Hydroxy Vitamin D, mshCG, IL-8, MMP-8 and maternal serum AFP were also shown to have a significant correlation with preterm delivery in twins; however, their use in the clinical settings may be limited, especially in countries with

limited resources, due to the high expenses involved. Further research into the association of these novel biomarkers with premature delivery in twins is recommended, as the existing data is sparse. The development of prediction models incorporating known risk factors, biomarkers, and biophysical parameters, is also recommended to predict preterm birth in twin pregnancies better.

4.6: Conclusion

Fetal fibronectin is significantly associated with a higher risk of preterm delivery among twin gestations in either symptomatic or asymptomatic women for preterm labour and in women without symptoms. A relationship with preterm birth was observed among other biomarkers; however, further studies are recommended to consolidate their use. Developing prediction models using biomarkers will provide added value in predicting preterm birth in twin pregnancies.

Chapter 5: Association Between Chorionicity and Preterm Birth in Twin Pregnancies

5.1: Abstract

Background

It has been observed that twins' perinatal morbidity and mortality rates depend on chorionicity. Monochorionicity is believed to lead to a higher risk of preterm birth among twins; however, a systematic review has not been performed to assess the relationship, and this systematic review aims to bridge the gap.

Methods

The electronic databases were searched for relevant studies published between January 1990 and July 2019 without any language restrictions. All studies exploring the association between preterm birth in twins and chorionicity were included. We reported the findings as odds ratios (OR) with a 95% confidence interval (CI). Random-effects meta-analysis was used to pool the estimates.

Results

Thirty-nine studies (29,864 pregnancies) were included out of 13,156 citations. In women either symptomatic or asymptomatic for preterm labour, a significant association was found between monochorionicity and preterm delivery at ≤28 weeks, ≤32 weeks ≤34 weeks, and ≤37 weeks' gestation. (OR 2.14, 95% CI 1.52-3.02 I^2=46%, OR 1.55, 95% CI 1.27-1.89 I^2=68%, OR 1.47, 95% CI 1.27-1.69, I^2=60%, OR 1.66, 95% CI 1.43-1.93, I^2=65%). In asymptomatic women, increased odds of preterm birth were seen with monochorionicity at ≤34 weeks (OR 1.85, 95% CI 1.42-2.40, I^2=25%) and at <37 weeks (OR 1.75, 95% CI 1.22-2.53, I^2=61%). Sensitivity analysis demonstrated a significantly elevated risk of spontaneous preterm birth at gestations ≤34

weeks (OR 1.25, 95% CI 1.01-1.55, I^2=0%) and <37 weeks (OR 1.41, 95% CI 1.13-1.78, I^2=0%) for monochorionicity.

Conclusion

Monochorionicity is significantly associated with preterm delivery at all gestations among twin pregnancies.

Publication arising from this chapter

Marleen S, Dias C, Nandasena R, MacGregor R, Allotey J, Aquilina J, Khalil A, Thangaratinam S, Association between chorionicity and preterm birth in twin pregnancies: A systematic review involving 29,864 twin pregnancies. British journal of obstetrics and gynecology. 2021; 128(5): 788-796.

Poster presentation and resulting peer-reviewed abstract publications

Marleen, S, Dias, C, Nandasena, R, MacGregor, R, Allotey, J, Aquilina, J, Khalil, A, Thangaratinam, S. Association between chorionicity and preterm birth in twin pregnancies: a systematic review involving 29 864 twin pregnancies. RCOG virtual world congress 2021

5.2: Introduction

It has been shown that perinatal morbidity and mortality rates in twins depend on chorionicity. Monochorionic twins experience higher rates of prematurity, perinatal mortality, stillbirth, and admission to neonatal intensive care units compared to dichorionic twins, as shown by several studies. [114, 115, 116, 117] The sharing of the placenta by the two foetuses and the placental vascular anastomoses, which are almost invariably present, lead to unique complications such as selective fetal growth restriction (sFGR), twin-to-twin transfusion syndrome (TTTS), twin anaemia-

polycythaemia sequence, twin reversed arterial perfusion sequence and single intrauterine death. (105)

Even though it is widely accepted that monochorionic twin pregnancies carry a higher risk of preterm delivery, a systematic review has not been conducted yet assessing the relationship between preterm delivery among twins and chorionicity. The individual studies include widely ranging sample sizes, and the risk estimates lack precision. Therefore, this systematic review aimed to establish the risk of preterm delivery among twin gestations indicated by chorionicity.

5.3: Methods

This review was performed using a prospective protocol (PROSPERO ID – CRD42019147871, https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42019147871), and the findings are reported according to the PRISMA guidelines.

Literature search

We searched the electronic databases MEDLINE, CINAHL, LILACS, EMBASE for relevant studies published from 1st January 1990 to July 2019, without any language restrictions. The search terms used were 'twin pregnancy', 'multiple pregnancies', combined with terms for outcomes like 'prematurity', 'preterm', 'premature birth' or 'preterm birth'. Additional terms for predictors, including 'chorionicity', 'dichorionic' and 'monochorionic', were combined with the above terms. Where applicable, we "exploded" the search terms. The reference lists of all primary studies and previously published systematic reviews were manually searched to locate more articles. The search strategy is depicted in Appendix 12.

Study selection

The identified citations were first screened by their title and abstract to select potentially relevant studies. The selected citations were then subjected to full-text evaluation. The studies which fulfilled the inclusion criteria were included in the review. Two independent reviewers (SM and CD) undertook study selection, and any disagreements were resolved by consensus after discussing with a third reviewer (ST).

At the time of the PROSPERO registration, it was planned to evaluate ultrasound predictors of preterm birth in twin pregnancies. However, an IPD meta-analysis on cervical length as a predictor of preterm birth in twin pregnancies was published soon after the PROSPERO registration. Additionally, it was felt that data on a vast number of ultrasound predictors was too large to analyse in a single review. Therefore, the analysis was limited to chorionicity. Studies on dichorionic or monochorionic twin pregnancies which assessed the risk of iatrogenic or spontaneous preterm delivery at ≤ 28 weeks, ≤ 32 weeks, ≤ 34 weeks or < 37 weeks of gestation were included in the review. The minimal sample size was not considered. [227] In vitro studies, animal studies, case reports and case series were excluded. The authors' definitions, thresholds or stratifications for the factors evaluated were accepted. Assessment of gestational age by all methods was accepted.

Study quality assessment and data extraction

The Newcastle Ottawa Scale (NOS) [227] was used by two independent reviewers (SM and CD) to assess the methodological quality of the studies. Criteria were developed with regard to selection, comparability and outcome assessment of cohorts to assess the risk of bias, and stars

were allocated to each study based on them. [143] Further details on study quality assessment are provided in the methodology chapter of this thesis.

Data extraction was carried out in duplicate by two reviewers (SM and CD), and the data was recorded in a customized data extraction form. If the potentially acceptable articles had insufficient data, we contacted the authors by email for the required information. Only the most recent study was included in our review if several articles had been published from the same cohorts for the same outcomes.

Statistical analysis

The estimates of the individual studies were pooled with random-effects meta-analysis. The summary estimates were reported as odds ratios (OR) with 95% confidence intervals (CI). I^2 statistics were used to evaluate the heterogeneity of the studies. Sensitivity analysis was performed with data on twin pregnancies with spontaneous preterm birth, and preterm birth excluding twin-twin transfusion syndrome. Funnel plot asymmetry was evaluated to detect potential small-study effects where more than ten studies were available. [144] The software RevMan [202] and Stata 13.0 [203] was used to carry out all analyses.

5.4: Results

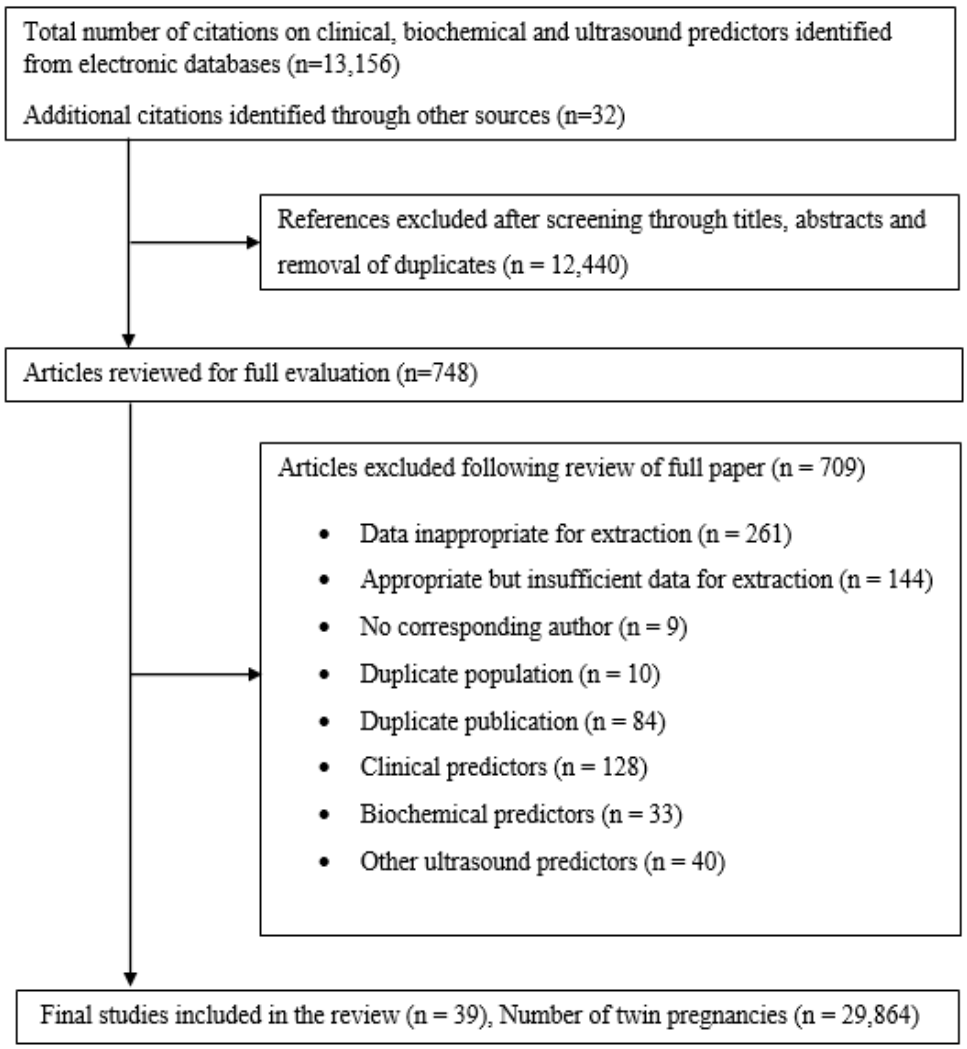
Out of 13,156 citations, 39 studies (29,864 pregnancies) were included in our analysis. The study selection process is indicated in detail in Figure 5.

5.4.1: Characteristics of the included studies

Of the 39 studies included, 29 were retrospective cohort studies, [51, 79, 112, 114, 165, 217, 228, 229, 230-249] 8 were prospective cohort studies, [52, 151, 153, 155, 250-253] and 2 were cohorts within randomized

controlled trials. [194, 254] Thirty-two studies were based in high-income countries (USA 6, Portugal 5, UK 6, Italy 4, Denmark 3, Germany 1, Korea 2, Israel 1, Austria 1, Taiwan 1, Canada 1, Poland 1, Sweden 1, France 1, Netherlands 1). Five studies were conducted in upper-middle-income countries (China 2, Brazil 2, Iran 1), while two were conducted in Pakistan, a middle-income country. All studies were published after 2000. The study populations ranged from 70 [250] to 3862 [238].

Figure 5: Study selection process in the systematic review of the association between chorionicity and preterm birth in twin pregnancies



Twenty-five studies (64%) excluded complicated twin pregnancies, including those with monoamnionicity (16 studies), [114, 151, 194, 231, 232, 234, 236, 240, 242, 243, 246-248, 251, 252, 254] twin-twin transfusion syndrome (12 studies), [79, 151, 194, 232, 234, 239, 240, 246, 247, 249, 251, 253] chromosomal anomalies in the foetus (9 studies), [79, 194, 217, 228, 231, 234, 244, 246, 247] major congenital anomalies (15 studies), [52, 79, 151, 194, 217, 228, 231, 232, 234, 239, 240, 246, 247, 253, 254] selective fetal reduction (6 studies), [194, 228, 232, 242, 245, 247] twin anaemia-polycythaemia sequence (1 study), [249] selective intrauterine growth restriction (1 study), [249] and twin reversed arterial perfusion sequence (2 studies). [240, 249]

Seven studies (18%) [151, 194, 234, 236, 244, 247, 254] included data only on women asymptomatic for preterm labour, while thirty-one studies included that of women both asymptomatic and symptomatic for preterm labour. [51, 52, 79, 114, 153, 155, 165, 217, 228-231, 233, 235, 237-243, 245, 246, 248-253, 255, 256] 18% of the studies (7/39) [194, 245, 246, 248, 250, 252, 254] reported on prophylaxis or treatment for preterm labour. Thirty-one studies (80%) included cases of iatrogenic and spontaneous preterm birth in their study population, [51, 79, 112, 114, 217, 228-231, 234, 235-245, 255, 256] and ten studies (26%) [151, 153, 155, 165, 194, 237, 246, 247, 252, 254] reported only on spontaneous preterm delivery. The different gestation cut-offs used to report data on preterm birth were, ≤28 weeks (11/39, 28%), [114, 153, 234, 237, 247, 249, 251, 252, 255, 256] ≤32 weeks (23/39, 59%), [51, 112, 114, 151, 217, 228-230, 233, 234, 237, 238, 240, 245-249, 251-254, 256] ≤34 weeks (22/39, 56%), [51, 52, 114, 151, 153, 155, 165, 194, 229, 233, 234, 238-242, 247, 251, 252, 254-256] and <37 weeks (26/39, 67%). [51, 52, 79, 112, 114, 153, 194, 217, 229, 230, 231, 233, 235-237, 239, 240, 243-245, 247, 248, 250-252, 256] The complete description of characteristics of the included studies is provided in Appendix 13.

5.4.2: Quality of the included studies

38 studies out of 39 (97%) were low risk for study selection, [51, 52, 79, 112, 114, 153, 155, 165, 194, 217, 228, 229-231, 233-247, 249-257] while 4 studies (10%) were low risk for comparability of cohorts. [165, 240, 253, 256] 100% (39/39) of the studies were low risk for outcome evaluation. [51, 52, 79, 112, 114, 153, 155, 165, 194, 217, 228-231, 233-239, 241-258] 3% of the studies (1/39) indicated a medium risk of bias for study selection, [248] while 32 studies showed a medium risk for comparability of cohorts. [51, 52, 79, 112, 114, 153, 155, 194, 217, 228, 229, 231, 234-239, 241-252, 254, 257] No studies indicated a medium risk of bias for outcome evaluation. Three studies showed a high risk of bias for comparability, [230, 233, 255] but no studies were at high risk of bias for study selection and outcome assessment. Appendix 14 depicts the quality of the studies included in the review.

5.4.3: Chorionicity as a predictor of preterm birth among women asymptomatic and symptomatic for preterm labour

The association between monochorionicity and preterm birth in twin pregnancies was assessed for births at ≤28 weeks' gestation in 11 studies (10484 pregnancies), [114, 153, 234, 237, 239, 247, 249, 251, 252, 255, 256] at ≤32 weeks' gestation in 23 studies (19783 pregnancies), [51, 112, 114, 217, 228-230, 233, 234, 237, 238, 240, 245-249, 251-254, 256, 257] at ≤34 weeks' gestation in 22 studies (21181 pregnancies), [51, 52, 114, 153, 155, 165, 194, 229, 233, 234, 238-242, 247, 251, 252, 254-257] and at <37 weeks' gestation in 26 studies (15997 pregnancies). [51, 52, 79, 112, 114, 153, 194, 217, 229-231, 233, 235-237, 239, 243-245, 247, 248, 250-252, 256] In women both symptomatic and asymptomatic for preterm labour, monochorionic gestations were observed to be at significantly higher odds of preterm delivery at all four gestation cut-offs. The findings are summarized in Table 7.

Table 7: Pooled odds ratios (OR) for preterm birth (PTB) in the systematic review of association between chorionicity and preterm birth in twin pregnancies.

Symptomatic and asymptomatic women								
Outcome: PTB	**No of studies**	**Monochorionicity**		**Dichorionicity**		**OR (95%CI)**	**P value**	**I^2 (%)**
		No of PTB	**No of women**	**No of PTB**	**No of women**			
≤28 weeks	11	121	2,372	206	8,112	2.14 (1.52-3.02)	0.0001	46
≤32 weeks	23	680	4,571	1,575	15,211	1.55 (1.27-1.89)	0.0001	68
≤34 weeks	22	1,266	4,649	3,320	16,532	1.47 (1.27-1.69)	0.00001	60
<37 weeks	26	2,455	3,917	6,437	12,080	1.66 (1.43-1.93)	0.00001	65
Asymptomatic women								
Outcome: PTB	**No of studies**	**Monochorionicity**		**Dichorionicity**		**OR (95%CI)**	**P value**	**I^2 (%)**
		No of PTB	**No of women**	**No of PTB**	**No of women**			
≤28 weeks	2	22	698	49	2,830	1.63 (0.97-2.72)	0.06	0
≤32 weeks	4	84	826	257	3,264	1.29 (0.99-1.68)	0.06	0
≤34 weeks	5	197	854	435	3,450	1.85 (1.42-2.40)	0.00001	25
<37 weeks	4	318	518	590	1,230	1.75 (1.22-2.53)	0.003	61

5.4.4: Chorionicity as a predictor of preterm birth among women asymptomatic for preterm labour

Significantly increased odds of premature delivery were observed at gestations ≤34 weeks (OR 1.85, 95 % CI 1.42-2.40, I^2=25%) [194, 234, 247, 254, 257] and <37 weeks (OR 1.75, 95 % CI 1.22-2.53, I^2=61%). [194, 236, 244, 247] There was no significant association between monochorionicity and preterm birth at gestations of ≤28 weeks [234, 247] and ≤32 weeks [234, 247, 254, 257] among women asymptomatic for preterm labour. The results are shown in Table 7.

5.4.5: Sensitivity analysis

Sensitivity analysis was conducted for spontaneous preterm delivery and for preterm birth reported in studies that had excluded cases of twin-twin transfusion syndrome. Monochorionicity was observed to impart a significantly higher risk of spontaneous preterm birth at <37 weeks (OR 1.41, 95% CI 1.13-1.78, I^2=0%) and at ≤34 weeks (OR 1.25, 95% CI 1.01-1.55, I^2=0%).

The data was reported in 5 studies (1999 pregnancies) [153, 194, 237, 247, 252] and in 8 studies (3048 pregnancies) [153, 155, 165, 194, 247, 252, 254, 257] respectively. Such a significant relationship was not observed for preterm birth at ≤28 weeks (3 studies, 1641 pregnancies) [153, 247, 252] and ≤32 weeks (5 studies, 2465 pregnancies). [246, 247, 252, 254, 257] The pooled odds ratios for spontaneous preterm delivery are shown in Table 8 and Appendix 15.

Table 8: Pooled odds ratios (OR) for spontaneous preterm birth (SPTB) in the systematic review of association between chorionicity and preterm birth in twin pregnancies.

Symptomatic and asymptomatic women								
Outcome: SPTB	**No of studies**	**Monochorionicity**		**Dichorionicity**		**OR (95%CI)**	**P value**	**I^2 (%)**
		No of SPTB	**No of women**	**No of SPTB**	**No of women**			
≤28 weeks	3	31	392	67	1249	1.90 (0.79-4.60)	0.15	65
≤32 weeks	5	54	677	144	1788	1.05 (0.65-1.70)	0.83	43
≤34 weeks	8	162	748	420	2300	1.25 (1.01-1.55)	0.04	0
<37 weeks	5	272	469	718	1530	1.41 (1.13-1.78)	0.003	0

Among studies that excluded twin-twin transfusion syndrome, 6 studies reported on any preterm birth occurring at ≤28 weeks (8315 pregnancies), [234, 239, 247, 249, 251, 255] 8 studies at ≤32 weeks (8658 pregnancies), [234, 240, 246, 247, 249, 251, 253, 257] 8 studies at ≤34 weeks (9342 pregnancies), [194, 234, 239, 240, 247, 251, 255, 257] and 6 studies on births occurring before 37 weeks (4650 pregnancies). [79, 194, 239, 240, 247, 251]

The sensitivity analysis excluding twin-twin transfusion syndrome showed that the risks of any preterm birth at gestations of ≤28 weeks (OR 2.05, 95% CI 1.42-2.95, I^2=37%) and ≤34 weeks

(OR 1.58, 95% CI 1.10-2.27, I^2=0%) were significantly increased with monochorionicity. The risk of spontaneous preterm birth among twin gestations excluding twin-twin transfusion syndrome was significantly increased only for births occurring at ≤34 weeks (OR 1.26, 95% CI 1.02-1.54, I^2=61%), as calculated by data given in 3 studies. [194, 247, 257] A significant association was not demonstrable between monochorionicity and spontaneous preterm delivery at ≤32 weeks [246, 247, 257] and at <37 weeks. [194, 247] The risk of spontaneous preterm birth at ≤28 weeks' gestation was not assessed due to the small number of studies. The findings are shown in Table 9 and Appendix 16.

Table 9: Pooled odds ratios (OR) for preterm birth (PTB) in studies excluding TTTS in the systematic review of association between chorionicity and preterm birth in twin pregnancies.

Any preterm birth (PTB)								
Outcome: PTB	**No of studies**	**Monochorionicity**		**Dichorionicity**		**OR (95%CI)**	**P value**	**I^2 (%)**
		No of PTB	**No of women**	**No of PTB**	**No of women**			
≤28 weeks	6	121	2,372	206	8,112	2.05 (1.42-2.95)	0.0001	37
≤32 weeks	8	680	4,572	1,575	15,111	1.23 (0.96-1.58)	0.10	49
≤34 weeks	8	1,253	4,649	3,295	16,532	1.26 (1.02-1.54)	0.03	61
<37 weeks	6	2,455	3,917	6,437	12,080	1.17 (0.57-2.41)	0.068	96
Spontaneous preterm birth (SPTB)								
Outcome: SPTB	**No of studies**	**Monochorionicity**		**Dichorionicity**		**OR (95%CI)**	**P value**	**I^2 (%)**
		No of SPTB	**No of women**	**No of SPTB**	**No of women**			
≤32 weeks	3	46	551	103	1,159	1.03 (0.52-2.04)	0.93	63
≤34 weeks	3	60	249	119	735	1.58 (1.10-2.27)	0.01	0
<37 weeks	2	115	204	283	594	1.35 (0.98-1.88)	0.07	0

5.4.6: Small study effects

Egger's method was used to assess Funnel plot asymmetry. Small study effect was not evident for any of the outcomes in the symptomatic and asymptomatic groups. (Appendix 17)

5.5: Discussion

Summary of main findings

The current review has established the relationship between preterm birth in twin pregnancies and monochorionicity with precise quantitative estimates. In both symptomatic and asymptomatic women for preterm labour, monochorionicity was observed to impart a significantly higher risk of preterm birth at gestations of ≤28 weeks, ≤32 weeks, ≤34 weeks, and <37 weeks. An increased risk was seen for birth occurring at ≤34 weeks and at <37 weeks in women asymptomatic for preterm labour. The odds for spontaneous preterm birth were significantly higher at gestations ≤34 weeks and at <37 weeks among monochorionic twin gestations. In studies that excluded twin-twin transfusion syndrome cases, there was a higher risk of any preterm birth at gestations ≤28 weeks, ≤34 weeks and spontaneous preterm birth at ≤34 weeks among monochorionic twin pregnancies.

Comparison with existing studies

All guidelines have constantly emphasised the need for robust evidence in predicting the likelihood of premature delivery in twin pregnancies. The entities explored the most in this regard are cervical length assessment and fetal fibronectin. Existing evidence recommends cervical length assessment to predict preterm delivery at 18-24 weeks in women asymptomatic for preterm labour, [198, 199, 259] while fetal fibronectin is currently not recommended for regular use due to its low predictive accuracy. [260] This is the first systematic review and meta-analysis conducted to analyse the association between chorionicity and preterm birth, searching for a more accurate predictor of preterm birth in twin pregnancies.

Strengths and limitations

This review is the first attempt at comprehensively evaluating the association between preterm birth in twins and chorionicity. The extensive study population allowed us to arrive at more accurate conclusions. A prospective protocol was followed during our review, and the literature search was conducted without any language restrictions, which ensured that all pertinent studies were included in the review. The sources of heterogeneity were explored. A thorough assessment of the methodological quality of the studies and checking how the quality affected the results was performed. After executing a robust methodology, we demonstrated a positive relationship between preterm birth in twins and chorionicity.

Limitations in our review mainly arose from the dissimilarities between the inclusion and exclusion criteria in different studies. Most studies reported on both spontaneous and iatrogenic preterm delivery, but some only included data on spontaneous preterm birth. Certain studies excluded twin pregnancies complicated with issues particular to monochorionicity, such as monoamnionicity, twin-twin transfusion syndrome, twin anaemia-polycythaemia sequence, and twin reversed arterial perfusion sequence. In contrast, some studies excluded complications observed among both monochorionic and dichorionic twin pregnancies, such as major congenital anomalies, chromosomal anomalies, selective fetal growth restriction, and selective fetal reduction. Individual studies also provided limited data on unit policies regarding the timing of delivery of uncomplicated monochorionic twin pregnancies.

Only a small number of studies reported on prophylactic and therapeutic measures for preterm labour. Such interventions may have resulted in underestimating the actual number of premature

births. A large proportion of the studies were based in high-income countries, while only seven were conducted in countries with an upper-middle income or a middle income.

This review did not show a significant relationship between monochorionicity and spontaneous preterm birth or preterm birth among women asymptomatic for preterm labour at gestations ≤28 weeks and ≤32 weeks. The small number of studies reporting data for these gestations may have contributed to this finding. A sensitivity analysis could not be performed for twin gestations excluding all complications particular to monochorionicity, as only one such study was reported. Although a sensitivity analysis was performed on studies where twin-twin transfusion syndrome was excluded, a significant association between monochorionicity and preterm delivery was not demonstrable at ≤32 weeks and at <37 weeks' gestation, possibly due to the small number of studies. A meta-analysis for spontaneous preterm birth at ≤28 weeks could not be performed for this group as there was an inadequate number of studies reported.

A more robust diagnosis would have been obtained if the individual studies had executed a strict follow-up protocol regarding twin-twin transfusion syndrome. However, such a protocol was not followed in any studies, and the authors' diagnosis of the condition was accepted. Therefore, the diagnosis may have been missed in some of the studies. We could not perform a meta-analysis for adjusted prognostic effect estimates due to the lack of information in published studies.

Clinical and research implications

The higher risk of preterm birth in monochorionic twins has been a widely accepted concept worldwide, but this is the first time a systematic review has statistically proven the relationship. This review demonstrated a significant association between monochorionicity and preterm birth

in twin pregnancies at all gestations in women either symptomatic or asymptomatic for preterm labour.

It is recommended that monochorionic twins should be delivered as early as 34 weeks' gestation, even when there are no complications, and certainly by 37 weeks. [261, 262] NICE guidelines recommend planned delivery of uncomplicated monochorionic diamniotic twin pregnancies from 36^{+0} weeks. [105] Iatrogenic prematurity originating from such recommendations may have led to the overall rise in the preterm delivery rates observed in monochorionic twin pregnancies. Our review demonstrated that the risk of spontaneous preterm birth was significantly higher among monochorionic pregnancies only at ≤34 weeks and <37 weeks. Even when cases of twin-twin transfusion syndrome were excluded from the study population, the risk of spontaneous preterm birth was significantly higher at the gestation of ≤34 weeks, implicating that monochorionicity by itself imparts a high risk of spontaneous preterm birth.

Women carrying monochorionic twin pregnancies should be counselled and managed appropriately to anticipate this inherent risk of premature delivery, even when the pregnancy is not complicated. This systematic review could establish the association between preterm birth and chorionicity with aggregate data meta-analysis. Other factors which may affect preterm birth can be investigated through individual participant data meta-analysis; therefore, additional research employing such methodology to further the understanding in this area is recommended.

5.6: Conclusion

There is a significant association between preterm delivery at all gestations among asymptomatic and symptomatic women for preterm labour and monochorionicity. The risk of preterm delivery

at ≤34 weeks and <37 weeks' gestation was significantly higher in monochorionic twin pregnancies among women asymptomatic for preterm labour and where preterm birth occurred spontaneously.

Early recognition of monochorionicity through ultrasonography allows clinicians to identify women at a higher risk of premature birth and counsel them appropriately. They can be managed with prompt referral, closer surveillance, and available interventions to prevent preterm birth so that the complications arising from prematurity can be minimised.

Chapter 6: Maternal Outcomes in Twin Pregnancies Following Assisted Reproduction

6.1: Abstract

Background

Assisted reproductive techniques (ART) significantly contribute to the global twinning rate. Twin pregnancies are associated with poorer maternal outcomes in comparison to singleton pregnancies. However, it remains to be assessed if ART twin pregnancies have additional maternal risks than non-ART and naturally conceived twin pregnancies.

Methods

We searched electronic databases for relevant studies published between January 1990 and June 2021 without any language restrictions. All cohort studies on maternal outcomes for ART twin pregnancies compared to non-ART twin pregnancies were included. Findings were reported as odds ratios with 95% confidence intervals. Random-effects meta-analysis was used to pool the estimates.

Results

Out of 4,282 citations, 93 studies (142,511 pregnancies) were included. ART twin pregnancies were observed to have a higher risk of preterm birth <37 weeks (OR 1.28, 95% CI 1.18-1.37), preterm birth <34 weeks (OR 1.33, 95% CI 1.13-1.57), gestational diabetes mellitus (OR 1.55, 95% CI 1.38-1.75), hypertensive disorders in pregnancy (OR 1.20, 95% CI 1.05-1.38), antepartum haemorrhage (OR 1.77, 95% CI 1.26-2.47), placenta praevia (OR 2.22, 95% CI 1.82-2.70), postpartum haemorrhage (OR 1.45, 95% CI 1.21-1.75) and caesarean section (OR 1.83, 95% CI 1.65-2.02) compared to non-ART twins. The increase in risk for the above outcomes was also observed among ART mothers compared to natural conception. A significantly higher

magnitude of association was seen in studies published before 2010 between ART and preterm birth <28 weeks, <34 weeks, <37 weeks, and GDM when comparing ART with non-ART groups. Spontaneous preterm birth <34 weeks was increased among ART compared to non-ART and natural conception. Many of the studied maternal risks were higher among ART twin pregnancies even after monochorionicity was excluded.

Conclusion

Twin pregnancies conceived by ART carry an increased risk of adverse maternal outcomes than those conceived by non-ART methods and natural conception.

Oral presentations resulting in peer-reviewed abstract publications

Marleen, S; Nandasena, R; Kodithuwakku, W; Mohideen, S; Aquilina, J; Khalil, A; Bhide, P; Thangaratinam, S. Maternal and offspring outcomes in twin pregnancies following assisted reproduction. BJOG: an international journal of obstetrics and gynaecology. Oral presentation at RCOG Virtual World Congress 2021 – Top 500 Abstracts

6.2: Introduction

Assisted Reproductive Technology (ART) is one of the most important contributors to the global increase in twin pregnancies. [120, 263] It is established that twin pregnancies are associated with higher adverse outcomes for mothers, including intensive care unit admission, hysterectomy, blood transfusion and maternal death, compared to singleton pregnancies. [16, 264] ART pregnancies have also been associated with poorer maternal outcomes in singleton pregnancies, including gestational diabetes mellitus (GDM), hypertensive disorders, placental disorders, and caesarean delivery. [265, 266] Therefore, it is crucial to determine whether twin pregnancies conceived by ART impart additional risks for the mother.

Individual studies assessing the maternal outcomes in ART twin pregnancies report conflicting data. Many studies observed worse outcomes in ART mothers than in non-ART mothers, [125, 267-269] while some reported no significant differences between the two groups regarding maternal risks. [270-272] Few studies have even reported higher preterm birth rates among spontaneously-conceived twins than twins conceived by ART. [273, 274] Previous systematic reviews and meta-analyses performed on this topic were limited in their number of studies, evaluated a limited number of maternal outcomes, and sometimes have pooled all multiple pregnancies together in their analyses. [275-277]

This systematic review aimed to quantify the risks ART twin pregnancies carry for women compared to non-ART methods and natural conception. A higher number of primary studies, a more extensive study population and more maternal outcomes were included for evaluation than previously published systematic reviews to provide an up-to-date and comprehensive review.

6.3: Methods

This review was performed using a prospective protocol (PROSPERO ID – CRD42020185228, https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020185228), and the findings are reported according to the PRISMA guidelines.

Literature search

The electronic databases MEDLINE and EMBASE were searched for relevant studies published from January 1990 to June 2021, without any language restrictions. The search terms used were 'twin pregnancy', 'multiple pregnancies', combined with terms for ART including 'In Vitro Fertilization', 'Intra Cytoplasmic Sperm Injection', 'Zygote Intra Fallopian Transfer' and

'Gamete Intra Fallopian Transfer'. Additional terms for maternal outcomes were used in combination with the above terms. Where applicable, the search terms were "exploded". The reference lists of all primary studies and previously published systematic reviews were manually searched to locate more articles. The search strategy is outlined in Appendix 18.

Study selection

First, the identified citations were screened by their title and abstract to select potentially relevant studies. The selected citations were then subjected to full-text evaluation. Studies which fulfilled the inclusion criteria were included in the review. Two independent reviewers (SM and RN) undertook study selection, and any disagreements were resolved by consensus after discussion with a third reviewer (ST).

All cohort studies which assessed the maternal outcomes of monochorionic and dichorionic twin pregnancies conceived following ART compared to non-ART twins were included in the review. All pregnancies where both oocytes and sperm were manipulated in vitro were included in the ART group. This included processes such as in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT) and intracytoplasmic sperm injection (ICSI). Twin pregnancies conceived following fertility treatment methods other than ART, such as intrauterine insemination (IUI) with or without controlled ovarian hyperstimulation, ovulation induction and those conceived naturally were grouped as 'non-ART'. Twin pregnancies conceived without any fertility treatment were included in the 'natural conception' group.

Case-control studies, case reports, case series, in-vitro studies and animal studies were excluded from our review. Studies where ovulation induction and IUI with or without controlled ovarian hyperstimulation were included in the ART category, and studies where data was unavailable for

ART separately, were also excluded. Those that presented maternal outcomes as the number of neonates were excluded. The authors' definitions, thresholds or stratifications for the factors evaluated were accepted. Assessment of gestational age by all methods was accepted. Patients were not involved in developing this review, and a core outcome set was not utilized.

Study quality assessment and data extraction

The Newcastle Ottawa Scale (NOS) [227] was used by two independent reviewers (SM and CD) to assess the methodological quality of the studies. Criteria were developed regarding the selection, comparability, and outcome assessment of cohorts to assess the risk of bias, and stars were allocated to each study. [143] Further details on study quality assessment are provided in the methodology chapter of this thesis.

Data extraction was carried out in duplicate by two reviewers (SM and WK), and the data was recorded in a customized data extraction form. Dichotomous data was extracted onto 2x2 tables. The authors were contacted by email for the required information if the articles had insufficient data. Only the most recent study was included if several articles had been published from the same cohorts for the same outcomes.

Statistical analysis

The estimates of the individual studies were pooled with random-effects meta-analysis. The summary estimates were reported as odds ratios (OR) with 95% confidence intervals (CI). I^2 statistics were used to evaluate the heterogeneity of the studies. Sensitivity analysis was performed with data on spontaneous preterm birth and dichorionic twin pregnancies. A subgroup analysis was performed comparing studies published before 2010 with studies published after

2010, considering the significant improvements in ART techniques over time. A further subgroup analysis was undertaken to compare fresh embryo transfer with frozen embryo transfer cycles. Using Egger's test and funnel plots, publication bias and small-study effects were assessed. [278] Meta-regression analysis was performed to adjust the estimated effect on specific maternal outcomes by confounders such as maternal age and parity. The software RevMan [202] and Stata 13.0 [203] was used to carry out all analyses.

6.4: Results

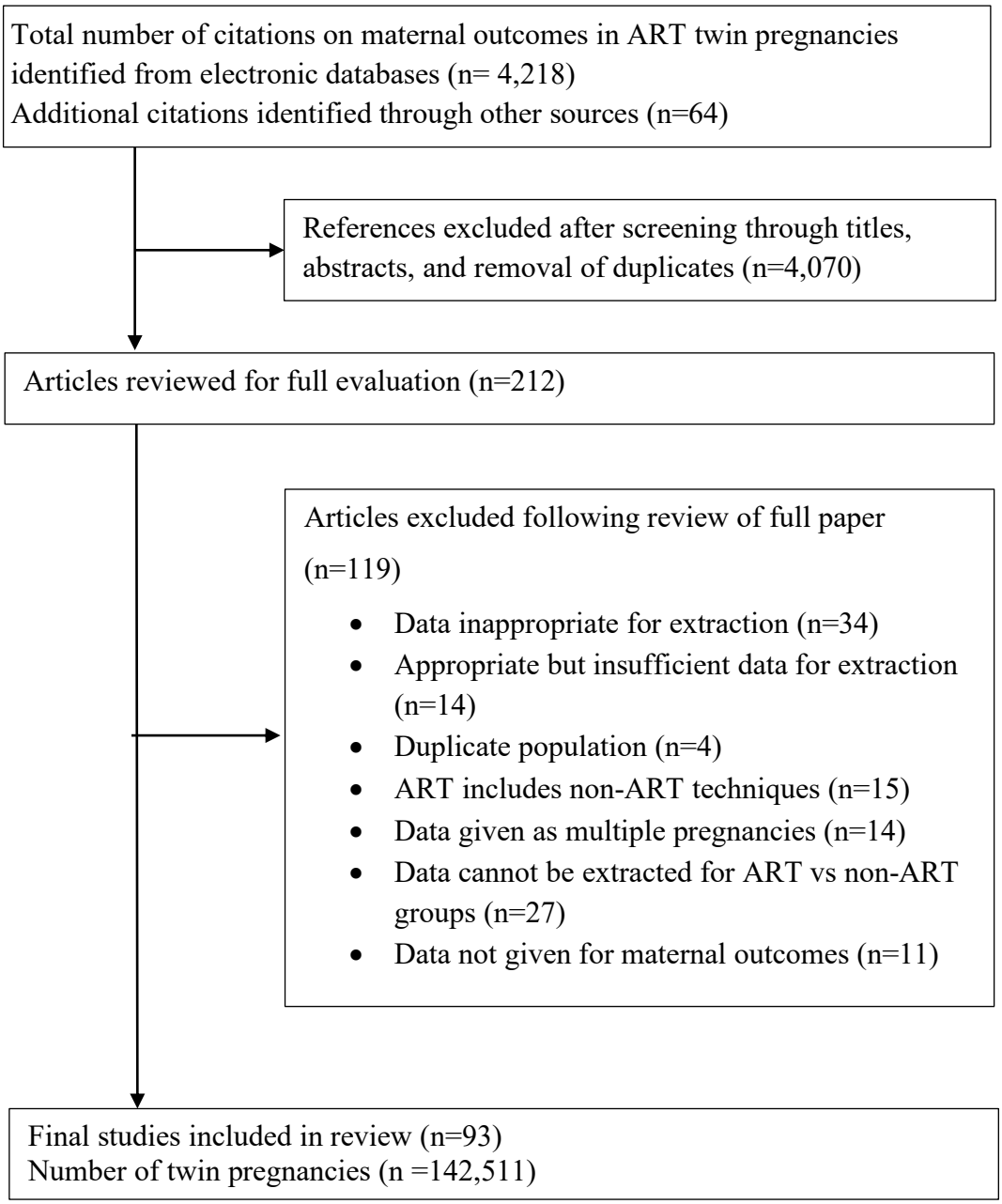
Out of 4,282 citations, 93 studies (142,511 pregnancies) were included. Figure 6 depicts the study selection process.

6.4.1: Characteristics of the included studies

Out of the 93 studies, 78 were retrospective cohorts, [124, 125, 185, 233, 241, 268, 279-350] 14 studies were prospective cohorts, [49, 153, 252, 351-361] and one study was a secondary analysis of a randomised controlled trial. [362]

Eighty-three out of ninety-three studies were based in high-income countries, while nine were performed in upper-middle-income countries. [153, 294, 299, 340, 343, 351, 356, 363, 364] The country with the highest number of studies was the USA (10/93), followed by China (9/93) and Denmark (7/93). The majority (89%) of studies (83/93) were published after the year 2000. The sample sizes of the study population ranged from 32 pregnancies [360] to 19,941 pregnancies. [321]

Figure 6: Study selection process in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.



Forty-five (48%) of the included studies specifically reported exclusion of twin pregnancies complicated with monochorionicity (23 studies), [73, 124, 283, 284, 289, 291, 293, 294, 297, 298, 301, 305, 308, 316, 327, 329, 334, 342, 356, 359, 362, 364, 365] monoamnionicity (27 studies), [73, 124, 252, 280, 283, 288, 290, 291, 293, 294, 297, 298, 301, 305, 308, 316, 324, 327, 334, 342, 353, 356, 357, 359, 362, 364, 365] major fetal anomalies (11 studies), [185, 290, 291, 292, 294, 297, 315, 324, 328, 337, 362] twin to twin transfusion syndrome (5 studies), [292, 315, 324, 357, 362] selective fetal reduction (25 studies), [124, 280, 281, 285, 286, 289, 291, 294, 298, 308, 312, 315, 316, 319, 322, 324, 328, 334, 343, 354, 356, 362, 365-367] chromosomal abnormalities (2 studies), [291, 362] and still birth (8 studies). [153, 292-294, 316, 322, 334, 357] Majority of studies have included an unselected population of women who were both symptomatic and asymptomatic for preterm labour (90/93).

80% of studies (74 /93) included IVF with or without ICSI in the ART group. GIFT was included in 4 studies. [285, 302, 317, 327] Two studies had reported using only frozen embryo transfer cycles, [335, 339] while three studies stated the use of only fresh embryo transfer cycles. [295, 307, 341] Only one study reported data separately for fresh and frozen embryo transfer cycles. [332] The remainder of the studies did not mention the use of either frozen or fresh embryo transfer cycles or both. Three studies excluded cases of oocyte donation from their analysis. [280, 286, 301] Preterm birth <37 weeks was the most commonly reported maternal outcome (72%, 67/93).

The details of the characteristics of the included studies are given in Appendix 19.

6.4.2: Quality of the included studies

Seventy-four studies out of 93 (80%) were low risk for study selection, [49, 125, 153, 185, 233, 241, 252, 267, 268, 279-284, 286, 288-290, 292-299, 301-305, 309, 310, 312-314, 316-324, 326-328, 330, 331, 333-337, 341-343, 351-355, 357, 358, 360, 362, 363, 365, 367-370] forty-three (46%) were low risk for comparability, [124, 268, 280, 283, 285-287, 289, 290, 292, 293, 295, 298, 300-302, 305, 307, 308, 310, 312, 313, 315, 317, 318, 320, 321, 324, 327, 328, 330, 331, 333, 334, 337, 352, 354, 355, 368-371] and ninety-one (98%) of the studies were low risk for study outcome. Nineteen studies (20%) had

medium risk of bias for study selection, [73, 285, 287, 291, 300, 306-308, 311, 315, 325, 329, 332, 340, 356, 366, 371-373] 14 studies (15%) for comparability [153, 284, 297, 329, 341-343, 351, 353, 356, 363, 365, 372, 373] and two studies (2%) [241, 306] had medium risk of bias for outcome. None of the studies showed high risk of bias for selection and outcome. However, 39 studies (35%) [49, 125, 185, 233, 241, 252, 279, 281, 282, 288, 291, 294, 296, 299, 303, 304, 306, 309, 311, 314, 316, 319, 322, 323, 325, 326, 332, 335, 336, 340, 347, 357, 358, 360, 362, 366] were found to have a high risk of bias with regard to comparability. Details of the quality of the included studies are depicted in Appendix 20.

6.4.3: Maternal outcomes in twin pregnancies following assisted reproduction

The odds of preterm birth <34 weeks were significantly increased among ART twins compared to non-ART twin pregnancies as assessed in 28 studies (OR 1.33, 95% CI 1.13-1.57, I^2=74%) and among ART twins compared to naturally-conceived twin pregnancies as assessed in 20 studies (OR 1.24, 95% CI 1.02-1.49, I^2=67%). Higher odds of preterm birth at <37 weeks was also seen among ART twin pregnancies compared to non-ART twin pregnancies as evaluated in 67 studies (OR 1.28, 95% CI 1.18-1.37, I^2=77%) and naturally conceived twins as assessed in 50 studies (OR 1.34, 95% CI 1.21-1.48, I^2=74%).

Gestational hypertension was assessed in 33 studies, pre-eclampsia in 22 studies, and hypertensive disorders in pregnancy which included gestational hypertension, pre-eclampsia and chronic hypertension in 55 studies. Compared to non-ART twin pregnancies, ART mothers were at a higher risk of gestational hypertension (OR 1.32, 95% CI 1.15-1.53, I^2=75%), pre-eclampsia (OR 1.37, 95% CI 1.20-1.57, I^2=19) and hypertensive disorders in pregnancy (OR 1.20, 95% CI 1.05-1.38, I^2=83%). Similar results were seen for ART twins compared to those conceived naturally for gestational hypertension (OR 1.33, 95% CI 1.12-1.58, I^2= 76%), pre-eclampsia (OR

1.27, 95% CI 1.10-1.47, I^2=12%), and hypertensive disorders in pregnancy (OR 1.40, 95% CI 1.11-1.76, I^2=77%).

Increased odds of GDM and diabetes in pregnancy, which included GDM and pre-existing diabetes, were seen among ART twin pregnancies compared to non-ART twins as evaluated in 45 studies (OR 1.55, 95% CI 1.38-1.75, I^2=41%) and 48 studies (OR 1.58, 95% CI 1.39-1.79, I^2= 49%) respectively. A similar result was also seen for GDM (OR 1.65, 95% CI 1.44-1.89, I^2=41%) and diabetes in pregnancy (OR 1.61, 95% CI 1.42-1.84, I^2=31%) comparing ART with naturally-conceived twin pregnancies.

Antepartum haemorrhage was evaluated in 10 studies comparing ART versus non-ART twin pregnancies and in 7 studies comparing ART to naturally conceived twin pregnancies. The odds were significantly increased among ART twin pregnancies compared to both non-ART twins (OR 1.77, 95% CI 1.26-2.47, I^2=59%) and naturally conceived twins (OR 2.55, 95% CI 1.86-3.50, I^2=5%).

Placenta praevia was reported in 25 studies comparing ART and non-ART twin pregnancies, and in 20 studies comparing ART and naturally conceived twin pregnancies. A significantly increased risk of placenta praevia was seen for ART twin pregnancies compared to non-ART twins (OR 2.22, 95% CI 1.82-2.70, I^2=8%) and twins conceived naturally (OR 2.22, 95% CI 1.64-3.02, I^2=29%). As for placental abruption, ART twin pregnancies also showed significantly higher odds compared to non-ART twins as evaluated in 27 studies (OR 1.20, 95% CI 1.01-1.44, I^2=0%) and naturally conceived twins as evaluated in 24 studies (OR 1.33, 95% CI 1.11-1.60, I^2=0%).

Postpartum haemorrhage was evaluated in 20 studies comparing ART versus non-ART twins where the odds were increased for ART twin pregnancies (OR 1.45, 95% CI 1.21-1.75, I^2=40%) with similar increase in risk for ART twins when compared to naturally conceived twins as assessed in 19 studies (OR 1.37, 95% CI 1.15-1.63, I^2=27%).

Caesarean section was also increased among ART twins compared with non-ART twins as evaluated in 65 studies (OR 1.83, 95% CI 1.65-2.02, I^2=86%) and naturally conceived twins as evaluated in 52 studies (OR 2.03, 95% CI 1.78-2.32, I^2=85%). The above results are summarized in Table 10-11.

Table 10: Pooled odds ratios (OR) for maternal outcomes comparing ART vs non-ART in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART*		Non-ART'' (including Natural)		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
PTB <28 weeks	20	280	8,769	676	24,356	0.49	1.13 [0.80, 1.59]	67
PTB <32 weeks	45	2,540	27,548	6,198	71,158	0.07	1.11 [0.99, 1.25]	66
PTB <34 weeks	28	1,609	7,289	3,415	16,942	0.0007	1.33 [1.13, 1.57]	74
PTB <37 weeks	67	18,332	34,545	45,829	93,864	<0.00001	1.28 [1.18, 1.37]	77
Gestational hypertension	33	3,338	17,456	5,720	36,619	0.0001	1.32 [1.15, 1.53]	75
Pre-eclampsia	21	846	7,736	1,304	12,830	0.00001	1.37 [1.20, 1.57]	19
Hypertensive disorders in pregnancy	55	4,481	21,227	8,018	43,819	0.010	1.20 [1.05, 1.38]	83
GDM	45	1,766	16,967	3,016	36,547	<0.00001	1.55 [1.38, 1.75]	41
Diabetes in pregnancy	48	1,819	17,790	3,076	37,028	<0.00001	1.58 [1.39, 1.79]	49
APH	10	321	6,885	532	17,234	0.0009	1.77 [1.26, 2.47]	59
Placenta praevia	25	385	15,191	257	33,062	<0.00001	2.22 [1.82, 2.70]	8
Placental abruption	27	257	11,253	424	19,979	0.04	1.20 [1.01, 1.44]	0
PPH	20	953	7,431	1,289	9,399	<0.001	1.45 [1.21, 1.75]	40
Caesarean delivery	65	20,749	32,773	44,308	81,285	<0.00001	1.83 [1.65, 2.02]	86

**ART - pregnancies where both oocytes and sperm were manipulated in vitro (e.g.: IVF, GIFT, ZIFT, ICSI)*

'' *Non-ART - Twin pregnancies conceived following fertility treatment methods other than ART, such as intrauterine insemination (IUI) with or without controlled ovarian hyperstimulation, ovulation induction and those conceived naturally*

Table 11: Pooled odds ratios (OR) for maternal outcomes comparing ART vs Natural conception in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART*		Natural^		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
PTB <28 weeks	15	128	2,859	268	6,109	0.47	1.21 [0.72, 2.04]	73
PTB <32 weeks	36	1,433	15,223	2,850	27,528	0.25	1.09 [0.94, 1.26]	64
PTB <34 weeks	20	1,075	5,497	1,911	10,027	0.03	1.24 [1.02, 1.49]	67
PTB <37 weeks	50	10,067	17,381	17,556	32,863	<0.00001	1.34 [1.21, 1.48]	74
Gestational hypertension	27	2,896	13,707	4,144	22,792	0.001	1.33 [1.12, 1.58]	76
Pre-eclampsia	18	713	6,898	813	8,362	0.001	1.27 [1.10, 1.47]	12
Hypertensive disorders in pregnancy	24	1,871	7,762	2,369	11,530	0.004	1.40 [1.11, 1.76]	77
GDM	41	1,475	13,839	1,946	23,791	<0.00001	1.65 [1.44, 1.89]	41
Diabetes in pregnancy	43	1,448	13,887	1,803	22,315	<0.00001	1.61 [1.42, 1.84]	31
APH	7	140	3,946	102	6,778	<0.00001	2.55 [1.86, 3.50]	5
Placenta praevia	20	323	8,928	133	12,443	<0.00001	2.22 [1.64, 3.02]	29
Placental abruption	24	252	10,155	294	16,133	0.002	1.33 [1.11, 1.60]	0
PPH	19	904	7,128	1,041	7,673	0.0004	1.37 [1.15, 1.63]	27
Caesarean delivery	52	12,352	18,287	19,245	35,207	<0.00001	2.03 [1.78, 2.32]	85

**ART - pregnancies where both oocytes and sperm were manipulated in vitro (e.g.: IVF, GIFT, ZIFT, ICSI)*
^Natural - twin pregnancies conceived without any fertility treatment

The forest plots of pooled odds ratios of certain maternal outcomes are depicted in Appendix 21.

6.4.4: Subgroup analysis

Subgroup analysis performed comparing studies published before versus after 2010 showed a significantly higher magnitude of association in studies published before 2010 between ART and preterm birth <28 weeks, <34 weeks, <37 weeks, and GDM when comparing ART with non-ART groups. There was no significant difference between the two subgroups for all other maternal outcomes studied when comparing ART versus non-ART twins. The findings are summarized in Table 12.

In the ART versus natural conception comparison, a significant difference was observed only for the association between ART and preterm birth before 37 weeks, where an increase was seen for both before and after 2010, with the difference being more significant before 2010. There were no other subgroup differences for any other maternal outcomes studied. The findings are given in Table 13.

A further subgroup analysis was performed comparing fresh embryo transfer cycles with frozen embryo transfer cycles. There was no significant difference between fresh and frozen embryo transfer cycles for any maternal outcomes considered between ART versus non-ART twins and ART versus naturally conceived twins. Table 14 and Table 15 provide the relevant findings.

Table 12: Subgroup analysis by year for maternal outcomes comparing ART vs non-ART in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	After 2010		Before 2010		Before 2010 vs After 2010
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
PTB <28 weeks	14	0.91 [0.57, 1.44]	6	1.80 [1.34, 2.42]	0.01
PTB <32 weeks	28	1.04 [0.90, 1.20]	17	1.29 [1.09, 1.52]	0.05
PTB <34 weeks	18	1.14 [0.93, 1.39]	11	1.64 [1.40, 1.92]	0.006
PTB <37 weeks	37	1.17 [1.09, 1.26]	30	1.45 [1.23, 1.72]	0.02
Gestational hypertension	18	1.29 [1.09, 1.52]	15	1.36 [1.02, 1.83]	0.73
Pre-eclampsia	15	1.41 [1.20, 1.67]	7	1.25 [1.03, 1.50]	0.32
Hypertensive disorders in pregnancy	33	1.34 [1.18, 1.53]	22	0.91 [0.57, 1.44]	0.11
GDM	31	1.46 [1.30, 1.64]	15	2.11 [1.55, 2.87]	0.03
Diabetes in pregnancy	32	1.47 [1.31, 1.65]	16	1.80 [1.24, 2.62]	0.31
APH	2	1.84 [0.88, 3.83]	8	1.72 [1.24, 2.37]	0.87
Placenta previa	17	2.12 [1.65, 2.71]	8	2.52 [1.46, 4.35]	0.57
Placental abruption	17	1.29 [0.96, 1.73]	10	1.05 [0.72, 1.55]	0.42
PPH	17	1.47 [1.21, 1.78]	3	1.22 [0.42, 3.50]	0.73
Caesarean delivery	34	1.82 [1.63, 2.03]	32	1.75 [1.48, 2.06]	0.7

Table 13: Subgroup analysis by year for maternal outcomes comparing ART vs Natural conception in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	After 2010		Before 2010		After 2010 vs Before 2010
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
PTB <28 weeks	11	0.99 [0.53, 1.87]	4	2.04 [1.36, 3.06]	0.06
PTB <32 weeks	21	1.04 [0.85, 1.27]	15	1.21 [1.02, 1.43]	0.26
PTB <34 weeks	13	1.13 [0.93, 1.38]	7	1.41 [1.02, 1.95]	0.24
PTB <37 weeks	27	1.17 [1.05, 1.30]	23	1.55 [1.30, 1.85]	0.008
Gestational hypertension	15	1.21 [1.00, 1.47]	13	1.44 [1.03, 2.00]	0.39
Pre-eclampsia	13	1.34 [1.11, 1.63]	5	1.11 [0.81, 1.52]	0.31
Hypertensive disorders in pregnancy	18	1.36 [1.07, 1.73]	7	1.68 [1.10, 2.55]	0.4
GDM	28	1.56 [1.36, 1.78]	14	2.19 [1.55, 3.10]	0.07
Diabetes in pregnancy	29	1.55 [1.36, 1.76]	14	1.84 [1.25, 2.71]	0.41
APH	1	2.96 [2.15, 4.08]	6	1.92 [1.14, 3.25]	0.17
Placenta previa	15	2.13 [1.50, 3.02]	6	2.73 [1.15, 6.49]	0.6
Placental abruption	15	1.41 [1.15, 1.74]	10	1.07 [0.72, 1.58]	0.21
PPH	16	1.38 [1.15, 1.66]	3	1.49 [0.57, 3.89]	0.87
Caesarean delivery	27	2.12 [1.75, 2.56]	27	1.84 [1.52, 2.22]	0.31

Table 14: Subgroup analysis by type of Embryo transfer (ET) for maternal outcomes comparing ART vs non-ART in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	Fresh ET		Frozen ET		Fresh Vs Frozen ET
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
PTB <28 weeks	1	2.04 [0.18, 23.29]	1	0.33 [0.01, 8.22]	0.37
PTB <32 weeks	3	0.87 [0.29, 2.59]	2	1.14 [1.09, 1.19]	0.63
PTB <37 weeks	4	1.18 [1.11, 1.26]	4	0.93 [0.57, 1.53]	0.35
Caesarean delivery	3	1.24 [1.16, 1.32]	3	0.95 [0.50, 1.80]	0.42

Table 15: Subgroup analysis by type of Embryo Transfer (ET) for maternal outcomes comparing ART vs Natural conception in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	Fresh ET		Frozen ET		Fresh Vs Frozen ET
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
PTB <28 weeks	1	2.04 [0.18, 23.29]	1	0.33 [0.01, 8.22]	0.37
PTB <32 weeks	2	0.63 [0.05, 8.04]	1	1.00 [0.24, 4.25]	0.75
PTB <37 weeks	1	1.56 [0.68, 3.60]	4	1.34 [1.09, 1.64]	0.72
Caesarean delivery	2	1.15 [0.61, 2.16]	2	0.68 [0.46, 1.01]	0.17

6.4.5: Sensitivity analysis

Sensitivity analysis was performed for studies that reported on spontaneous preterm birth as summarized in Table 16 and Table 17. The odds of spontaneous preterm birth were found to be significantly increased at <34 weeks' gestation among ART twin pregnancies when compared to non-ART conception (OR 1.84, 95%CI 1.40- 2.42, I^2=2%) [49, 252, 324, 357] and natural conception (OR 1.83, 95% CI 1.23-2.71, I^2=NA). [324]

Table 16: Sensitivity analysis for spontaneous preterm birth (PTB) comparing ART vs non-ART in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction

Spontaneous preterm birth	No of studies	ART		Non-ART		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
<28 weeks	2	10	379	22	672	0.08	0.90 [0.40, 2.01]	0
<32 weeks	5	301	3,328	555	5,273	0.97	0.99 [0.67, 1.46]	67
<34 weeks	4	187	532	187	906	<0.0001	1.84 [1.40, 2.42]	2
<37 weeks	4	1,719	5,023	3,956	12,187	0.18	0.90 [0.78, 1.05]	56

Table 17: Sensitivity analysis for spontaneous preterm birth comparing ART vs Natural conception in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Spontaneous preterm birth	No of studies	ART		Natural		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
<28 weeks	1	5	158	18	510	0.83	0.89 [0.33, 2.45]	N/A
<32 weeks	3	258	3,009	423	4,433	0.79	0.96 [0.73, 1.27]	38
<34 weeks	1	52	158	108	510	0.003	1.83 [1.23, 2.71]	N/A
<37 weeks	2	1,074	2,608	1,478	3,644	0.48	0.84 [0.52, 1.35]	76

Sensitivity analysis performed after excluding monochorionic twin pregnancies showed significantly increased odds of adverse maternal outcomes such as preterm birth <37 weeks (OR 1.19, 95% CI 1.03-1.37, I^2=64%), pre-eclampsia (OR 1.29, 95% CI 1.07-1.55, I^2=24%), hypertensive disorders in pregnancy (OR 1.38, 95% CI 1.13-1.69, I^2=71%), GDM (OR 1.56, 95% CI 1.30-1.87, I^2=0%), diabetes in pregnancy (OR 1.61, 95% CI 1.32-1.96, I^2=0%), placenta previa (OR 2.11, 95% CI 1.45-3.07, I^2=0%), and caesarean delivery (OR 1.86, 95% CI 1.42-2.32, I^2=79%) among ART twin pregnancies compared with non-ART. Similarly, the risk of preterm birth <37 weeks (OR 1.24, 95% CI 1.03-1.50, I^2=54%), pre-eclampsia (OR 1.24, 95% CI 1.02-1.50, I^2=20%), hypertensive disorders in pregnancy (OR 1.46, 95% CI 1.06-2.01, I^2=77%), GDM (OR 1.59, 95% CI 1.31-1.94, I^2=0%), diabetes in pregnancy (OR 1.69, 95% CI 1.42-2.02, I^2=0%), placenta praevia (OR 1.87, 95% CI 1.22-2.88, I^2=0%), and caesarean delivery (OR 1.97, 95% CI 1.45-2.68, I^2=87%) was higher among ART twin pregnancies excluding

monochorionicity compared to naturally-conceived twins. The above findings are summarized in Tables 18 and 19.

Table 18: Sensitivity analysis for maternal outcome in studies excluding monochorionicity comparing ART vs non-ART in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Non-ART		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
PTB <28 weeks	12	106	2,742	141	4,416	0.23	1.33 [0.83, 2.12]	49
PTB <32 weeks	16	647	7,024	1,352	16,185	0.08	1.25 [0.98, 1.60]	70
PTB <34 weeks	10	542	2,639	648	4,130	0.10	1.28 [0.95, 1.72]	74
PTB <37 weeks	17	3,365	6,323	8,510	17,329	0.02	1.19 [1.03, 1.37]	64
Gestational hypertension	7	911	3,727	1,632	5,576	0.23	1.27 [0.86, 1.87]	77
Pre-eclampsia	9	547	4,423	634	6,076	0.008	1.29 [1.07, 1.55]	24
Hypertensive disorders in pregnancy	20	1,720	6,586	2,718	10,143	0.001	1.38 [1.13, 1.69]	71
GDM	15	328	3,391	241	3,469	<0.00001	1.56 [1.30, 1.87]	0
Diabetes in pregnancy	14	274	3,009	207	3,154	<0.00001	1.61 [1.32, 1.96]	0
APH	1	28	514	68	2,067	0.02	1.69 [1.08, 2.66]	N/A
Placenta previa	8	85	2,467	49	3,749	<0.0001	2.11 [1.45, 3.07]	0
Placental abruption	9	56	1,994	35	1,800	0.08	1.49 [0.95, 2.33]	0
PPH	10	541	4,448	718	6,198	0.03	1.27 [1.02, 1.58]	27
Caesarean delivery	15	2,738	5,803	3,232	9,286	<0.00001	1.86 [1.49, 2.32]	79

Table 19: Sensitivity analysis for maternal outcomes in studies excluding monochorionicity comparing ART vs Natural conception in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Natural		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
PTB <28 weeks	8	71	1,669	60	1,771	0.20	1.54 [0.79, 2.98]	58
PTB <32 weeks	12	407	4,888	521	5,857	0.17	1.25 [0.91, 1.71]	65
PTB <34 weeks	7	306	1,829	302	1,802	0.56	1.12 [0.95, 1.31]	34
PTB <37 weeks	13	2,084	3,879	2,650	5,118	0.02	1.24 [1.03, 1.50]	54
Gestational hypertension	6	859	3,424	1,241	4,178	0.43	1.17 [0.79, 1.73]	66
Pre-eclampsia	7	506	4,120	496	4,678	0.03	1.24 [1.02, 1.50]	20
Hypertensive disorders in pregnancy	10	1,352	4,359	1,727	5,141	0.02	1.46 [1.06, 2.01]	77
GDM	14	302	3,088	213	3,052	<0.00001	1.59 [1.31, 1.94]	0
Diabetes in pregnancy	14	342	3,388	312	4,447	<0.00001	1.69 [1.42, 2.02]	0
Placenta previa	7	74	1,953	33	1,634	0.004	1.87 [1.22, 2.88]	0
Placental abruption	8	56	1,691	32	1,383	0.04	1.63 [1.02, 2.59]	0
PPH	9	492	4,145	563	4,858	0.12	1.11 [0.97, 1.26]	0
Caesarean delivery	13	3,107	4,986	3,142	6,088	<0.0001	1.97 [1.45, 2.68]	87

6.4.6: Small study effects

Funnel plot asymmetry was assessed for outcomes with at least ten studies using Egger's test of asymmetry. Effects of small studies were significant for caesarean section ($p = 0.002$) and preterm birth <37 weeks ($p = 0.033$) when ART twin pregnancies were compared with non-ART twin pregnancies. In the comparison with natural conception, small study effects were significantly seen for hypertensive disorders in pregnancy ($p = 0.0012$), preterm birth <37 weeks ($p = 0.004$), diabetes in pregnancy ($p = 0.047$), caesarean section ($p = 0.002$) and postpartum haemorrhage ($p = 0.045$). (Appendix 22)

6.4.7: Meta-regression analysis

This was carried out to adjust for confounding factors such as maternal age and parity. The influence of ART on GDM, hypertensive disorders of pregnancy, preterm birth <34 weeks and preterm birth <37 weeks was not dependent on maternal age. However, the effect of assisted reproduction on caesarean section was observed to increase when the average maternal age difference between ART and non-ART increased. Nulliparity was not observed to affect the effect of ART on preterm birth <34 weeks, hypertensive disorders of pregnancy and caesarean section. However, when the proportion of nulliparity in the ART group was higher, the effect of ART on preterm birth <37 weeks and GDM also increased. (Appendix 23)

6.5: Discussion

Summary of main findings

This systematic review indicates that twin pregnancies conceived following ART have significantly higher adverse maternal outcomes than non-ART twin pregnancies for majority of

the outcomes studied. The ART group are more likely to encounter preterm delivery, medical complications such as gestational diabetes mellitus and hypertensive disorders in pregnancy, placental disorders, and delivery by caesarean section. These observations remained largely consistent when comparing ART twin pregnancies with twins born by natural conception. ART has continued to impose a higher risk of unfavourable outcomes for the mother except for preterm birth <28 weeks, <34 weeks, <37 weeks and GDM throughout the years, despite new developments in assisted reproductive techniques. Even after monochorionic twins are excluded, ART twin pregnancies show poorer maternal outcomes than non-ART twin pregnancies.

Comparison with existing studies/reviews

Numerous systematic reviews have compared maternal outcomes among ART and non-ART singleton pregnancies. Preterm birth <37 weeks, <32 weeks, caesarean delivery, antepartum haemorrhage, GDM, premature rupture of membranes and hypertensive disorders in pregnancy were significantly higher among ART singletons than non-ART singleton pregnancies. [374, 375, 376] The findings from our review for twin pregnancies follow the same risks observed among ART singleton pregnancies regarding maternal outcomes.

A systematic review that assessed the risk of adverse pregnancy outcomes in twins subsequent to high-technology subfertility treatment, published in 2016, reported that GDM, gestational hypertension and preterm birth were significantly higher among ART twins than in naturally-conceived twins. [377] Compared to this review, our study evaluated a higher number of maternal outcomes and included studies without language restrictions, allowing a more extensive analysis of maternal outcomes among twins conceived by ART.

A higher risk of caesarean delivery and preterm birth <37 weeks was reported among ART twins when controlled for maternal characteristics in a systematic review and meta-analysis published in 2011. [378] The risk of preterm birth <37 weeks was found to be increased among ART twins when unlike-sex twins were assessed separately. Our review, in comparison, evaluated more maternal outcomes and a larger study population. Another systematic review published in 2005 reported a higher risk of preterm birth and caesarean delivery among ART twin pregnancies than twins of natural conception when matched for parity and maternal age. [276] The inclusion criteria and the reported adverse maternal outcomes in this study were similar to our observations, although the study population was notably smaller, with only eleven studies included. Individual studies have differed in the observed maternal risks among twin pregnancies conceived by ART. Some studies have shown that twins conceived following ART and those conceived spontaneously do not significantly differ in maternal outcomes. [284, 286, 289, 292, 303, 329, 339] On the contrary, other studies have reported worse pregnancy outcomes in ART twins compared to non-ART twins. [125, 268, 280, 283, 290, 296, 299, 310, 314, 325, 331, 379, 380] Some studies have also demonstrated lower preterm birth rates among ART twins than naturally conceived twins. [274, 304]

However, many previously published systematic reviews support our observation of an increased risk of adverse obstetric outcomes among twin pregnancies following ART. [276, 277, 378, 381, 382] The higher maternal risks among ART twin pregnancies may be attributable to multiple factors. Women going through assisted reproductive techniques tend to be older than women who conceive naturally, thereby elevating the risks of medical complications during pregnancy. Increased anxiety among subfertile women and the clinicians' inclination to avoid vaginal delivery and its complications in what is considered a "precious" pregnancy might contribute to higher rates of caesarean delivery and preterm birth among ART twins. In our review, after 2010,

ART twin pregnancies did not show significantly higher preterm delivery rates indicating a possible change in practice and increased confidence among obstetricians in managing ART twin pregnancies. However, the differences in populations and study designs might also have contributed to the differences noted between these two groups.

Strengths and limitations

The current systematic review presents an in-depth analysis of maternal outcomes in twin pregnancies conceived by assisted reproduction, involving the most extensive study population to date. A thorough literature search was conducted without language restrictions on a prospective protocol, increasing the chances of incorporating all relevant primary studies. An extensive list of clinically relevant maternal outcomes was included in the review. A comprehensive assessment of study quality was carried out, and a robust methodology was followed. Maternal outcomes in ART twin pregnancies compared to non-ART twins and naturally conceived twins were assessed to quantify the effects caused by ART.

As monochorionicity is known to cause worse pregnancy outcomes than dichorionic twins, a sensitivity analysis was performed comparing ART versus non-ART after the exclusion of monochorionicity. A subgroup analysis was conducted to assess whether the association between ART and maternal outcomes changed over the years. In addition, meta-regression analysis was performed to evaluate the effect of maternal age and parity, and the analysis results did not deviate significantly from the main findings of the review. In the meta-regression analysis, the effect of ART on caesarean section was shown to increase when the average maternal age difference between ART and non-ART groups increased, which is on par with what is already known. This finding can be considered to validate the results of our analysis.

Limitations in the review included heterogeneity in the study population, type of ART, comparison and outcomes. The individual studies varied in their inclusion criteria. Some studies had excluded monoamniotic and monochorionic twins, twin-twin transfusion syndrome, fetal chromosomal or structural anomalies, multifetal pregnancy reduction, and twins conceived following intrauterine insemination or ovulation induction. The definitions given by primary study authors for the outcomes were accepted, although they sometimes varied across different studies. Certain studies included twin pregnancies conceived by ovulation induction and intrauterine insemination under the ART category, which were excluded from the review. Heterogeneity among studies was also observed in the non-ART group; some included twins conceived naturally, twins conceived following IUI or ovulation induction. Most studies lacked information on donor eggs, a factor likely to have influenced the outcomes. Data on fresh or frozen embryo transfer cycles were also limited. Most studies were from high-income countries, warranting further research to make these findings more applicable to low-income countries. The main aim of the review was to compare ART with the Non-ART group. Many studies had collectively included OI and IUI in the Non-ART group without providing data for them separately. In contrast, some studies had included OI and IUI in the ART group and were not included in the review. Data insufficiency did not allow a comparison between the IUI and OI groups.

Clinical and research implications

Due to the high rate of twin pregnancies following ART, accurate determination of the risks following assisted reproductive techniques holds great importance. This information can be utilised to counsel women seeking ART, including optimising the management of ART twin

pregnancies. Despite the higher maternal risks observed among ART twin pregnancies, present guidelines on managing twin pregnancies do not identify twin pregnancies conceived by ART as a cohort that requires special attention.[383] The findings of this review emphasize the need for management guidelines to recognize ART twin pregnancies as a separate entity that requires closer monitoring to reduce maternal morbidity and mortality.

Additional research with more refined inclusion and exclusion criteria is recommended to identify which aspect of ART contributes to adverse maternal outcomes and determine ways to minimize these risks. Subgroup analysis between frozen and fresh embryo transfer may provide and different assisted reproductive techniques may provide further clarity. Given the increased maternal morbidity associated with ART, we recommend long-term follow up of women undergoing ART to evaluate the effects of such treatment on their health.

6.6: Conclusion

Twin pregnancies conceived by ART impart significantly higher maternal risks compared to non-ART twin pregnancies. Therefore, women seeking and undergoing assisted reproduction should be counselled regarding the higher adverse maternal risks associated with ART and placed under closer monitoring for early detection and management of complications.

Chapter 7: Perinatal Outcomes in Twin Pregnancies Following Assisted Reproduction

7.1: Abstract

Background

Assisted reproductive techniques (ART) have contributed significantly to the exponential rise in the incidence of twin pregnancies worldwide. Perinatal outcomes among twin pregnancies are known to be worse in comparison to that of singleton pregnancies. It is essential to establish whether offspring of ART twin pregnancies have additional complications than non-ART twin pregnancies.

Methods

We searched electronic databases for relevant studies published between January 1990 and June 2021 without any language restrictions. All cohort studies on perinatal outcomes following ART twin pregnancies compared with non-ART and naturally conceived twins were included. Findings were reported as odds ratios with 95% confidence intervals. Random-effects meta-analysis was used to pool the estimates.

Results

Out of 4,282 citations, 66 studies (442,247 neonates) were included. Twins born by ART were observed to be at a significantly increased risk for birth weight discordance >25% (OR 1.31, 95% CI 1.05-1.63), congenital malformations (OR 1.18, 95% CI 1.06-1.31), respiratory distress syndrome (OR 1.35, 95% CI 1.07-1.70), necrotizing enterocolitis (OR 1.78, 95% CI 1.06-3.01), neurological complications (OR 1.61, 95% CI 1.04-2.48) and neonatal intensive care unit admission (OR 1.25, 95% CI 1.12-1.41) compared to non-ART twins. All the above outcomes except congenital malformations were also higher among ART twins than twins conceived

naturally. A risk reduction was seen among ART twins for stillbirth (OR 0.83, 95% CI 0.70-0.99), twin-twin transfusion syndrome (OR 0.45, 95% CI 0.25-0.82) and small for gestation age <10th centile (OR 0.91, 95% CI 0.87-0.96) compared to non-ART twins. The above outcomes were also lower in the ART group than in natural conception. Monochorionicity excluded, the odds of neonatal intensive care unit admission, birth weight discordance >25% and respiratory distress syndrome were increased among ART twins compared to non-ART and natural conception groups.

Conclusion

Twins conceived following ART are at a significantly higher risk of adverse perinatal outcomes than twins conceived by non-ART and natural conception.

Oral presentations resulting in peer-reviewed abstract publications

Marleen, S; Nandasena, R; Kodithuwakku, W; Mohideen, S; Aquilina, J; Khalil, A; Bhide, P; Thangaratinam, S. Maternal and offspring outcomes in twin pregnancies following assisted reproduction. BJOG: an international journal of obstetrics and gynaecology. Oral presentation at RCOG Virtual World Congress 2021 – Top 500 Abstracts

7.2: Introduction

Over six million neonates have been born across the world from Assisted Reproductive Technology (ART) since 1978. [384] According to a survey done by The International Committee for Monitoring Assisted Reproductive Technologies (ICMART), more than 1.9 million ART cycles were conducted in seventy-six countries in 2014 alone. [385] Likewise, the number of twin pregnancies has increased worldwide, with ART being one of the main contributors to the increased twinning rates. [263] [386] Singleton newborns conceived by ART have been identified to

be at a higher risk of adverse perinatal outcomes than non-ART singletons. [265, 266, 387] Additionally, twin neonates are also at a significantly higher risk of adverse neonatal outcomes. [388, 389] Therefore, it is essential to determine whether offspring of twin pregnancies conceived by ART are at an added risk of adverse outcomes.

Individual studies report conflicting data on perinatal outcomes following ART. Some studies report that ART twins are at a higher risk of poor outcomes than non-ART twins, [125, 267, 361, 390] while others have not observed any difference in neonatal outcomes between ART twins and non-ART twins. [338, 358, 391, 392] Some studies have even reported fewer congenital anomalies, higher birth weight, less infant mortality and less neonatal morbidity among twins born by ART than those born by non-ART methods. [241, 273, 274, 304]

Previously conducted systematic reviews and meta-analyses on this topic were limited in their study population and number of evaluated outcomes and sometimes included multiple births as a collective in the analysis. [275, 276, 277] This review aimed to determine and quantify the risk of adverse perinatal outcomes in ART twin pregnancies incorporating a larger study population and a more extensive list of outcomes than in previously published systematic reviews.

7.3: Methods

The review was performed using a prospective protocol (PROSPERO ID – CRD42020185228, https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020185228), and the findings are reported according to the PRISMA guidelines.

Literature search

The electronic databases MEDLINE and EMBASE were searched for relevant studies published from January 1990 to June 2021, without any language restrictions. The search terms used were 'twin pregnancy', 'multiple pregnancies', combined with terms for ART including 'In Vitro Fertilization', 'Intra Cytoplasmic Sperm Injection', 'Zygote Intra Fallopian Transfer' and 'Gamete Intra Fallopian Transfer'. Additional terms for perinatal outcomes were used in combination with the above terms. Where applicable, the search terms were "exploded". The reference lists of all primary studies and previously published systematic reviews were manually searched to identify more articles. The search strategy is outlined in Appendix 24.

Study selection

First, the identified citations were screened by their title and abstract to select potentially relevant studies. The selected citations were then subjected to full-text evaluation. The studies which fulfilled the inclusion criteria were included in the review. Two independent reviewers (SM and RN) undertook study selection, and any disagreements were resolved by consensus after discussion with a third reviewer (ST).

All cohort studies which assessed the perinatal outcomes of monochorionic and dichorionic twins conceived following ART compared to non-ART twins were included in this review. All twins conceived following manipulation of oocytes and sperm in vitro such as in vitro fertilization (IVF), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT) and intracytoplasmic sperm injection (ICSI) were included in the ART group. Twin neonates conceived following fertility treatment methods other than ART, such as intrauterine insemination (IUI) with or without controlled ovarian hyperstimulation and ovulation induction,

or naturally were grouped as ‘non-ART’. Twin pregnancies conceived without any fertility treatment were included in the ‘natural conception’ group.

Case-control studies, case reports, case series, in-vitro studies and animal studies were excluded. Studies where ovulation induction and IUI with or without controlled ovarian hyperstimulation were included in the ART category where data was not extractable for ART separately, were also excluded. Studies that presented perinatal outcomes as number of mothers were excluded. The authors’ definitions, thresholds or stratifications for the factors evaluated were accepted. Assessment of gestational age by all methods was accepted. Patients were not involved in developing this review, and a core outcome set was not utilised.

Study quality assessment and data extraction

The Newcastle Ottawa Scale (NOS) [(227)] was used by two independent reviewers (SM and RN) to assess the methodological quality of the studies. Criteria were developed concerning selection, comparability, and outcome assessment of cohorts to assess the risk of bias, and stars were allocated to each study. [(143)] Further details on study quality assessment are provided in the methodology chapter of this thesis.

Data extraction was carried out in duplicate by two reviewers (SM and WK), and the data was recorded in a customized data extraction form. Dichotomous data were extracted onto 2x2 tables. The authors were contacted by email for the required information if the articles had insufficient data. Only the most recent study was included in the review if several articles had been published from the same cohorts for the same outcomes.

Statistical analysis

The estimates of the individual studies were pooled with random-effects meta-analysis. The summary estimates were reported as odds ratios (OR) with 95% confidence intervals (CI). I^2 statistics were used to evaluate the heterogeneity of the studies. Sensitivity analysis was performed with data pertaining to dichorionic twin pregnancies. Subgroup analysis to compare studies published before 2010 with studies published after 2010 was performed as ART techniques have seen remarkable changes over the years. A further subgroup analysis comparing fresh embryo transfer with frozen embryo transfer cycles was also performed. Using Egger's tests and funnel plots, publication bias and small-study effects were assessed. [278] Meta-regression analysis evaluated the estimated effect on specific perinatal outcomes by confounders such as maternal age and parity. The software RevMan [202] and Stata 13.0 [203] was used to carry out all analyses.

7.4: Results

Out of 4,282 citations, 66 studies (442,247 pregnancies) were included. Figure 7 depicts the study selection process.

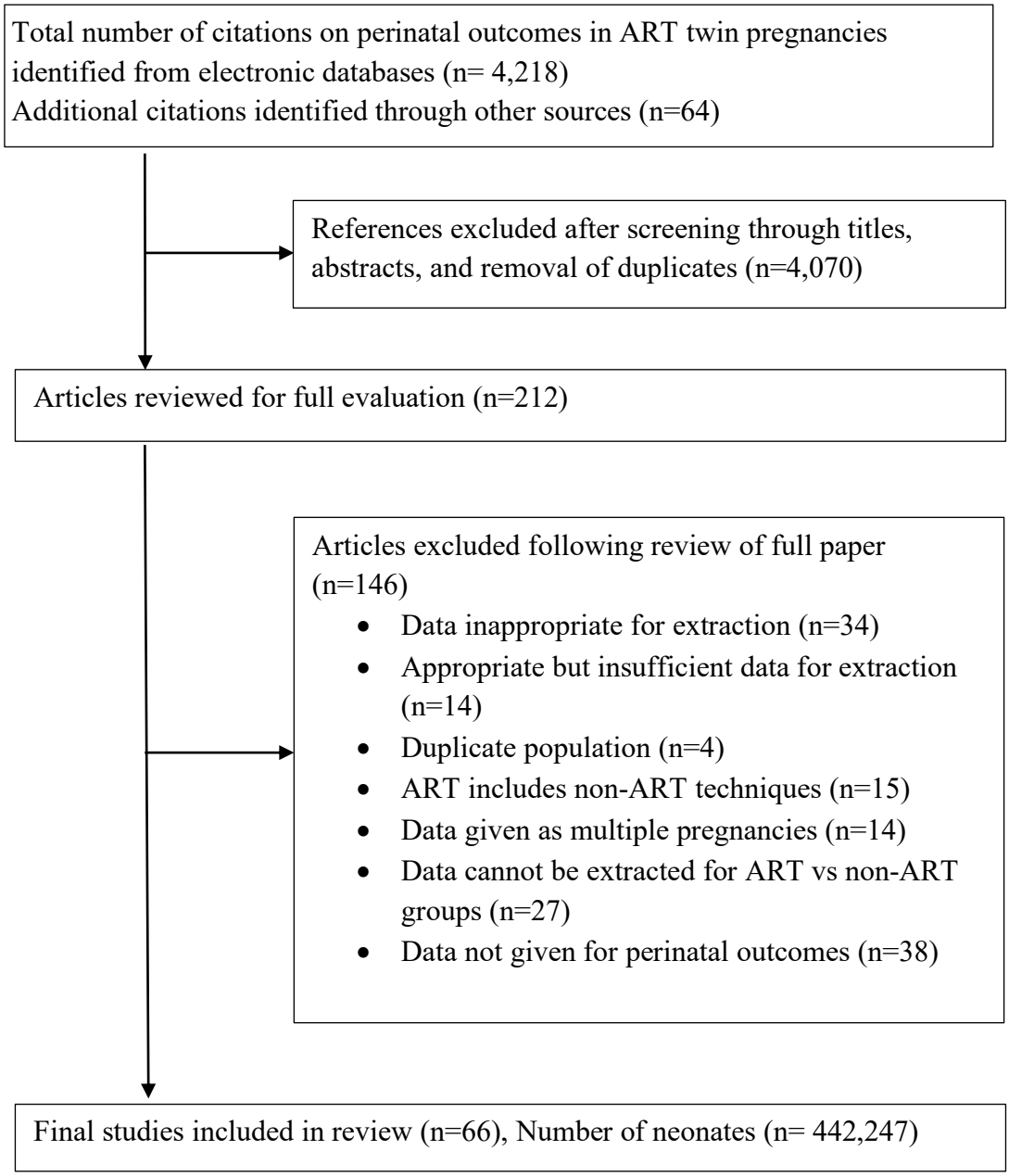
7.4.1: Characteristics of the included studies

Of the 66 studies, 58 were retrospective cohorts and 8 were prospective cohorts. [352, 353, 355, 356, 358, 359, 401, 402]

Sixty-two out of sixty-six studies (94%) were based in high-income countries, while three were conducted in upper-middle-income countries. [343, 356, 364] USA, Denmark, Israel and China each

had five studies. The majority (89%) of studies (59/66) were published after year 2000. The sample sizes of the study population ranged from 120 neonates [342] to 160,661 neonates. [394]

Figure 7: Study selection process in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.



Thirty-one (47%) of the included studies specifically reported exclusion of twin pregnancies complicated with monochorionicity (18 studies), [124, 283, 284, 289, 291, 293, 298, 301, 305, 308, 316, 329, 334, 342, 356, 359, 364, 402] monoamnionicity (19 studies), [73, 124, 280, 283, 288, 290, 291, 293, 298, 301, 305, 308, 316, 334, 342, 353, 356, 359, 364] major fetal anomalies (5 studies), [290, 291, 328, 337, 402] chromosomal abnormalities (1 study), [291] selective fetal reduction (15 studies), [124, 280, 286, 289, 291, 298, 308, 316, 319, 322, 328, 334, 343, 356, 367] and still birth (4 studies). [293, 316, 322, 334]

The majority of studies (92%, 61/66) included IVF with or without ICSI in the ART group. GIFT was included in 4 studies. [302, 317, 396, 402] Three studies had reported the use of only frozen embryo transfer cycles, [339, 394, 398] while four studies stated the use of only fresh embryo transfer cycles. [295, 341, 401, 402] Two studies reported data separately for fresh and frozen embryo transfer cycles. [332, 396] The remainder of the studies did not mention the use of either fresh or frozen embryo transfer cycles or both. Five studies excluded cases of oocyte donation from their analysis. [280, 286, 301, 395, 396] Any congenital malformation was the most commonly reported perinatal outcome (36/66, 55%). The details of the characteristics of the included studies are given in Appendix 25.

7.4.2: Quality of the included studies

Fifty-seven studies out of 66 (86%) were low risk for study selection, [124, 125, 233, 241, 268, 279, 280, 283, 284, 286, 288-290, 293, 295, 296, 298, 301-305, 309, 310, 313, 314, 316-320, 322, 326, 328, 331, 334, 337, 341, 342, 343, 352, 353, 355, 358, 367-370, 393-397, 399, 401-403] thirty-six (55%) were low risk for comparability [124, 268, 280, 283, 286, 289, 290, 293, 295, 298, 301, 302, 305, 308, 310, 313, 317, 318, 320, 328, 331, 334, 337, 352, 355, 367, 368-370, 371, 394-397, 399, 403] and sixty-four (97%) of the studies were low risk for study outcome. Medium risk of bias was seen among nine studies (14%) for study selection, [291, 308, 325, 329, 332, 356, 371, 398, 404] 9 studies (14%) for

comparability, [284, 329, 341-343, 353, 356, 402, 404] and two studies (3%) [241, 393] for outcome. None of the studies showed a high risk of bias for study selection and outcome. However, 21 studies (32%) [125, 233, 241, 279, 288, 291, 296, 303, 304, 309, 314, 316, 319, 322, 325, 326, 332, 358, 393, 398, 401] were observed to have a high risk of bias for comparability. Details of the quality of the included studies is depicted in Appendix 26.

7.4.3: Perinatal outcomes in twin pregnancies following assisted reproduction

The risk of any congenital malformation was significantly higher among ART twins compared to non-ART twins as evaluated in 36 studies (OR 1.18, 95% CI 1.06-1.31, I^2=61%) while there was no difference in risk between ART and natural conception groups (OR 1.15, 95% CI 0.96-1.37, I^2=64%, 31 studies).

An increase in risk of NICU admission was observed among ART twins in comparison to non-ART as seen in 30 studies (OR 1.25, 95% CI 1.12-1.41, I^2=88%) and naturally conceived twins as assessed in 27 studies (OR 1.23, 95% CI 1.08-1.40, I^2=88%).

Increased risk was seen for neonatal RDS (OR 1.35, 95% CI 1.07-1.70, I^2= 64%, 14 studies), neurological complications (OR 1.61, 95% CI 1.04-2.48, I^2=0%, 2 studies) and NEC (OR 1.78, 95% CI 1.06-3.01, I^2=0%, 6 studies) among ART twins when compared to non-ART twins. A similarly increased risk was seen for RDS (OR 1.32, 95% CI 1.03-1.69, I^2= 70%, 12 studies), NEC (OR 1.79, 95% CI 1.06-3.05, I^2=0%, 6 studies) and neurological complications (OR 1.95, 95% CI 1.23-3.09, I^2=0%, 2 studies) when compared with twins born by natural conception as well.

Birth weight discordance >25% was seen to be significantly more frequent among ART twins both when compared to non-ART twins (OR 1.31, 95% CI 1.05-1.63, I^2=0%, 7 studies) and to naturally-conceived twins (OR 1.32, 95% CI 1.06-1.64, I^2=0%, 7 studies).

Interestingly, a reduction of risk was observed for stillbirth among ART twins in comparison to non-ART twins as assessed in 33 studies (OR 0.83, 95% CI 0.70-0.99, I^2=49%) and to naturally conceived twins as analysed in 25 studies (OR of 0.78, 95% CI 0.65-0.95, I^2=29%).

The risk of SGA <10th centile was also reduced in ART twins when compared with non-ART twins as assessed in 25 studies (OR 0.91, 95% CI 0.87-0.96, I^2=21%) and with naturally conceived twins as seen in 24 studies (OR 0.91, 95% CI 0.87-0.95, I^2=7%).

TTTS was reported in 9 studies comparing ART to non-ART twins and in seven studies comparing ART to naturally conceived twins. There was a significant risk reduction for TTTS among the ART group compared to both non-ART twins (OR 0.45, 95% CI 0.25-0.82, I^2=25%) and twins of natural conception (OR 0.35, 95% CI 0.14-0.87, I^2=28%).
There was no significant difference between ART twins and non-ART twins for neonatal death (OR 1.03, 95% CI 0.85-1.25, I^2=57%, 28 studies), perinatal mortality (OR 0.92, 95% CI 0.74-1.15, I^2=75%, 21 studies), SGA <5th centile (OR 0.88, 95% CI 0.69-1.12, I^2=70%, 4 studies), major congenital malformation (OR 1.26, 95% CI 0.99-1.61, I^2=69%, 8 studies), APGAR <7 at 5 minutes (OR 1.07, 95% CI 0.87-1.31, I^2=75%, 28 studies), neonatal sepsis (OR 1.12, 95% CI 0.82-1.53, I^2=31%, 11 studies) and mechanical ventilation (OR 1.17, 95% CI 0.89-1.55, I^2=75%, 7 studies).

There were also no significant outcome differences when comparing ART with the natural conception for neonatal death (OR 1.05, 95% CI 0.82-1.34, I^2=49%, 22 studies), perinatal

mortality(OR 0.94, 95% CI 0.71-1.24, I^2=76%, 17 studies), SGA <5th centile (OR 0.88, 95% CI 0.69-1.12, I^2=70%, 4 studies), major congenital malformations (OR 1.22, 95% CI 0.56-2.68, I^2=86%, 4 studies), APGAR <7 at 5 minutes (OR 1.05, 95% CI 0.83-1.32, I^2=76%, 27 studies), mechanical ventilation (OR 1.15, 95% CI 0.87-1.52, I^2=74%, 7 studies) and neonatal sepsis (OR 1.11, 95% CI 0.78-1.58, I^2=44%, 9 studies). These findings are summarized in Tables 20 and 21. The forest plots of pooled OR of certain perinatal outcomes are given in Appendix 27.

Other offspring morbidities in twin pregnancies following assisted reproduction

Intraventricular haemorrhage (IVH), neonatal hypoglycaemia, neonatal jaundice, hypoxic-ischemic (HIE) and umbilical cord pH <7.2 were grouped as other offspring morbidities. A significant reduction in risk for HIE was observed when comparing ART to non-ART twins (OR 0.43, 95%CI 0.20-0.92, I^2=NA). None of the other evaluated outcomes showed a significant difference when ART neonates were compared with non-ART twins [Intraventricular haemorrhage (OR 1.36, 95%CI 0.57-3.24, I^2=73%, 6 studies), neonatal jaundice (OR 1.06, 95% CI 0.80-1.41, I^2=84%, 10 studies), neonatal hypoglycaemia (OR 1.12, 95%CI 0.70-1.79, I^2=76%, 7 studies), and umbilical cord pH <7.2 (OR 0.78, 95%CI 0.53-1.16, I^2=0%, 4 studies)] and naturally conceived twins [IVH (OR 1.35, 95%CI 0.56-3.26, I^2=74%, 6 studies), neonatal jaundice (OR 0.95, 95%CI 0.69-1.30, I^2=78%, 8 studies), neonatal hypoglycaemia (OR 1.05, 95%CI 0.68-1.62, I^2=71%, 7 studies), and umbilical cord pH <7.2 (OR 0.72, 95%CI 0.37-1.41, I^2=0%, 3 studies)]. The above findings are summarized in Tables 22 and 23.

Table 20: Pooled odds ratios (OR) for perinatal outcomes comparing ART vs non-ART in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART*		Non-ART'' (including natural)		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
Stillbirth	33	568	52,375	2,431	152,985	0.04	0.83 [0.70, 0.99]	49
Neonatal death	28	627	41,295	1,936	118,754	0.76	1.03 [0.85, 1.25]	57
Perinatal mortality	21	904	36,886	2,668	89,099	0.46	0.92 [0.74, 1.15]	75
SGA <10th centile	25	6,695	35,965	16080	80,434	0.0003	0.91 [0.87, 0.96]	21
SGA <5th centile	4	4,696	30,714	20,919	132,777	0.30	0.88 [0.69, 1.12]	70
Birth weight discordance >25%	7	157	1,862	348	6,020	0.01	1.31 [1.05, 1.63]	0
TTTS	9	19	1,209	224	4,178	0.009	0.45 [0.25, 0.82]	25
Any congenital malformation	36	3,749	65,291	11,272	216,224	0.003	1.18 [1.06, 1.31]	61
Major congenital malformations	8	1,818	33,319	6,226	145,385	0.06	1.26 [0.99, 1.61]	69
APGAR <7 at 5 min	28	1,191	23,122	2,118	48,979	0.53	1.07 [0.87, 1.31]	75
NICU admission	30	15,132	30,735	36,477	69,451	0.0001	1.25 [1.12, 1.41]	88
RDS	14	644	5,414	728	7,573	0.01	1.35 [1.07, 1.70]	64
Mechanical ventilation	7	1,337	12,477	4,019	33,743	0.25	1.17 [0.89, 1.55]	75
Neonatal sepsis	11	149	3,838	215	4,838	0.47	1.12 [0.82, 1.53]	31
NEC	6	37	2,990	27	3,476	0.03	1.78 [1.06, 3.01]	0
Neurological complications	2	42	1,104	43	1,780	0.03	1.61 [1.04, 2.48]	0

**ART - pregnancies where both oocytes and sperm were manipulated in vitro (e.g.: IVF, GIFT, ZIFT, ICSI)*

''Non-ART - Twin pregnancies conceived following fertility treatment methods other than ART, such as intrauterine insemination (IUI) with or without controlled ovarian hyperstimulation, ovulation induction and those conceived naturally

Table 21: Pooled odds ratios (OR) for perinatal outcomes comparing ART vs Natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART*		Natural^		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
Stillbirth	25	316	34,752	1,167	90,577	0.01	0.78 [0.65, 0.95]	29
Neonatal death	22	341	24,747	960	62,006	0.70	1.05 [0.82, 1.34]	49
Perinatal mortality	17	615	25,474	1,365	49,272	0.66	0.94 [0.71, 1.24]	76
SGA < 10^{th} centile	24	6,682	35,893	14,981	74,705	<0.00001	0.91 [0.87, 0.95]	7
SGA < 5^{th} centile	4	4,696	30,714	20,919	132,777	0.30	0.88 [0.69, 1.12]	70
Birth weight discordance >25%	7	157	1,862	322	5,542	0.01	1.32 [1.06, 1.64]	0
TTTS	7	6	893	120	2,946	0.02	0.35 [0.14, 0.87]	28
Any congenital malformation	31	999	24,472	1,694	43,032	0.14	1.15 [0.96, 1.37]	64
Major congenital malformations	4	173	2,310	334	6,781	0.62	1.22 [0.56, 2.68]	86
APGAR <7 at 5 min	27	1,022	18,542	1,414	30,705	0.71	1.05 [0.83, 1.32]	76
NICU admission	27	12,037	25,025	23,797	45,949	0.002	1.23 [1.08, 1.40]	88
RDS	12	630	5,272	696	7,125	0.03	1.32 [1.03, 1.69]	70
Mechanical ventilation	7	1,337	12,477	3,444	28,835	0.32	1.15 [0.87, 1.52]	74
Neonatal sepsis	9	145	3,696	204	4,390	0.56	1.11 [0.78, 1.58]	44
NEC	6	37	2,990	25	3,308	0.03	1.79 [1.06, 3.05]	0
Neurological complications	2	42	1,104	34	1,708	0.004	1.95 [1.23, 3.09]	0

**ART - pregnancies where both oocytes and sperm were manipulated in vitro (e.g.: IVF, GIFT, ZIFT, ICSI)*

^Natural - twin pregnancies conceived without any fertility treatment

Table 22: Pooled odds ratios (OR) for other offspring morbidity comparing ART vs non-ART in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Non-ART		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
IVH	6	61	2,096	84	2,580	0.49	1.36 [0.57, 3.24]	73
Neonatal Jaundice	10	1,041	5,880	3,448	21,306	0.67	1.06 [0.80, 1.41]	84
Neonatal Hypoglycemia	7	163	2,918	229	4,190	0.65	1.12 [0.70, 1.79]	76
HIE	1	22	992	10	200	0.03	0.43 [0.20, 0.92]	NA
Umbilical cord pH <7.2	4	112	2,214	44	1,518	0.22	0.78 [0.53, 1.16]	0

Table 23: Pooled odds ratios (OR) for other offspring morbidity comparing ART vs Natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Natural		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
IVH	6	61	2,096	82	2,518	0.50	1.35 [0.56, 3.26]	74
Neonatal Jaundice	8	466	2,720	690	3,434	0.74	0.95 [0.69, 1.30]	78
Neonatal hypoglycaemia	7	163	2,918	213	3,814	0.83	1.05 [0.68, 1.62]	71
Umbilical cord pH <7.2	3	17	1,222	22	1,318	0.34	0.72 [0.37, 1.41]	0

7.4.4: Subgroup analysis

Subgroup analysis was performed comparing studies published after 2010 with those published before 2010. A significant difference between subgroups was observed only for SGA <5th centile, where it was significantly higher among ART twins before 2010 than non-ART twins. The rest of the outcomes studied comparing ART and non-ART twins and all outcomes comparing ART and naturally conceived twins did not show any significant subgroup difference before and after 2010. Subgroup analysis was also performed for other offspring morbidity, comparing ART vs non-ART and ART vs natural conception, where differences were not observed for any outcomes. (Tables 24-27) A further subgroup analysis was conducted comparing fresh and frozen embryo transfer cycles. No subgroup difference was seen for ART versus non-ART twins. Significantly increased odds of major congenital malformations were observed for frozen embryo transfer cycles when comparing ART with naturally conceived twins, but only one study was available for comparison. (Tables 28 and 29).

Table 24: Subgroup analysis by year for perinatal outcomes comparing ART vs non-ART in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	After 2010		Before 2010		After 2010 vs Before 2010
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
Stillbirth	23	0.78 [0.64, 0.95]	10	0.97 [0.70, 1.33]	0.26
Neonatal death	17	0.99 [0.80, 1.23]	11	1.04 [0.68, 1.60]	0.85
Perinatal mortality	9	0.78 [0.60, 1.01]	12	1.02 [0.68, 1.52]	0.28
SGA <10th centile	16	0.92 [0.88, 0.96]	9	0.96 [0.77, 1.19]	0.71
SGA <5th centile	3	0.86 [0.65, 1.14]	1	3.67 [2.10, 6.41]	<0.00001
Birth weight discordance >25%	4	1.38 [1.03, 1.84]	4	1.23 [0.88, 1.71]	0.61
TTTS	7	0.49 [0.23, 1.06]	2	0.31 [0.13, 0.77]	0.45
Any congenital malformation	22	1.17 [1.04, 1.32]	14	1.13 [0.86, 1.48]	0.8
Major congenital malformations	5	1.44 [1.01, 2.05]	3	1.04 [0.80, 1.35]	0.15
APGAR <7 at 5 minutes	18	0.99 [0.77, 1.28]	10	1.26 [0.90, 1.76]	0.27
NICU admission	17	1.31 [1.13, 1.52]	13	1.17 [0.96, 1.43]	0.37
RDS	8	1.51 [1.32, 1.74]	6	1.28 [0.66, 2.46]	0.62
Mechanical ventilation	4	0.99 [0.78, 1.25]	3	2.04 [0.70, 5.94]	0.19
Neonatal sepsis	6	1.08 [0.67, 1.74]	5	1.24 [0.86, 1.79]	0.65
NEC	4	1.35 [0.69, 2.63]	2	2.76 [1.19, 6.40]	0.19

Table 25: Subgroup analysis by year for perinatal outcomes comparing ART vs Natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	After 2010		Before 2010		After 2010 vs Before 2010
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
Stillbirth	19	0.72 [0.59, 0.88]	7	0.85 [0.59, 1.23]	0.44
Neonatal death	14	0.97 [0.78, 1.20]	9	1.10 [0.71, 1.70]	0.61
Perinatal mortality	9	0.83 [0.60, 1.15]	8	1.01 [0.59, 1.76]	0.54
SGA <10th centile	16	0.91 [0.88, 0.95]	9	0.95 [0.77, 1.17]	0.71
Birth weight discordance >25%	4	1.38 [1.03, 1.84]	4	1.36 [1.00, 1.85]	0.96
TTTS	6	0.37 [0.13, 1.02]	1	0.14 [0.01, 2.46]	0.53
Any congenital malformation	18	1.21 [0.96, 1.52]	13	1.06 [0.77, 1.45]	0.51
Major congenital malformations	2	0.91 [0.03, 5.49]	2	0.97 [0.64, 1.48]	0.97
APGAR <7 at 5 minutes	17	1.00 [0.73, 1.35]	10	1.18 [0.83, 1.66]	0.48
NICU admission	16	1.32 [1.10, 1.59]	11	1.10 [0.90, 1.34]	0.19
RDS	8	1.56 [1.35, 1.80]	5	0.98 [0.50, 1.90]	0.18
Mechanical ventilation	4	0.96 [0.77, 1.19]	3	2.04 [0.70, 5.94]	0.18
Neonatal sepsis	6	1.12 [0.66, 1.91]	4	1.18 [0.82, 1.71]	0.88
NEC	4	1.35 [0.68, 2.67]	2	2.76 [1.19, 6.40]	0.19

Table 26: Subgroup analysis by year for other offspring morbidities comparing ART vs non-ART in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	After 2010		Before 2010		After 2010 vs Before 2010
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
IVH	3	1.19 [0.48, 2.95]	3	1.81 [0.20, 16.40]	0.73
Neonatal Jaundice	7	0.99 [0.71, 1.38]	3	1.37 [0.74, 2.56]	0.36
Neonatal Hypoglycemia	5	1.16 [0.73, 1.82]	2	0.96 [0.13, 7.02]	0.85
Umbilical cord pH <7.2	2	0.70 [0.34, 1.48]	2	0.81 [0.51, 1.29]	0.74

Table 27: Subgroup analysis by year for other offspring morbidities comparing ART vs Natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	After 2010		Before 2010		After 2010 vs Before 2010
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
IVH	3	1.19 [0.48, 2.95]	3	1.77 [0.18, 17.27]	0.75
Neonatal Jaundice	6	0.95 [0.64, 1.41]	3	1.01 [0.72, 1.40]	0.83
Neonatal Hypoglycemia	5	1.16 [0.74, 1.82]	2	0.72 [0.15, 3.38]	0.56
Umbilical cord pH < 7.2	2	0.70 [0.34, 1.48]	1	0.80 [0.16, 3.91]	0.89

Table 28: Subgroup analysis by type of Embryo Transfer (ET) for perinatal outcomes comparing ART vs non-ART in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	Fresh ET		Frozen ET		Fresh Vs Frozen ET
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
Stillbirth	2	0.80 [0.44, 1.45]	1	0.70 [0.22, 2.21]	0.84
Neonatal death	2	0.97 [0.82, 1.15]	1	0.24 [0.03, 1.75]	0.17
Perinatal mortality	2	1.69 [0.36, 7.87]	1	0.40 [0.13, 1.17]	0.13
SGA <10th centile	2	1.05 [0.85, 1.29]	1	0.63 [0.36, 1.10]	0.09
SGA <5th centile	1	0.94 [0.47, 1.87]	3	0.80 [0.52, 1.21]	0.69
Any congenital malformation	4	1.24 [0.77, 1.98]	3	1.69 [0.83, 3.42]	0.48
Major congenital malformations	1	0.73 [0.43, 1.27]	3	1.73 [0.89, 3.36]	0.05
APGAR <7 at 5 minutes	2	1.15 [0.55, 2.40]	1	0.77 [0.31, 1.88]	0.50

Table 29: Subgroup analysis by type of Embryo Transfer (ET) for perinatal outcomes comparing ART vs Natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	Fresh ET		Frozen ET		Fresh Vs Frozen ET
	No of studies	OR (95% CI)	No of studies	OR (95% CI)	P-value for subgroup difference
Stillbirth	1	1.12 [0.70, 1.77]	1	0.69 [0.22, 2.20]	0.45
Neonatal death	1	0.88 [0.52, 1.48]	1	0.24 [0.03, 1.71]	0.21
SGA <10th centile	2	1.05 [0.86, 1.29]	1	0.63 [0.36, 1.11]	0.09
SGA <5th centile	1	0.94 [0.47, 1.87]	1	1.00 [0.51, 1.98]	0.9
Any congenital malformation	3	1.59 [0.50, 5.08]	1	3.34 [2.02, 5.53]	0.25
Major congenital malformations	1	0.73 [0.43, 1.27]	1	3.34 [2.02, 5.53]	<0.0001
APGAR <7 at 5 minutes	2	1.14 [0.53, 2.43]	1	0.75 [0.31, 1.84]	0.49

7.4.5: Sensitivity analysis

Sensitivity analysis was carried out for all perinatal outcomes after excluding monochorionic twins. There was a significantly higher risk of birthweight discordance >25% (OR 1.38, 95% CI 1.03-1.84, I^2=0%, 4 studies), NICU admission (OR 1.37, 95% CI 1.01-1.84, I^2=93%, 14 studies), and RDS (OR 1.53, 95% CI 1.27-1.83, I^2=0%, 7 studies) among ART neonates when comparing with non-ART following exclusion of monochorionic twins. A similar risk increase was seen among ART twins for birthweight discordance >25% (OR 1.38, 95% CI 1.03-1.84, I^2=0%, 4 studies), NICU admission (OR 1.36, 95% CI 1.01-1.84, I^2=93%, 14 studies), and RDS (OR 1.57, 95% CI 1.31-1.90, I^2=0%, 7 studies) compared to naturally conceived neonates. (Tables 30 and 31). As for other offspring morbidity, a significantly higher risk was seen only for neonatal jaundice (OR 1.22, 95% CI 1.12-1.34, I^2=0, 5 studies) among ART twins when compared to non-ART twins (Tables 32 and 33).

7.4.6: Small study effects

Funnel plot asymmetry was evaluated for outcomes reported in ten or more studies. Small study effects were evident only for APGAR score <7 at 5 minutes (p = 0.04) in the comparison between ART twins and twins born by natural conception. (Appendix 28)

Table 30: Sensitivity analysis for perinatal outcomes excluding monochorionicity comparing ART vs non-ART in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Non-ART		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
Stillbirth	9	27	3,288	53	3,798	0.54	0.85 [0.52, 1.41]	0
Neonatal death	9	41	3,162	85	3,982	0.98	0.99 [0.49, 2.01]	51
Perinatal mortality	5	144	6,556	229	10,112	0.56	1.20 [0.66, 2.18]	49
SGA <10th centile	9	1,891	8,598	2,624	11,930	0.53	0.98 [0.91, 1.05]	0
Birth weight discordance >25%	4	109	1,052	111	1,254	0.03	1.38 [1.03, 1.84]	0
Any congenital malformation	12	317	8,080	478	12,390	0.20	1.20 [0.91, 1.57]	41
APGAR <7 at 5 mins	9	696	8,892	754	12,468	0.55	1.13 [0.76, 1.67]	86
NICU admission	14	2,079	9,608	2393	13,616	0.04	1.37 [1.01, 1.84]	93
RDS	7	339	2,702	283	2,738	<0.00001	1.53 [1.27, 1.83]	0
Mechanical ventilation	3	66	1,434	23	858	0.06	2.68 [0.95, 7.53]	56
Neonatal sepsis	4	41	1,752	49	1,356	0.60	1.13 [0.71, 1.81]	0
NEC	3	15	1,670	6	1,192	0.28	1.74 [0.64, 4.72]	0
Neurological complications	1	9	276	6	414	0.12	2.29 [0.81, 6.51]	NA

Table 31: Sensitivity analysis for perinatal outcomes in studies excluding monochorionicity comparing ART vs Natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Natural		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
Stillbirth	8	22	2,682	41	3,060	1.00	1.00 [0.56, 1.77]	0
Neonatal death	8	40	2,556	71	3,244	0.68	1.14 [0.60, 2.16]	39
Perinatal mortality	5	144	6,556	168	8,150	0.45	1.23 [0.72, 2.12]	40
SGA <10th centile	9	1,891	8,598	2,194	9,872	0.40	0.97 [0.90, 1.04]	0
Birth weight discordance >25%	4	109	1,052	111	1,254	0.03	1.38 [1.03, 1.84]	0
Any congenital malformation	12	317	8,080	394	10,106	0.32	1.15 [0.87, 1.51]	37
APGAR <7 at 5 mins	9	696	8,892	628	10,506	0.54	1.14 [0.75, 1.71]	87
NICU admission	14	2,079	9,608	2148	11,558	0.05	1.36 [1.01, 1.84]	93
RDS	7	339	2,702	272	2,642	<0.00001	1.57 [1.31, 1.90]	0
Mechanical ventilation	3	66	1,434	21	762	0.10	3.24 [0.80, 13.03]	60
Neonatal sepsis	4	41	1,752	46	1,260	0.58	1.15 [0.70, 1.88]	0
NEC	3	15	1,670	6	1,096	0.45	1.47 [0.54, 3.98]	0
Neurological complications	1	9	276	6	414	0.12	2.29 [0.81, 6.51]	N/A

Table 32: Sensitivity analysis for other offspring morbidity in studies excluding monochorionicity comparing ART vs non-ART in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Non-ART		P value	OR (95% CI)	I^2
		Events	Total	Events	Total			
IVH	2	25	980	28	682	0.08	1.76 [0.94, 3.30]	0
Neonatal Jaundice	5	802	4,408	2,938	18,638	<0.0001	1.22 [1.12, 1.34]	0
Neonatal Hypoglycemia	3	77	1,516	60	1,668	0.45	1.36 [0.62, 3.00]	77
Umbilical cord pH <7.2	1	14	898	12	518	0.31	0.67 [0.31, 1.45]	N/A

Table 33: Sensitivity analysis for other offspring morbidity in studies excluding monochorionicity comparing ART vs Natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

Outcome	No of studies	ART		Natural		P-value	OR (95% CI)	I^2
		Events	Total	Events	Total			
IVH	2	25	980	28	682	0.08	1.76 [0.94, 3.30]	0
Neonatal Jaundice	4	228	1,318	238	1,288	0.16	1.17 [0.94, 1.45]	0
Neonatal Hypoglycemia	3	77	1,516	60	1,668	0.45	1.36 [0.62, 3.00]	77
Umbilical cord pH <7.2	1	14	898	12	518	0.31	0.67 [0.31, 1.45]	N/A

7.4.7: Meta-regression analysis

Meta-regression analysis was carried out to adjust for confounding factors like maternal age and parity. The effects of ART on stillbirth, SGA <10th centile and NICU admission was independent of maternal age and parity. Interestingly, when the average maternal age difference between ART and non-ART groups increased, the effect of ART on congenital malformations was observed to be significantly reduced. (Appendix 29)

7.5: Discussion

Summary of main findings

The current review emphasises that twins born following ART are at a higher risk for many adverse outcomes evaluated than their non-ART counterparts. ART newborns are at an increased risk of birth weight discordance, congenital malformation, neonatal morbidity, and neonatal intensive care unit admissions. However, our findings indicate that twins born by ART carry a reduced chance of stillbirth, small for gestation age, and twin-twin transfusion syndrome. These observations were generally consistent when ART twins were compared with naturally conceived twins. The association between ART and poor perinatal outcomes has been observed throughout the years, despite advances in assisted reproductive techniques. ART twins were at higher risk of adverse outcomes than non-ART and naturally conceived twins even after monochorionicity was excluded.

Comparison with existing studies/reviews

Many systematic reviews have compared perinatal outcomes of ART and non-ART singletons. The risk of birth weight <1500g, birth weight <2500g, small for gestational age, congenital

malformation, perinatal mortality, and NICU admission were significantly increased among ART singletons. [375, 376, 405] When comparing the results of these studies with our review, it can be safely assumed that perinatal outcomes of ART twin pregnancies follow a similar trend.

A systematic review and meta-analysis published in 2011 assessed thirteen studies on maternal and neonatal outcomes following assisted reproduction. [378] This review observed a higher risk of perinatal death among ART twins when controlled for basic maternal characteristics. Analysis of unlike-sex twins separately showed a higher risk of birth weight of 1500-2500 g and perinatal death among ART twins than in twins born by natural conception. Our review, however, did not demonstrate a significant increase in perinatal mortality among ART twins as observed in the above review despite having included a more extensive study population.

Another systematic review that evaluated the risk of adverse outcomes in twins after high-technology subfertility treatment, published in 2016, reported an increased risk of low birth weight in pregnancies following ART than in natural conception. [377] All meta-analytic data included in this review showed no significant difference in perinatal mortality between ART twins and naturally conceived twins, similar to the observations in our review. Our study included a higher number of perinatal outcomes, allowing a more comprehensive analysis than this review.

A systematic review published in 2005, with inclusion criteria similar to our study, matched for maternal age and parity, found that the risk of NICU admission was increased among ART twins compared to naturally conceived twins. [276] Although the observed risks were similar to what we reported, the list of perinatal outcomes and the study population in this review was notably smaller, with only 11 studies included.

Existing individual studies have demonstrated conflicting findings for adverse outcomes in twin pregnancies following ART. Many studies have reported worse outcomes in twins conceived by ART than in spontaneously conceived twins, [125, 290, 302, 314, 341, 342, 398, 402] while others failed to demonstrate a significant difference in outcomes between ART twins and non-ART twins. [283, 284, 289, 303, 308, 329, 334, 359]. Interestingly, some studies have reported better outcomes among ART twins than spontaneously conceived twins, such as fewer congenital anomalies, fewer infant death, fewer babies of very low birth weight and a reduced risk of admission to neonatal intensive care units. [274, 304, 334] However, most previously published systematic reviews and the current review are similar in concluding a higher risk of adverse outcomes among twins following ART. [276, 277, 378, 381, 382]

The higher risks observed for twins born by ART may be due to a constellation of reasons. Women who conceive by ART are generally older than women who conceive spontaneously, and the former are at higher risk of medical complications during the antenatal period. These medical issues may indirectly contribute to increased perinatal risks among their offspring. Higher rates of caesarean delivery and preterm birth among ART twins also contribute to higher perinatal morbidity.

The observed reduction in stillbirth among ART twins may be attributed to close fetal surveillance in this group. A likely reason for the observed lower rates of SGA <10th centile among ART twins may be due to the higher rates of preterm birth among this group, reducing the occurrence of late-onset fetal growth restriction. The lower risk of twin-twin transfusion syndrome among ART twins could be attributed to the significantly fewer monochorionic twins

in this group. [406] However, differences in populations and study designs may also have contributed to the differences noted between these two groups.

Strengths and limitations

This review provides a detailed analysis of perinatal outcomes in twin pregnancies conceived by assisted reproduction, involving the largest study population to date. A comprehensive literature search was performed without language restrictions using a prospective protocol, increasing the chances of including all relevant studies. The relationship between ART and many clinically relevant perinatal outcomes was assessed in this review. A comprehensive assessment of study quality was carried out, and a comprehensive methodology was followed. Perinatal outcomes in ART twin pregnancies compared to non-ART twins and naturally conceived twins were assessed to quantify the effects caused explicitly by ART. As monochorionicity is known to cause worse pregnancy outcomes than dichorionicity, a sensitivity analysis was performed comparing ART versus non-ART after excluding monochorionicity. In addition, subgroup analysis was aimed at assessing if the association between ART and perinatal outcomes changed over the years.

Observations based on the meta-regression analysis did not significantly digress from the main findings, except where the risk of congenital malformations among ART twins reduced when the average age difference between ART and non-ART women increased. One can presume that older women undergoing ART are more likely to conceive via donor oocytes, which may have therefore reduced the risk of congenital malformation. A sensitivity analysis excluding oocyte donation should have ideally backed this assumption. However, due to paucity of data, a sensitivity analysis excluding donor oocytes was not performed.

The heterogeneity in the study population limited the findings in this systematic review. Primary studies varied in their inclusion criteria. Some studies had excluded cases of monoamniotic and monochorionic twins, twin-twin transfusion syndrome, fetal chromosomal or anatomic anomalies, multifetal pregnancy reduction, and twins conceived following intrauterine insemination or ovulation induction. The definitions given by the primary study authors for the evaluated outcomes also varied across studies.

Some studies grouped twin pregnancies conceived by ovulation induction and intrauterine insemination under the ART category, which had to be excluded from the review. There was heterogeneity in the non-ART group which included twins conceived naturally, following IUI or ovulation induction. The main aim of the review was to compare ART with the Non-ART group. Additionally, many studies had collectively included OI and IUI in the Non-ART group without providing data for them separately. Data insufficiency did not allow a comparison between the IUI and OI groups.

Most studies lacked information on donor eggs, a factor likely to have influenced the outcomes. Furthermore, data on fresh or frozen embryo transfer cycles was limited. As most studies were conducted in high-income countries, further research is warranted to make the review findings more applicable to low-income countries.

Clinical and research implications

Due to the high numbers of twin pregnancies worldwide, it is essential to accurately quantify ART's risks to neonates to enable counselling and proper management. Current management guidelines on twin pregnancies do not identify ART twins as a group requiring special care. The higher risk of adverse perinatal outcomes demonstrated in this review emphasises the need for

ART twin pregnancies to be recognised in guidelines as a separate high-risk group so that closer surveillance can be offered to minimise morbidity and mortality.

Further research is recommended with more refined inclusion and exclusion criteria to identify how ART contributes to this observed increase in perinatal risks. Subgroup analysis between fresh versus frozen embryo transfer cycles and between different ART techniques is likely to provide more insight.

7.6: Conclusion

Twins conceived by ART are at a significantly higher risk of adverse perinatal outcomes than non-ART twins. Women undergoing assisted reproduction should therefore be counselled regarding the increased risks among ART twins and should be offered closer monitoring in addition to what is routinely practised.

Chapter 8: Conclusion

8.1: Summary of findings

This chapter summarizes the results described in the individual chapters previously. More detailed accounts of the findings can be found in each chapter. I was able to address the objectives pre-specified at the start of this thesis. Accordingly, I evaluated maternal clinical predictors and biochemical markers that indicate a higher risk of preterm birth in twin pregnancies. The risk of preterm birth at different gestations was quantified for the various predictors evaluated. I also determined the association between chorionicity and preterm birth in twin pregnancies. I was able to assess an extensive list of adverse maternal and perinatal outcomes and quantify their risks in ART twin pregnancies compared to non-ART and naturally conceived twin pregnancies. The following table summarizes the objectives of my thesis and the findings from each chapter.

Table 34: Summary of the thesis

Chapter 3: Maternal clinical predictors of preterm birth in twin pregnancies
Population: Twin pregnancies
Intervention: Maternal clinical predictors • Maternal age • Maternal BMI • Race • Parity • History of smoking • History of preterm birth in previous pregnancies • Pre-existing or new-onset conditions such as diabetes mellitus, anaemia and hypertensive disorders of pregnancy
Outcomes: Preterm birth at gestations • <34 weeks • <37 weeks
Research design: Systematic review and meta-analysis

Results:

- Age <20 years significantly increased both early (OR 1.81, 95% CI 1.68–1.95, I^2 = 0%) and late (OR 1.36, 95% CI 1.18-1.57, I^2 =57%) preterm birth. Age >35 years significantly lowered the risk of early preterm birth (OR 0.89, 95% CI 0.82-0.96, I2 = 37%).
  [15 studies, 386,421 pregnancies]
- BMI >35 kg/m^2 was associated with a significantly higher risk of both early (OR 1.63, 95% CI 1.30–2.05, I^2 = 52%) and late (OR 1.18, 95% CI 1.02–1.35, I^2 = 46%) preterm birth.
  [10 studies, 44,052 pregnancies]
- Non-white women compared to white women (OR 1.31, 95% CI 1.20–1.43, I^2 = 0%) and black women compared to non-black women (OR 1.38, 95% CI 1.07–1.77, I^2 = 98%) had a higher risk of early preterm birth.
  [13 studies, 1,468,584 pregnancies]
- Women who smoked were at a higher risk of early preterm birth (OR 1.30, 95% CI 1.23–1.37, I^2 = 0%)
  [15 studies, 83 955 pregnancies]
- Nulliparous women were at a higher risk of both early (OR 1.51, 95% CI 1.38–1.65, I^2 = 73%) and late (OR 1.41, 95% CI 1.23– 1.62, I^2 = 68%) preterm birth.
  [28 studies, 508 021 pregnancies]
- A history of previous preterm birth was associated with an increased risk of early (OR 2.67, 95% CI 2.16–3.29, I^2 = 0%) and late preterm birth (OR 3.08, 95% CI 2.10–4.51, I^2 = 73%).
  [19 studies, 9,924 pregnancies]
- Diabetes in pregnancy was associated with a higher risk of early preterm birth (OR 1.73, 95% CI 1.29–2.33, I^2 = 0%).
  [5 studies, 425,918 pregnancies]
- Hypertensive disorders in pregnancy were associated with an increased risk for late preterm birth (OR 1.49, 95% CI 1.20-1.86, I^2= 52%).
  [5 studies, 281,376 pregnancies]
- No significant association was noted between maternal anaemia and preterm birth in twin pregnancies.
- Sensitivity analysis showed that the risk of both early (OR 2.83, 95% CI 2.00–4.00, I^2 = 0%) and late (OR 2.77, 95% CI 1.66-4.63, I^2 = 85%) spontaneous preterm birth was higher among women with a past history of preterm birth. The risk of early (OR 0.14, 95% CI 0.03 – 0.58) and late (OR 0.53, 95% CI 0.31– 0.90) spontaneous preterm birth was significantly reduced among women over 40 years of age [one study]. A reduction in risk of spontaneous late preterm birth was observed among black women compared to non-black women (OR 0.48, 95% CI 0.24 – 0.98) [one study].

Chapter 4: Biochemical predictors of preterm birth in twin pregnancies

Population: Twin pregnancies

Intervention: Biochemical predictors

- Cervicovaginal Fetal fibronectin
- Maternal serum PAPP-A
- Maternal serum hCG
- Maternal serum Relaxin
- Maternal serum Alfa fetoprotein
- Maternal serum 25 hydroxyvitamin D
- Maternal cervical PhIGFBP-1
- Amniotic fluid MMP-8

- Maternal cervical Granulocyte elastase
- Maternal cervicovaginal Interleukin-8
- Intrauterine infection (chorioamnionitis, positive amniotic culture)
- Bacterial vaginosis

Outcomes: Preterm birth at gestations

- <28 weeks
- <32 weeks
- <34 weeks
- <37 weeks

Research design: Systematic review and meta-analysis

Results:

- A positive fetal fibronectin test in women either asymptomatic or symptomatic for preterm labour was associated with an increased risk of preterm birth <28 weeks (OR 12.06, 95 % CI 4.90-29.70, I^2 = 0%), <32 weeks (OR 10.03, 95 % CI 6.11-16.47, I^2 = 0%), <34 weeks (OR 6.26, 95 % CI 3.85-10.17, I^2 = 30 %), <37 weeks (OR 5.34, 95 % CI 3.68-7.76, I^2 = 15 %), and within 14 days of testing (OR 13.95, 95 % CI 4.33-44.98, I^2 = 0%). Measurement of fFN was done at various gestational ages.
  [16 studies, 2,347 pregnancies]
- A significant association was not observed between maternal PAPP-A levels and preterm birth in twin pregnancies.
- An elevated maternal serum beta- hCG level was associated with a higher risk of birth before 37 weeks (OR 1.51, 95 % CI 1.07-2.13, I^2 = 0%)
- [4 studies, 2,042 pregnancies]
- No significant association was seen between maternal serum relaxin level and preterm birth.
- Maternal serum AFP >3.5 MoM was associated with a higher risk of preterm birth <34 weeks (OR 2.35, 95 % CI 1.12-4.96, I^2=NA)
- [1 study, 267 pregnancies]
- 25-hydroxy vitamin D <75 nmol/l was associated with a higher risk of preterm birth at <32 weeks (OR 3.01, 95 % CI 1.26-7.19, I^2=NA) and at <37 weeks (OR 2.59, 95 % CI 1.35-4.95, I^2=NA).
  [1 study, 211 pregnancies]
- A positive phIGFBP-1 test was associated with a significant increase in risk for preterm birth <37 weeks (OR 4.23, 95 % CI 1.97-9.09, I^2 = 0%)
- [3 studies, 388 pregnancies]
- A positive MMP-8 test was significantly associated with a higher risk of birth within 7 days (OR 10.59, 95 % CI 3.70–30.29, I^2=NA)
- [1 study, 88 pregnancies]
- The risk of preterm birth <37 weeks was associated with an IL-8 level exceeding 1.75 ng/g of mucus (OR 3.13, 95 % CI 1.18-8.34, I^2=NA)
  [1 study, 101 pregnancies]
- There was no significant association between Granulocyte Elastase, intrauterine infection or bacterial vaginosis and preterm birth of twins.
- Sensitivity analysis – the risk of preterm birth <32 weeks (OR 10.54, 95 % CI 5.66-19.64, I^2 = 19 %), <34 weeks (OR 8.07, 95 % CI 5.28-12.33, I^2 = 0%) and <37 weeks (OR 6.21, 95 % CI 4.34-8.87, I^2 = 0%) was significantly higher among women asymptomatic for preterm labour with a positive fFN test. In women symptomatic for preterm labour with a positive fFN test, the risk of birth before 32 weeks (OR 28.47, 95 %

CI 1.32-612.60, I^2=NA), birth before 34 weeks (OR 3.07, 95 % CI 1.44-6.57, I^2 = 0%) and delivery within fourteen days of testing (OR 13.95, 95 % CI 4.33-44.98, I^2 = 0%) was significantly increased.

Chapter 5: Association between chorionicity and preterm birth in twin pregnancies

Population: Twin pregnancies

Intervention: Chorionicity

- Monochorionicity
- Dichorionicity

Outcomes: Preterm birth at gestations

- <28 weeks
- <32 weeks
- <34 weeks
- <37 weeks

Research design: Systematic review and meta-analysis

Results:

- Among women both asymptomatic and symptomatic for preterm labour, the risk of preterm birth was significantly higher with monochorionicity at ≤28 weeks (OR 2.14, 95% CI 1.52-3.02, I^2 = 46%) [11 studies, 10,484 pregnancies], ≤32 weeks (OR 1.55, 95% CI 1.27-1.89, I^2 = 68%) [23 studies, 19,783 pregnancies], ≤34 weeks (OR 1.47, 95% CI 1.27-1.69, I^2 = 60%) [22 studies, 21,181 pregnancies] and <37 weeks (OR 1.66, 95% CI 1.43-1.93, I^2 = 65%) [26 studies, 15,997 pregnancies].
- Among women asymptomatic for preterm labour, the risk of preterm birth was significantly increased in monochorionic twin pregnancies at ≤34 weeks (OR 1.85, 95% CI 1.42-2.40, I^2 = 25%) [5 studies, 4,304 pregnancies] and <37 weeks (OR 1.75, 95% CI 1.22-2.53, I^2 = 61%) [4 studies, 1,748 pregnancies].
- Sensitivity analysis for spontaneous preterm birth showed a significantly higher risk in monochorionicity at ≤34 weeks (OR 1.25, 95% CI 1.01-1.55, I^2 = 0%) [8 studies, 3,048 pregnancies] and at <37 weeks (OR 1.41, 95% CI 1.13-1.78, I^2 = 0%) [5 studies, 1,999 pregnancies].
- Sensitivity analysis excluding TTTS showed significantly higher risk of any preterm birth for monochorionicity at ≤28 weeks (OR 2.05, 95% CI 1.42-2.95, I^2 = 37%) [6 studies, 8,315 pregnancies] and at ≤34 weeks (OR 1.58, 95% CI 1.10-2.27, I^2 = 0%) [8 studies, 9,342 pregnancies]. The risk of spontaneous preterm birth was significantly higher with monochorionicity at ≤34 weeks (OR 1.26, 95% CI 1.02-1.54, I^2 = 61%) [3 studies].

Chapter 6: Maternal outcomes in twin pregnancies following assisted reproductive technology

Population: Twin pregnancies conceived via spontaneous conception or via assisted reproductive techniques

Intervention: Assisted reproductive techniques

- In vitro fertilization (IVF)
- Intracytoplasmic sperm injection (ICSI)
- Fresh or frozen embryo transfer
- Gamete intrafallopian transfer (GIFT)

Outcomes: Maternal outcomes

- Preterm birth at gestational ages: <28 weeks, <32 weeks, <34 weeks, <37 weeks
- Gestational hypertension
- Pre-eclampsia
- Hypertensive disorders in pregnancy

- Gestational diabetes mellitus
- Diabetes in pregnancy
- Antepartum haemorrhage
- Placenta previa
- Placental abruption
- Postpartum haemorrhage
- Caesarean delivery

Research design: Systematic review and meta-analysis

Results:

- The risk of preterm birth <34 weeks was higher among ART twins compared to non-ART (OR 1.33, 95% CI 1.13-1.57, I^2=74%) [28 studies, 24,731 pregnancies] and naturally conceived twins (OR 1.24, 95% CI 1.02-1.49, I^2=67%) [20 studies, 15,524 pregnancies].
- Preterm birth <37 weeks was higher among ART twins compared to non-ART (OR 1.28, 95% CI 1.18-1.37, I^2=77%) [67 studies, 128,409 pregnancies] and naturally conceived twins. (OR 1.34, 95% CI 1.21-1.48, I^2=74%) [50 studies, 50,244 pregnancies]
- Risk of gestational hypertension was higher among ART mothers compared to non-ART (OR 1.32, 95% CI 1.15-1.53, I^2=75%) [33 studies, 54,075 pregnancies] and naturally conceived twins (OR 1.33, 95% CI 1.12-1.58, I^2= 76%) [27 studies, 36,499 pregnancies].
- ART mothers were at a higher risk of pre-eclampsia compared to non-ART mothers (OR 1.37, 95% CI 1.20-1.57, I^2=19%) [22 studies, 23,147 pregnancies] and naturally conceived twin pregnancies (OR 1.27, 95% CI 1.10-1.47, I^2=12%) [18 studies, 15,260 pregnancies].
- The risk of hypertensive disorders in pregnancy was increased among ART mothers compared to non-ART (OR 1.20, 95% CI 1.05-1.38, I^2=83%) [55 studies, 65,046 pregnancies] and natural conception (OR 1.40, 95% CI 1.11-1.76, I^2=77%) [24 studies, 19,292 pregnancies].
- The risk of GDM was higher among ART twin pregnancies than non-ART (OR 1.55, 95% CI 1.38-1.75, I^2=41%) [45 studies, 53,514 pregnancies] and naturally conceived twin pregnancies (OR 1.65, 95% CI 1.44-1.89, I^2=41%) [41 studies, 37,630 pregnancies].
- Diabetes in pregnancy was significantly increased among ART mothers compared to non-ART (OR 1.58, 95% CI 1.39-1.79, I^2= 49%) [48 studies, 54,818 pregnancies] and natural conception (OR 1.61, 95% CI 1.42-1.84, I^2=31%) [43 studies, 36,202 pregnancies].
- APH was significantly higher among ART mothers compared to non-ART (OR 1.77, 95% CI 1.26-2.47, I^2=59%) [10 studies, 24,119 pregnancies] and natural conception (OR 2.55, 95% CI 1.86-3.50, I^2=5%) [7 studies, 10,724 pregnancies].
- Placenta praevia was significantly higher among ART twin pregnancies compared to non-ART twins (OR 2.22, 95% CI 1.82-2.70, I^2=8%) [25 studies, 48,253 pregnancies] and twins conceived naturally (OR 2.22, 95% CI 1.64-3.02, I^2=29%) [20 studies, 21,371 pregnancies].
- The risk of placental abruption was higher among ART mothers than non-ART (OR 1.20, 95% CI 1.01-1.44, I^2=0%) [27 studies, 31,232 pregnancies] and naturally conceived twin pregnancies (OR 1.33, 95% CI 1.11-1.60, I^2=0%) [24 studies, 26,288 pregnancies].
- PPH was significantly higher among ART mothers than non-ART (OR 1.45, 95% CI 1.21-1.75, I^2=40%) [20 studies, 16,830 pregnancies] and natural conception (OR 1.37, 95% CI 1.15-1.63, I^2=27%) [19 studies, 14,801 pregnancies].
- The risk of caesarean delivery was increased among ART twin pregnancies compared to non-ART (OR 1.83, 95% CI 1.65-2.02, I^2=86%) [65 studies, 114,058 pregnancies] and naturally conceived twin pregnancies (OR 2.03, 95% CI 1.78-2.32, I^2=85%) [52 studies, 53,494 pregnancies].

- When studies published before 2010 were compared with studies published after 2010 in the subgroup analysis, the risk of preterm birth <28 weeks, <34 weeks and <37 weeks in ART twins was significantly higher before 2010 when compared with non-ART twins. The risk of GDM among ART mothers was significantly higher than non-ART mothers before 2010.
- There was no significant difference in any outcomes when fresh embryo transfer cycles were compared with frozen embryo transfer cycles.
- Sensitivity analysis done limiting for studies reporting on spontaneous preterm birth showed a higher risk of spontaneous preterm birth at <34 weeks among ART twins compared to non-ART twins (OR 1.84, 95% CI 1.40-2.42, I^2=2%) [4 studies, 1,438 pregnancies] and naturally conceived twins (OR 1.83, 95% CI 1.23-2.71, I^2=NA) [1 study, 668 pregnancies].
- When monochorionicity was excluded, ART twin pregnancies were at higher risk of preterm birth <37 weeks, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes in pregnancy, placenta praevia and caesarean delivery when compared to non-ART and naturally conceived twin pregnancies.

Chapter 7: Perinatal outcomes in twin pregnancies following assisted reproductive technology

Population: Twin pregnancies conceived via spontaneous conception or via assisted reproductive techniques

Intervention: Assisted reproductive techniques

- In vitro fertilization (IVF)
- Intracytoplasmic sperm injection (ICSI)
- Fresh or frozen embryo transfer
- Gamete intrafallopian transfer (GIFT)

Outcomes: Perinatal outcomes

- Stillbirth
- Neonatal death
- Perinatal mortality
- Small for gestational age <10th centile
- Small for gestational age <5th centile
- Birth weight discordance >25%
- Twin-twin transfusion syndrome
- Any congenital malformation
- Major congenital malformations
- APGAR score <7 at 5 minutes
- Admission to the neonatal intensive care unit
- Respiratory distress syndrome (RDS)
- Mechanical ventilation
- Neonatal sepsis
- NEC
- Neurological complications
- Other offspring morbidity: IVH, Neonatal jaundice, Neonatal hypoglycaemia, HIE, Umbilical cord pH <7.2

Research design: Systematic review and meta-analysis

Results:

- The risk of any congenital malformation was higher among ART twins compared to non-ART twins (OR 1.18, 95% CI 1.06-1.31, I^2=61%) [36 studies, 281,515 neonates]. A significant difference was not observed between ART and naturally conceived twins.
- ART twins were at a higher risk of NICU admission when compared to non-ART twins (OR 1.25, 95% CI 1.12-1.41, I^2=88%) [30 studies, 100,186 neonates] and naturally conceived twins (OR 1.23, 95% CI 1.08-1.40, I^2=88%) [27 studies, 70,974 neonates].
- The risk of RDS was significantly increased among ART twins in comparison to non-ART (OR 1.35, 95% CI 1.07-1.70, I^2= 64%) [14 studies, 12,987 neonates] and naturally conceived twins (OR 1.32, 95% CI 1.03-1.69, I^2= 70%) [12 studies, 12,397 neonates].
- ART twins were at a higher risk of neurological complications compared to non-ART twins (OR 1.61, 95% CI 1.04-2.48, I^2=0%) [2 studies, 2,884 neonates] and those conceived naturally (OR 1.95, 95% CI 1.23-3.09, I^2=0%) [2 studies, 2,812 neonates].
- The risk of NEC was significantly higher among ART twins than non-ART twins (OR 1.78, 95% CI 1.06-3.01, I^2=0%) [6 studies, 6466 neonates] and naturally conceived twins (OR 1.79, 95% CI 1.06-3.05, I^2=0%) [6 studies, 6,298 neonates].
- Birthweight discordance >25% was significantly higher in the ART group compared to the non-ART (OR 1.31, 95% CI 1.05-1.63, I^2=0%) [7 studies, 7,882 neonates] and naturally conceived groups (OR 1.32, 95% CI 1.06-1.64, I^2=0%) [7 studies, 7,404 neonates].
- A reduced risk of stillbirth was seen for ART twins when compared to both non-ART twins (OR 0.83, 95% CI 0.70-0.99, I^2=49%) [33 studies, 205,360 neonates] and naturally conceived twins (OR of 0.78, 95% CI 0.65-0.95, I^2=29%) [25 studies, 125,329 neonates].
- The risk of SGA <10^{th} centile was also reduced among ART twins in comparison to non-ART (OR 0.91, 95% CI 0.87-0.96, I^2=21%) [25 studies, 116,399 neonates] and naturally conceived ones (OR 0.91, 95% CI 0.87-0.95, I^2=7%) [24 studies, 110,598 neonates].
- The risk of TTTS was lower in the ART group compared to both non-ART twins (OR 0.45, 95% CI 0.25-0.82, I^2=25%) [9 studies, 5,387 pregnancies] and naturally conceived twins (OR 0.35, 95% CI 0.14-0.87, I^2=28%) [7 studies, 3,839 pregnancies].
- There was no significant difference between ART twins and non-ART twins or between the ART and natural conception groups regarding neonatal death, perinatal mortality, SGA <5^{th} centile, major congenital malformations, neonatal sepsis, APGAR <7 at 5 minutes and mechanical ventilation.
- The risk of HIE was less in the ART group compared to non-ART twins (OR 0.43, 95%CI 0.20-0.92, I^2=NA) [1 study, 1,192 neonates].
- IVH, neonatal jaundice, neonatal hypoglycaemia and umbilical cord pH <7.2 were not significantly different between ART and non-ART or ART and natural conception groups.
- In the subgroup analysis, a significant difference was only observed for SGA <5^{th} centile between studies published before 2010 and after 2010 where the said outcome was higher among ART twins before 2010 compared to non-ART twins.
- When comparing ART and non-ART twins for fresh vs frozen embryo transfer cycles, none of the outcomes showed a significant difference.
- When monochorionicity was excluded, ART twins were at a higher risk for birthweight discordance >25% (OR 1.38, 95% CI 1.03-1.84, I^2=0%) [4 studies, 2,306 neonates], NICU admission (OR 1.37, 95% CI 1.01-1.84, I^2=93%) [14 studies, 23,224 neonates], RDS (OR 1.53, 95% CI 1.27-1.83, I^2=0%) [7 studies, 5,440 neonates] and neonatal jaundice (OR 1.22, 95% CI 1.12-1.34, I^2=0) [5 studies, 23,046 neonates] than non-ART twins.

- Monochorionicity excluded, when compared with naturally conceived twins, ART twins were at a higher risk of birthweight discordance >25% (OR 1.38, 95% CI 1.03-1.84, I^2=0%) [4 studies, 2,306 neonates], NICU admission (OR 1.36, 95% CI 1.01-1.84, I^2=93%) [14 studies, 21,166 neonates] and RDS (OR 1.57, 95% CI 1.31-1.90, I^2=0%) [7 studies, 5,344 neonates].

8.1.1: Maternal clinical predictors of preterm birth in twin pregnancies

The increased number of twins worldwide has contributed to a rise in prematurity, which is the leading cause of perinatal morbidity and mortality. Various prediction models incorporating maternal clinical characteristics to predict preterm birth have been used for singletons, but such data has been limited for twin pregnancies. I conducted the first systematic review evaluating the relationship between various maternal clinical characteristics and preterm birth in twin pregnancies.

Through this systematic review on maternal clinical predictors, I was able to demonstrate that age <20 years, BMI >35 kg/m^2, nulliparity, a history of preterm delivery and diabetes in pregnancy placed women with twin pregnancies at an increased risk of both early and late preterm birth compared to those who do not have these risk factors. Additionally, maternal smoking and being black or non-white in race imposed a higher risk for early preterm birth in twins. Hypertensive disorders in pregnancy increased the risk of late preterm birth. Out of all maternal clinical characteristics assessed, the presence of a previous preterm delivery was identified as the strongest predictor for early and late preterm birth, including spontaneous preterm birth.

8.1.2: Biochemical predictors of preterm birth in twin pregnancies

Although studies have been published on biochemical markers which predict preterm birth in twins, they have mainly focused on fetal fibronectin. I conducted a systematic review and meta-analysis on a more extensive list of biochemical predictors of preterm birth in twin pregnancies. I demonstrated that a positive fFN test is associated with an increased risk of preterm birth at gestations <28 weeks, <32 weeks, <34 weeks, <37 weeks and within 14 days of testing in women either asymptomatic or symptomatic for preterm labour. A positive fFN test indicated a higher risk of preterm delivery in women asymptomatic for preterm labour at <32 weeks, <34 weeks, and <37 weeks. Among symptomatic women, a positive fFN test was associated with a higher risk of premature delivery at <32 weeks, <34 weeks and within 14 days of testing. I also demonstrated that elevated maternal cervical phIGFBP-1 levels, mshCG levels, cervicovaginal IL-8 levels, and a maternal serum level of 25-hydroxy vitamin D <75 nmol/l were associated with an increased risk of preterm birth before 37 weeks. A positive amniotic fluid MMP-8 test was significantly associated with a higher risk of delivery within seven days of testing, and elevated maternal serum AFP was associated with a higher risk of birth before 34 weeks.

8.1.3: Association between chorionicity and preterm birth in twin pregnancies

Monochorionicity is associated with higher perinatal mortality rates and prematurity among twins. However, a systematic review has not been conducted to date to quantify the relationship between chorionicity and preterm birth in twin pregnancies.

In my systematic review and meta-analysis, I established that monochorionicity contributed to a significantly increased risk of preterm birth in twins at gestations ≤28 weeks, ≤32 weeks, ≤34 weeks and <37 weeks among women either asymptomatic or symptomatic for preterm labour. In

women asymptomatic for preterm labour, monochorionicity was associated with higher odds of preterm birth at <37 weeks and ≤34 weeks. The risk of spontaneous preterm birth was higher among monochorionic twin pregnancies at ≤34 weeks and <37 weeks. When cases of twin-twin transfusion syndrome were excluded from the analysis, monochorionicity was identified as a risk factor for any preterm birth at <28 weeks and ≤34 weeks, including spontaneous preterm birth at ≤34 weeks' gestation.

8.1.4: Maternal outcomes in twin pregnancies following assisted reproductive technology

The use of assisted reproductive technology has expanded worldwide, contributing to the global rise in twinning rates. Individual studies differ in the reported risks on maternal outcomes for twin pregnancies conceived by ART. An up-to-date and extensive evaluation was conducted to quantify women's risks with ART.

In my systematic review and meta-analysis, I was able to demonstrate that the risk of preterm birth <37 weeks, preterm birth <34 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes in pregnancy, antepartum haemorrhage, placenta praevia, placental abruption, postpartum haemorrhage, and caesarean delivery were significantly higher among ART twin pregnancies than non-ART twin pregnancies. The same outcomes were higher among the ART group when compared with naturally conceived twin pregnancies as well.

Subgroup analysis comparing studies published before 2010 with those published after 2010 showed that all evaluated outcomes continued to be more frequent among women conceiving by ART except for GDM, preterm birth <28 weeks, <34 weeks and <37 weeks despite dramatic advances in assisted reproductive technology. Spontaneous preterm birth at <34 weeks was also significantly higher among ART twins than non-ART twins. Excluding cases of monochorionic

twin pregnancies from the analysis, I was able to show that ART twin pregnancies were still at a higher risk of pre-eclampsia, preterm birth <37 weeks, GDM, hypertensive disorders in pregnancy, caesarean delivery, and placenta praevia in comparison to both non-ART and naturally conceived twin pregnancies.

8.1.5: Perinatal outcomes in twin pregnancies following assisted reproductive technology

Similar to maternal outcomes, the risk of perinatal outcomes in twin pregnancies conceived following ART has been reported inconsistently. I conducted an up-to-date systematic review with an extensive list of perinatal outcomes to quantify the risks faced by ART twins.

This systematic review demonstrated that the risk of any congenital malformation, NICU admission, neonatal RDS, neurological complications, birthweight discordance >25% and NEC is significantly higher among ART twins than non-ART twins. The same outcomes, except for any congenital malformation, were higher in ART twins when compared to naturally conceived twins. The risk of stillbirth, TTTS and SGA <10th centile was lower among the ART group when compared to both non-ART and naturally conceived twins. Subgroup analysis comparing studies published after 2010 with those published before 2010 showed that except for SGA <5th centile, which was significantly higher before 2010 among ART twins than non-ART twins, there were no other subgroup differences. After excluding cases of monochorionicity, the risk of birthweight discordance >25%, NICU admission, RDS and neonatal jaundice were still higher among ART twins than non-ART twins. Except for neonatal jaundice, all the above outcomes were higher among ART twins than naturally conceived twins.

8.2: Strengths and limitations

This thesis's systematic reviews were carried out according to standard guidelines and a prospective protocol. PROSPERO registration was done for all the reviews at the outset. While two of my reviews were never conducted before, the others included a study population more extensive than those in the previous reviews on the same topic. Multiple databases were searched extensively without language restrictions to find relevant citations, and reference lists of selected studies were manually screened to find more relevant studies. Two independent reviewers always did study selection, extraction of data and assessment of the quality to improve the accuracy of the data. Meta-analyses were performed to quantify the associations being studied. Heterogeneity was assessed using I^2 statistics and meta-regression to minimise the effect of confounders. Small study effects were also evaluated to improve the accuracy of the findings. Sensitivity and subgroup analyses were performed where relevant for improved interpretation of the data by re-analysing data under different assumptions and according to clinically relevant subgroups.

Data heterogeneity was a significant limitation as the definitions and cut-offs for many outcomes differ across different studies. The primary authors' definitions and thresholds were accepted, along with any method they used to determine the gestational age, but this invariably led to heterogeneity across studies. The difference in the inclusion and exclusion criteria between studies likely contributed to the heterogeneity. Whenever possible, meta-regression analysis was carried out to lessen the effects of diversified data. The paucity of data on specific outcomes might have lessened the accuracy of the findings. Many individual studies were of retrospective observational nature. In most reviews, the study population, albeit large, was mostly limited to the high-income or upper-middle-income countries, reducing the applicability of the findings to the poorer regions of the world.

8.3: Implications for clinical practice, research, and policy

Attempts to predict preterm birth in twin pregnancies have been limited to assessing cervical length and fetal fibronectin levels in cervicovaginal fluid. However, they are not universally accepted and useful predictors. For instance, cervical length assessment is of poor predictive value in women symptomatic for preterm labour, [197] while fetal fibronectin has shown better negative predictive value than positive predictive value. [83] Also, NICE guidelines advise against using fetal fibronectin alone to predict the risk of spontaneous preterm birth in twin pregnancies. [105]

All obstetric units are not equipped financially and logistically to test every twin pregnancy with expensive markers. However, clinical predictors of preterm birth in twin pregnancies are at the clinician's disposal. Based on the findings of this thesis, identification of high-risk twin pregnancies by way of clinical predictors, using history and examination alone, will be especially useful in resource-limited settings. Although biochemical markers such as fetal fibronectin, phIGFBP-1, IL-8 and MMP-8 were associated with a higher risk of preterm birth in twins, they are expensive to test and not universally available. Therefore, future research should focus on establishing biochemical predictors which are both reliable and less costly.

A standard scoring tool including maternal clinical characteristics and less costly biochemical markers can be constructed and disseminated for a more accurate prediction of preterm birth in twin gestations so that appropriate and timely intervention can be undertaken to minimise neonatal morbidity and mortality.

NICE guidelines recommend uncomplicated monochorionic diamniotic twin pregnancies to be delivered from 36+0 weeks. [105] While these recommendations may have contributed to the

observed increase in risk for preterm birth among monochorionic twins at <37 weeks, my review demonstrated that the risk of preterm birth was increased among monochorionic twins at all gestations studied while spontaneous preterm birth was significantly higher at ≤34 weeks and <37 weeks. This is the first systematic review to establish the association between preterm birth and monochorionicity. The information from this review should be used to counsel women carrying a monochorionic twin pregnancy regarding their risks of preterm birth, even when the pregnancy is apparently uncomplicated.

I quantified the higher risk of adverse maternal and offspring outcomes in twin pregnancies conceived following ART. Although this association has been shown in previously published reviews, it was consolidated using the findings of the extensive and up to date systematic review in this thesis. Guidelines on managing twin pregnancies do not recognise ART twin pregnancies as a group that needs special attention. Guidelines should therefore be updated to emphasise the increased maternal and offspring risks associated with ART and provide recommendations on counselling ART twin mothers, closer surveillance, and timely intervention to minimise the morbidity both to mothers and their offspring.

APPENDICES

APPENDIX 1: My role in the thesis

Chapter 3

I was involved in the conception of the research question and designing the protocol. Dr R. Dandeniya and I performed the literature search, study selection, and data extraction. I undertook the data analysis with the assistance from Dr J. Aquilina, Prof. A. Khalil, and Prof S Thangaratinam. I designed the figures, tables, and appendices and drafted the original manuscript.

Chapter 4

I was involved in the conception of the research question and designing the protocol. Dr C. Dias and I performed the literature search, study selection, and data extraction. I undertook the data analysis with the help from J. Allotey, Prof A. Khalil, and Prof S Thangaratinam. I designed the figures, tables, and appendices and drafted of the original manuscript.

Chapter 5

I was involved in the conception of the research question and designing the protocol. Dr C. Dias and I performed the literature search, study selection, and data extraction. I undertook the data analysis with the assistance from J. Allotey, and Prof S Thangaratinam. I designed the figures, tables, and appendices and drafted the original manuscript.

Chapter 6

I was involved in the conception of the research question and designing the protocol. Dr R. Nandasena and I performed the literature search, study selection, and data extraction. I undertook the data analysis with the assistance from Prof Javier Xamora and Dr J. Allotey. I designed the figures, tables, and appendices and wrote the first draft of the manuscript.

Chapter 7

I was involved in the conception of the research question and designing the protocol. Dr W. Kodithuwakku and I performed the literature search, study selection, and data extraction. I undertook the data analysis with the assistance from Prof Javier Xamora and Dr J. Allotey. I designed the figures, tables, and appendices and drafted the original manuscript.

APPENDIX 2: Search strategy used in the systematic review of maternal clinical predictors of preterm birth in twin pregnancies.

1. Twin*.mp. Or exp Twins
2. "multiple pregnan$".mp.
3. Multiple pregnancy.mp. Or exp Pregnancy, Multiple
4. 1 or 2 or 3
5. Exp Obstetric Labor, Premature/ or exp Premature Birth
6. Premat*.mp.
7. Preterm*.mp.
8. 5 or 6 or 7
9. 4 and 8
10. Fibronectin.mp. Or exp Fibronectins
11. Estriol.mp. Or exp Estriol
12. Oestriol.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
13. 11 or 12
14. Home uterine activity monitor$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
15. HUAM.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
16. Uterine contractions.mp. Or exp Uterine Contraction
17. 14 or 15 or 16
18. Interleukin$.mp. Or exp Interleukins
19. Cervical funnel*.mp.
20. Exp Uterine Cervical Incompetence/ or exp Cervical Length Measurement/ or cervical length.mp.
21. Exp Fetal Movement/ or fetal breathing movement.mp.
22. Fetal breath*.mp.
23. Fetal breath*.mp.
24. 21 or 22 or 23
25. Cervical assessment.mp.
26. Bishop score.mp.
27. Cervi* score.mp.
28. Vaginal exam*.mp.
29. Cervical digital exam*.mp.
30. 25 or 26 or 27 or 28 or 29
31. Serum biomarker.mp. Or exp Biological Markers
32. HCG.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
33. Insulin-like growth factor.mp. Or exp Somatomedins

34. Exp alpha-Fetoproteins/ or AFP.mp.
35. Exp C-Reactive Protein/ or CRP.mp.
36. Exp Corticotropin-Releasing Hormone/ or CRH.mp.
37. Exp Insulin-Like Growth Factor Binding Protein 1/ or exp Insulin-Like Growth Factor I/ or IGFPB.mp. Or exp Insulin-Like Growth Factor Binding Proteins
38. MMP.mp. Or exp Matrix Metalloproteinases
39. Relaxin.mp. Or exp Relaxin
40. Periodontal screen*.mp. Or exp Periodontitis
41. Exp Urinary Tract Infections/ or UTI.mp. Or exp Bacteriuria
42. Asymptomatic bacteriuria.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
43. MSU culture.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
44. Mid-stream urine.mp.
45. 41 or 42 or 43 or 44
46. Exp Premature Birth/ or exp Obstetric Labor, Premature
47. Exp Medical History Taking/ or patient history.mp.
48. Medical history.mp.
49. Past history.mp.
50. Previous history.mp.
51. 47 or 48 or 49 or 50
52. 46 and 51
53. Exp Abdomen/ and exp Palpation
54. Abdominal palpation.mp.
55. 53 or 54
56. Prolactin.mp. Or exp Prolactin
57. Rheobase.mp.
58. Mammary stimulation test.mp.
59. Mammary stim*.mp.
60. 58 or 59
61. Cervicovaginal glycoproteins.mp.
62. Glycoprotein.mp. Or exp Glycoproteins
63. Exp Hormones/ or endocrine hormone.mp.
64. Inflammatory markers.mp.
65. Bacterial vaginosis.mp. Or exp Vaginosis, Bacterial
66. 10 or 13 or 17 or 18 or 19 or 20 or 24 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 45 or 52 or 55 or 56 or 57 or 60 or 61 or 62 or 63 or 64 or 65
67. Exp Twins, Dizygotic/ or exp Twins, Monozygotic/ or chorionicity.mp.
68. Exp Ovulation Induction/ or exp Fertilization in Vitro/ or IVF.mp.
69. Method of conception.mp. Or exp Insemination, Artificial
70. Amniotic fluid.mp. Or exp Amniotic Fluid
71. Oocyte donation.mp. Or exp Oocyte Donation
72. PAPP-A.mp. Or exp Pregnancy-Associated Plasma Protein-A
73. Interpregnancy interval.mp.

74. Exp Birth Intervals/ or inter-pregnancy interval.mp.
75. 73 or 74
76. Vaginal bleed*.mp.
77. Antepartum hemorrhage.mp.
78. 76 or 77
79. Exp Crown-Rump Length/ or crown rump length discordance.mp.
80. CRL discordance.mp.
81. 79 or 80
82. Exp Nuchal Translucency Measurement/ or nuchal translucency discordance.mp.
83. VEGF.mp. Or exp Vascular Endothelial Growth Factor A
84. Fundal length.mp.
85. Fundal height.mp.
86. Exp Anthropometry
87. 84 or 85 or 86
88. Endotoxins.mp. Or exp Endotoxins
89. Exp Hispanic Americans/ or exp European Continental Ancestry Group/ or exp African Americans/ or maternal race.mp. Or exp African Continental Ancestry Group
90. Paternal race.mp.
91. Race.mp. Or exp Continental Population Groups
92. 89 or 90 or 91
93. Exp Socioeconomic Factors
94. Exp Educational Status/ or maternal education.mp.
95. Maternal status.mp.
96. Exp Body Mass Index/ or BMI.mp.
97. Smoking.mp. Or exp Smoking
98. Parity.mp. Or exp Parity
99. Fetal sex.mp.
100. Fetal sex.mp.
101. 99 or 100
102. Exp Progesterone/ or progesterone.mp.
103. Assisted reproductive technology.mp. Or exp Reproductive Techniques, Assisted
104. Marital status.mp. Or exp Marital Status
105. Prenatal care.mp. Or exp Prenatal Care
106. Exp Leptin/ or leptins.mp.
107. Exp Fetal Growth Retardation/ or discordant growth.mp.
108. Growth discordance.mp.
109. 67 or 68 or 69 or 70 or 71 or 72 or 75 or 78 or 81 or 82 or 83 or 87 or 88 or 92 or 93 or 94 or 95 or 96 or 97 or 98 or 101 or 102 or 103 or 104 or 105 or 106 or 107 or 108
110. 66 or 109
111. 9 and 110
112. 111 and 1990:2017. (sa_year).

APPENDIX 3: Study characteristics in the systematic review of maternal clinical predictors of preterm birth in twin pregnancies.

No	Study, Year Country	Study design	Number of women	Inclusion period (Months)	Inclusion criteria	Exclusion criteria	Maternal clinical predictors	Outcome (preterm delivery)
1	**Ananth, 2008** USA	Retrospective cohort	2329	96	Women whose first two successive pregnancies consisted of a singleton live birth followed by a gestation resulting in a live twin birth from Missouri maternal linked files	Pregnancies that ended as a stillbirth, missing gestational age, gestational age < 20 completed weeks	Age <20 years Age ≥35 years BMI <19.8 BMI ≥ 30 BMI ≥ 35 Black race Previous preterm delivery Smoking	<37 weeks
2	**Bergelin, 2003** Sweden	Prospective cohort	20	Not known	Healthy, Swedish speaking, twin pregnancies ≥ 18 years of age, recruited in the first half of the pregnancy	Pregnancy complications	Nulliparity	<36 weeks
3	**Berkovitz, 2010** Israel	Prospective cohort	243	48	All twin pregnancies including multiple order pregnancies reduced to twins in a fertility center conceived by ART and ovulation induction after completion of the first trimester	Egg donation pregnancies	Nulliparity	<32 weeks
4	**Blackwell, 2012** USA	Cohort nested in RCT	640	Not known	Not known	Maternal diabetes, hypertension, asthma, medical conditions requiring medications	BMI ≥ 30	<32 weeks <35 weeks

5	**Branum, 2005** USA	Retrospective cohort	175658	48	Matched twin records of 24- 48 weeks gestation for women ≥ 20 years, with complete gestational age and parity information, pregnancies with two live births	Any twin pregnancy that had different gestational ages reported, twin births to women <20 years old, implausible birth weight and gestational age combinations	Nulliparity Age ≥35 years Age ≥40 years	<33 weeks
6	**Branum, 2006** USA	Retrospective cohort	72283	60	Matched multiple birth data, Twin live births	Records with implausible gestational age-birth weight combinations, records with missing birth weight or gestational age information, any twin pregnancy that had different gestational ages, women >24 years	Black Age ≤ 18 years Age ≤16 years	<33 weeks
7	**Cooperstock, 1998** USA	Retrospective cohort	10015	144	All twin deliveries from Missouri successive Pregnancy Birth Data Set which provided maternally linked birth certificate data	Fetal death in one or both twins or missing infant gender or gestation weeks	Age < 20 years	≤33 weeks <37 weeks
8	**Delbaere, 2008** Belgium	Retrospective cohort	1180	48	All nulliparous women ≥35 years and nulliparous women 25-29 years from a regional population-based database	Twins with birth weight < 500g	Age ≥ 35 years	<32 weeks <37 weeks

9	**Dickey, 2012** USA	Retrospective cohort	10225	24	All twin births following fresh non-donor oocytes transfer from the SART CROS dataset	Stillbirths, gestational age < 20 weeks or >44 weeks, >2 heart rates initially, height <107 cm or >213 cm, weight <27 kg or ≥150 kg, BMI <12 kg/m2 or ≥ 70 kg/m2	BMI ≥ 30 BMI ≥ 35	<28 weeks <32 weeks <37 weeks
10	**Dickey, 2013** USA	Retrospective cohort	19549	24	All twin births following fresh non-donor oocytes transfer from the SART CROS dataset	Stillbirths, gestational age < 20 weeks or >44 weeks, >2 heart rates initially, height <107 cm or >213 cm, weight <27 kg or ≥150 kg, BMI <12 kg/m2 or ≥ 70 kg/m2	BMI ≥ 30 BMI ≥ 35	<28 weeks <32 weeks <37 weeks
11	**Do, 2016** USA	Prospective Cohort	1280	Not known	All twin pregnancies assigned to 17 OHP STTARS	Fetal anomalies	BMI ≥ 30 BMI ≥ 35	<37 weeks
12	**Easters, 2016** USA	Cohort nested in RCT	342	60	All mothers included in MFM randomised placebo-controlled trial of 17 alfa-hydroxyprogesterone caproate in twins which enrolled healthy women with twins at 16 & 20 weeks	Selective fetal reduction of a twin, termination of pregnancy	Previous preterm delivery	<28 weeks <34 weeks <37 weeks

13	**Erez, 2008** Israel	Retrospective Cohort	2601	168	All twin deliveries after 24 weeks gestation	Patients who lacked minimal prenatal care (<3 visits), pregnancies complicated by congenital anomalies	Nulliparity	≤34 weeks ≤37 weeks
14	**Fichera, 2014** Italy	Prospective Cohort	197	24	Consecutive twin pregnancies	Indicated preterm delivery <34 weeks for maternal or fetal indications, cervical cerclage, Arabin cervical pessary insertions	Nulliparity Previous preterm delivery	<34 weeks
15	**Francesca, 2008** USA	Retrospective Cohort	293	120	All patients who delivered a singleton followed by a twin pregnancy of greater than 20 weeks of gestation	Iatrogenic preterm delivery, prophylactic cerclage, fetus with a major anomaly or an intrauterine death	Previous preterm delivery	<37 weeks
16	**Fox, 2010** USA	Retrospective Cohort	297	48	Patients with a recorded pre-pregnancy weight, maternal height and maternal weight measurements during pregnancy	Patients who were transferred to the practice after 20 weeks, monoamniotic twins, pregnancies with major fetal anomalies	BMI ≥ 30	<28 weeks <32 weeks <35 weeks <37 weeks
17	**Fox, 2015** USA	Retrospective Cohort	647	108	All twin pregnancies delivered by single maternal-fetal medicine practice	Twin-twin transfusion syndrome, major fetal anomalies, monochorionic monoamniotic	Nulliparity Previous singleton preterm delivery	<28 weeks <34 weeks <37 weeks

						placentation, the most recent previous twin pregnancy		
18	**Goldenberg, 1996** USA	Prospective Cohort	147	22	All twin pregnancies available for study visits at 22 to 24 weeks gestation	Cervical cerclage, placenta previa, severe fetal anomaly	Age <18 years BMI <19.8 Race Smoking Previous spontaneous preterm delivery	<32 weeks <35 weeks <37 weeks
19	**Haghighi, 2013** Iran	Prospective Cohort	678	15	All women with a twin pregnancy without any contraindications to continuation of pregnancy, presenting to the prenatal clinic/ward	Deliveries < 26 weeks, intrauterine fetal death, previous history of preterm birth, presence of clinical infection, medical or fetal indications for delivery (pre-eclampsia, non-reassuring fetal status, intrauterine growth restriction)	Nulliparity	<28 weeks <34 weeks <37 weeks
20	**Hannoun, 2011** USA	Retrospective Cohort	841	168	All twin gestations who delivered beyond 24 weeks	Higher order multiples, congenital malformations incompatible with life in either or both twins	Nulliparity	<34 weeks <37 weeks

21	**Hediger, 2005** USA	Retrospective Cohort	1612	276	Delivery ≥ 28 weeks, both twins born alive, at least two ultrasound evaluations of fetal growth for estimation of growth rates from 20-28 weeks and /or 28 weeks to delivery	Delivery < 28 weeks, missing ultrasound evaluations of growth	Race Nulliparity Smoking Gestational Diabetes Pre-eclampsia /gestational hypertension	<31 weeks <33 weeks <37 weeks
22	**Hong, 1996** USA	Retrospective Cohort	267	81	Twin pregnancies with at least one maternal serum alpha-fetoprotein (MSAFP) test	Multifetal pregnancy reductions, neural tube defects, abdominal wall defects, low MSAFP levels, missing MSAFP results, chronic maternal conditions, death of at least one fetus	Nulliparity Previous preterm delivery	<34 weeks
23	**Houlton, 1982** South Africa	Prospective Cohort	129	13	Twin pregnancies in black African women assessed at least on three occasions at weekly intervals either at the clinic or after admission to hospital, < 36 weeks gestation by dates	Twin pregnancies greater than 36 weeks when first seen	Age < 20 years, Nulliparity	<37 weeks
24	**Imseis, 1997** USA	Retrospective Cohort	85	87	Twin Gestations	Indicated preterm deliveries, cervical cerclage before 24 weeks	Race Nulliparity Smoking Previous spontaneous preterm delivery	<34 weeks

25	**James, 2009** USA	Retrospective Cohort	316983	60	All twin birth data from 1995-2000 from public access matched multiple birth file produced by National Centre for Health Statistics	Chromosomal or congenital anomalies, gestational age at birth not available, births <20 weeks gestation	Nulliparity	<32 weeks <36 weeks
26	**Joon Oh, 2012** Korea	Prospective Cohort	190	78	Women with a viable twin gestation, CL >25 mm at 20-24 weeks, no history of prophylactic cerclage, intact amniotic membranes, absence of regular uterine contractions, no evidence of major fetal anomalies or suspected twin-to-twin transfusion syndrome or evidence of monoamniotic placenta or placenta previa, well documented obstetric dates	Spontaneous preterm delivery before follow-up measurement, loss to follow up, one fetal demise, incomplete data set	Nulliparity Previous preterm delivery <37 weeks	<34 weeks
27	**Kalish, 2001** USA	Retrospective Cohort	289	12	Dichorionic twin pregnancies	Not known	Previous singleton preterm delivery	<37 weeks
28	**Laskov, 2013** Israel	Retrospective Cohort	141	108	Study - all women carrying a twin pregnancy who were ≥45 years at the time of delivery conceived by IVF with ovum donation, delivered ≥20 weeks Control - IVF pregnancies in women aged < 40 years	Triplets	Age ≥ 45 years	<32 weeks <37 weeks

29	**Lim, 2017** UK	Prospective Cohort	98	17	All twin mothers older than 16 years, chorionicity and gestation established on ultrasound before 16 weeks, recruited before 20 weeks of gestation, without known serious congenital abnormalities and intending to deliver at a single center	Positive for HIV, Hepatitis B or C, on progesterone therapy, gestational length of less than 26 weeks or fewer than 3 blood or saliva measurements	Nulliparity Smoking Gestational Hypertension	<34 weeks <37 weeks
30	**Lisonkova, 2011** Canada	Retrospective Cohort	1819	48	All twins born in British Columbia Canada, Mothers > 35 years & mothers 25-34 years old	Twins born at <20 weeks gestation	Maternal age >35 years	<28 weeks <33 weeks <37 weeks
31	**Luke, 2005** USA	Retrospective Cohort	3036	144	Both twins born alive, >28 weeks of gestation, documented screening glucose concentration between 24-28 weeks gestation, prenatal weight at each visit, documented genders and birth weights of both twins	Pregestational or gestational diabetes, major congenital anomalies	Race	<32 weeks <36 weeks
32	**Luo, 2011** USA	Retrospective Cohort	422068	36	All twin pregnancies	Births at extreme gestational ages (<20 weeks & >45 weeks), birth weights <500 g or >6000g, implausible with weight for gestational age based on the algorithm of Alexander et al,	Diabetes in pregnancy	<37 weeks

						missing values for plurality, infant sex, gestational age or birth weight, births with missing data for diabetes in pregnancy, congenital anomalies or neonatal survival, births to mothers with other major illnesses (chronic hypertension, heart disease, lung disease, genital herpes, renal disease, Rh sensitization)		
33	**Luo, 2014** USA	Retrospective Cohort	278821	60	Births in twin pregnancies with non-missing value for gestational hypertension, pregnancies without chronic hypertension, gestational age at delivery between 20-42 inclusive	Births with missing data on birth weight	Gestational hypertension	<37 weeks
34	**McPherson, 2013** USA	Retrospective Cohort	2106	216	All consecutive twin pregnancies at 17-22 weeks presenting for a routine sonographic anatomic survey, twin pregnancies with complete outcome information	Monoamniotic twins, TTTS, placenta previa, singletons, higher order multiples	Nulliparity	<34 weeks

35	**Michaluk, 2013** Canada	Retrospective Cohort	576	168	Women who had a singleton delivery immediately before their twin pregnancy	Having a twin or multiple birth in the immediate preceding delivery, iatrogenic preterm twin delivery for medical reasons (maternal or fetal) not encountered in a previous singleton pregnancy, TTTS, fetal chromosomal or structural anomalies, fetal death of one twin, transfer from another hospital with incomplete medical data	Race Smoking Chronic or gestational HT Insulin-dependent diabetes Previous preterm delivery	<37 weeks
36	**Oliveira, 1998** Brazil	Prospective Cohort	52	26	All twin pregnancies between 24 and 34 weeks of gestation	Preterm birth for medical reasons, use of tocolytics to inhibit preterm labour	Race Nulliparity	<37 weeks
37	**Pagani, 2016** Italy	Retrospective Cohort	940	151	Twin pregnancies with cervical length measurement done by TVS at 18-23 weeks	At least one fetus with structural or chromosomal abnormalities, higher order pregnancy, monoamniotic twins, pregnancy referred after 16 weeks, TTTS, those that required intrauterine therapy,	Nulliparity Smoking Previous preterm delivery	<32 weeks

						pregnancies with indicated preterm birth, follow-up data unavailable		
38	**Pinzauti, 2016** Italy	Retrospective Cohort	430	60	Nulliparity, Dichorionic diamniotic twin pregnancies, ART conception	Multiparty, monochorionic twins, spontaneous conception, pregnancies conceived by ovulation induction and intrauterine insemination	Age ≥40 years	<32 weeks <37 weeks
39	**Pollack, 2000** USA	Retrospective Cohort	76636	12	Individual-level 1995 data from the National Centre for Health Statistics Perinatal Mortality Data Set, birth records that included maternal smoking data	Records that lacked infants with matching sibling	Smoking	<33 weeks
40	**Rafael, 2012** USA	Retrospective Cohort	255	168	Women with a twin delivery who subsequently had a singleton delivery	Iatrogenic preterm birth at <37 weeks in either pregnancy, previous preterm birth preceding the twin delivery, intrauterine death of one or both twins, major fetal anomaly, unclear or incomplete data	Race Nulliparity	<37 weeks

41	**Rode, 2012** Denmark	Cohort nested in RCT	218	29	Women whom all had dried blood spot samples collected from capillary blood in the prevention of preterm delivery in twin gestations study	Women who never took project medicine, who did not want blood samples to be collected, samples collected from venous blood, blood samples unavailable	Nulliparity Smoking Previous preterm delivery	<34 weeks <37 weeks
42	**Rolett, 2000** USA	Retrospective Cohort	567796	72	All live births from 1990-1995 using US birth records	Missing gestational age	Race	<35 weeks
43	**Sauber-Schatz, 2012** USA	Retrospective Cohort	8746	24	Births that occurred in Florida during 2004-2006, recorded with the 2004 revised Florida birth certificate, live births resulting from ART	Missing pre-pregnancy BMI, higher order multiples	BMI ≥ 30 BMI <18.5	<34 weeks <37 weeks
44	**Shamshirsaz, 2014** USA	Retrospective Cohort	570	120	Twin gestations with two liveborn infants delivered at 23+6 weeks gestation that had a documented pre-pregnancy BMI along with maternal height and weight measurements during pregnancy	Major fetal anomalies, TTTS, Rh alloimmunization, monoamnionicity, pre-pregnancy BMI <18.5 Kg/m2	BMI ≥ 30	<32 weeks <37 weeks
45	**Shumpert, 2004** USA	Retrospective Cohort	80495	24	Study group - mothers ≤ 19 years with twin pregnancies, Control group - mothers with twin pregnancies between 20-29 years	Not known	Age <20 years Anaemia	<33 weeks <37 weeks

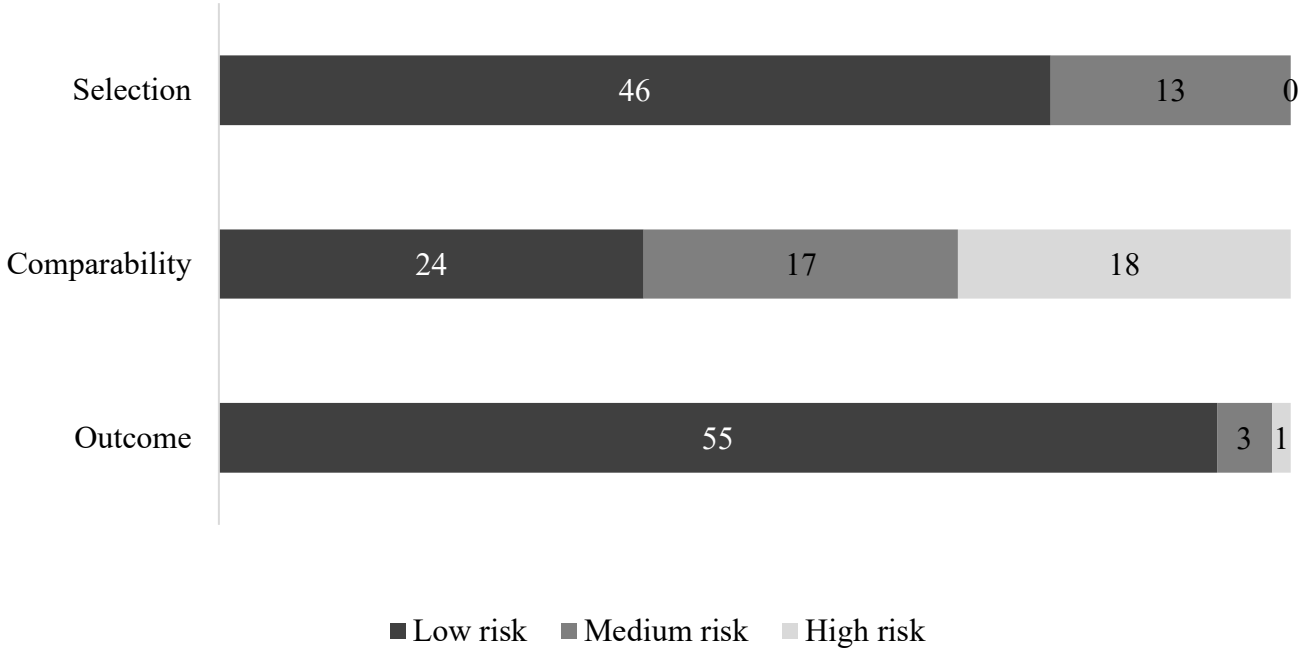
46	**Skentou, 2001** UK	Retrospective Cohort	434	NK	Women with twin pregnancies attending for the 23-week fetal anatomy and growth scan	Patients with no follow-up, iatrogenic delivery before 33 weeks, cervical suture placed for short cervix <2cm	Age ≥ 35 years Nulliparity Smoking Previous preterm delivery	<33 weeks
47	**Soriano, 2002** Israel	Prospective Cohort	44	12	Twin pregnancies, first pregnancy, no history of in utero Diethylstilbesterol (DES) exposure or previous cervical cone biopsy, conceived following infertility treatment, who had a routine second trimester (18-24 weeks) ultrasonographic anatomic survey	Uterine anomalies	Smoking	<34 weeks
48	**Suzuki, 2007** Japan	Retrospective Cohort	131	48	Dichorionic twin pregnancies	Not known	Age ≥ 35 years	<32 weeks <37 weeks
49	**Suzuki, 2010** Japan	Case-control	269	96	Cases - women with dichorionic twin pregnancies who delivered <32 weeks Controls - dichorionic twin pregnancies delivered at 37-40 weeks	Not known	Age ≥ 35 years BMI ≥30 Nulliparity Smoking Hypertension Diabetes mellitus	<32 weeks
50	**Tarter, 2002** USA	Retrospective Cohort	1268	59	Patients enrolled in outpatient preterm labour surveillance program at < 24 weeks, documented pregnancy outcome	Not known	Nulliparity Previous preterm delivery	<35 weeks

51	**To, 2006** UK	Prospective Cohort	1135	72	Women with twin pregnancies, two live fetuses, attending for a 2nd-trimester scan, consenting for TVS for cervical length	Major fetal anomalies, painful regular uterine contractions, history of ruptured membranes, cervical cerclage in situ, severe TTTS, treated with cervical cerclage or progesterone pessary	Nulliparity Previous preterm delivery	<34 weeks
52	**Tudela, 2016** USA	Retrospective Cohort	666	108	All patients with twin pregnancies ≥22 weeks delivered by a single maternal-fetal medicine practice	Monochorionic, Monoamniotic pregnancies, fetuses with major anomalies discovered before or after birth, TTTS	Nulliparity	<32 weeks <37 weeks
53	**Tward, 2016** Canada	Retrospective Cohort	1393	134	All twin pregnancies who underwent screening for Gestational diabetes mellitus (GDM) in a single tertiary referral center	Pre GDM, incomplete or nonstandard screening for GDM, delivery < 28 weeks, complicated MCDA twins, MCMA twins, stillbirth, reduction of ≥1fetuses, genetic or structural fetal anomalies	Gestational diabetes mellitus	<34 weeks
54	**Vintzileos, 2003** USA	Retrospective Cohort	779387	132	Twin live births that occurred at ≥ 20 completed weeks of gestation	Congenital or chromosomal anomalies, missing data on gestational	Race	<32 weeks <37 weeks

						age after imputations, gestational age < 20 weeks, BW < 500g, missing data regarding presence or absence of prenatal care or maternal race, nonwhite and nonblack women		
55	**Wennerholm, 1998** Sweden	Prospective Cohort	101	18	Twin pregnancies recruited before 20 weeks gestation	Induced preterm deliveries indicated for maternal or fetal conditions	Nulliparity Smoking Prior preterm delivery	<37 weeks
56	**Wisborg, 2001** Denmark	Prospective Cohort	401	77	All Danish speaking pregnant women	Not known	Nulliparity Smoking Prior preterm delivery	<34 weeks <37 weeks
57	**Xiong, 2013** USA	Retrospective Cohort	40961	48	Twin pregnancies resulting from fresh non-donor IVF cycles between 20-44 completed weeks	Triplets, quadruplets, women with mixed or multiple race ethnicity	Race	<32 weeks <37 weeks
58	**Xiong, 2015** USA	Retrospective Cohort	40961	60	All births resulting from fresh non-donor embryo transfers	Triplets, quadruplets	Age ≥ 35 years Age ≥ 40 years	<32 weeks <37 weeks

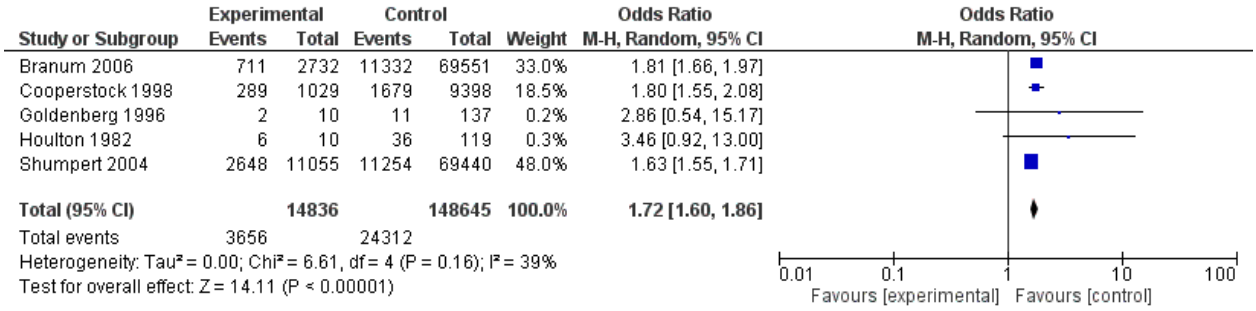
59	**Yang, 2000** USA	Prospective Cohort	65	36	All twin pregnancies	Preterm labour, prophylactic cervical cerclage, placenta praevia or bleeding, induced preterm delivery unrelated to preterm labour or preterm rupture of membranes, cervical sonography not performed before 26 weeks, lost to follow-up	Race Parity Smoking Prior preterm delivery	<32 weeks <37 weeks

APPENDIX 4: Quality assessment using the Newcastle Ottawa Scale in the systemic review of maternal clinical predictors of preterm birth in twin pregnancies.

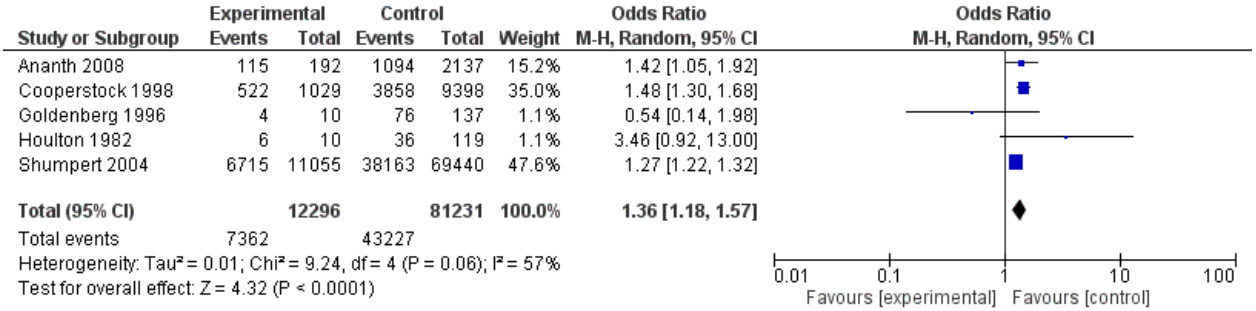


APPENDIX 5: Forest plots of pooled odds ratios (OR) for early (<34 weeks) and late (<37 weeks) preterm birth (PTB) in the systemic review of association between maternal clinical predictors and preterm birth in twin pregnancies.

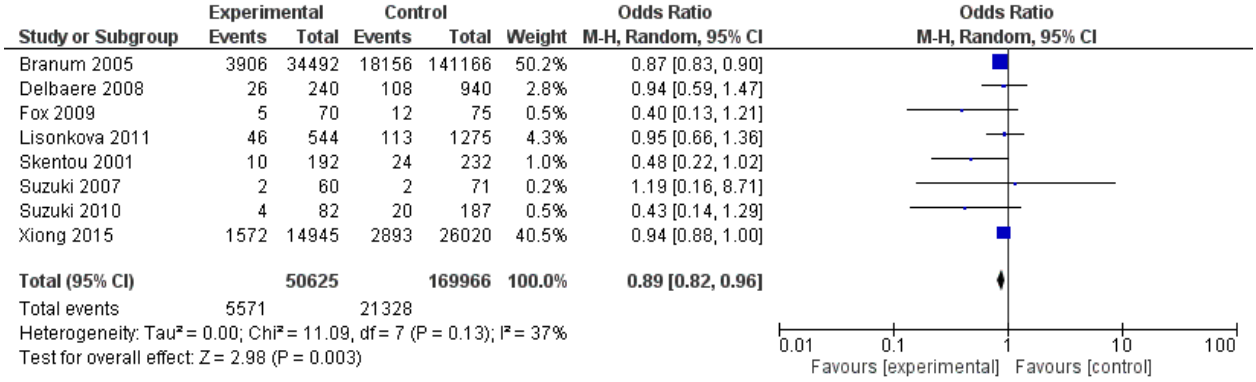
1. Age
    a. <20 years – PTB <34 weeks



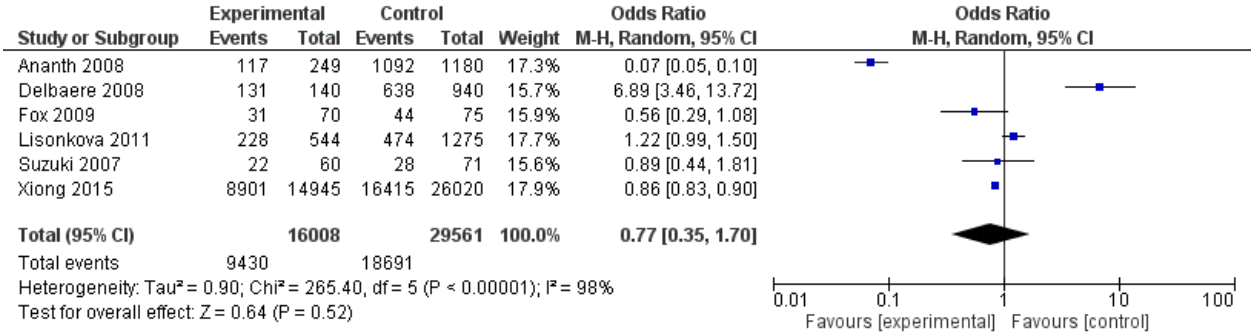
    b. <20 years – PTB <37 weeks



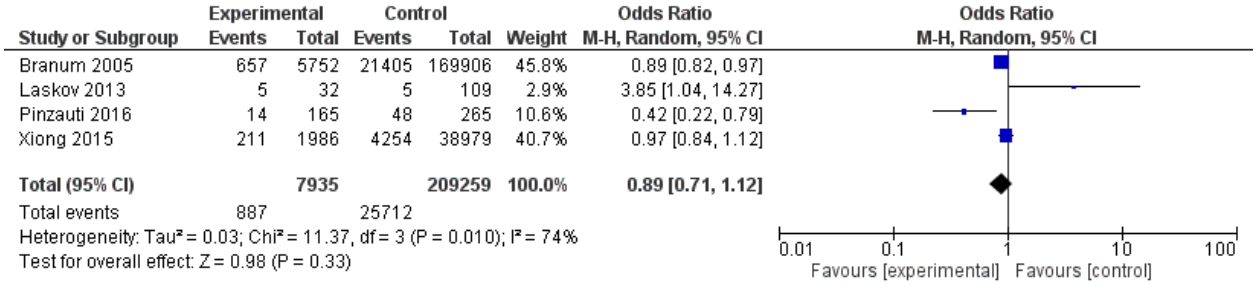
    c. >35 years – PTB <34 weeks



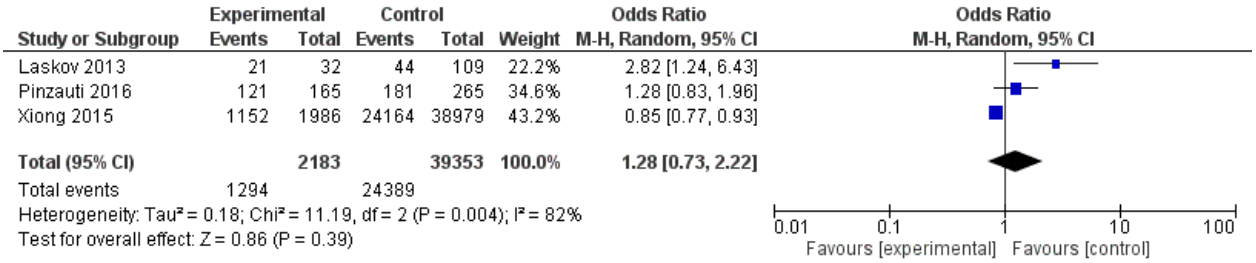
d. >35 years – PTB <37 weeks



e. >40 years – PTB <34 weeks

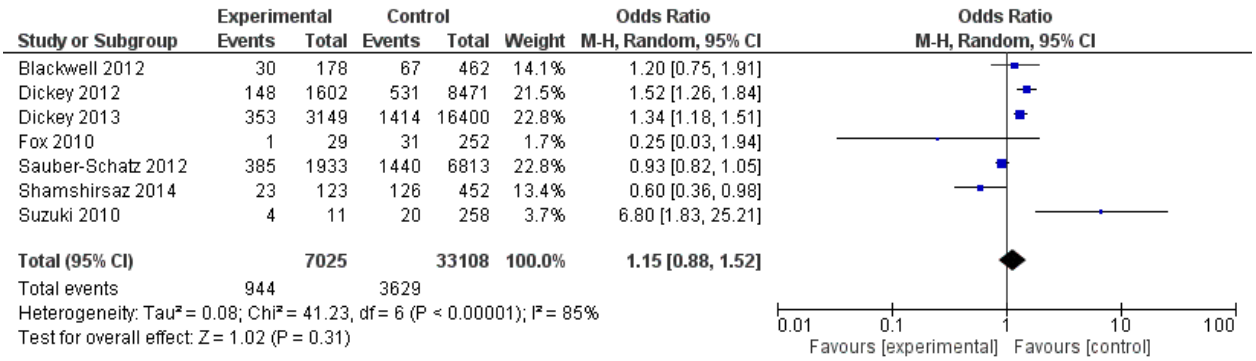


f. >40 years – PTB <37 weeks



2. BMI

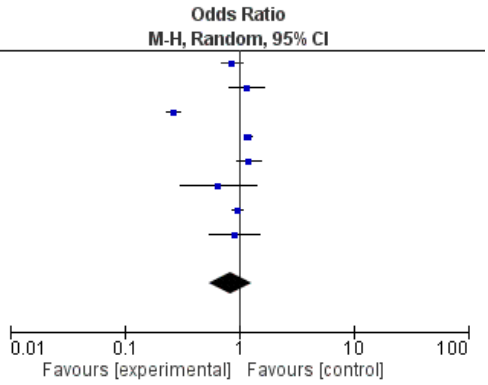
a. >30 kg/m^2 – PTB <34 weeks



b. >30 kg/m^2 – PTB <37 weeks

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ananth 2008	198	403	1011	1926	13.2%	0.87 [0.71, 1.08]
Blackwell 2012	74	178	175	462	12.5%	1.17 [0.82, 1.66]
Dickey 2012	244	1602	3392	8471	13.4%	0.27 [0.23, 0.31]
Dickey 2013	1998	3149	9729	16400	13.6%	1.19 [1.10, 1.29]
Do 2016	152	360	346	920	13.0%	1.21 [0.95, 1.55]
Fox 2010	14	29	148	252	9.4%	0.66 [0.30, 1.42]
Sauber-Schatz 2012	1227	1933	4381	6813	13.5%	0.96 [0.87, 1.07]
Shamshirsaz 2014	99	123	366	447	11.4%	0.91 [0.55, 1.52]
Total (95% CI)		**7777**		**35691**	**100.0%**	**0.83 [0.54, 1.28]**
Total events	4006		19548			

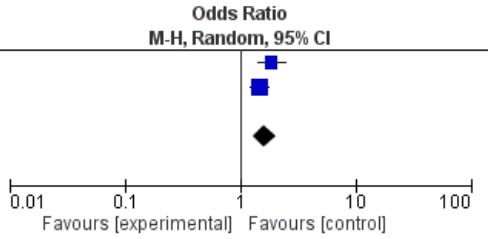
Heterogeneity: Tau² = 0.35; Chi² = 335.41, df = 7 (P < 0.00001); I² = 98%
Test for overall effect: Z = 0.83 (P = 0.41)



c. >35 kg/m^2 – PTB <34 weeks

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Dickey 2012	66	576	613	9497	40.6%	1.88 [1.43, 2.45]
Dickey 2013	151	1208	1616	18341	59.4%	1.48 [1.24, 1.77]
Total (95% CI)		**1784**		**27838**	**100.0%**	**1.63 [1.30, 2.05]**
Total events	217		2229			

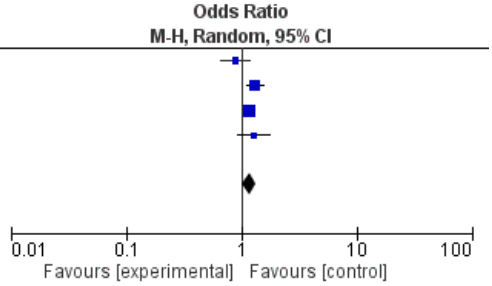
Heterogeneity: Tau² = 0.01; Chi² = 2.09, df = 1 (P = 0.15); I² = 52%
Test for overall effect: Z = 4.17 (P < 0.0001)



d. >35 kg/m^2 – PTB <37 weeks

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ananth 2008	94	191	1115	2138	15.9%	0.89 [0.66, 1.20]
Dickey 2012	244	576	3392	9497	30.6%	1.32 [1.12, 1.57]
Dickey 2013	766	1208	10961	18341	39.7%	1.17 [1.03, 1.32]
Do 2016	76	172	422	1110	13.9%	1.29 [0.93, 1.79]
Total (95% CI)		**2147**		**31086**	**100.0%**	**1.18 [1.02, 1.35]**
Total events	1180		15890			

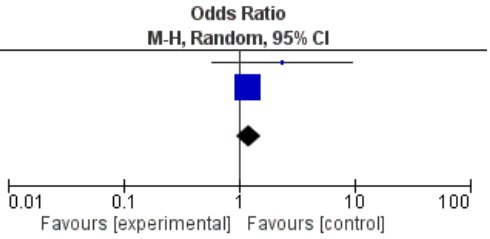
Heterogeneity: Tau² = 0.01; Chi² = 5.54, df = 3 (P = 0.14); I² = 46%
Test for overall effect: Z = 2.31 (P = 0.02)



e. <19.8 or 18.5 kg/m^2 – PTB <34 weeks

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Goldenberg 1996	3	18	10	129	3.2%	2.38 [0.59, 9.63]
Sauber-Schatz 2012	82	345	1743	8401	96.8%	1.19 [0.92, 1.53]
Total (95% CI)		**363**		**8530**	**100.0%**	**1.22 [0.95, 1.56]**
Total events	85		1753			

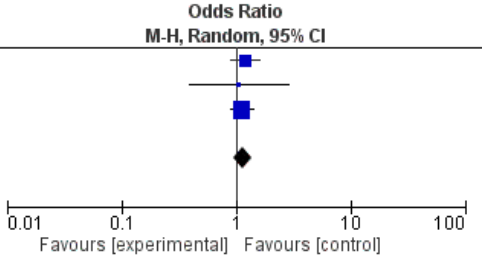
Heterogeneity: Tau² = 0.00; Chi² = 0.91, df = 1 (P = 0.34); I² = 0%
Test for overall effect: Z = 1.55 (P = 0.12)



f. <19.8 or 18.5 kg/m^2 – PTB <37 weeks

Study or Subgroup	Experimental Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Ananth 2008	105	187	1104	2142	35.3%	1.20 [0.89, 1.63]
Goldenberg 1996	10	18	70	129	3.3%	1.05 [0.39, 2.84]
Sauber-Schatz 2012	230	345	5378	8401	61.4%	1.12 [0.89, 1.41]
Total (95% CI)		**550**		**10672**	**100.0%**	**1.15 [0.96, 1.37]**
Total events	345		6552			

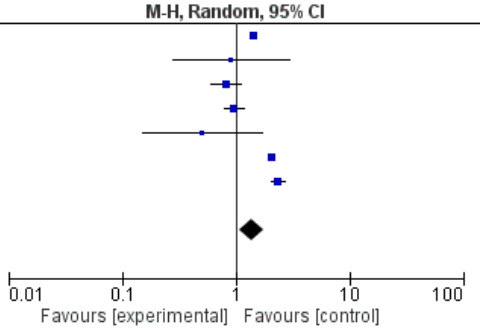
Heterogeneity: Tau² = 0.00; Chi² = 0.16, df = 2 (P = 0.92); I² = 0%
Test for overall effect: Z = 1.52 (P = 0.13)



3. Ethnicity
    a. Black – PTB <34 weeks

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Branum 2006	4378	21247	5607	36776	20.1%	1.44 [1.38, 1.51]
Goldenberg 1996	8	87	5	50	3.7%	0.91 [0.28, 2.95]
Hediger 2005	72	584	150	1028	15.6%	0.82 [0.61, 1.11]
Luke 2005	173	1215	268	1821	17.8%	0.96 [0.78, 1.18]
Skentou 2001	3	67	31	367	3.5%	0.51 [0.15, 1.71]
Vintzileos 2003	22441	82240	55750	367045	20.2%	2.10 [2.06, 2.13]
Xiong 2013	283	1316	2563	24729	19.1%	2.37 [2.06, 2.72]
Total (95% CI)		**106756**		**431816**	**100.0%**	**1.38 [1.07, 1.77]**
Total events	27358		64374			

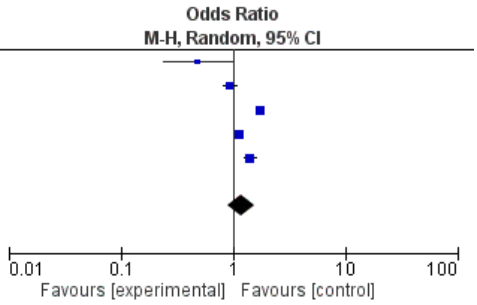
Heterogeneity: Tau² = 0.08; Chi² = 333.14, df = 6 (P < 0.00001); I² = 98%
Test for overall effect: Z = 2.52 (P = 0.01)



    b. Black – PTB <37 weeks

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Goldenberg 1996	47	97	33	50	9.3%	0.48 [0.24, 0.98]
Luke 2005	495	1215	770	1821	21.9%	0.94 [0.81, 1.09]
Rolett 2000	37757	107876	108734	459920	23.3%	1.74 [1.71, 1.76]
Vintzileos 2003	80728	140528	345564	638859	23.3%	1.15 [1.13, 1.16]
Xiong 2013	919	1316	15316	24729	22.3%	1.42 [1.26, 1.60]
Total (95% CI)		**251032**		**1125379**	**100.0%**	**1.17 [0.89, 1.55]**
Total events	119946		470417			

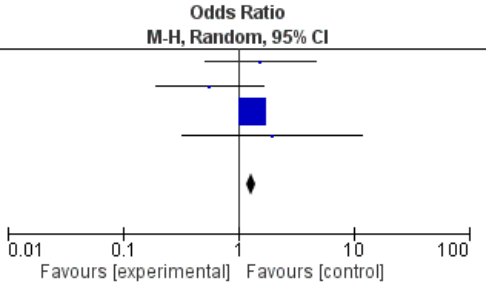
Heterogeneity: Tau² = 0.09; Chi² = 2009.19, df = 4 (P < 0.00001); I² = 100%
Test for overall effect: Z = 1.12 (P = 0.26)



    c. Non-white – PTB <34 weeks

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Imseis1997	7	28	10	57	0.6%	1.57 [0.52, 4.68]
Skentou 2001	4	80	30	354	0.7%	0.57 [0.19, 1.66]
Xiong 2013	759	5780	2087	20265	98.4%	1.32 [1.20, 1.44]
Yang 2000	2	15	4	55	0.2%	1.96 [0.32, 11.91]
Total (95% CI)		**5903**		**20731**	**100.0%**	**1.31 [1.20, 1.43]**
Total events	772		2131			

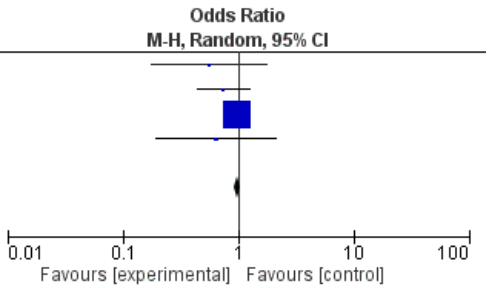
Heterogeneity: Tau² = 0.00; Chi² = 2.63, df = 3 (P = 0.45); I² = 0%
Test for overall effect: Z = 6.04 (P < 0.00001)



    d. Non-white – PTB <37 weeks

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Oliveira 1998	8	18	20	34	0.3%	0.56 [0.18, 1.77]
Rafael 2012	46	89	98	166	1.3%	0.74 [0.44, 1.25]
Xiong 2013	3590	5780	12645	20265	98.2%	0.99 [0.93, 1.05]
Yang 2000	5	15	24	55	0.2%	0.65 [0.19, 2.14]
Total (95% CI)		**5902**		**20520**	**100.0%**	**0.98 [0.92, 1.04]**
Total events	3649		12787			

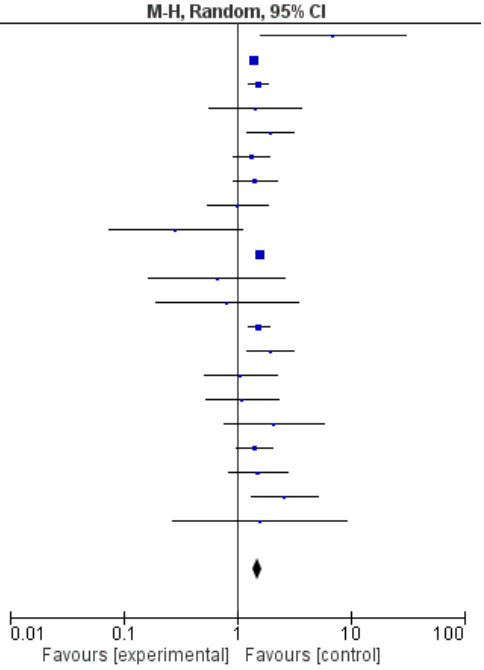
Heterogeneity: Tau² = 0.00; Chi² = 2.54, df = 3 (P = 0.47); I² = 0%
Test for overall effect: Z = 0.61 (P = 0.54)



4. Parity
    a. Nulliparity – PTB <34 weeks

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Berkovitz 2010	24	161	2	82	0.4%	7.01 [1.61, 30.43]
Branum 2005	9917	66021	12145	109637	24.2%	1.42 [1.38, 1.46]
Erez 2008	185	666	387	1935	10.7%	1.54 [1.26, 1.88]
Fichera 2014	13	106	8	91	0.9%	1.45 [0.57, 3.67]
Fox 2015	90	423	27	224	3.1%	1.97 [1.24, 3.14]
Hannoun 2011	69	333	83	508	4.9%	1.34 [0.94, 1.91]
Hediger 2005	44	695	41	917	3.5%	1.44 [0.93, 2.24]
Hong 1996	33	163	21	104	1.9%	1.00 [0.54, 1.85]
Imseis1997	3	32	14	53	0.4%	0.29 [0.08, 1.10]
James 2008	19179	127011	18807	189792	24.5%	1.62 [1.58, 1.65]
Joon Oh 2012	8	148	3	38	0.4%	0.67 [0.17, 2.64]
Lim 2017	4	54	4	45	0.4%	0.82 [0.19, 3.48]
McPherson 2013	168	580	318	1526	9.8%	1.55 [1.25, 1.93]
Pagani 2016	51	473	27	467	2.9%	1.97 [1.21, 3.20]
Rode 2012	20	121	15	97	1.4%	1.08 [0.52, 2.25]
Skentou 2001	11	131	23	303	1.3%	1.12 [0.53, 2.36]
Suzuki 2010	19	176	5	93	0.7%	2.13 [0.77, 5.90]
To 2006	85	566	58	526	4.9%	1.43 [1.00, 2.04]
Tudela 2016	39	415	16	251	1.9%	1.52 [0.83, 2.79]
Wisborg 2001	36	224	12	177	1.5%	2.63 [1.33, 5.23]
Yang 2000	4	37	2	28	0.2%	1.58 [0.27, 9.28]
Total (95% CI)		**198536**		**306894**	**100.0%**	**1.51 [1.38, 1.65]**
Total events	30002		32018			

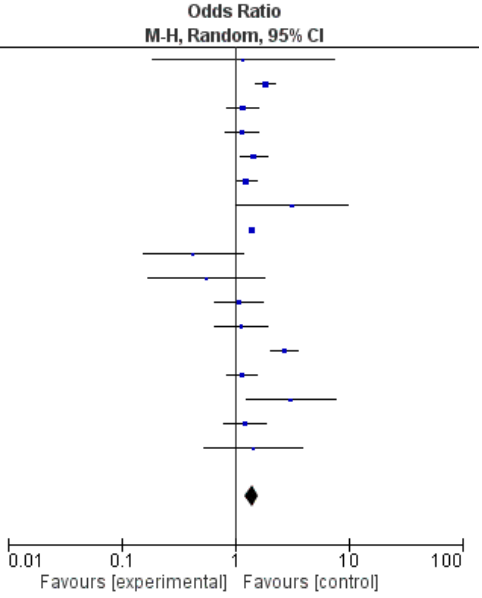
Heterogeneity: Tau² = 0.01; Chi² = 72.97, df = 20 (P < 0.00001); I² = 73%
Test for overall effect: Z = 9.18 (P < 0.00001)



    b. Nulliparity – PTB <37 weeks

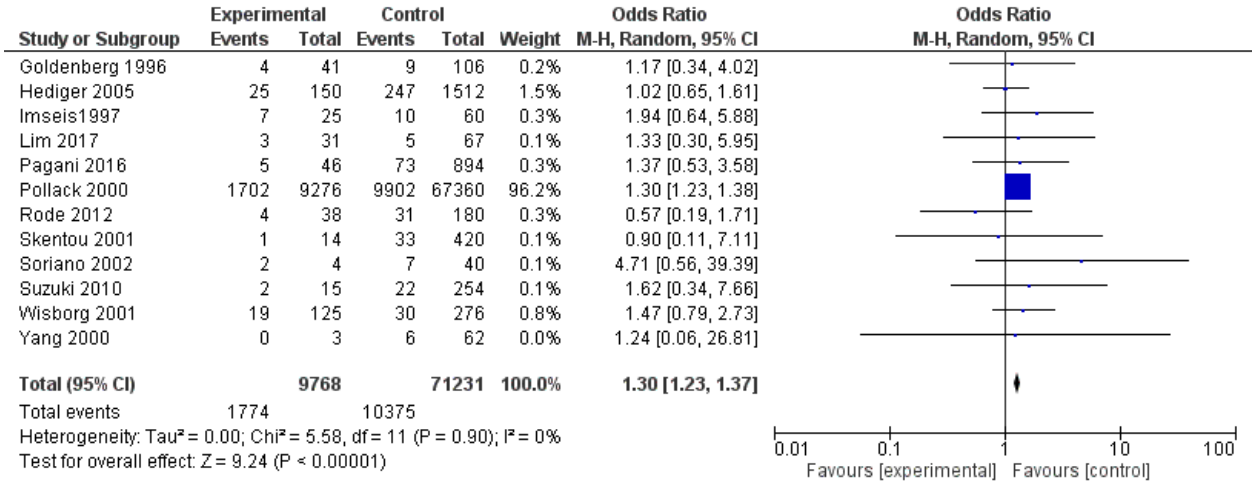
Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Bergelin 2003	5	12	3	8	0.6%	1.19 [0.19, 7.46]
Erez 2008	513	666	1243	1935	10.6%	1.87 [1.52, 2.29]
Fox 2015	260	423	129	224	7.8%	1.17 [0.84, 1.63]
Haghighi 2013	272	355	239	323	7.4%	1.15 [0.81, 1.63]
Hannoun 2011	181	333	229	508	8.9%	1.45 [1.10, 1.91]
Hediger 2005	397	695	471	917	10.7%	1.26 [1.03, 1.54]
Houlton 1982	8	14	34	115	1.4%	3.18 [1.02, 9.85]
James 2008	57409	127011	69910	189972	13.7%	1.42 [1.40, 1.44]
Lim 2017	29	54	19	26	1.7%	0.43 [0.15, 1.18]
Oliveira 1998	7	16	21	36	1.3%	0.56 [0.17, 1.83]
Rafael 2012	87	152	57	103	4.9%	1.08 [0.65, 1.79]
Rode 2012	55	121	41	97	4.5%	1.14 [0.66, 1.95]
Tarter 2002	209	635	96	633	9.0%	2.74 [2.09, 3.61]
Tudela 2016	252	415	144	251	8.0%	1.15 [0.84, 1.58]
Wennerholm 1998	27	59	9	42	2.1%	3.09 [1.26, 7.59]
Wisborg 2001	74	224	51	177	6.0%	1.22 [0.79, 1.87]
Yang 2000	18	37	11	28	1.7%	1.46 [0.54, 3.96]
Total (95% CI)		**131222**		**195395**	**100.0%**	**1.41 [1.23, 1.62]**
Total events	59803		72707			

Heterogeneity: Tau² = 0.04; Chi² = 50.02, df = 16 (P < 0.0001); I² = 68%
Test for overall effect: Z = 4.82 (P < 0.00001)

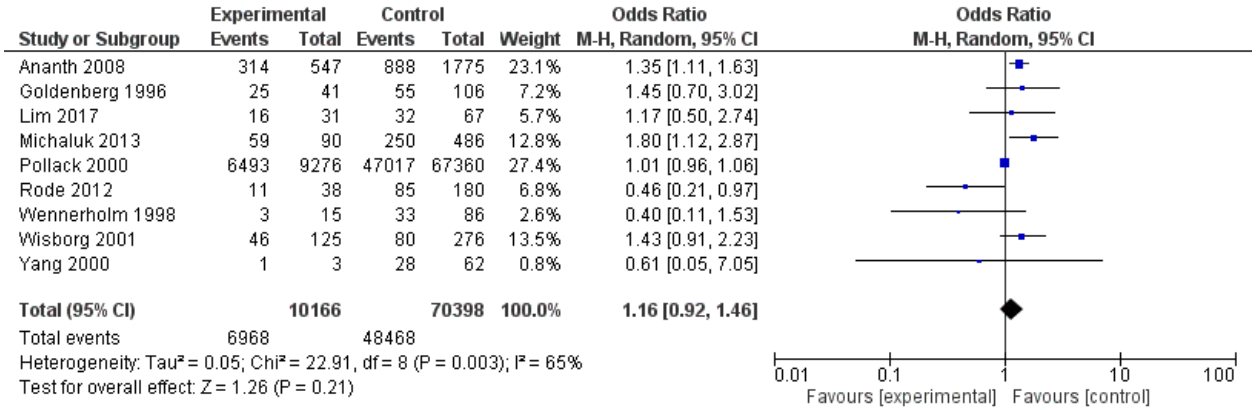


5. Maternal smoking

a. Maternal smoking – PTB <34 weeks

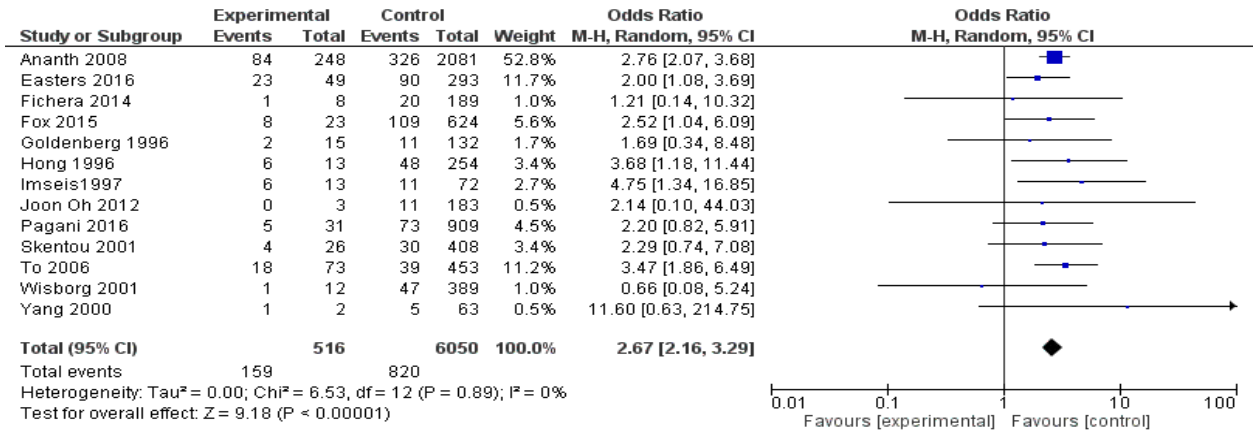


b. Maternal smoking – PTB <37 weeks

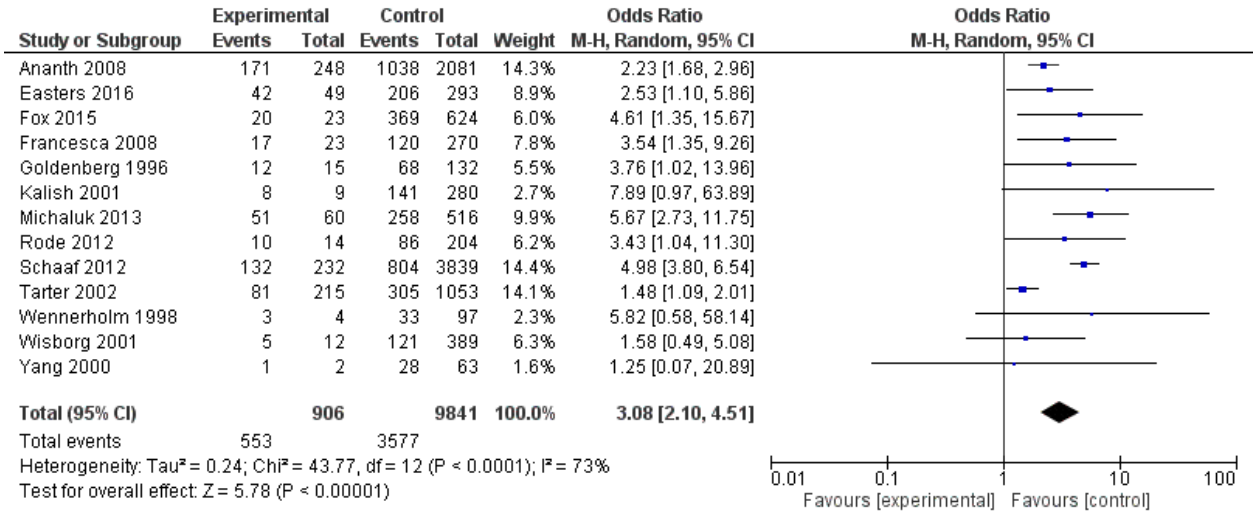


6. Past history of preterm delivery

a. Past history of preterm delivery - PTB <34 weeks



b. Past history of preterm delivery – PTB <37 weeks



APPENDIX 6: Funnel plots for meta-analysis with more than 10 included studies in the systemic review of maternal clinical predictors of preterm birth in twin pregnancies.

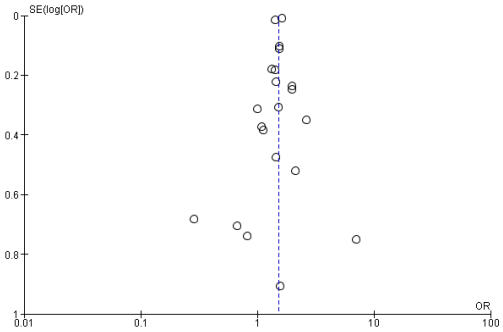


Figure 1. Funnel plot for comparison of nulliparity and odds of early preterm birth

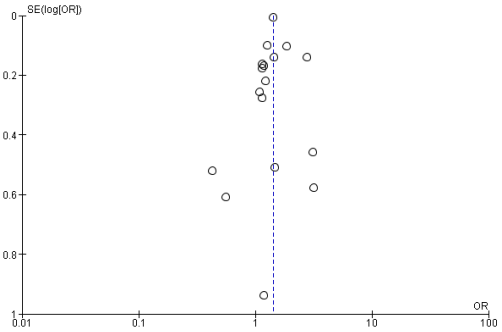


Figure 2. Funnel plot for comparison of nulliparity and odds of late preterm birth

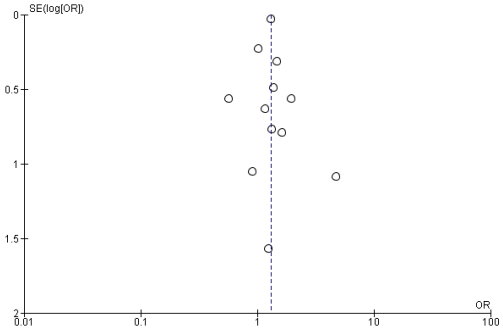


Figure 3. Funnel plot for comparison of maternal smoking and odds of early preterm birth

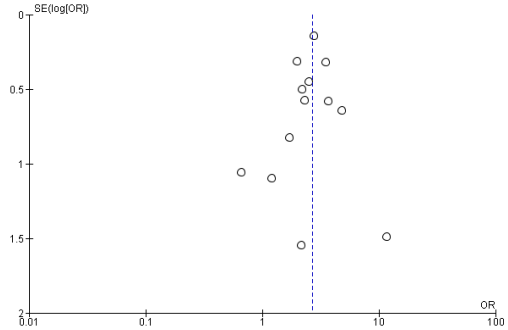


Figure 4. Funnel plot for comparison of previous history of preterm delivery and early preterm birth

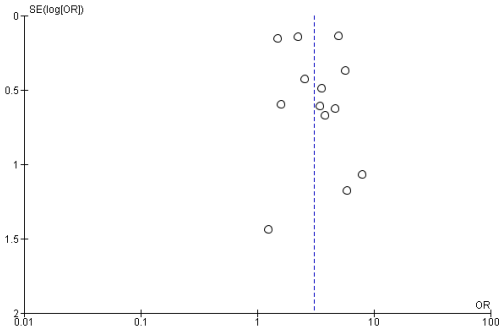


Figure 5. Funnel plot for comparison of previous history of preterm delivery and late preterm birth

APPENDIX 7: Search strategy used in the systematic review of biochemical predictors of preterm birth in twin pregnancies.

1. Twin*.mp. Or exp Twins
2. "multiple pregnan$".mp.
3. Multiple pregnancy.mp. Or exp Pregnancy, Multiple
4. 1 or 2 or 3
5. Exp Obstetric Labor, Premature/ or exp Premature Birth
6. Premat*.mp.
7. Preterm*.mp.
8. 5 or 6 or 7
9. 4 and 8
10. Fibronectin.mp. Or exp Fibronectins
11. Estriol.mp. Or exp Estriol
12. Oestriol.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
13. 11 or 12
14. Home uterine activity monitor$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
15. HUAM.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

16. Uterine contractions.mp. Or exp Uterine Contraction

17. 14 or 15 or 16

18. Interleukin$.mp. Or exp Interleukins

19. Cervical funnel*.mp.

20. Exp Uterine Cervical Incompetence/ or exp Cervical Length Measurement/ or cervical length.mp.

21. Exp Fetal Movement/ or fetal breathing movement.mp.

22. Fetal breath*.mp.

23. Fetal breath*.mp.

24. 21 or 22 or 23

25. Cervical assessment.mp.

26. Bishop score.mp.

27. Cervi* score.mp.

28. Vaginal exam*.mp.

29. Cervical digital exam*.mp.

30. 25 or 26 or 27 or 28 or 29

31. Serum biomarker.mp. Or exp Biological Markers

32. HCG.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

33. Insulin-like growth factor.mp. Or exp Somatomedins

34. Exp alpha-Fetoproteins/ or AFP.mp.

35. Exp C-Reactive Protein/ or CRP.mp.

36. Exp Corticotropin-Releasing Hormone/ or CRH.mp.

37. Exp Insulin-Like Growth Factor Binding Protein 1/ or exp Insulin-Like Growth Factor I/ or IGFPB.mp. Or exp Insulin-Like Growth Factor Binding Proteins

38. MMP.mp. Or exp Matrix Metalloproteinases

39. Relaxin.mp. Or exp Relaxin

40. Periodontal screen*.mp. Or exp Periodontitis

41. Exp Urinary Tract Infections/ or UTI.mp. Or exp Bacteriuria

42. Asymptomatic bacteriuria.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

43. MSU culture.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

44. Mid-stream urine.mp.

45. 41 or 42 or 43 or 44

46. Exp Premature Birth/ or exp Obstetric Labor, Premature

47. Exp Medical History Taking/ or patient history.mp.

48. Medical history.mp.

49. Past history.mp.

50. Previous history.mp.

51. 47 or 48 or 49 or 50

52. 46 and 51

53. Exp Abdomen/ and exp Palpation

54. Abdominal palpation.mp.

55. 53 or 54

56. Prolactin.mp. Or exp Prolactin

57. Rheobase.mp.

58. Mammary stimulation test.mp.

59. Mammary stim*.mp.

60. 58 or 59

61. Cervicovaginal glycoproteins.mp.

62. Glycoprotein.mp. Or exp Glycoproteins

63. Exp Hormones/ or endocrine hormone.mp.

64. Inflammatory markers.mp.

65. Bacterial vaginosis.mp. Or exp Vaginosis, Bacterial

66. 10 or 13 or 17 or 18 or 19 or 20 or 24 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 45 or 52 or 55 or 56 or 57 or 60 or 61 or 62 or 63 or 64 or 65

67. Exp Twins, Dizygotic/ or exp Twins, Monozygotic/ or chorionicity.mp.

68. Exp Ovulation Induction/ or exp Fertilization in Vitro/ or IVF.mp.

69. Method of conception.mp. Or exp Insemination, Artificial

70. Amniotic fluid.mp. Or exp Amniotic Fluid

71. Oocyte donation.mp. Or exp Oocyte Donation

72. PAPP-A.mp. Or exp Pregnancy-Associated Plasma Protein-A

73. Interpregnancy interval.mp.

74. Exp Birth Intervals/ or inter-pregnancy interval.mp.

75. 73 or 74

76. Vaginal bleed*.mp.

77. Antepartum hemorrhage.mp.

78. 76 or 77

79. Exp Crown-Rump Length/ or crown rump length discordance.mp.

80. CRL discordance.mp.

81. 79 or 80

82. Exp Nuchal Translucency Measurement/ or nuchal translucency discordance.mp.

83. VEGF.mp. Or exp Vascular Endothelial Growth Factor A

84. Fundal length.mp.

85. Fundal height.mp.

86. Exp Anthropometry

87. 84 or 85 or 86

88. Endotoxins.mp. Or exp Endotoxins

89. Exp Hispanic Americans/ or exp European Continental Ancestry Group/ or exp African Americans/ or maternal race.mp. Or exp African Continental Ancestry Group

90. Paternal race.mp.

91. Race.mp. Or exp Continental Population Groups

92. 89 or 90 or 91

93. Exp Socioeconomic Factors

94. Exp Educational Status/ or maternal education.mp.

95. Maternal status.mp.

96. Exp Body Mass Index/ or BMI.mp.

97. Smoking.mp. Or exp Smoking

98. Parity.mp. Or exp Parity

99. Fetal sex.mp.

100. Fetal sex.mp.

101. 99 or 100

102. Exp Progesterone/ or progesterone.mp.

103. Assisted reproductive technology.mp. Or exp Reproductive Techniques, Assisted

104. Marital status.mp. Or exp Marital Status

105. Prenatal care.mp. Or exp Prenatal Care

106. Exp Leptin/ or leptins.mp.

107. Exp Fetal Growth Retardation/ or discordant growth.mp.

108. Growth discordance.mp.

109. 67 or 68 or 69 or 70 or 71 or 72 or 75 or 78 or 81 or 82 or 83 or 87 or 88 or 92 or 93 or 94 or 95 or 96 or 97 or 98 or 101 or 102 or 103 or 104 or 105 or 106 or 107 or 108

110. 66 or 109

111. 9 and 110

112. 111 and 1990:2019.(sa_year)

APPENDIX 8: Study characteristics in the systematic review of biochemical predictors of preterm birth in twin pregnancies.

No	Study, Year Country	Study design	Number of women	Inclusion period (Months)	Inclusion criteria	Exclusion criteria	Biochemical predictors	Cutoff	Gestational age at collection	Outcome (Preterm delivery)
1	**Adeyemi, 2010** UK	Retrospective Cohort	40	36	Twin pregnancies	Women who had a spontaneous miscarriage, women who did not have rapid IGFBP-1.	Positive IGFBP-1	Two blue lines on the dipstick	26 weeks	<34 weeks <35 weeks <36 weeks <37 weeks
2	**Bang, 2015** Korea	Retrospective Cohort	300	36	All placentas collected from Samsung medical center, Seoul.	Not known	Chronic chorioamnionitis	Lymphocytic infiltration into the chorionic trophoblast layer and/or chorioamniotic connective tissue		<37 weeks
3	**Bodnar, 2013** USA	Prospective RCT	211	12	Women with twin pregnancies 16 to 20 weeks & 3 days, randomized into weekly injections of either 17 alpha hydroxyprogesterone caproate or placebo until 34 weeks & 6 days or delivery, 25 Hydroxy Vitamin D measured from 24-28 weeks.	Not known	Maternal serum 25 Hydroxy Vitamin D	<75 nmol/l	24-28 weeks	<32 weeks <35 weeks

4	**Combs, 2014** USA	Prospective Cohorts nested in RCT	198	57	Women with uncomplicated dichorionic-diamniotic twin pregnancy enrolled at 15 to 23 weeks of gestation and randomly assigned to receive a weekly intramuscular injection of either 17 Hydroxy progesterone caproate or an identical appearing placebo.	Women <18 years, those who had taken progestins at >15 weeks of gestation, had symptomatic uterine contractions, rupture of the fetal membranes, any contraindication to prolonging the pregnancy, any pre-existing condition that might be worsened by progesterone, pre-existing medical condition carrying a high risk of preterm delivery.	Fetal Fibronectin	≥50ng/ml	24-26 weeks	<32 weeks <34 weeks <37 weeks
5	**Fichera, 2014** Italy	Prospective Cohort	197	24	Consecutive twin pregnancies	Indicated preterm delivery for maternal or fetal indications, cervical cerclage, Arabin cervical pessary insertion.	PhIGFBP-1	>10 µg/l	At the time of anomaly scan	<28 weeks <30 weeks <32 weeks <34 weeks
6	**Fox, 2015** USA	Retrospective Cohort	611	96	All patients with twin pregnancies >22 weeks delivered by a single maternal fetal medicine practice.	Monochorionic monoamniotic placentation, major fetal congenital anomalies discovered	Fetal Fibronectin	≥50ng/ml	22-32 weeks	<28 weeks <32 weeks <34 weeks <37 weeks

						before or after birth, TTTS, major uterine anomalies, cerclage, Fetal Fibronectin not done, Intra-uterine fetal demise.				
7	**Fuchs, 2018** France	Prospective Cohort	40	6	Women older than 18 years with a monochorionic or dichorionic twin pregnancy between 24 weeks to 33 weeks & 6 days gestation with symptoms of preterm labour and intact membranes.	Monoamniotic twin pregnancy, confirmed rupture of membranes, cervical dilatation >3cm, prolapsed membranes bulging in the vagina, cervical cerclage, vaginal bleeding, placenta praevia, placental abruption, severe intrauterine growth restriction, polyhydramnios, fetal malformation, preeclampsia, medically indicated preterm delivery before 34 weeks.	Fetal Fibronectin	>50ng/ml	24-33+6	<32 weeks <34 weeks <37 weeks Within 7 days of testing Within 14 days of testing

8	**Goldenberg, 2006** USA	Prospective Cohort	147	22	All twin pregnancies <22 to 24 weeks gestation.	Cervical cerclage, placenta praevia, severe fetal anomalies.	Fetal Fibronectin Bacterial vaginosis	≥50ng/ml pH >4.5 in addition to a Gram stain score of 7 to 10 according to the criteria of Nugent et al. to grade the presence of bacterial morphotypes.	28 weeks 28 weeks	<32 weeks <35 weeks <37 weeks
9	**Gonzalez, 2003** France	Prospective Cohort	44	47	Singleton or twin pregnancy, gestational age <34 weeks of amenorrhoea, determined from the date of the last period and confirmed by a 1st trimester ultrasound scan. Intact amniotic membranes.	Pregnancies associated with cerclage or maternal or fetal pathology contra-indicating the continuation of pregnancy.	Fetal Fibronectin	Not mentioned	<34 weeks	<34 weeks <37 weeks
10	**Hershkovitz, 2005** Israel	Retrospective Cohort	279	36	All twin pregnancies with maternal serum hCG determined between 16-18 weeks.	Patients with no prenatal care, unknown chorionicity, abnormal karyotype, elevated AFP (>2.5 MoM) and fetal malformations.	Maternal serum hCG	> 2.5 MoM	16-18 weeks	<32 weeks 32-36 weeks <37 weeks

11	**Hong, 1996** USA	Retrospective Cohort	267	82	Twin pregnanicies who have had at least one maternal serum AFP test.	Multifetal pregnancy reductions, neural tube defects, abdominal wall defects, low maternal serum AFP levels, missing AFP results, maternal chronic conditions, death of at least one fetus.	Maternal serum Alpha fetoprotein	>3.5 MoM	15-20 weeks	<34 weeks
12	**Iams, 2001** USA	Prospective Cohort	188	22	Women attending prenatal clinics of participating institutions, who have spontaneously conceived and identified as having a twin gestation before 24 weeks.	Placenta praevia, major fetal anomalies, ovulation induction, fetal reduction to twins.	Maternal serum relaxin	> 90th centile	24 weeks	<32 weeks <35 weeks <37 weeks
13	**Iskender, 2013** Turkey	Retrospective Cohort	104	84	All twin pregnancies that gave birth during the study duration.	Not known	PAPP-A hCG	< 10th centile > 90th centile	11-14 weeks 11-14 weeks	<34 weeks
14	**Joon Oh, 2016** Korea	Prospective Cohort	88	Not known	Patients with preterm labour with intact membranes.	Not known	Matrix metalloprotein 8	Measured by commercially available point of care test	≤34 weeks	Delivery within 7 days

15	**Joon Oh, 2019** Korea	Prospective Cohort	90	239	Twin gestation with live fetuses who had preterm labour with intact membranes, and underwent amniocentesis between 20 weeks and 34 weeks and 6 days gestation.	Rupture of membranes	Intra-amniotic infection	Positive microorganisms in amniotic fluid culture	20 to 34+6 weeks	<34 weeks <36 weeks
16	**Kosinska-Kaczynska, 2018** Poland	Prospective Cohort	151	36	Women prior to 16 weeks of twin gestation with gestational age and chorionicity confirmed in the 1st trimester ultrasound scan.	Monoamniotic pregnancy, miscarriage, twin-to-twin transfusion syndrome, cervical length <25 mm between 20-24 weeks and had Arabin pessary inserted and/ or vaginal progesterone administered, insertion of a secure cerclage for protruding amniotic membranes through external os, PPROM prior to 24 weeks of gestation, intrauterine fetal demise, delivered before 37 weeks due to	PhIGFBP-1	>10 µg/l	20-24 weeks	<28 weeks <32 weeks <34 weeks <37 weeks

						medical indications.				
17	**Kurtzman, 2014** USA, UK	Prospective Cohort	76	Not known	Asymptomatic patients with twins	Not known	Fetal Fibronectin	≥50ng/ml	22 to 27+6 weeks	<30 weeks <34 weeks <36 weeks
18	**Laughon, 2009** USA	Retrospective Cohort	70	24	All twin gestations with crown rump length 45-84 cm who had first trimester screening with PAPP-A, beta hCG, nuchal translucency.	Elective multifetal pregnancy reduction, incomplete records	Free beta hCG PAPP-A	>75th centile ≤25th centile	11-14 weeks	<32 weeks <37 weeks
19	**Lepage, 2002** USA	Retrospective Case control	1589	36	Twin pregnancies who were offered second trimester triple-marker maternal serum screening.	Chromosomal abnormalities, fetal structural abnormalities, insulin dependent diabetes mellitus and induced abortion	Maternal serum hCG	<5 MoM	15-20 weeks	<37 weeks
20	**Lockwood, 1993** USA	Prospective Cohort	7	33	Estimated date of confinement established by ultrasound scan <24 weeks or at <14 weeks or two concordant ultrasound scan between 14 and 24 weeks.	Ultrasound scan evidence of placenta praevia after 24 weeks, elective or spontaneous termination before 24 weeks, delivered elsewhere or loss to follow up, underwent indicated deliveries at <37	Fetal Fibronectin	≥50ng/ml	24-36 weeks	<37 weeks

						weeks because of preeclampsia and fetal growth retardation with or without evidence of fetal distress				
21	**Matthews, 2017** USA	Retrospective Cohort	155	132	All patients with twin pregnancies, test results from 22 to 28 weeks, all women with a shortened cervical length ≤25mm.	Symptomatic patients, monoamniotic twins, twin-twin transfusion syndrome, pregnancies affected by aneuploidy, any major fetal congenital anomalies, medically indicated preterm birth.	Fetal Fibronectin	≥50ng/ml	22-28 weeks	<28 weeks <30 weeks <32 weeks <34 weeks <35 weeks <37 weeks
22	**Mazor, 1996** Israel	Prospective Cohort	74	48	Twin gestations diagnosed with preterm labour and intact membranes.	Patients with an unreliable LMP, lack of prenatal evaluation (never seen by a nurse, midwife or obstetrician).	Amniotic fluid infection	Positive amniotic fluid culture +/- Gram stain examination and/or possible amniotic fluid culture for aerobic, anaerobic or Mycoplasma species.	On admission with preterm labour	<37 weeks
23	**Oliveira, 1998** Brazil	Prospective Cohort	52	26	All twin pregnancies between 24- and 34-weeks gestation.	Preterm delivery for medical reasons, use of tocolytics to	Fetal Fibronectin	≥50ng/ml	24-34 weeks	<34 weeks <37 weeks

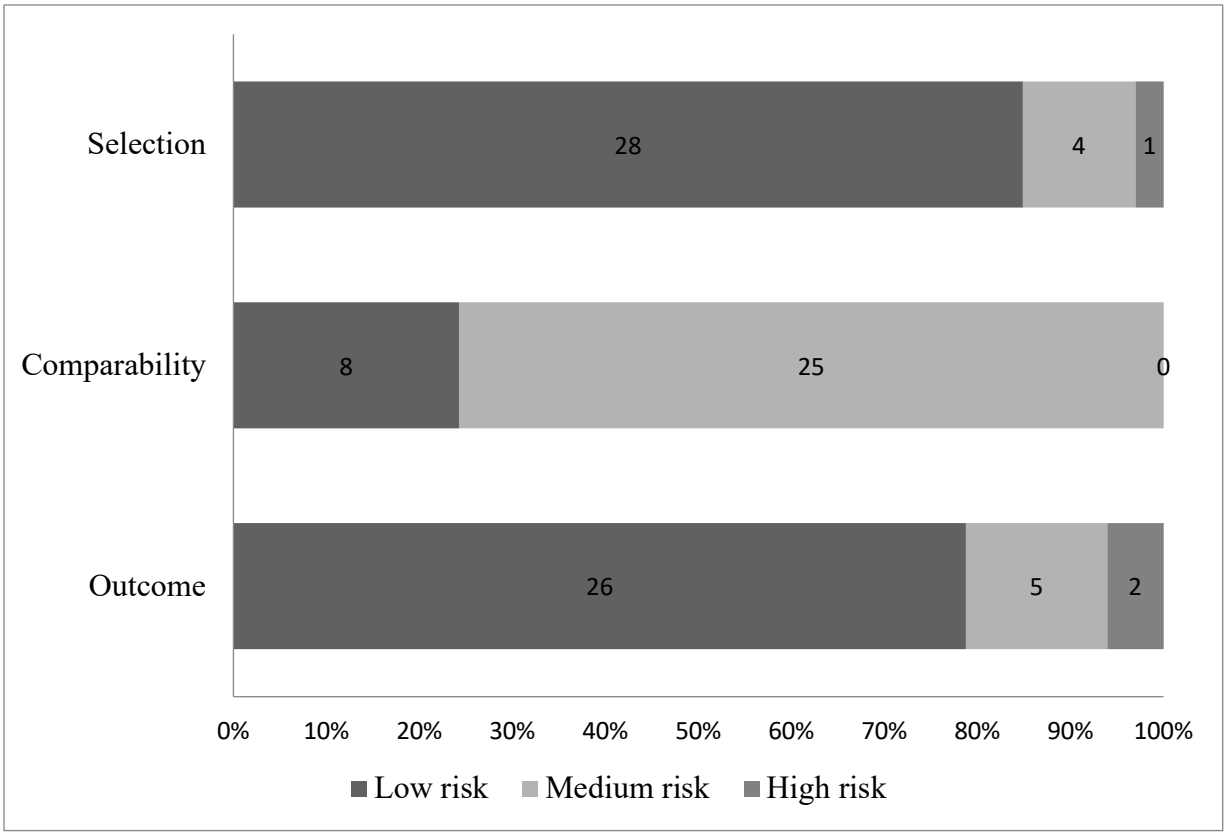
						inhibit preterm labour				
24	**Peaceman, 1997** USA	Prospective Cohort	38	Not known	Patients who made unscheduled visits to the ten participating hospitals with symptoms of preterm labour between 24 and 34+6 weeks of gestation with no history of spontaneous rupture of membranes and no history of tocolysis. Cervical dilation <3 cm.	Placenta praevia, cervical cerclage, trauma, spontaneous rupture of membranes, history of tocolysis, cervical dilation >3cm	Fetal Fibronectin	≥50ng/ml	24- 34+6/7 weeks	<37 weeks
25	**Platek, 1997** USA	Prospective Cohort	28	Not known	Spontaneous twin pregnancies ≤32 weeks. (Pilot study)	Not known	Maternal serum relaxin	≥1.4ng/ml	≤32 weeks	≤35 weeks
26	**Ramirez, 1999** USA	Prospective Cohort	65	16	All twin pregnancies from 24 through 34 weeks of gestation.	Not known	Fetal Fibronectin	≥50ng/ml	24-34 weeks	<36 weeks
27	**Ruiz, 2004** USA	Prospective Cohort	48	Not known	Twin pregnancies <22 weeks	Cervical incompetence, uterine malformations, intra-uterine fetal demise, positive for monilia vaginitis	Fetal Fibronectin	≥50ng/ml	Anytime during pregnancy	<35 weeks

28	**Singer, 2007** USA	Retrospective Cohort	87	60	Patients presenting with complaints concerning preterm labour such as contractions, pelvic pressure and lower back pain, fFN testing between 24 and 34.9 weeks of gestation, intact membranes, cervical dilatation of <3cm.	Women who have had intercourse, a vaginal examination or a vaginal ultrasound within 24 hours of fFN testing, cervical cerclage, required preterm delivery within 14 days of testing due to maternal or fetal complications.	Fetal Fibronectin	≥50ng/ml	24- 34+6 weeks	<34 weeks Delivery within 14 days of testing
29	**Spiegelman, 2016** USA	Retrospective Cohort	635	104	All patients with twin pregnancies delivered by a single maternal fetal medicine practice.	Patients with cerclage, monochorionic monoamniotic placentation, major fetal congenital anomalies detected before or after birth, TTTS, indicated preterm birth.	Fetal Fibronectin	≥50ng/ml	22 0/7 to 25 6/7 weeks	<35 weeks

30	**Tanaka, 2017** Japan	Prospective Cohort	54	78	All women with asymptomatic twin pregnancies who received perinatal care and delivered at the tertiary perinatal centre, cervical length was less than 25mm between 22-29 weeks of gestation with intact fetal membranes.	Uterine anomalies, major fetal anomalies, intrauterine fetal death, TTTS, cervical cerclage placement, medically indicated delivery including preeclampsia prior to 34 weeks, symptomatic women with gross cervical bleeding, cervical change (effacement of at least 50% or dilatation of at least 2cm) or painful and regular uterine contractions.	Granulocyte elastase	>1.6 µg/ml	22-29 weeks	<34 weeks
31	**Terrone, 1998** USA	Prospective Cohort. Combined from two prospective multicentre trials	43	Not known	Not known	Not known	Fetal Fibronectin	Not known	Not known	Delivery within ≤7 days of testing
32	**Wennerholm, 1997** Sweden	Prospective Cohort	101	18	Twin pregnancies	Induced preterm deliveries	Fetal Fibronectin	≥50ng/ml	Anytime between 24-34 weeks	<35 weeks <37 weeks

33	**Wennerholm, 1998** Sweden	Prospective Cohort	101	18	Twin pregnancies recruited before 20 weeks of gestation.	Induced preterm delivery indicated for maternal or fetal conditions (pre-eclampsia, IUGR, placental abruption, placenta praevia, polyhydramnios, twin to twin transfusion syndrome, abdominal pain)	Bacterial vaginosis Chorioamnionitis IL-8	Presence of clue cells in any concentration in the vaginal smear preparation Maternal fever >38°C and either uterine tenderness or unexplained fetal tachycardia > 1.75ng /g mucus	24-34 weeks	<37 weeks

APPENDIX 9: Quality assessment using the Newcastle Ottawa Scale in the systemic review of biochemical predictors of preterm birth in twin pregnancies.

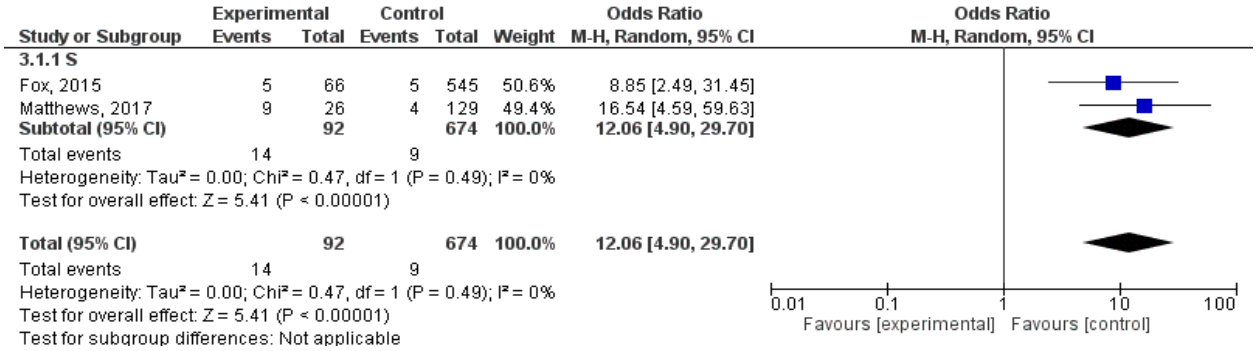


APPENDIX 10: Forest plots of pooled odds ratios (OR) for preterm birth (PTB) in the systematic review of biochemical predictors of preterm birth in twin pregnancies.

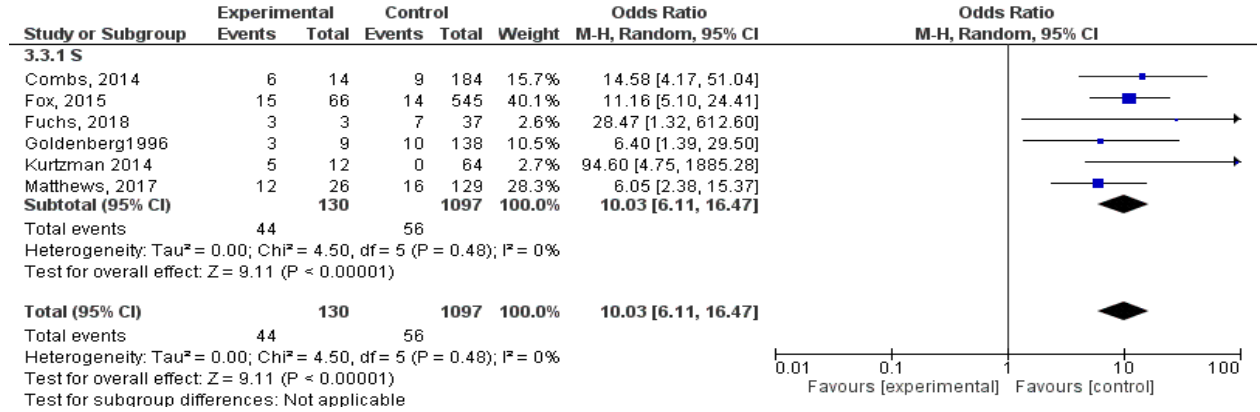
Maternal cervicovaginal factors

1. Fetal Fibronectin (fFn)

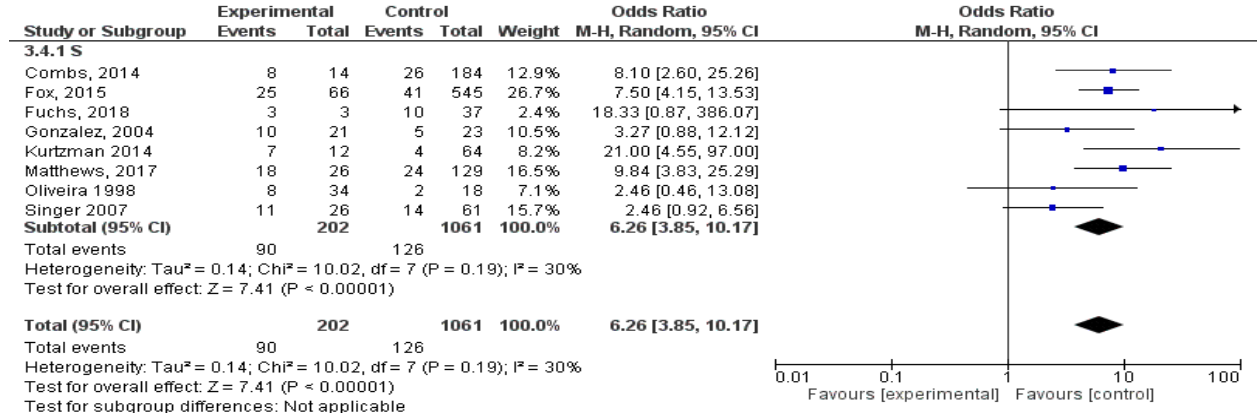
(a) Elevated fFn – PTB <28 weeks



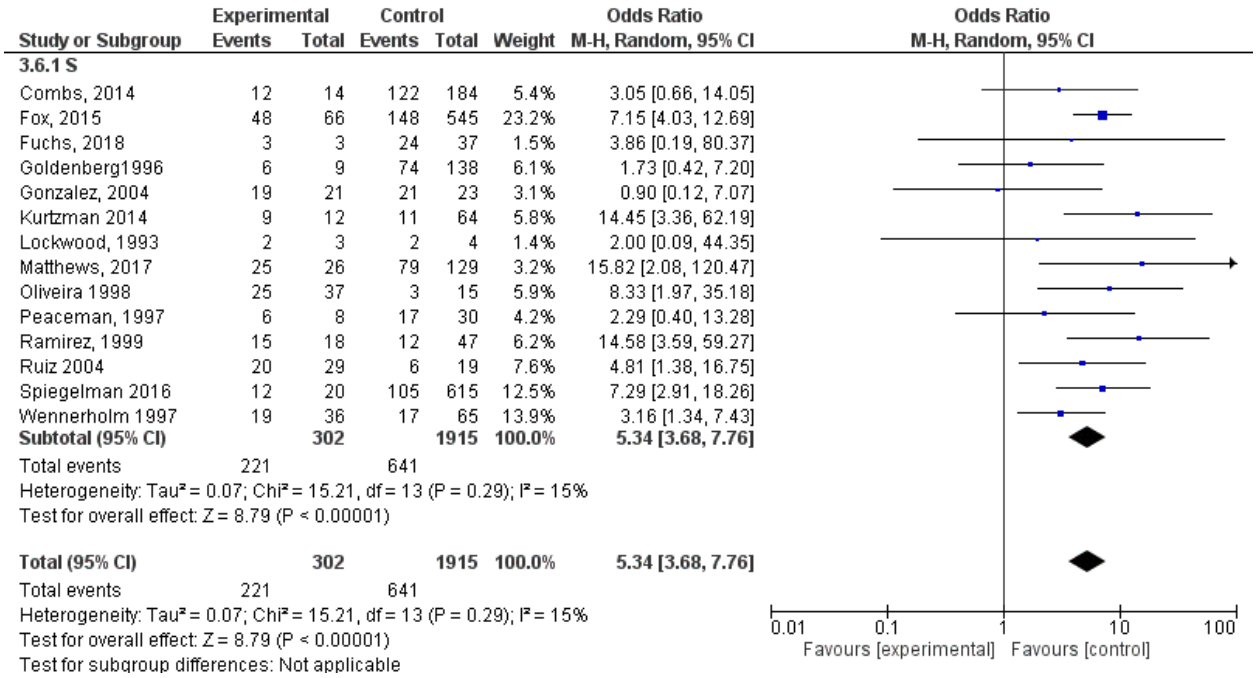
(b) Elevated fFn – PTB <32 weeks



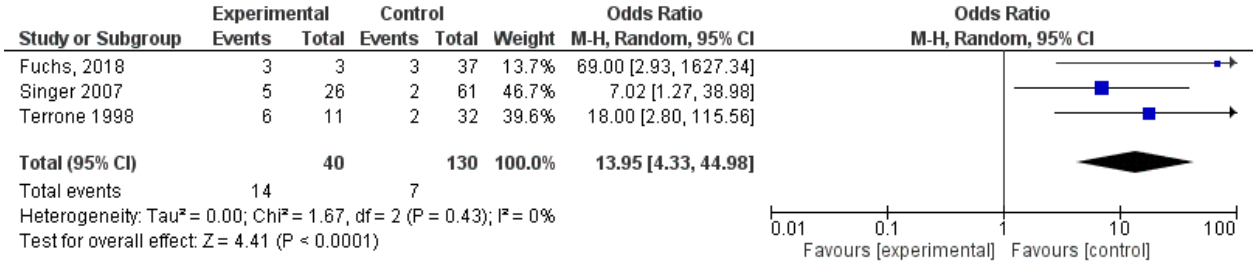
(c) Elevated fFn – PTB <34 weeks



(d) Elevated fFn – PTB <37 weeks

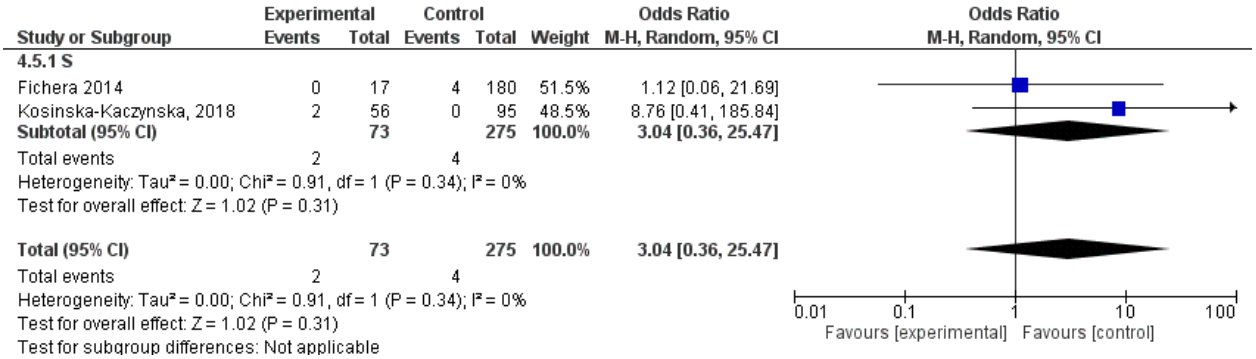


(e) Elevated fFn – Delivery within 14 days of testing

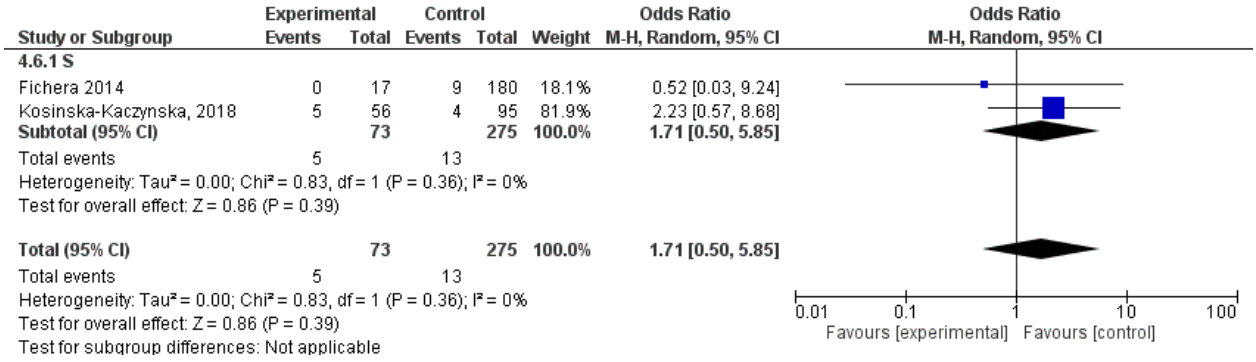


2. phIGFBP-1

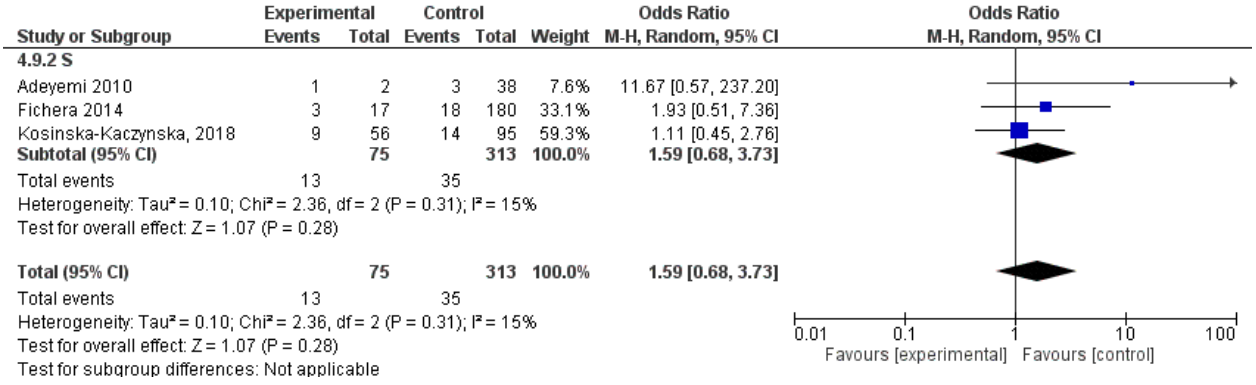
(a) Elevated phIGFBP-1 – PTB <28 weeks



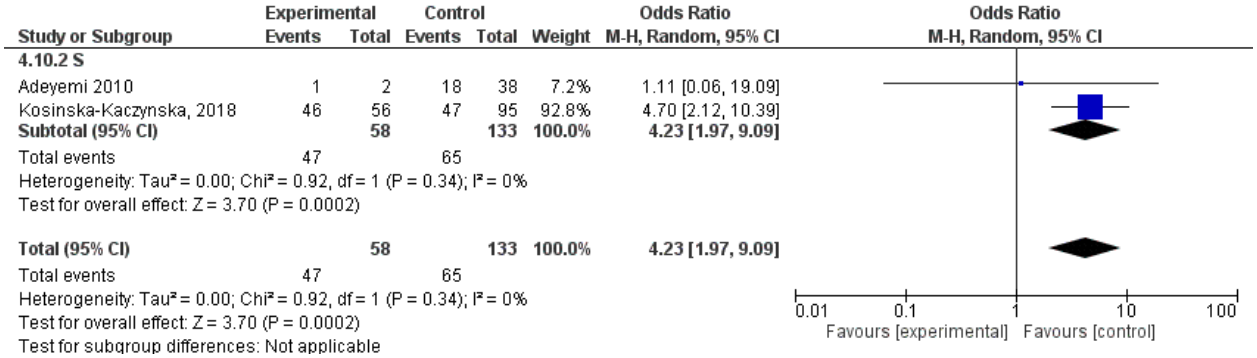
(b) Elevated phIGFBP-1 – PTB <32 weeks



(c) Elevated phIGFBP-1 – PTB <34 weeks

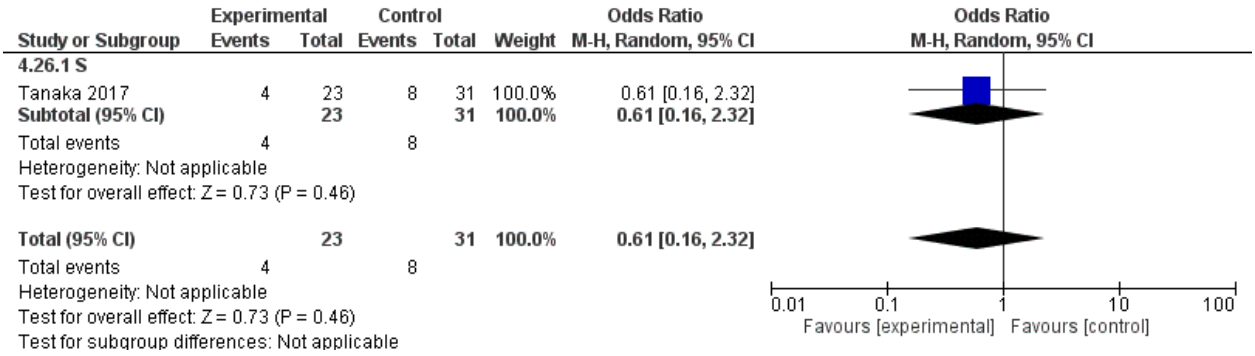


(d) Elevated phIGFBP-1 – PTB <37 weeks



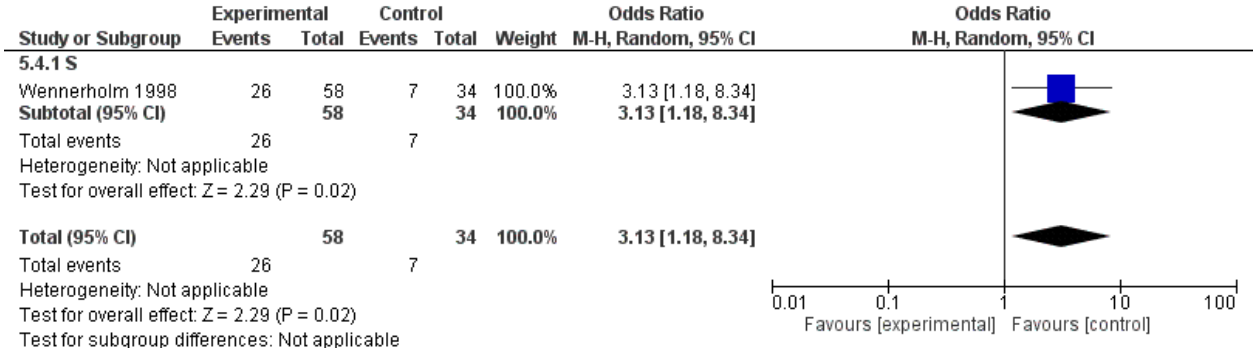
3. Granulocyte elastase

(a) Elevated Granulocyte elastase – PTB <34 weeks



4. IL-8

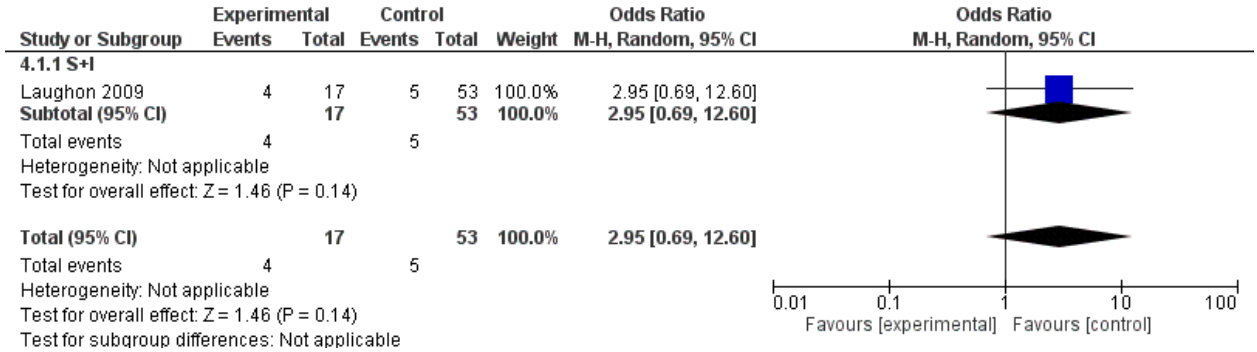
(a) Elevated IL-8 – PTB <37 weeks



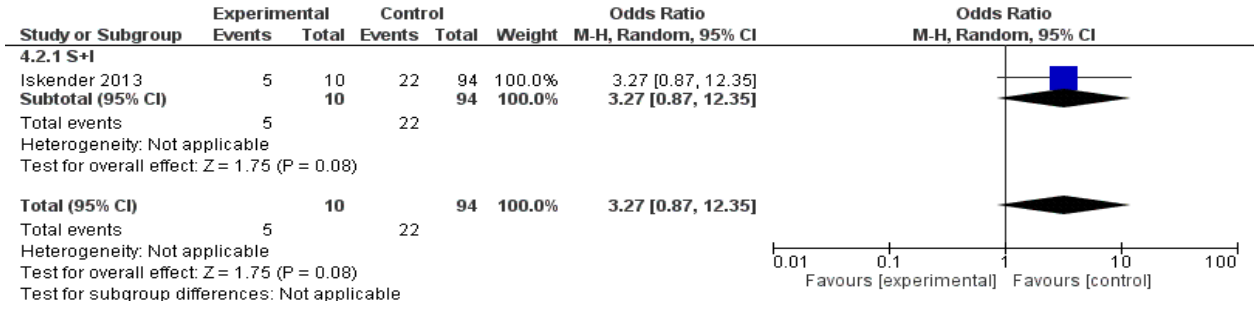
Maternal serum factors

1. PAPP-A

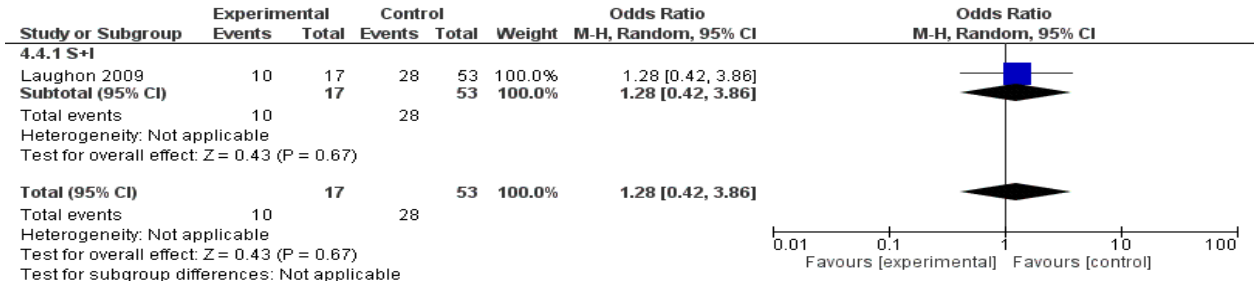
(a) Reduced PAPP-A – PTB <32 weeks



(b) Reduced PAPP-A – PTB <34 weeks

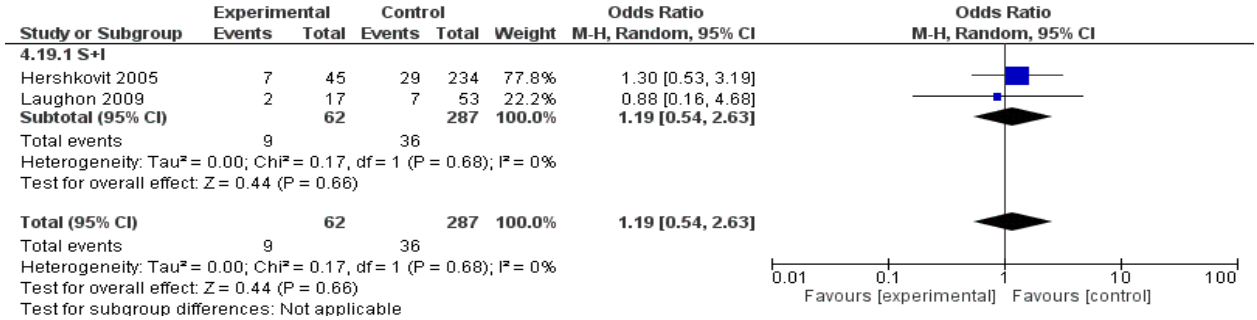


(c) Reduced PAPP-A – PTB <37 weeks

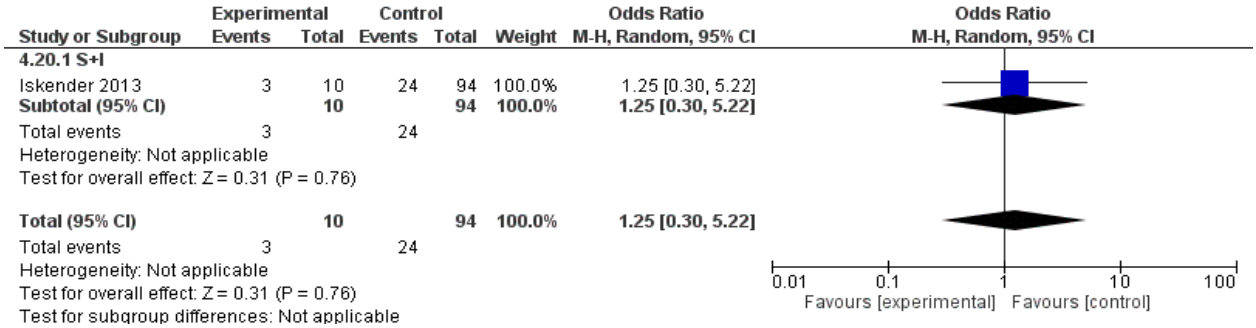


2. hCG

(a) Elevated hCG – PTB <32 weeks

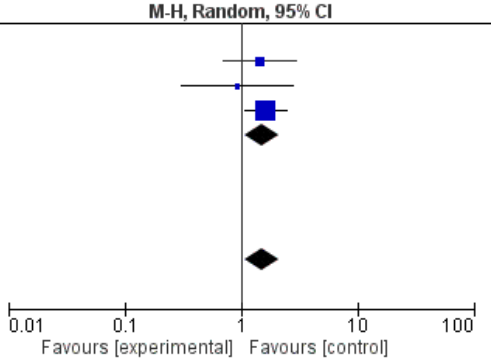


(b) Elevated hCG – PTB <34 weeks



(c) Elevated hCG – PTB <37 weeks

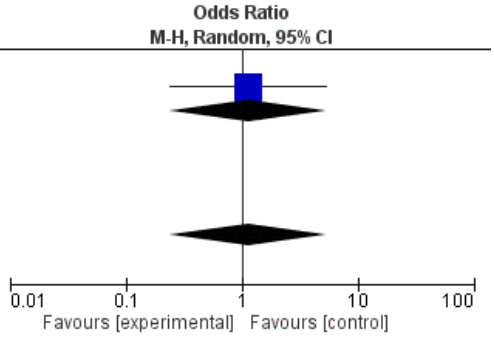
Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
4.21.1 S+I						
Hershkovit 2005	33	45	153	234	23.3%	1.46 [0.71, 2.97]
Laughon 2009	9	17	29	53	9.9%	0.93 [0.31, 2.78]
Lepage 2002	51	93	637	1496	66.8%	1.64 [1.07, 2.49]
Subtotal (95% CI)		**155**		**1783**	**100.0%**	**1.51 [1.07, 2.13]**
Total events	93		819			
Heterogeneity: Tau² = 0.00; Chi² = 0.90, df = 2 (P = 0.64); I² = 0%						
Test for overall effect: Z = 2.33 (P = 0.02)						
Total (95% CI)		**155**		**1783**	**100.0%**	**1.51 [1.07, 2.13]**
Total events	93		819			
Heterogeneity: Tau² = 0.00; Chi² = 0.90, df = 2 (P = 0.64); I² = 0%						
Test for overall effect: Z = 2.33 (P = 0.02)						
Test for subgroup differences: Not applicable						



3. Relaxin

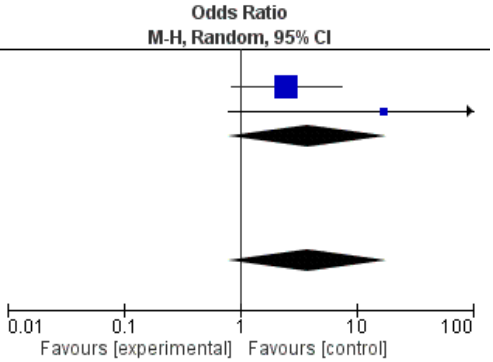
(a) Elevated Relaxin – PTB <32 weeks

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
4.15.1 S						
Iams 2014	2	17	18	171	100.0%	1.13 [0.24, 5.36]
Subtotal (95% CI)		**17**		**171**	**100.0%**	**1.13 [0.24, 5.36]**
Total events	2		18			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.16 (P = 0.87)						
Total (95% CI)		**17**		**171**	**100.0%**	**1.13 [0.24, 5.36]**
Total events	2		18			
Heterogeneity: Not applicable						
Test for overall effect: Z = 0.16 (P = 0.87)						
Test for subgroup differences: Not applicable						



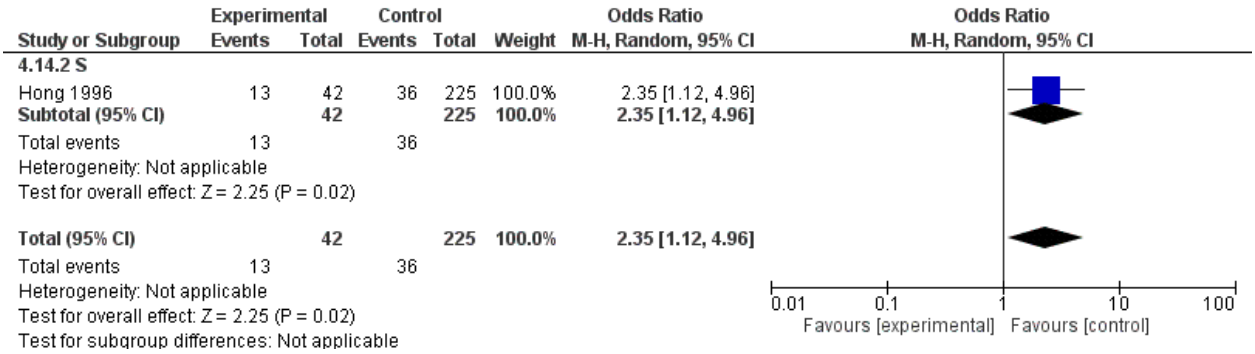
(b) Elevated Relaxin – PTB <37 weeks

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
4.16.1 S						
Iams 2014	12	17	84	171	78.6%	2.49 [0.84, 7.36]
Platek 1997	3	3	7	25	21.4%	17.27 [0.79, 376.54]
Subtotal (95% CI)		**20**		**196**	**100.0%**	**3.77 [0.79, 18.05]**
Total events	15		91			
Heterogeneity: Tau² = 0.51; Chi² = 1.36, df = 1 (P = 0.24); I² = 27%						
Test for overall effect: Z = 1.66 (P = 0.10)						
Total (95% CI)		**20**		**196**	**100.0%**	**3.77 [0.79, 18.05]**
Total events	15		91			
Heterogeneity: Tau² = 0.51; Chi² = 1.36, df = 1 (P = 0.24); I² = 27%						
Test for overall effect: Z = 1.66 (P = 0.10)						
Test for subgroup differences: Not applicable						



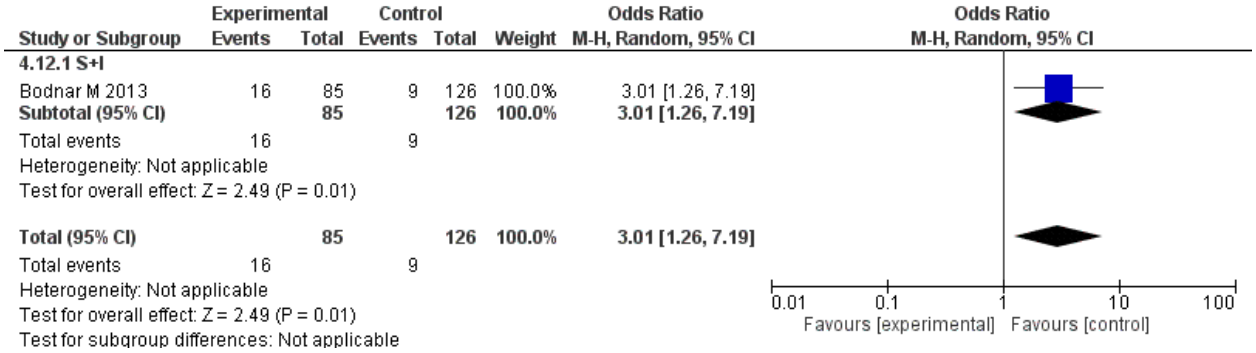
4. Alpha feto protein (AFP)

(a) Elevated AFP – PTB <34 weeks

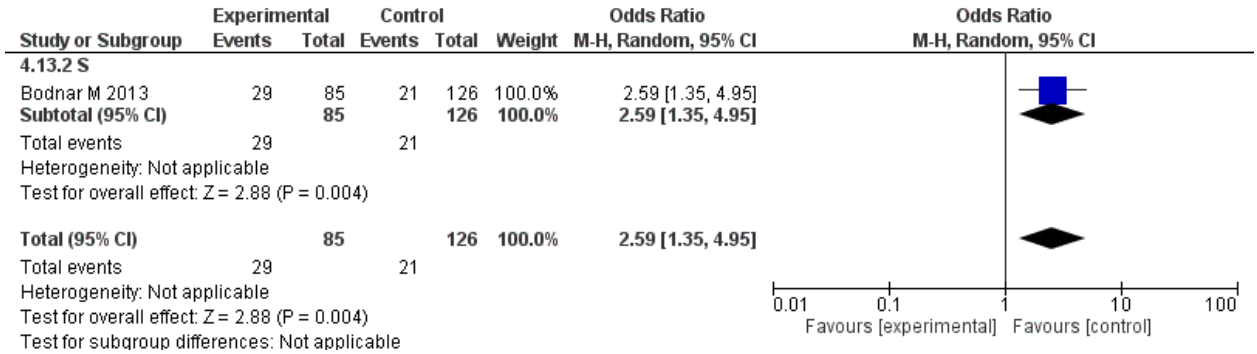


5. 25-hydroxy vitamin D

(a) Reduced 25-hydroxy vitamin D – PTB <32 weeks



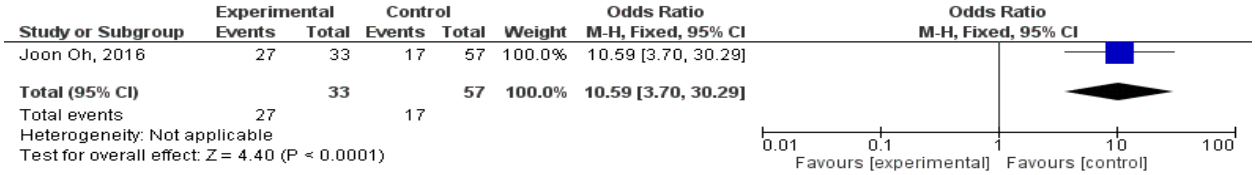
(b) Reduced 25-hydroxy vitamin D – PTB <37 weeks



Amniotic fluid markers

6. Amniotic fluid MMP-8

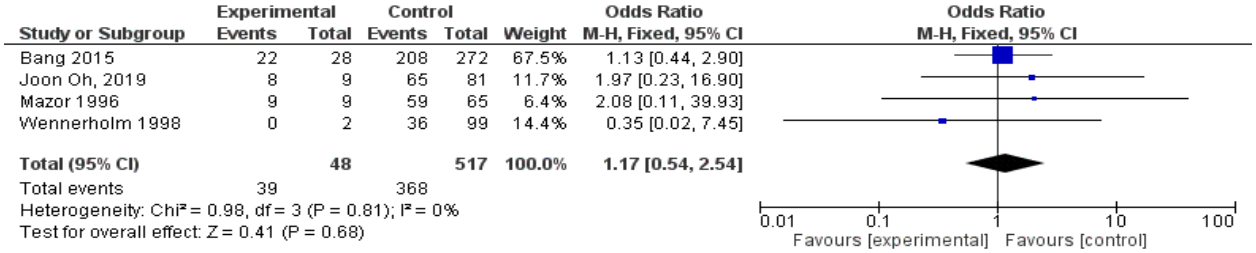
(a) MMP-8 positive status – delivery within 7 days of testing



Maternal infection

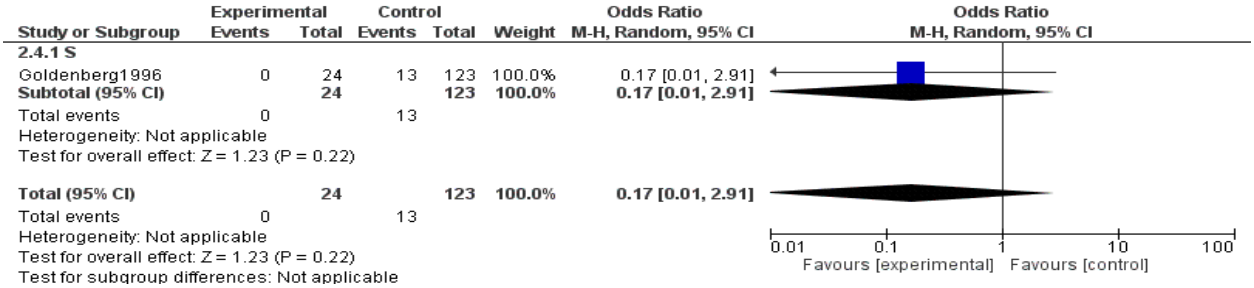
1. Intrauterine infection

(a) Confirmed intrauterine infection – PTB <37 weeks

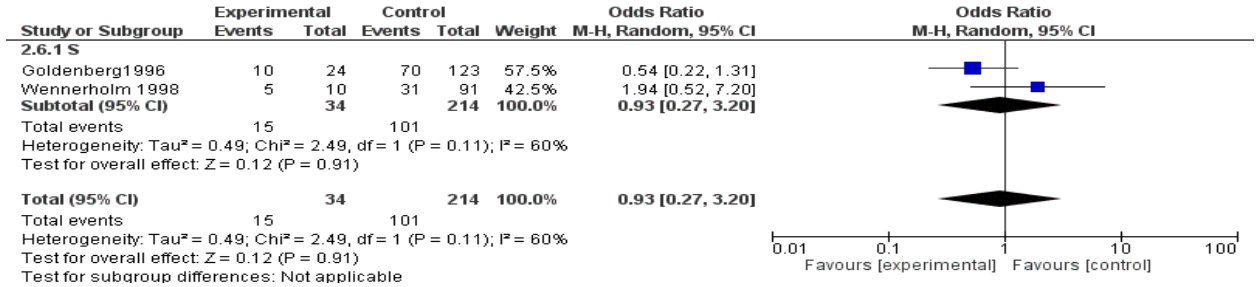


2. Bacterial vaginosis

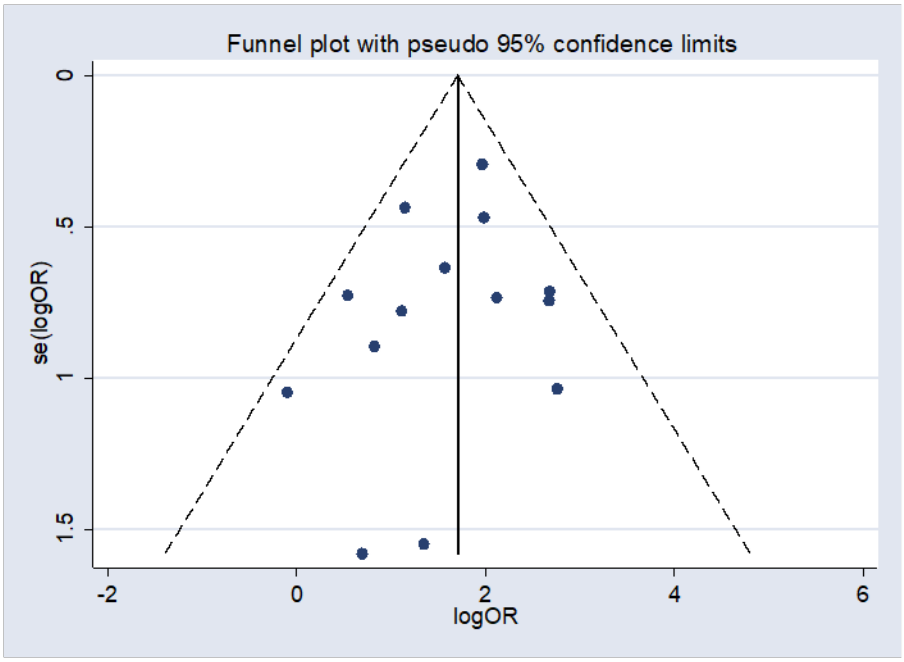
(a) Confirmed bacterial vaginosis – PTB <32 weeks



(b) Confirmed bacterial vaginosis – PTB <37 weeks



APPENDIX 11: Funnel plot for meta-analyses with more than 10 included studies in the systemic review of biochemical predictors of preterm birth in twin pregnancies.



FFN and PTB <37 weeks (symptomatic and asymptomatic)

p = 0.358

Funnel plot asymmetry was assessed using Egger's method. There was no evidence of small study effect for preterm birth <37 weeks and FFN in the symptomatic and asymptomatic group.

APPENDIX 12: Search strategy used in the systematic review of association between chorionicity and preterm birth in twin pregnancies.

1. Twin*.mp. Or exp Twins
2. "multiple pregnan$".mp.
3. Multiple pregnancy.mp. Or exp Pregnancy, Multiple
4. 1 or 2 or 3
5. Exp Obstetric Labor, Premature/ or exp Premature Birth
6. Premat*.mp.
7. Preterm*.mp.
8. 5 or 6 or 7
9. 4 and 8
10. Fibronectin.mp. Or exp Fibronectins
11. Estriol.mp. Or exp Estriol
12. Oestriol.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
13. 11 or 12
14. Home uterine activity monitor$.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
15. HUAM.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
16. Uterine contractions.mp. Or exp Uterine Contraction
17. 14 or 15 or 16
18. Interleukin$.mp. Or exp Interleukins
19. Cervical funnel*.mp.
20. Exp Uterine Cervical Incompetence/ or exp Cervical Length Measurement/ or cervical length.mp.
21. Exp Fetal Movement/ or fetal breathing movement.mp.

22. Fetal breath*.mp.
23. Fetal breath*.mp.
24. 21 or 22 or 23
25. Cervical assessment.mp.
26. Bishop score.mp.
27. Cervi* score.mp.
28. Vaginal exam*.mp.
29. Cervical digital exam*.mp.
30. 25 or 26 or 27 or 28 or 29
31. Serum biomarker.mp. Or exp Biological Markers
32. HCG.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
33. Insulin-like growth factor.mp. Or exp Somatomedins
34. Exp alpha-Fetoproteins/ or AFP.mp.
35. Exp C-Reactive Protein/ or CRP.mp.
36. Exp Corticotropin-Releasing Hormone/ or CRH.mp.
37. Exp Insulin-Like Growth Factor Binding Protein 1/ or exp Insulin-Like Growth Factor I/ or IGFPB.mp. Or exp Insulin-Like Growth Factor Binding Proteins
38. MMP.mp. Or exp Matrix Metalloproteinases
39. Relaxin.mp. Or exp Relaxin
40. Periodontal screen*.mp. Or exp Periodontitis
41. Exp Urinary Tract Infections/ or UTI.mp. Or exp Bacteriuria
42. Asymptomatic bacteriuria.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
43. MSU culture.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
44. Mid-stream urine.mp.
45. 41 or 42 or 43 or 44

46. Exp Premature Birth/ or exp Obstetric Labor, Premature
47. Exp Medical History Taking/ or patient history.mp.
48. Medical history.mp.
49. Past history.mp.
50. Previous history.mp.
51. 47 or 48 or 49 or 50
52. 46 and 51
53. Exp Abdomen/ and exp Palpation
54. Abdominal palpation.mp.
55. 53 or 54
56. Prolactin.mp. Or exp Prolactin
57. Rheobase.mp.
58. Mammary stimulation test.mp.
59. Mammary stim*.mp.
60. 58 or 59
61. Cervicovaginal glycoproteins.mp.
62. Glycoprotein.mp. Or exp Glycoproteins
63. Exp Hormones/ or endocrine hormone.mp.
64. Inflammatory markers.mp.
65. Bacterial vaginosis.mp. Or exp Vaginosis, Bacterial
66. 10 or 13 or 17 or 18 or 19 or 20 or 24 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 45 or 52 or 55 or 56 or 57 or 60 or 61 or 62 or 63 or 64 or 65
67. Exp Twins, Dizygotic/ or exp Twins, Monozygotic/ or chorionicity.mp.
68. Exp Ovulation Induction/ or exp Fertilization in Vitro/ or IVF.mp.
69. Method of conception.mp. Or exp Insemination, Artificial
70. Amniotic fluid.mp. Or exp Amniotic Fluid
71. Oocyte donation.mp. Or exp Oocyte Donation
72. PAPP-A.mp. Or exp Pregnancy-Associated Plasma Protein-A
73. Interpregnancy interval.mp.
74. Exp Birth Intervals/ or inter-pregnancy interval.mp.
75. 73 or 74

76. Vaginal bleed*.mp.
77. Antepartum hemorrhage.mp.
78. 76 or 77
79. Exp Crown-Rump Length/ or crown rump length discordance.mp.
80. CRL discordance.mp.
81. 79 or 80
82. Exp Nuchal Translucency Measurement/ or nuchal translucency discordance.mp.
83. VEGF.mp. Or exp Vascular Endothelial Growth Factor A
84. Fundal length.mp.
85. Fundal height.mp.
86. Exp Anthropometry
87. 84 or 85 or 86
88. Endotoxins.mp. Or exp Endotoxins
89. Exp Hispanic Americans/ or exp European Continental Ancestry Group/ or exp African Americans/ or maternal race.mp. Or exp African Continental Ancestry Group
90. Paternal race.mp.
91. Race.mp. Or exp Continental Population Groups
92. 89 or 90 or 91
93. Exp Socioeconomic Factors
94. Exp Educational Status/ or maternal education.mp.
95. Maternal status.mp.
96. Exp Body Mass Index/ or BMI.mp.
97. Smoking.mp. Or exp Smoking
98. Parity.mp. Or exp Parity
99. Fetal sex.mp.
100. Fetal sex.mp.
101. 99 or 100
102. Exp Progesterone/ or progesterone.mp.
103. Assisted reproductive technology.mp. Or exp Reproductive Techniques, Assisted
104. Marital status.mp. Or exp Marital Status
105. Prenatal care.mp. Or exp Prenatal Care

106. Exp Leptin/ or leptins.mp.
107. Exp Fetal Growth Retardation/ or discordant growth.mp.
108. Growth discordance.mp.
109. 67 or 68 or 69 or 70 or 71 or 72 or 75 or 78 or 81 or 82 or 83 or 87 or 88 or 92 or 93 or 94 or 95 or 96 or 97 or 98 or 101 or 102 or 103 or 104 or 105 or 106 or 107 or 108
110. 66 or 109
111. 9 and 110
112. and 1990:2019.(sa_year).

APPENDIX 13: Study characteristics in the systematic review of association between chorionicity and preterm birth in twin pregnancies.

No	Study, Year Country	Study design	Number of women	Inclusion period (Months)	Inclusion criteria	Exclusion criteria	Maternal ultrasound predictors	Outcome (preterm delivery)
1	**Adedayo, 2005** UK	Retrospective Cohort	179	84	All twins delivered between 24-34 weeks of gestation	Fetal aneuploidy, major congenital malformations, intrauterine demise of both twins, triplets, fetocide, embryo reduction, incomplete data sets	Monochorionicity	<30 weeks
2	**Alsam, 2010** Pakistan	Prospective Cohort	70	12	All cases of twin pregnancies presenting at first and second trimester	Twin pregnancies presenting at third trimester, all other cases of multiple pregnancies	Monochorionicity	<37 weeks
3	**Assuncao, 2010** Brazil	Retrospective Cohort	283	47	All twin deliveries >20 weeks	Incomplete data in records	Monochorionicity	<32 weeks <34 weeks <37 weeks
4	**Bamberg, 2012** Germany	Retrospective Cohort	1239	129	All consecutive twin pregnancies delivered at >24 weeks	Twin pregnancies after fetal reduction	Monochorionicity	<32 weeks <37 weeks
5	**Brizot, 2015** Brazil	Prospective Cohort	372	77	Naturally-conceived diamniotic twin pregnancies, no history of preterm delivery and gestational age 18-21+6 at random assignment, absence of major fetal anomalies, no allergies to progesterone or peanuts, absence of hepatic dysfunction, porphyria,	Subsequent diagnosis of major fetal anomaly and presence of ovular infection or loss to follow up	Monochorionic diamniotic	<32 weeks <34 weeks

					otosclerosis, malignant disease, severe depressive state, current or previous thromboembolic disease, uterine malformations and cervical cerclage			
6	**Burgess, 2014** USA	Retrospective Cohort	768	324	All dichorionic and monochorionic twins at ≥34 weeks gestation who were delivered at the Medical University of South Carolina from 1987-2010	Gestational age ,34 weeks, monoamnionicity, aneuploidy, fetal anomalies that require prolonged hospitalization or immediate surgery, co twin death at <34 weeks, unknown chorionicity	Monochorionicity	<37 weeks
7	**Carter, 2015** USA	Retrospective Cohort	1856	240	Patients with a confirmed viable twin gestation, all twin pregnancies undergoing routine 2nd trimester (15-22 weeks) ultrasound scan for anomaly survey	Fetal anomalies, TTTS, monoamnionicity, selective reduction	Monochorionicity	<28 weeks <34 weeks
8	**Choi, 2006** South Korea	Retrospective Cohort	537	111	All twin pregnancies >24 weeks	Not known	Monochorionicity	<30 weeks <34 weeks <37 weeks
9	**D'Antonio, 2017** UK	Retrospective Cohort	2948	120	All twin pregnancies booked for antenatal care in 9 hospitals in the STORK. All women up to 11 weeks of gestation registering for routine antenatal care	Pregnancies affected by TTTS, termination of pregnancy, fetal/chromosomal abnormality, pregnancies of unknown chorionicity, monochorionic monoamniotic pregnancies, higher order multiple gestations, pregnancies complicated by miscarriages occurring before 24 weeks,	Monochorionicity	<28 weeks <32 weeks <34 weeks

						those with double fetal loss at time of diagnosis, single intra uterine death		
10	**D'Arpe, 2014** Italy	Retrospective Cohort	196	63	Multiple pregnancies that delivered at the Department of Gynecology, Obstetrics and Urology of the University of Rome Sapienza	Gestational age <24 weeks at delivery and early or late miscarriages	Monochorionicity	<37 weeks
11	**Feng, 2018** China	Retrospective Cohort	559	59	Twin pregnancies	Presence of mental diseases, serious primary diseases with brain, heart, liver, kidney and haemopoietic system problems. Monamniotic twin pregnancy	Monochorionicity	<37 weeks
12	**Ferreira, 2005** USA	Retrospective Cohort	140	36	All twin deliveries	Lack of information about the type of placentation	Monochorionicity	<28 weeks <32 weeks <35 weeks
13	**Fichera, 2014** Italy	Prospective Cohort	197	24	Consecutive twin pregnancies	Indicated preterm delivery <34 weeks for maternal or fetal indications, cervical cerclage, Arabin cervical pessary insertions	Monochorionicity	<34 weeks
14	**Glinianaia, 2011** UK	Retrospective Cohort	3862	108	Twin pregnancies delivered during 1998-2007 to mothers resident in the former Northern region of England, non-malformed twins in pregnancies resulting in registered births of both twins	Spontaneous losses of one or both twins before 24 weeks due to congenital anomalies, termination of pregnancy	Monochorionicity	≤30 weeks ≤32 weeks ≤34 weeks

15	**Hack, 2006** Netherlands	Retrospective Cohort	603	372	Twin pregnancies recorded in Utrecht, Rotterdam and surrounding areas	All fetuses smaller than 500g, all fetuses delivered <24 weeks	Monochorionicity	<32 weeks <37 weeks
16	**Haghighi, 2013** Iran	Prospective Cohort	678	15	All women with a twin pregnancy without any contraindications to continuation of pregnancy, presenting to the prenatal clinic/ward	Deliveries < 26 weeks, intrauterine fetal death, previous history of preterm birth, presence of clinical infection, medical or fetal indications for delivery (pre-eclampsia, non-reassuring fetal status, intrauterine growth restriction)	Male-male sex Female-female sex Male-female sex Monochorionicity	<28 weeks <34 weeks <37 weeks
17	**Harper, 2012** USA	Retrospective Cohort	1145	Not known	Consecutive twin pregnancies undergoing ultrasound scan between 15-22 weeks at a single tertiary centre	Birth weight of either twin ≤10% on Alexander growth standard, TTTS, fetal anomalies, delivery at <24 weeks	Monochorionicity	<28 weeks <34 weeks <37 weeks
18	**Hediger, 2005** USA	Retrospective Cohort	1612	276	Delivery ≥ 28 weeks, both twins born alive, at least two ultrasound evaluations of fetal growth for estimation of growth rates from 20-28 weeks and /or 28 weeks to delivery	Delivery < 28 weeks, missing ultrasound evaluations of growth	Monochorionicity	<31 weeks <33 weeks <37 weeks
19	**Hernandez, 2012** USA	Retrospective Cohort	1863	149	All women with twin pregnancies who delivered live born neonates	Monamniotic pregnancies, TTTS, twin reversed arterial perfusion sequence, stillborn fetuses ≥24 weeks, fetal anomalies	Monochorionicity	≤32 weeks ≤34 weeks ≤36 weeks

20	**Hershkovitz, 2005** Israel	Retrospective Cohort	279	36	All twin pregnancies, maternal serum hCG determined between 16-18 weeks between 1998-2001	No prenatal care, unknown chorionicity, abnormal karyotype, elevated alfa Feto protein >2.5MoM, fetal malformations	Monochorionicity	<32 weeks 32-36 weeks <37 weeks
21	**Ho, 2005** Taiwan	Retrospective Cohort	159	24	All twin pregnancies delivered at 23 or more weeks of gestation including higher order multiples reduced to twins	Not mentioned	Monochorionicity	<34 weeks
22	**Johansen, 2013** Denmark	Retrospective Cohort	1993	36	In pregnancies with a chorionicity determination and two live fetuses identified at the time of nuchal translucency scan	Pregnancies with unknown chorionicity, monochorionic monoamniotic pregnancies, pregnancy with a known reduction from a higher number of multiples	Monochorionicity	<34 weeks
23	**Joon Oh, 2012** Korea	Prospective Cohort	190	78	Women with a viable twin gestation, cervical length >25 mm at 20-24 weeks, no history of prophylactic cerclage, intact amniotic membranes, absence of regular uterine contractions, no evidence of major fetal anomalies or suspected twin-to-twin transfusion syndrome or evidence of monoamniotic placenta or placenta previa, well documented obstetric dates	Spontaneous preterm delivery before follow-up measurement, loss to follow up, one fetal demise, incomplete data set	Monochorionicity	<32 weeks <34 weeks

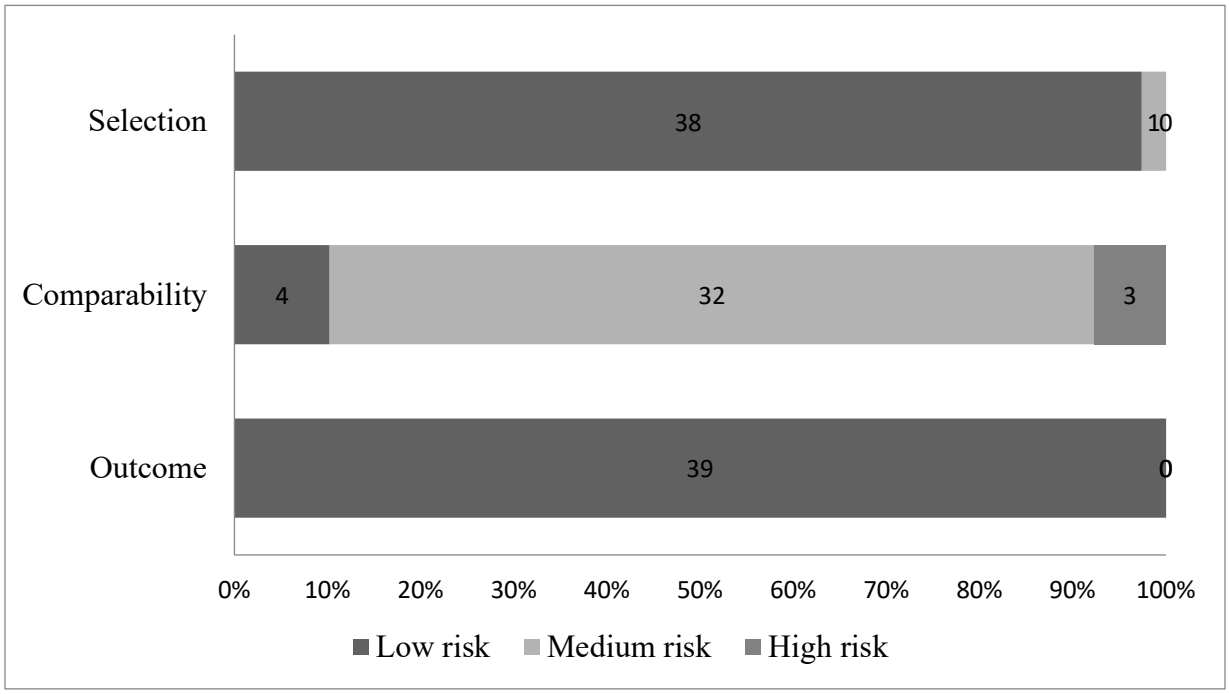
24	**Kosinska-Kaczynska, 2016** Poland	Retrospective Cohort	465	12	All twin deliveries in 2012 in five tertiary university obstetrics departments	Not mentioned	Monochorionicity	<28 weeks <30 weeks <32 weeks <34 weeks 34-36 weeks <37 weeks
25	**Lim, 2017** UK	Prospective Cohort	98	17	All twin mothers older than 16 years, chorionicity and gestation established on ultrasound before 16 weeks, recruited before 20 weeks of gestation, without known serious congenital abnormalities and intending to deliver at a single center	Positive for HIV, Hepatitis B or C, on progesterone therapy, gestational length of less than 26 weeks or fewer than 3 blood or saliva measurements	Monochorionicity	<34 weeks <37 weeks
26	**Machado, 2017** Portugal	Retrospective Cohort	540	120	All twins delivered at a level 3 hospital over 10 years	Monoamniotic twins	Monochorionicity	<37 weeks
27	**Manso, 2011** Portugal	Retrospective Cohort	504	120	Monochorionic and dichorionic twin pregnancies confirmed by ultrasound scan in 1st trimester	Monochorionic monoamniotic gestation	Monochorionicity	<28 weeks <32 weeks <34 weeks <37 weeks
28	**Masheer, 2015** Pakistan	Retrospective Cohort	391	153	Pregnant women with multiple pregnancies who were booked between 10-14 weeks of pregnancy, women where the outcome of pregnancy was known	Fetal aneuploidy, unknown chorionicity	Monochorionicity	Not known
29	**Michaluk, 2013** Canada	Retrospective Cohort	576	168	Women who had a singleton delivery immediately before their twin pregnancy	Having a twin or multiple birth in the immediate preceding delivery, iatrogenic preterm twin delivery for medical reasons (maternal or fetal) not encountered in a previous	Monochorionic diamniotic	<37 weeks

						singleton pregnancy, TTTS, fetal chromosomal or structural anomalies, fetal death of one twin, transfer from another hospital with incomplete medical data		
30	**Morcel, 2010** France	Retrospective Cohort	350	60	Twin pregnancies >22 weeks	Twin pregnancies reduced to singleton pregnancies; singleton deliveries complicated by early vanishing fetuses	Monochorionicity	<32 weeks <37 weeks
31	**Nunes, 2015** Portugal	Prospective Cohort	808	192	All twin pregnancies who delivered at the tertiary obstetrics centre over a period of 6 years	Gestations of more than 2 fetuses, monochorionic monoamniotic twin pregnancies, TTTS	Monochorionicity	<28 weeks ≤32 weeks ≤34 weeks ≤36 weeks
32	**Pagani, 2016** Italy	Retrospective Cohort	940	151	Twin pregnancies with cervical length measurement done by transvaginal sonography at 18-23 weeks	At least one fetus with structural or chromosomal abnormalities, higher order pregnancy, monoamniotic twins, pregnancy referred after 16 weeks, TTTS, those that required intrauterine therapy, pregnancies with indicated preterm birth, follow-up data unavailable	Monochorionicity	<32 weeks
33	**Rode, 2012** Denmark	Cohort nested in RCT	218	29	Women in the prevention of preterm delivery in twin gestations study, women with a live diamniotic twin pregnancy and chorionicity assessed by ultrasound scan before 16 weeks gestation.	Women with age <18 years, known allergy to progesterone or peanuts, history of hormone assocated thromboembolic disorders, rupture of membranes, treatment for or signs of TTTS, intentional fetal reduction, known	Monochorionicity	<34 weeks <37 weeks

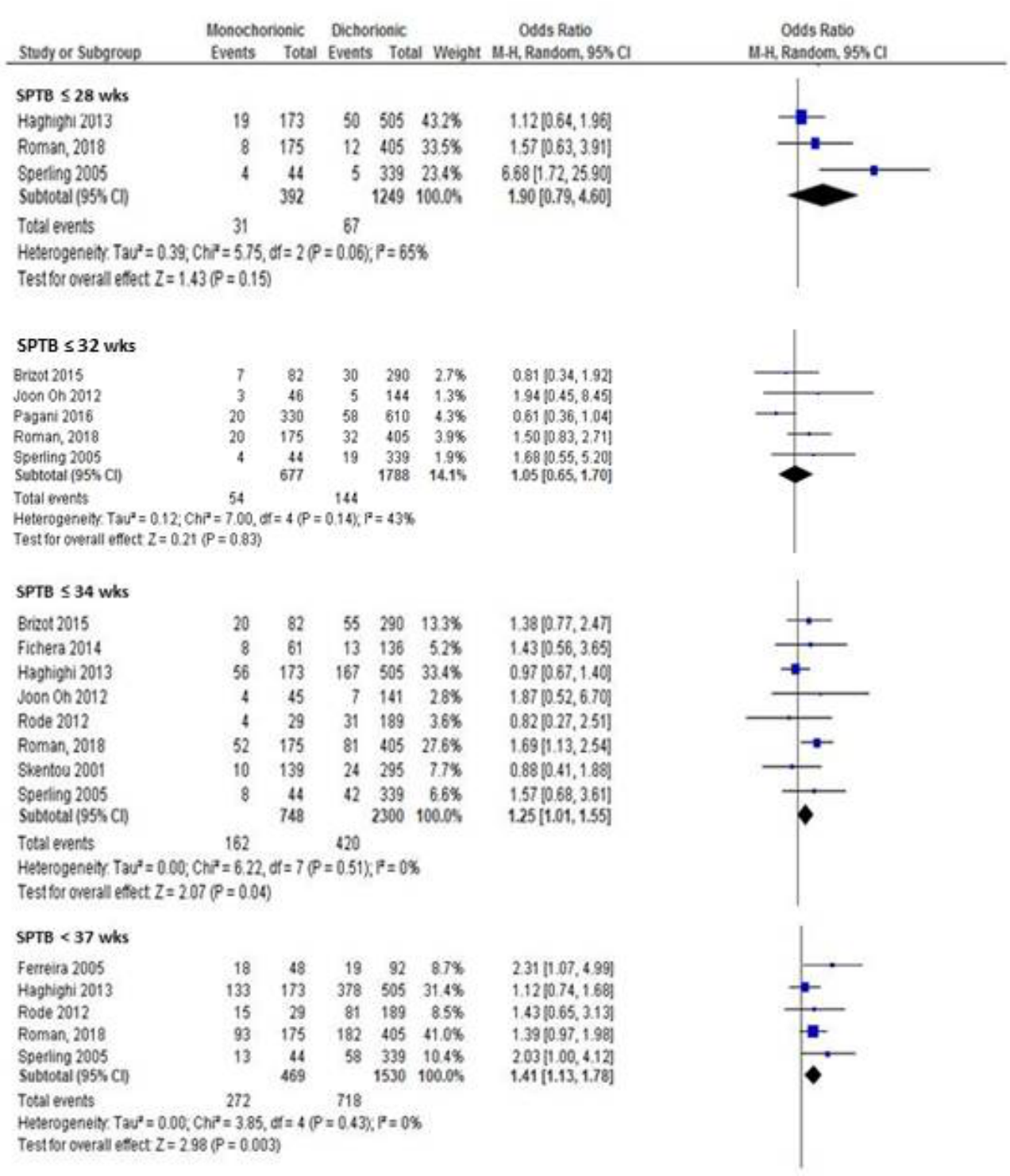
						major structural or chromosomal fetal abnormality, known or suspected malignancy in genitals or breasts, known liver disease, women with higher order multiple pregnancies, women who did not speak and understand Danish or German as appropriate.		
34	**Roman, 2018** Italy, USA	Retrospective Cohort	580	36	All consecutive asymptomatic twin pregnancies who underwent transvaginal sonography cervical length screening at relevant centres	Monoamniotic twins, twin pregnancies with TTTS, use of vaginal progesterone, pessary/cerclage in place,major fetal malformations oe genetic abnormalities at the time of transvaginal ultrasound cervical length measurement, fetal demise or selective reduction of any of the twins before delivery	Monochorionicity	<28 weeks <32 weeks <34 weeks <37 weeks
35	**Simoes, 2015** Portugal	Retrospective Cohort	345	244	Monochorionic and dichorionic twin pregnancies followed and delivered ≥24 weeks to mothers conceived by ART and spontaneously	Twin delivered but not followed up in the hospital. Monoamniotic pregnancies	Monochorionicity	<32 weeks <36 weeks
36	**Skentou, 2001** UK	Retrospective Cohort	434	Not mentioned	Women with twin pregnancies attending for the 23-week fetal anatomy and growth scan	Patients with no follow-up, iatrogenic delivery before 33 weeks, cervical suture placed for short cervix <2cm	Monochorionicity	<33 weeks
37	**Sperling, 2005**	Prospective Cohort	383	42	Twin pregnancies from 5 centres before 14+6 weeks of	Patients who underwent induction of labour, prior	Monochorionicity	<28 weeks <32weeks

	Denmark, Sweden				gestation after oral and written informed consent	cerclage, prior conization, monoamniotic twin pregnancies		<33 weeks <34 weeks <35 weeks
38	**Sun, 2016** China	Retrospective Cohort	1153	48	All twin pregnancies monitored prenatally and delivered at the Shanghai First Maternity Hospital between 2010-2014	TTTS, twin anaemia-polycythaemia sequence, twin reverse arterial perfusion sequence, selective IUGR type 2 or 3	Monochorionicity	<28 weeks <32 weeks
39	**Yu, 2002** UK	Prospective Cohort	351	27	All women with 2 live fetuses who had transvaginal scan to measure both cervical length and uterine artery Doppler	Major fetal anomaly, TTTS	Monochorionicity	<32 weeks

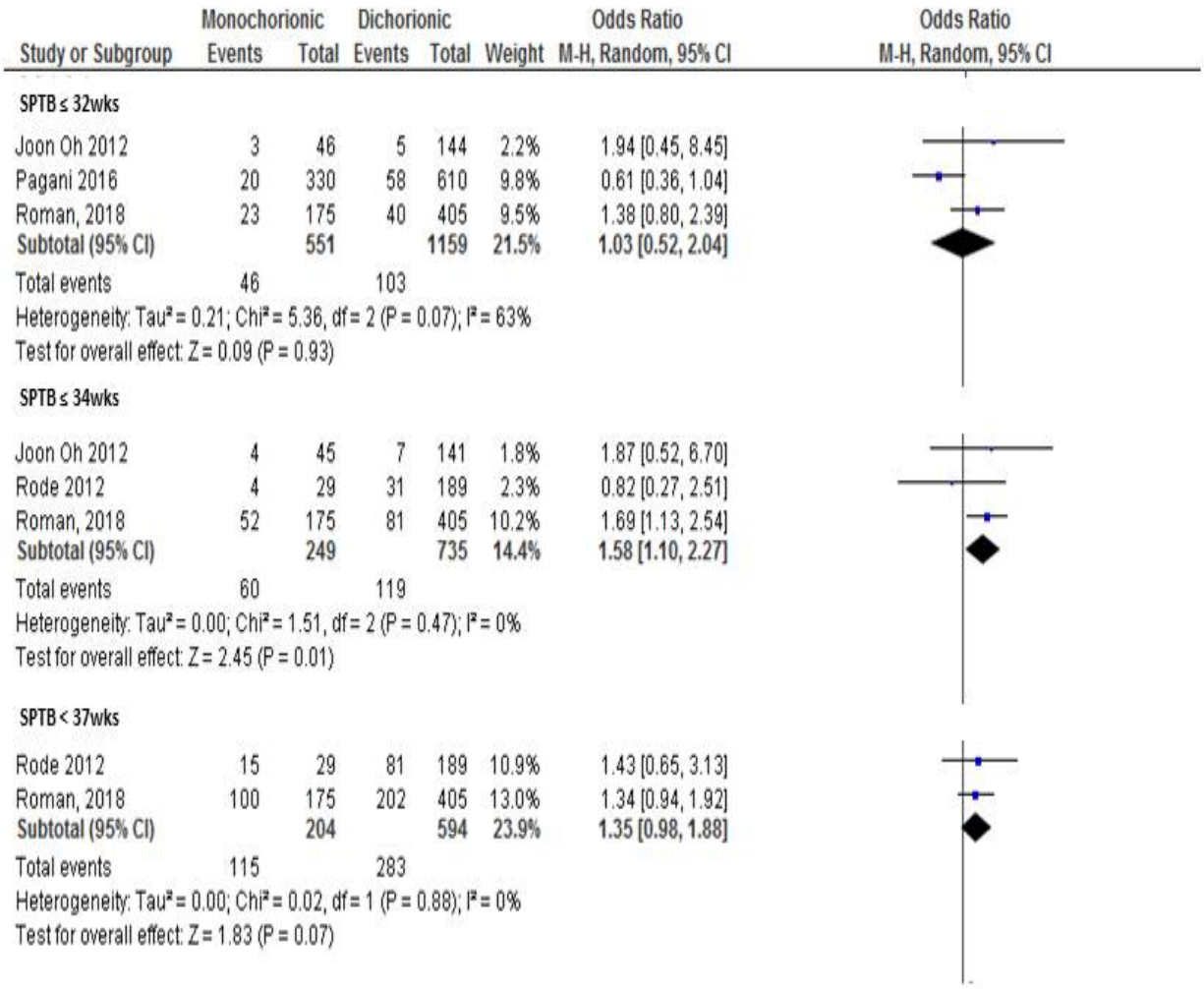
APPENDIX 14: Quality assessment using the Newcastle Ottawa Scale in the systemic review of association between chorionicity and preterm birth in twin pregnancies.



APPENDIX 15: Forest plots of pooled odds ratios (OR) for spontaneous preterm birth (SPTB) in the systemic review of association between chorionicity and preterm birth in twin pregnancies.

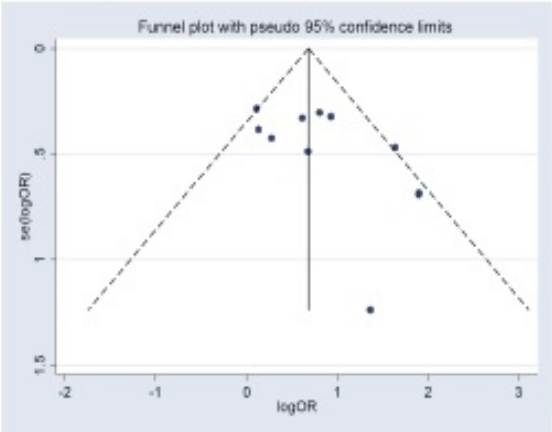


APPENDIX 16: Forest plots of pooled odds ratios (OR) for spontaneous preterm birth (SPTB) in studies excluding twin-twin transfusion syndrome in the systemic review of association between chorionicity and preterm birth in twin pregnancies.



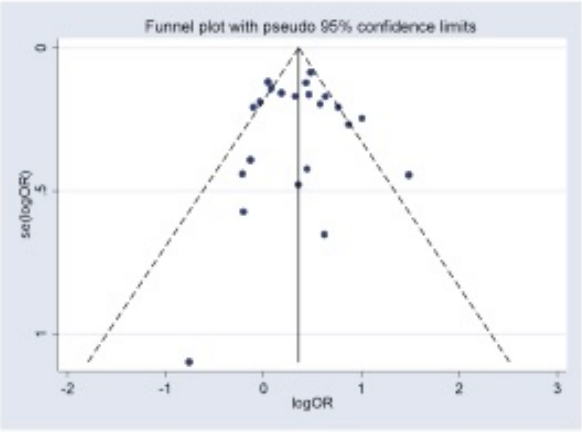
APPENDIX 17: Funnel plot for meta-analyses with more than 10 included studies in the systemic review of association between chorionicity and preterm birth in twin pregnancies.

Chorionicity and PTB ≤ 28 weeks



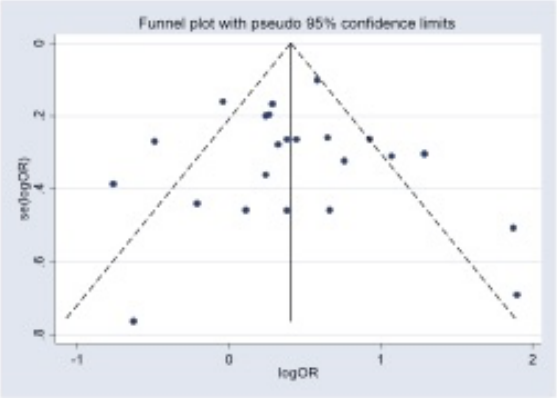
P = 0.066

Chorionicity and PTB ≤ 34 weeks



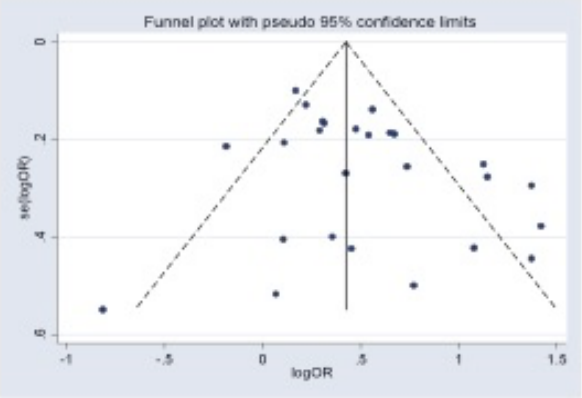
P = 0.948

Chorionicity and PTB ≤ 32 weeks



P = 0.837

Chorionicity and PTB < 37 weeks



P = 0.062

APPENDIX 18: Search strategy used in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

1. exp Twins/ or exp Twins, Monozygotic/ or exp Twins, Dizygotic/ or twins.mp.
2. twin$.mp.
3. multiple pregnan$.mp.
4. multiple pregnancy.mp. or exp Pregnancy, Multiple/ or exp Multiple Birth Offspring/
5. (multipl$ gestation or multifetal pregnancy or multifetal gestation).mp.
6. 1 or 2 or 3 or 4 or 5
7. Assisted reproductive techniques.mp. or exp Reproductive Techniques, Assisted/ or assisted reproductive technology.mp.
8. ART.mp.
9. Method of conception.mp. or exp Insemination, Artificial/ or infertility treatment$.mp.
10. infertility therap$.mp.
11. Intracytoplasmic Sperm Injection.mp. or exp Sperm Injections, Intracytoplasmic/
12. ICSI.mp.
13. Intracytoplasmic morphologically selected sperm injection.mp.
14. IMSI.mp.
15. (Gamete intrafallopian transfer or GIFT).mp.
16. exp Ovulation Induction/ or ovulation induction.mp.
17. in vitro fertilization.mp. or exp Fertilization in Vitro/ or IVF.mp.
18. ZIFT.mp. or exp Zygote Intrafallopian Transfer/
19. exp Embryo Culture Techniques/ or frozen embryo transfer.mp.
20. (spontaneous conception or Self-Fertilization).mp.
21. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
22. 6 and 21
23. exp Maternal Death/ or exp Maternal Health/ or exp Maternal Mortality/
24. (maternal morbidity or maternal complications or maternal outcomes or maternal mortality).mp.
25. (Prenatal outcomes or Prenatal morbidity or Prenatal mortality or Prenatal complications).mp.
26. exp Prenatal Care/ or exp Ultrasonography, Prenatal/ or exp Prenatal Diagnosis/ or exp Prenatal Injuries/
27. Antenatal complications.mp.
28. Postpartum complications.mp.
29. exp Obstetrics/
30. (Obstetric outcomes or Obstetric morbidity or Obstetric mortality).mp.
31. antepartum h$emorrhage.mp.
32. placenta previa.mp. or exp Placenta Previa/
33. placental abruption.mp. or exp Abruptio Placentae/
34. PROM.mp. or exp Fetal Membranes, Premature Rupture/
35. exp Fetal Membranes, Premature Rupture/ or pPROM.mp. or exp Obstetric Labor, Premature/
36. oligohydramnios.mp. or exp Oligohydramnios/
37. polyhydramnios.mp. or exp Polyhydramnios/

38. exp Cesarean Section/ or c$esarian section.mp. or C section.mp.
39. exp Postpartum Hemorrhage/ or PPH.mp. or postpartum haemorrhage.mp.
40. exp Fetal Growth Retardation/ or IUGR.mp. or fetal growth retardation.mp.
41. anemia in pregnancy.mp. or exp Pregnancy Complications, Hematologic/
42. (anaemia or iron deficiency).mp.
43. obstetric cholestasis.mp.
44. preterm birth.mp. or exp Premature Birth/
45. exp Hypertension, Pregnancy-Induced/ or PIH.mp. or hypertensive disorder.mp.
46. preeclampsia.mp. or exp Pre-Eclampsia/
47. HELLP syndrome.mp. or exp HELLP Syndrome/
48. exp Diabetes, Gestational/ or GDM.mp. or hyperglycemia in pregnancy.mp.
49. APH.mp.
50. 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49
51. exp Neonatal Abstinence Syndrome/ or exp Intensive Care, Neonatal/ or exp Neonatal Brachial Plexus Palsy/ or exp Neonatal Screening/ or exp Hyperbilirubinemia, Neonatal/ or exp Thrombocytopenia, Neonatal Alloimmune/ or exp Neonatal Nursing/ or exp Epilepsy, Benign Neonatal/ or exp Intensive Care Units, Neonatal/ or exp Anemia, Neonatal/ or exp Neonatal Sepsis/ or exp Jaundice, Neonatal/
52. (Neonatal outcomes or Neonatal morbidity or Neonatal mortality or Neonatal complications or neonatal death or offspring outcomes or offspring complications).mp.
53. (Perinatal outcomes or Perinatal morbidity or Perinatal mortality or Perinatal complications).mp.
54. exp Perinatal Death/ or exp Perinatal Care/ or exp Perinatal Mortality/
55. exp Child Health/ or exp Child/ or Child health outcomes.mp. or exp Infant, Newborn/
56. (Newborn morbidity or Newborn mortality).mp.
57. postnatal.mp. or exp Postnatal Care/
58. exp Pregnancy Outcome/ or exp Infant Mortality/ or stillbirth.mp. or exp Fetal Death/ or exp Stillbirth/
59. exp Infant, Small for Gestational Age/ or small for gestational age.mp.
60. intrauterine growth restriction.mp.
61. congenital malformation$.mp. or exp Congenital Abnormalities/
62. apgar score.mp. or exp Apgar Score/
63. Hypoxia-Ischemia, Brain/ or HIE.mp. or hypoxic ischemic encephalopathy.mp.
64. birth weight.mp. or exp Birth Weight/
65. gestational age.mp. or exp Gestational Age/
66. delivery.mp. or exp Delivery, Obstetric/
67. cord gas.mp. or Acidosis/
68. (pregnancy outcome or fetal outcome).mp.
69. exp Respiratory Distress Syndrome, Newborn/ or RDS.mp.
70. exp Respiration, Artificial/
71. exp Sepsis/ or Sepsis.mp. or exp Neonatal Sepsis/ or neonatal sepsis.mp.
72. intraventricular h$emorrhage.mp.
73. exp Cerebral Intraventricular Hemorrhage/ or IVH.mp.
74. exp Enterocolitis, Necrotizing/ or NEC.mp.
75. exp Hyperbilirubinemia, Neonatal/ or hyperbilirubinemia.mp. or Hyperbilirubinemia/

76. hypoglycemia.mp. or exp Hypoglycemia/
77. neurolog$ complications.mp.
78. exp Fetofetal Transfusion/ or Fetofetal Transfusion.mp.
79. twin to twin transfusion syndrome.mp.
80. transient tachypnoea of newborn.mp.
81. composite morbidity.mp.
82. 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81
83. Premature labor.mp. or exp Obstetric Labor, Premature/
84. premature birth.mp. or exp Premature Birth/
85. exp Infant, Premature/
86. (Premat* or Preterm*).mp.
87. 83 or 84 or 85 or 86
88. limit 22 to yr="1990 -Current"
89. 50 or 82 or 87
90. 88 and 89

APPENDIX 19: Study characteristics in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

No	Author, Year, Country	Study design	Type of ART	Inclusion period (months)	Inclusion criteria	Exclusion criteria	Pregnancies	Maternal outcomes
01	**Adler-Levy, 2007** Israel	Retrospective cohort	IVF, ICSI	168	All twin deliveries achieved by IVF, ovulation induction and spontaneously conceived twins, deliveries > 24 weeks of gestation	Vanishing twins	2365	Any preterm birth < 28 weeks, < 34 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placental abruption, caesarean delivery
02	**Agustsson, 1997** Iceland and Scotland	Retrospective cohort	IVF (+ 1 case of IUI+ 1 case of GIFT)	48	All twin pregnancies, where delivery occurred after 16 completed weeks of pregnancy (≥112 days)		522	Caesarean delivery

03	**Algeri, 2018** Italy	Retrospective cohort	Autologous ART; IVF with or without ICSI. Heterologous ART; egg/ embryo donation	98	Nulliparous and pluriparous diamniotic twin pregnancies managed and delivered at the study centre during the study period	Triplets, twin pregnancies resulted from selected fetal reductions/ termination of pregnancy, monoamnionicity, lack of data on conception method, use of conception techniques including IUI, induction of ovulation, evidence of pregestational chronic disease at the first antenatal visit and any maternal pregnancy-related complications such as gestational hypertension pre-eclampsia, cholestasis, or GDM	360	Any preterm birth < 28 weeks, < 32 weeks, < 34 weeks, PPH, caesarean delivery
04	**Almonte, 2012** USA	Retrospective cohort	Type of ART not mentioned	24	All twin deliveries at a single institution	Twins reduced from a higher-order multiple	346	Any preterm birth <37 weeks
05	**Andrijasevic, 2014** Serbia	Prospective cohort	IVF, ICSI	36	Women with twin pregnancies who were conceived at the study clinic		431	Hypertensive disorders in pregnancy, diabetes mellitus in pregnancy, caesarean delivery,

06	**Antsaklis, 2013** Greece	Retrospective cohort	IVF, ICSI	360	Twin deliveries at ≥ 24 weeks of gestation during the study period		1959	Any preterm birth < 34 weeks, <37 weeks, caesarean delivery
07	**Barda, 2017** Israel	Retrospective cohort	IVF	77	All consecutive dichorionic diamniotic twins delivered after gestational age > 20 weeks in a single centre	Monochorionic twins	708	Any preterm birth < 28 weeks, < 32 weeks, < 34 weeks, <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placental abruption, PPH, caesarean delivery
08	**Baxi, 2008** India	Prospective cohort	IVF, ICSI	24	Twin pregnancies achieved by ART delivered in a private infertility clinic, twin pregnancies conceived naturally and delivered at the same obstetric unit during the study period	Selective fetal reduction, spontaneous fetal resorption	174	Any preterm birth <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, APH, caesarean delivery
09	**Bensdorp, 2016** Netherland	Retrospective cohort	IVF, ICSI	156	All primiparous women with twin offspring of the opposite sex, offspring each weighing at least 500g after 22 weeks of gestation	Same-sex twins	6694	Any preterm birth < 32 weeks, <37 weeks, spontaneous preterm birth < 32 weeks, <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, PPH, caesarean delivery

10	**Bernasko, 1997** USA	Retrospective cohort	IVF, GIFT	72	All twin pregnancies delivered after 20 weeks gestation	Women who underwent ovulation induction only, multifetal pregnancy reduction, selective fetal termination	384	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, caesarean delivery
11	**Bordi, 2017** Italy	Retrospective cohort	IVF, ICSI, egg or embryo donation	186	All ART and spontaneously conceived pregnancies ending by spontaneous delivery by 24 weeks of gestation	Spontaneous gestation with a discrepancy in menstrual and USS gestational age more than 7 days, triplet pregnancies reduced to twins, insufficient clinical data	1097	Any preterm birth < 32 weeks, spontaneous preterm birth < 32 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, PPH, caesarean delivery
12	**Boulet, 2008** USA	Retrospective cohort	Type of ART not mentioned	48	All ART and non-ART twin births of Massachusetts residents from 1997-2000	Maternal age < 20 years, less high school education, unmarried, public or no health insurance for prenatal care or labour and delivery, inadequate or no prenatal care, third-trimester prenatal care initiation	4175	Any preterm birth < 32 weeks, <37 weeks, placental abruption, caesarean delivery

13	**Bregar, 2016** Slovenia	Retrospective cohort	Type of ART not mentioned	132	Monochorionic diamniotic twin pregnancies and dichorionic twins conceived by ART delivered at > 22 weeks of gestation or when the foetus weighs > 500g during the study period	Monoamniotic twins	483	Any preterm birth < 32 weeks, <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
14	**Caserta, 2014** Italy	Retrospective cohort	IVF, ICSI	54	Dichorionic diamniotic twins conceived via conventional IVF and ICSI	Monochorionic twins, twin pregnancies reduced to singleton births, pregnancies conceived via OI, IUI, egg donation. Spontaneous pregnancies with discrepancies in menstrual and ultrasound gestational age estimates, history of hypertension or diabetes mellitus before pregnancy	345	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, anaemia in pregnancy, placental abruption, PPH, caesarean delivery
15	**Chen, 2019** China	Retrospective cohort	IVF, ICSI	10	Dizygotic twin pregnancies delivered after 28 weeks gestation and only those who conceived following IVF/ICSI treatment at the study hospital	Those conceived by other forms of ART than IVF/ICSI. Twin gestations obtained after natural abortion or fetal reduction in multiple pregnancies	470	Hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, PPH, caesarean delivery

16	**Choi, 2006** South Korea	Retrospective cohort	IVF	100	All twin pregnancies > 24 weeks gestation following IVF and spontaneous fertilisation		537	Any preterm birth < 32 weeks, < 34 weeks, <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, caesarean delivery,
17	**Couck, 2020** Belgium	Retrospective cohort	IVF, ICSI, Egg donation	200	Ongoing MCDA twin pregnancies in the first trimester during the study period	Women referred for invasive testing because of an anomaly, pregnancies resulting from ovulation stimulation	654	Any preterm birth < 32 weeks, caesarean delivery
18	**Daniel, 2000** Israel	Retrospective cohort	IVF, ICSI	24	All twin pregnancies delivered at > 24 weeks gestation	Higher-order multiple pregnancies with or without intra uterine fetal demise, early vanishing twin pregnancies, twins reduced to singletons	297	Any preterm birth < 34 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, APH, PPH, caesarean delivery,
19	**Daskalakis, 2015** Greece	Retrospective cohort	Type of ART not mentioned	324	Dichorionic twin pregnancies conceived after ART or spontaneous conception which underwent chorionic villous sampling during the study period at the study hospital	Women having undergone embryo reduction, selective feticide; with fetal anatomic or chromosomal anomalies, with the demise of one twin at the time of the procedure, monochorionic twin pregnancy, cases that underwent a repeat	162	Any preterm birth < 28 weeks, < 32 weeks, <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy

						invasive procedure due to culture failure, incomplete data		
20	**Declercq, 2015** USA	Prospective cohort	Type of ART not mentioned	54	Twins with complete data in Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) and Pregnancy to Early Life Longitudinal (PELL) data systems		7248	Any preterm birth < 32 weeks, < 34 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
21	**Deltombe-Bodart, 2017** France	Retrospective cohort	IVF, ICSI	204	All twin births following infertility treatment	Twin pregnancies following egg donation, Cases of IUFD, TTTS, polymalformation syndrome	1561	Any preterm birth < 28 weeks, < 32 weeks, <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, PPH, caesarean delivery
22	**DeLuca, 2013** USA	Retrospective cohort	IVF	72	All infants of twin pregnancies > 20 weeks gestation who were born at the study hospital and delivered by a single maternal-fetal medicine practice	Incomplete data	378	Gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption

23	**Dhont, 1997** Belgium	Retrospective cohort	IVF, ICSI	60	Twins conceived after ART (IVF, ICSI), natural conception twins matched for maternal age, parity, zygosity, order of gestation	Higher-order multiples	115	Any preterm birth < 32 weeks, <37 weeks
24	**Dhont, 1999** Belgium	Retrospective cohort	IVF, ICSI	72	All twin pregnancies resulting in babies weighing > 500g conceived using ART (IVF/ICSI), natural conception matched for age, parity, fetal sex, order of gestation, the multiplicity of birth	Pregnancies resulting from OI, gestations of a higher order than twins	2482	Caesarean delivery
25	**Domingues, 2014** Portugal	Prospective cohort	IVF, ICSI	192	All twin pregnancies conceived spontaneously and those following an induction method (OI, IVF, ICSI)	Triplets and higher orders, monoamniotic multiple gestations	876	Any preterm birth < 32 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, anaemia in pregnancy, caesarean delivery

26	**Egic, 2014** Serbia	Retrospective cohort	IVF	48	All consecutive patients with a twin gestation who underwent routine second trimester (15-22 weeks) USS anatomical survey pregnancy control and gave birth from Jan 2010- Dec 2012	Monochorionic, Monoamniotic twins, IUFD at second trimester USS, twins with structural anomalies, Pregnancies resulting in selective reduction and incomplete outcome information	391	Any preterm birth < 28 weeks, < 34 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
27	**Eldar-Geva, 2014** Israel	Prospective cohort	IVF, ICSI	96	All pre-implantation genetic diagnosis pregnancies for which live births occurred during the study period. Comparison group 1: Twins born after consecutive ICSI treatment during the same time period to mothers matched for age, preconception BMI, and parity. Comparison group 2: Randomly selected twins born after spontaneous conception during the same time period to mothers matched for age and parity	Pregnancies from donated gametes, surgically retrieved sperm, live-born infants delivered from pregnancies with associated selective fetal reduction	204	Any preterm birth < 28 weeks, < 32 weeks, < 34 weeks, <37 weeks
28	**Erez,2008** Israel	Retrospective cohort	IVF	180	All twin deliveries > 24 weeks	Patients who lacked minimal prenatal care (< 3 visits to the prenatal clinic) and pregnancies complicated by congenital anomalies	2601	Any preterm birth < 34 weeks, <37 weeks

29	**Eskandar, 2007** Saudi Arabia	Prospective cohort	ICSI	24	ICSI conceived twins and spontaneously conceived twin pregnancies		108	Any preterm birth < 34 weeks, caesarean delivery
30	**Fedder, 2013** Denmark	Retrospective cohort	Conventional IVF. ICSI with epididymal, testicular or ejaculated sperm	180	Study - All Danish children born after ICSI with testicular or epididymal sperm. Control group - children conceived by ICSI with ejaculated sperm, IVF and natural conception.	Children born after transfer of frozen-thawed embryos	19906	Any preterm birth < 32 weeks, <37 weeks, caesarean delivery
31	**Feng, 2018** China	Retrospective cohort	IVF-ET	109	Twin pregnancies complicated with Intrahepatic cholestasis of pregnancy who were conceived natural or by IVF	Infertility factors except for tubal factor infertility, pregnancies after OI or artificial insemination, any of the causes of liver dysfunction: viral or autoimmune hepatitis, acute fatty liver of pregnancy, primary biliary cirrhosis, pre-eclampsia, haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, other hepatic imaging abnormalities	142	Any preterm birth <37 weeks, caesarean delivery

32	**Fichera, 2014** Italy	Prospective cohort	IVF	24	Consecutive twin pregnancies attending the clinic during the study period	Indicated preterm delivery < 34 weeks for maternal or fetal indications, cervical cerclage, Arabian pessary insertions	197	Any preterm birth < 34 weeks, spontaneous preterm birth < 34 weeks
33	**Geipel, 2001** Germany	Retrospective cohort	ICSI	54	Cases: All DCDA ICSI patients with colour Doppler studies of uterine artery at 18-24 weeks. Controls: Spontaneous DCDA twin pregnancies attending for routine examination. Patients with singletons and twins attending the routine examination.	All foetuses with malformations or other indications (suspected abnormality, growth retardation) than screening	64	Any preterm birth <37 weeks, pre-eclampsia, hypertensive disorders in pregnancy, placental abruption, caesarean delivery
34	**Geisler, 2014** Ireland	Retrospective cohort	IVF, ICSI, Oocyte donation	48	All viable DCDA twin pregnancies around 12 weeks gestation	Twin pregnancy complicated by early fetal loss (< 12 weeks gestation), 2nd-trimester loss of both twins, women conceived using IUI /OI, monochorionic twins	539	Any preterm birth < 28 weeks, < 32 weeks, < 34 weeks, <37 weeks, spontaneous preterm birth <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
35	**Gocmen, 2015** Turkey	Retrospective cohort	IVF	46	All twin pregnancies > 24 weeks gestation	Pregnancies resulting in singletons, triplets, deliveries < 24 weeks, pre-gestational diabetes, hypertension	84	Any preterm birth < 34 weeks, pre-eclampsia, hypertensive disorders in pregnancy, PPH

36	**Gui, 2019** China	Retrospective cohort	ICSI, fresh, frozen Embryo transfer	72	Non-smoking Han Chinese aged between 18- 35 years, BMI between 18-32 and delivery of at least one live foetus after 28 weeks. Pre-eclamptic and normotensive mothers who were matched for maternal age, pre-gestational BMI, gravidity and parity with the closest propensity score	Underlying diseases like SLE, APLS, PCOS, prior pre-eclampsia, pre-gestational diabetes and hypertension and chronic cardiac and nephritic disease	88	Any preterm birth < 34 weeks, <37 weeks, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, PPH, caesarean delivery
37	**Guilbaud, 2017** France	Retrospective cohort	IVF with autologous and donor oocytes	58	All women with twin pregnancies who gave birth after 24 weeks of gestation	Monochorionic, monoamniotic pregnancy, women transferred during pregnancy from another maternity unit due to maternal or fetal disease	672	Any preterm birth < 28 weeks, < 32 weeks, <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placental abruption, PPH, caesarean delivery
38	**Guney, 2006** Turkey	Retrospective cohort	IVF	84	All twin pregnancies delivered at the study clinic	Miscarriages < 24 weeks, Birth weight < 500g, pre-existing hypertension, pre GDM, renal disease and ICSI pregnancies	156	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, caesarean delivery

39	**Haghighi, 2013** Iran	Prospective cohort	Type of ART not mentioned	14	All women with a twin pregnancy to the prenatal clinic/ward following spontaneous or ART conception	Deliveries <26 weeks, IUFD, previous history of PTB, presence of clinical infection, medical or fetal indications for delivery (pre-eclampsia, non-reassuring fetal status, IUGR)	678	Any preterm birth <37 weeks
40	**Hansen, 2009** Australia	Retrospective cohort	IVF, ICSI, GIFT	84	Children born as twins up to the age of 3 years following ART or spontaneous conception	Aboriginal children	2398	Any preterm birth <37 weeks, < 32 weeks, caesarean delivery
41	**Ho, 2005** Taiwan	Retrospective cohort	IVF-ET, tubal embryo transfer	24	All twin pregnancies delivered at 23 or more weeks of gestation, including higher-order multiples reduced to twin		159	Any preterm birth < 34 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, APH, caesarean delivery
42	**Huang, 2006** Taiwan	Retrospective cohort	IVF, ICSI-ET	120	Twin births delivered at Taipei Medical University Hospital during 1992-2001	All patients with any history of hypertension, diabetes mellitus, abortions, foetuses with a birth age of < 24 gestational weeks, higher-order multiples, cases with incomplete data	194	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, PPH, caesarean delivery

43	**Isaksson, 2002** Finland	Retrospective cohort	IVF, ICSI, Frozen Embryo Transfer	75	Pregnancies achieved after IVF/ICSI in women with unexplained infertility ending with birth. Comparison group 1: women with non-assisted pregnancy, group 2: all women delivering after IVF, ICSI, frozen ET	Spontaneous abortion, triplet pregnancies	596	Any preterm birth <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, diabetes mellitus in pregnancy, placenta previa, placental abruption, caesarean delivery
44	**Joy, 2008** Ireland	Retrospective cohort	IVF, ICSI	24	All twin births with available records	Births conceived after ovulation induction	202	Any preterm birth < 32 weeks, gestational hypertension, hypertensive disorders in pregnancy, APH, caesarean delivery
45	**Kallen, 2010** Sweden	Retrospective cohort	IVF, ICSI	276	Cases: Complete dizygotic twin pairs born after IVF/ICSI. Controls: Dizygotic twins where no information on the presence of IVF existed	Twins with incomplete data	10220	Any preterm birth < 32 weeks, <37 weeks
46	**Kathiresan, 2010** USA	Retrospective cohort	IVF	84	Twin gestations conceived spontaneously and by IVF		1049	Any preterm birth <37 weeks, GDM, diabetes mellitus in pregnancy, caesarean delivery

47	**Kim, 2015** South Korea	Retrospective cohort	IVF	120	Women with IVF and natural conception	Selective fetal reduction, MCMA twins, delayed interval delivery, no available data on conception method or chorionicity	1337	Any preterm birth < 32 weeks, < 34 weeks, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, caesarean delivery
48	**Koivurova, 2002** Finland	Retrospective cohort	IVF	72	IVF exposed children and unexposed naturally conceived controls (All births after 22 weeks or birth weight > 500g)		206	Any preterm birth < 32 weeks, <37 weeks
49	**Koudstaal, 2000** Netherland	Retrospective cohort	IVF	Not mentioned	Ongoing twin pregnancies > 16 weeks Pregnancy before the end of 1992 and obstetric care provided by the hospital where the IVF care was provided. Spontaneous twin pregnancies from the same hospital as the cases, maternal age maximum 3 years apart from IVF mothers	IVF pregnancy after transfer of frozen embryos, embryo reduction	192	Any preterm birth <37 weeks, caesarean delivery

50	**Lehnen, 2011** Germany	Retrospective cohort	IVF, ICSI	111	Twins conceived by spontaneous conception and ART that have been delivered in the ICU of the study hospital		379	Any preterm birth <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, caesarean delivery
51	**Lei, 2019** China	Retrospective cohort	IVF, ICSI, fresh or frozen Embryo transfer	36	Live newborns after 28th week of gestation	Donor oocytes/ sperm, embryo recipients, ovulation induction, women applied preimplantation genetic diagnosis	904	Any preterm birth <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, PPH
52	**Liang, 2002** China	Retrospective cohort	Type of ART not mentioned		Twin pregnancies conceived by ART and spontaneous conception		277	Any preterm birth <37 weeks, diabetes mellitus in pregnancy, caesarean delivery

53	**Luke, 2004** USA	Retrospective cohort	Type of ART not mentioned	144	Both twins born alive, > 24 weeks gestation, documented sexes and birth weights of both infants in the pair, absence of major congenital malformations, maternal height, pregravid weight, and at least 3 prenatal weights with the first at or before 20 weeks and the last within 1 week of delivery	Multiparous, reduced twins	953	Any preterm birth < 30 weeks, pre-eclampsia, hypertensive disorders in pregnancy
54	**Luke, 2017** USA	Retrospective cohort	IVF, ICSI	78	All live twin births of > 22 weeks gestation and > 350g birth weight of residents of Massachusetts		10352	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, APH, placenta previa, placental abruption, caesarean delivery
55	**Manoura, 2004** Greece	Retrospective cohort	IVF	103	Twin pregnancies conceived by IVF and spontaneous conception	Higher-order multiples, pregnancies conceived after OI, twin pregnancies reduced to a singleton at 10^{th} week, early loss of one twin (12^{th} week), uncontrolled	221	Any preterm birth < 28 weeks, < 34 weeks, <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, APH, caesarean delivery

						diabetes mellitus type 1, SLE		
56	**Michaluk, 2013** Canada	Retrospective cohort	IVF	168	Women who had a singleton delivery immediately before their twin pregnancy	Having twins / multiple births in the immediately preceding delivery, iatrogenic preterm twin delivery for medical reasons (maternal or fetal) not encountered in a previous singleton pregnancy, TTTS, fetal chromosomal or structural anomalies, fetal death of one twin, transfer from another hospital with incomplete medical data	576	Any preterm birth <37 weeks
57	**Mohammed, 2012** Doha Qatar	Retrospective cohort	IVF	120	All eligible cases of DCDA twin pregnancies	IUFD, BW < 500g, < 24 weeks at delivery, higher-order multiples, monochorionic twins, singletons complicated by early vanishing foetus, twins reduced to singletons, triplets reduced to twins	320	Any preterm birth < 28 weeks, < 34 weeks, <37 weeks, pre-eclampsia, hypertensive disorders in pregnancy, placenta previa, placental abruption, PPH, caesarean delivery

58	**Moini, 2012** Iran	Prospective cohort	IVF, ICSI	33	All DCDA twin pregnancies to nulliparous women referred < 14 weeks and delivering > 22 weeks.	Twin pregnancies following infertility treatment due to PCOS and uterine factor, those who had experienced OHSS during controlled ovarian hyperstimulation protocols, history of medical diseases and surgery on pelvic organs, height < 150 cm, smokers, non-Iranian race, pregnancies conceived by OI, IUI and selective fetal reduction, pregnancies with vanishing embryos	400	Any preterm birth <28 weeks, < 32 weeks, <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placental abruption, PPH, caesarean delivery
59	**Moise, 1998** Israel	Retrospective cohort	IVF	66	All IVF twins, dizygotic pairs of twins		60	Any preterm birth <37 weeks, caesarean delivery
60	**Nassar, 2003** Lebanon	Retrospective cohort	IVF	72	All twin pregnancies that were delivered at ≥ 25 weeks of gestation	Women who underwent OI only, multifetal pregnancy reduction, underlying maternal disease (HTN, pre GDM, renal disease)	168	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, caesarean delivery

61	**Nunes, 2015** Portugal	Retrospective cohort	Type of ART not mentioned		DCDA twins conceived following ART, DCDA twins conceived by spontaneous conception	Monochorionic twin pregnancies, higher-order multiple pregnancies, fetal reduction due to malformations, ovulation induction techniques, IUI and those with incomplete access to clinical records	285	Any preterm birth < 32 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
62	**Ochsenkuhn, 2003** Germany	Retrospective cohort	IVF, GIFT	60	All twin and singleton pregnancies after GIFT/ IVF with live-born infants at least 24 weeks with more than 499g birth weight. Next respective pregnancy with a live birth after spontaneous conception who were matched for gestational age, maternal age and parity		156	Any preterm birth <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, APH, caesarean delivery
63	**Oh, 2012** South Korea	Prospective cohort	IVF	77	Women with twin pregnancies attending ANC at 20-24 weeks, viable twin gestation, CL > 25mm at 20-24 weeks, no history of prophylactic cerclage, intact amniotic membranes, absence of regular uterine contractions, no major fetal anomalies, no suspected TTTS, no evidence of monoamniotic placenta, no placenta previa, well	Occurrence of spontaneous PTB before follow up, loss to follow up, one fetal demise, incomplete data set	190	Any preterm birth < 32 weeks, < 34 weeks, spontaneous preterm birth < 32 weeks, < 34 weeks

					documented obstetric data			
64	**Okby, 2018** Israel	Retrospective cohort	IVF	276	Diagnosis of pre-eclampsia in twin pregnancies conceived via IVF and spontaneous conception during the study period	Women suffering from chronic hypertension or gestational hypertension, pregnancies conceived after ovulation induction	314	Any preterm birth < 34 weeks, <37 weeks, GDM, diabetes mellitus in pregnancy, placental abruption, caesarean delivery
65	**Olivennes, 1996** France	Retrospective cohort	IVF-ET	66	Deliveries that occurred after 28 weeks of gestation following IVF	Twin pregnancies resulting from selective fetal reduction	318	Any preterm birth < 32 weeks, < 34 weeks, <37 weeks, hypertensive disorders in pregnancy, caesarean delivery
66	**Peterson, 1995** Denmark	Prospective cohort	IVF	49	All deliveries after IVF at the study clinic, all consecutive pregnancies to women with normal fertility and infertility patients with singleton pregnancies	Prenatal death following an uneventful pregnancy	32	Any preterm birth <37 weeks

67	**Pinborg, 2004** Denmark	Retrospective cohort	IVF, ICSI	12	All twin pregnancies born after 24 weeks completed		1017	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
68	**Pinborg, 2014** Denmark	Retrospective cohort	IVF, ICSI	180	All ART deliveries between 1995-2009 in Denmark	Deliveries conceived by oocyte donation	19941	Any preterm birth < 28 weeks, < 32 weeks, <37 weeks, caesarean delivery, placenta previa
69	**Pourali, 2016** Iran	Prospective cohort	long protocol	60	Women with DCDA twin pregnancies	History of underlying disease before gestation such as overt diabetes mellitus, chronic hypertension, autoimmune diseases, monochorionic twins, incomplete data	127	Any preterm birth <37 weeks, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, PPH
70	**Putterman, 2003** USA	Retrospective cohort	IVF	24	All twin gestations where two live neonates were delivered after 20 weeks	Higher-order gestations reduced to twins or twins that were delivered in a single live birth	195	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placental abruption, caesarean delivery

71	**Raposo, 2013** Portugal	Retrospective cohort	Type of ART not mentioned	192	All twin pregnancies delivered in a tertiary obstetric centre during the study period		182	Caesarean delivery
72	**Rhode, 2012** Denmark, Austria	Secondary analysis of prospective RCT	Type of ART not mentioned	29	Women with a live diamniotic twin pregnancy, with chorionicity assessed by ultrasound before 10 weeks gestation	Age < 18, known allergy to progesterone/ peanuts, history of hormone associates thromboembolic disorders, rupture of membranes, treatment or signs of TTTS, intentional fetal reduction on known major structural/ chromosomal anomalies, known or suspected malignancy in genitals or breasts, known liver disease, women with higher-order multiples	218	Any preterm birth <37 weeks, < 34 weeks
73	**Saccone, 2017** Italy	Retrospective cohort	IVF, ICSI, third party assisted ART	24	All consecutive asymptomatic twin pregnancies who had undergone TVU CL with normal, viable twins	Women who had undergone IUI and who received only medical treatment but not IVF, Surrogates and gestational carriers	668	Any preterm birth < 28 weeks, < 32 weeks, < 34 weeks, spontaneous preterm birth < 28 weeks, < 32 weeks, < 34 weeks

74	**Saygan-Karamursel, 2006** Turkey	Retrospective cohort	ICSI	59	All twin pregnancies delivered ≥ 24 weeks.	OI and IUI pregnancies	622	Any preterm birth < 32 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
75	**Simoes, 2015** Portugal	Retrospective cohort	IVF, ET	244	Monochorionic twin pregnancies followed up and delivered ≥ 24 weeks gestation to mothers conceived by ART and spontaneous conception	Twin gestations delivered but not followed up at the hospital of study, monoamniotic pregnancies	508	Any preterm birth < 32 weeks, <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, caesarean delivery
76	**Skrypchenko, 2016** Ukraine	Retrospective cohort	Type of ART not mentioned	12	Twin pregnancies conceived by ART and natural conception		51	Any preterm birth <37 weeks, caesarean delivery
77	**Smithers, 2003** Australia	Retrospective cohort	IVF, ICSI, GIFT	96	Mixed-sex twins (dizygotic twins), births from 20 weeks gestation or births weighing 400g or more if gestation is unknown		2581	Any preterm birth < 28 weeks, < 34 weeks, <37 weeks, hypertensive disorders in pregnancy, APH, placenta previa, caesarean delivery
78	**Sperling, 2005** Denmark, Sweden	Prospective cohort	IVF	42	Twin pregnancies from 5 centres before 14+6 weeks gestation	Women who underwent IOL, prior cerclage, prior conization, monoamniotic twin pregnancies	383	Any preterm birth < 28 weeks, < 32 weeks, < 34 weeks, spontaneous preterm birth < 28 weeks, < 32 weeks, < 34 weeks

79	**Sun, 2016** China	Retrospective cohort	IVF, ICSI	60	All twin pregnancies undergoing serial US examinations at the study hospital	Structural anomalies, fetal reduction, feticide or termination	1153	Any preterm birth < 28 weeks, < 32 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, PPH
80	**Suzuki, 2010** Japan	Retrospective cohort	IVF	96	Women with dichorionic twin pregnancies who delivered at < 32 weeks, Dichorionic twin pregnancies delivered at 37-40 weeks		269	Any preterm birth < 32 weeks
81	**Szymusik, 2012** Poland	Retrospective cohort	IVF	60	Cases - IVF twin pregnancies from 2005-2009. Controls: Spontaneous conception DCDA from 2005-2009	Monochorionic twins, TOP < 22 weeks	126	Any preterm birth < 28 weeks, < 32 weeks, < 34 weeks, <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy
82	**Tan, 1992** England	Retrospective cohort	IVF	132	British residents who delivered live born/still born babies at > 28 weeks resulting from IVF, All sequential deliveries to primiparous women from 2 NHS hospitals		146	Any preterm birth <37 weeks, spontaneous preterm birth <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, APH, placenta previa, caesarean delivery

83	**Vasario, 2010** Italy	Prospective cohort	IVF, ICSI	48	Patients referred to study centre < 14 weeks and delivered > 22 weeks, DCDA twins	Triplets, monochorionic twins, twin pregnancies deriving from heterologous IVF or ART other than IVF, pregnancies referred > 14 weeks, delivery < 22 weeks, twin pregnancies obtained after fetocides undertaken in triplet or multiple pregnancies	223	Any preterm birth < 28 weeks, < 32 weeks, <37 weeks, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, PPH, caesarean delivery
84	**Verstraelen, 2005** Belgium	Retrospective cohort	IVF, ICSI	323	All twins with one of the children weighing at least 500g	Incomplete data sets	4368	Any preterm birth <37 weeks, caesarean delivery
85	**Wang, 2018** Australia	Retrospective cohort	Type of ART not mentioned	48	Twins born > 20 weeks gestation or > 400g birth weight		9831	Any preterm birth <37 weeks, spontaneous preterm birth <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, APH, caesarean delivery

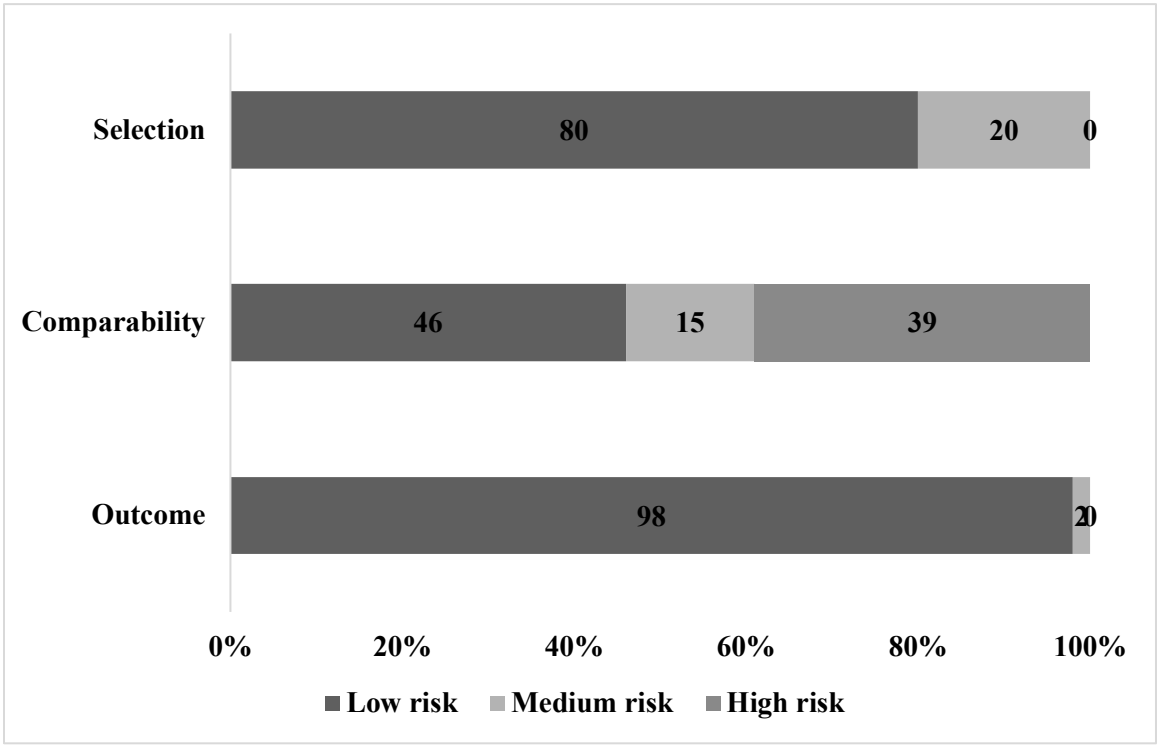
86	**Wennerholm, 1997** Sweden	Retrospective cohort	IVF, Cryopreserved fresh embryo transfer	61	Cases: Births conceived after IVF with cryopreserved-thawed embryos, all liveborn and stillborn infants after more than 28 weeks gestation. Controls: Group 1: Births after IVF with fresh embryos Group 2: Spontaneous pregnancies; Controls were matched according to maternal age, =/-5 years, parity, plurality and date of delivery	Parents who declined to participate	147	Any preterm birth < 28 weeks, < 32 weeks, <37 weeks, caesarean delivery
87	**Werder, 2013** USA	Retrospective cohort	Type of ART not mentioned	72	First twin pregnancies delivered ≥ 23 weeks of gestation		2532	Any preterm birth <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption
88	**Westergaard, 1999** Denmark	Retrospective cohort	IVF, ICSI	24	Cases: Births after IVF/ICSI, registered in Danish IVF registry. Controls: Births following non-ART conception matched by maternal age, child age, parity and multiplicity		854	Any preterm birth <37 weeks

89	**Yang, 2011** South Korea	Retrospective cohort	IVF	168	All dichorionic twin births after IVF or natural conception	IUFD, neonates with birth weight < 500g, or < 24 weeks gestation at delivery, higher order multiple pregnancies, singleton deliveries complicated by early vanishing foetuses, twin pregnancies reduced to singleton, triplet pregnancies reduced to twins	210	Any preterm birth < 32 weeks, < 34 weeks, <37 weeks, pre-eclampsia, hypertensive disorders in pregnancy, placenta previa, placental abruption
90	**Yang, 2014** China	Retrospective cohort	IVF, ICSI, frozen-thawed embryo transfer	12	Spontaneously conceived and ART pregnancies of 39 hospitals in 14 provinces of China	Infertility medication and treatments in which only sperms were handled in the laboratory (IUI)	1787	Any preterm birth <37 weeks, gestational hypertension, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption
91	**Zadori, 2003** Hungary	Retrospective cohort	IVF-ET	86	All pregnancies conceived spontaneously and by IVF-ET		72	Pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, PPH, caesarean delivery

92	**Zhang, 2008** China	Retrospective cohort	IVF-ET	76	Twin pregnancies conceived by IVF-ET and those conceived spontaneously		118	Any preterm birth <37 weeks, caesarean delivery
93	**Zhu, 2016** China	Retrospective cohort	IVF, ICSI	108	Live newborns after 28th week of gestation following ART and natural conception	Donor oocytes/sperms or embryo recipients, ovulation induction or women applied pre-implantation genetic diagnosis, chronic hypertension, diabetes, or heart disease, fetal anomalies	1071	Any preterm birth <37 weeks, gestational hypertension, pre-eclampsia, hypertensive disorders in pregnancy, GDM, diabetes mellitus in pregnancy, placenta previa, placental abruption, PPH

ART – Assisted Reproductive Techniques, IVF – In Vitro Fertilization, ICSI – Intra Cytoplasmic Sperm Injection, GIFT – Gamete Intra Fallopian Transfer, ZIFT – Zygote Intra Fallopian Transfer, ET – Embryo Transfer, OI – Ovulation Induction, IUI – Intra Uterine Insemination, IOL- Induction of Labour, TTTS – Twin-Twin Transfusion Syndrome, GDM- Gestational diabetes mellitus, APH- Antepartum haemorrhage, PPH- Postpartum haemorrhage, IUFD – Intra Uterine Fetal Demise, DCDA- Dichorionic Diamniotic, MCDA – Monochorionic Monoamniotic, PELL – Pregnancy to Early Life Longitudinal, SART CORS – Society of Assisted Reproductive Technology Clinical Outcomes Reporting System, PTB- Preterm Birth, TOP – Termination of Pregnancy, TVU– Transvaginal ultrasound, CL – Cervical length, USS- Ultrasonographic

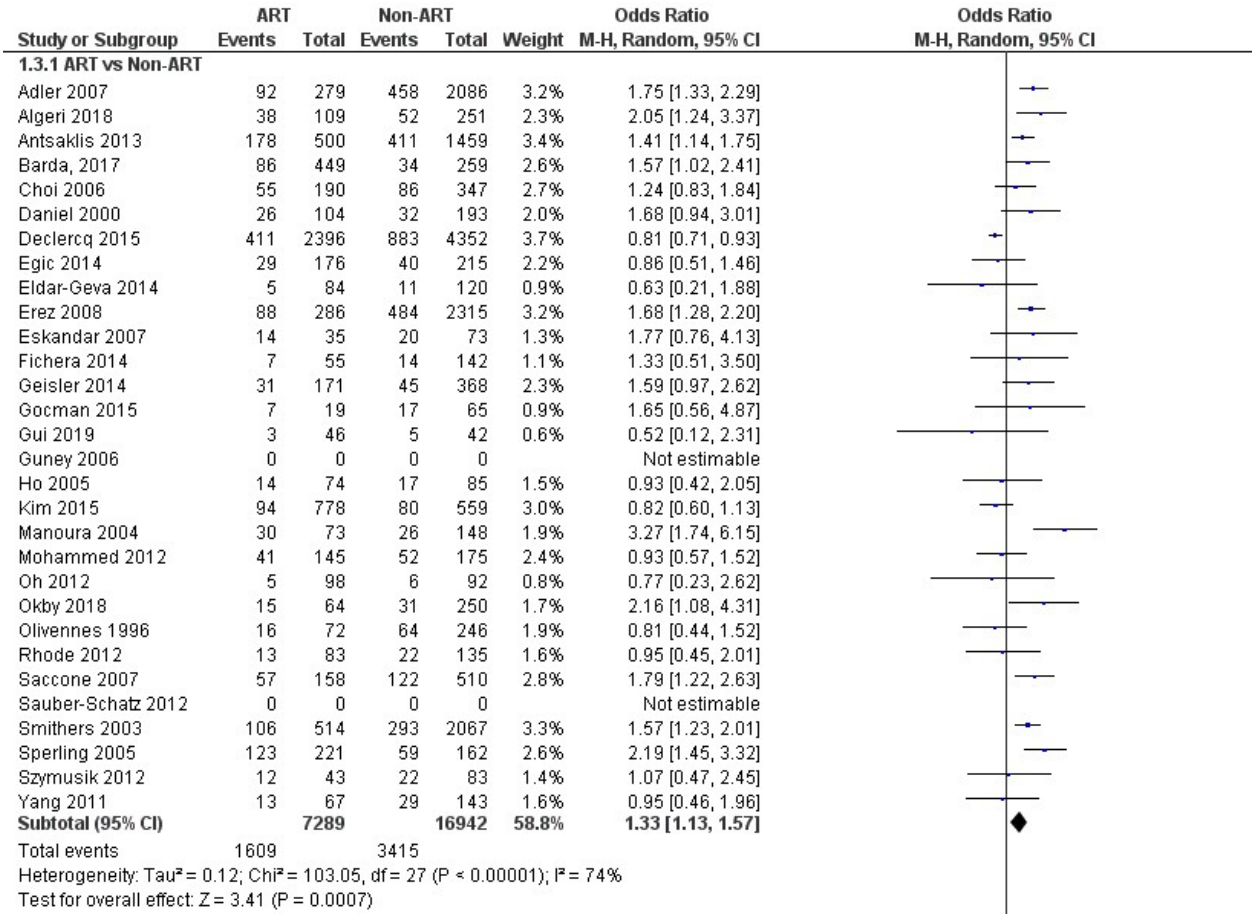
APPENDIX 20: Quality assessment using the Newcastle Ottawa Scale in the systemic review of maternal outcomes in twin pregnancies following assisted reproduction.



APPENDIX 21: Forest plots of pooled odds ratios (OR) for certain maternal outcomes comparing ART vs non-ART and ART vs natural conception in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

ART vs Non-ART

1. Preterm birth <34 weeks

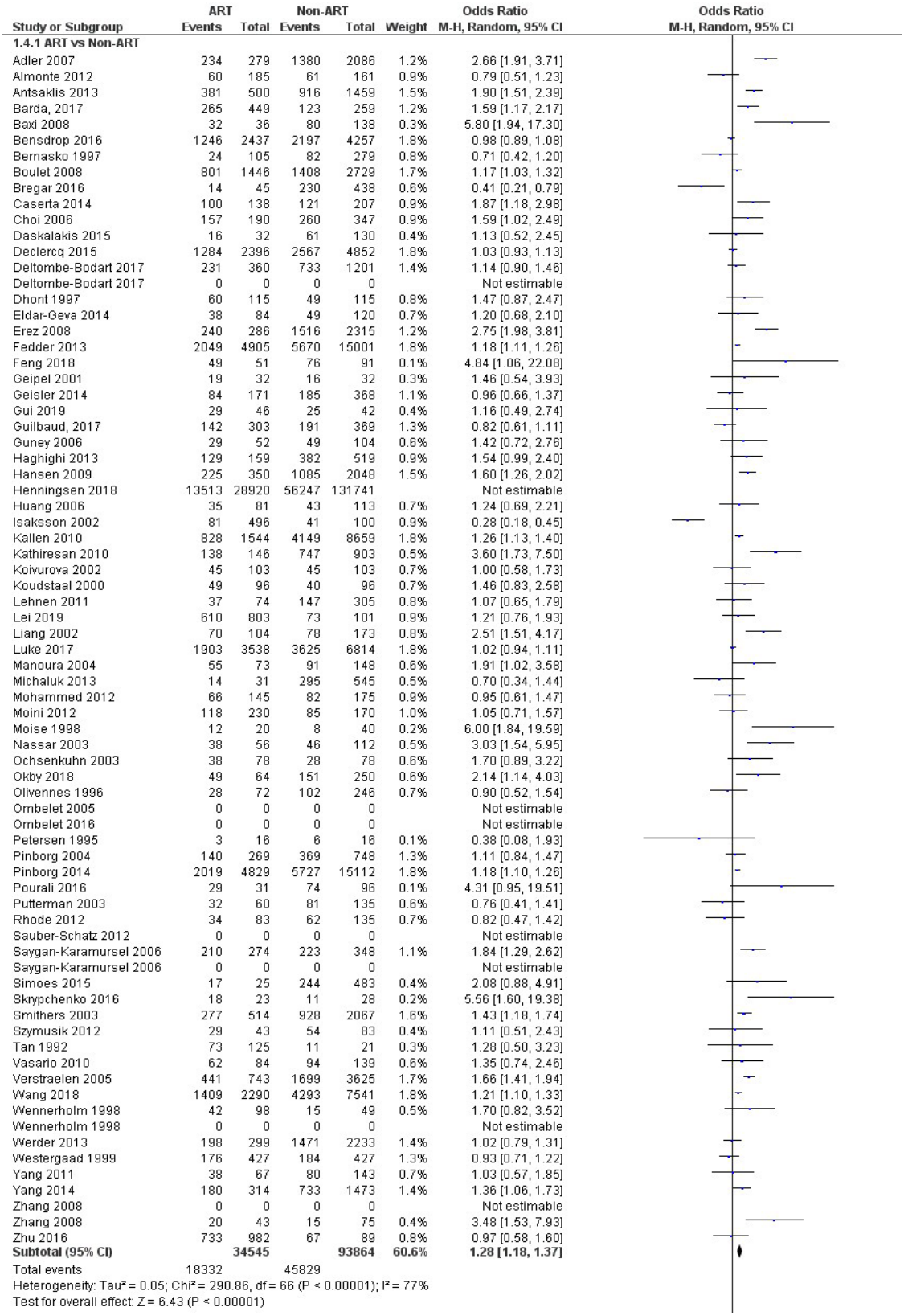


Study or Subgroup	ART Events	ART Total	Non-ART Events	Non-ART Total	Weight	Odds Ratio M-H, Random, 95% CI
1.3.1 ART vs Non-ART						
Adler 2007	92	279	458	2086	3.2%	1.75 [1.33, 2.29]
Algeri 2018	38	109	52	251	2.3%	2.05 [1.24, 3.37]
Antsaklis 2013	178	500	411	1459	3.4%	1.41 [1.14, 1.75]
Barda, 2017	86	449	34	259	2.6%	1.57 [1.02, 2.41]
Choi 2006	55	190	86	347	2.7%	1.24 [0.83, 1.84]
Daniel 2000	26	104	32	193	2.0%	1.68 [0.94, 3.01]
Declercq 2015	411	2396	883	4352	3.7%	0.81 [0.71, 0.93]
Egic 2014	29	176	40	215	2.2%	0.86 [0.51, 1.46]
Eldar-Geva 2014	5	84	11	120	0.9%	0.63 [0.21, 1.88]
Erez 2008	88	286	484	2315	3.2%	1.68 [1.28, 2.20]
Eskandar 2007	14	35	20	73	1.3%	1.77 [0.76, 4.13]
Fichera 2014	7	55	14	142	1.1%	1.33 [0.51, 3.50]
Geisler 2014	31	171	45	368	2.3%	1.59 [0.97, 2.62]
Gocman 2015	7	19	17	65	0.9%	1.65 [0.56, 4.87]
Gui 2019	3	46	5	42	0.6%	0.52 [0.12, 2.31]
Guney 2006	0	0	0	0		Not estimable
Ho 2005	14	74	17	85	1.5%	0.93 [0.42, 2.05]
Kim 2015	94	778	80	559	3.0%	0.82 [0.60, 1.13]
Manoura 2004	30	73	26	148	1.9%	3.27 [1.74, 6.15]
Mohammed 2012	41	145	52	175	2.4%	0.93 [0.57, 1.52]
Oh 2012	5	98	6	92	0.8%	0.77 [0.23, 2.62]
Okby 2018	15	64	31	250	1.7%	2.16 [1.08, 4.31]
Olivennes 1996	16	72	64	246	1.9%	0.81 [0.44, 1.52]
Rhode 2012	13	83	22	135	1.6%	0.95 [0.45, 2.01]
Saccone 2007	57	158	122	510	2.8%	1.79 [1.22, 2.63]
Sauber-Schatz 2012	0	0	0	0		Not estimable
Smithers 2003	106	514	293	2067	3.3%	1.57 [1.23, 2.01]
Sperling 2005	123	221	59	162	2.6%	2.19 [1.45, 3.32]
Szymusik 2012	12	43	22	83	1.4%	1.07 [0.47, 2.45]
Yang 2011	13	67	29	143	1.6%	0.95 [0.46, 1.96]
Subtotal (95% CI)		**7289**		**16942**	**58.8%**	**1.33 [1.13, 1.57]**
Total events	1609		3415			

Heterogeneity: Tau² = 0.12; Chi² = 103.05, df = 27 (P < 0.00001); I² = 74%

Test for overall effect: Z = 3.41 (P = 0.0007)

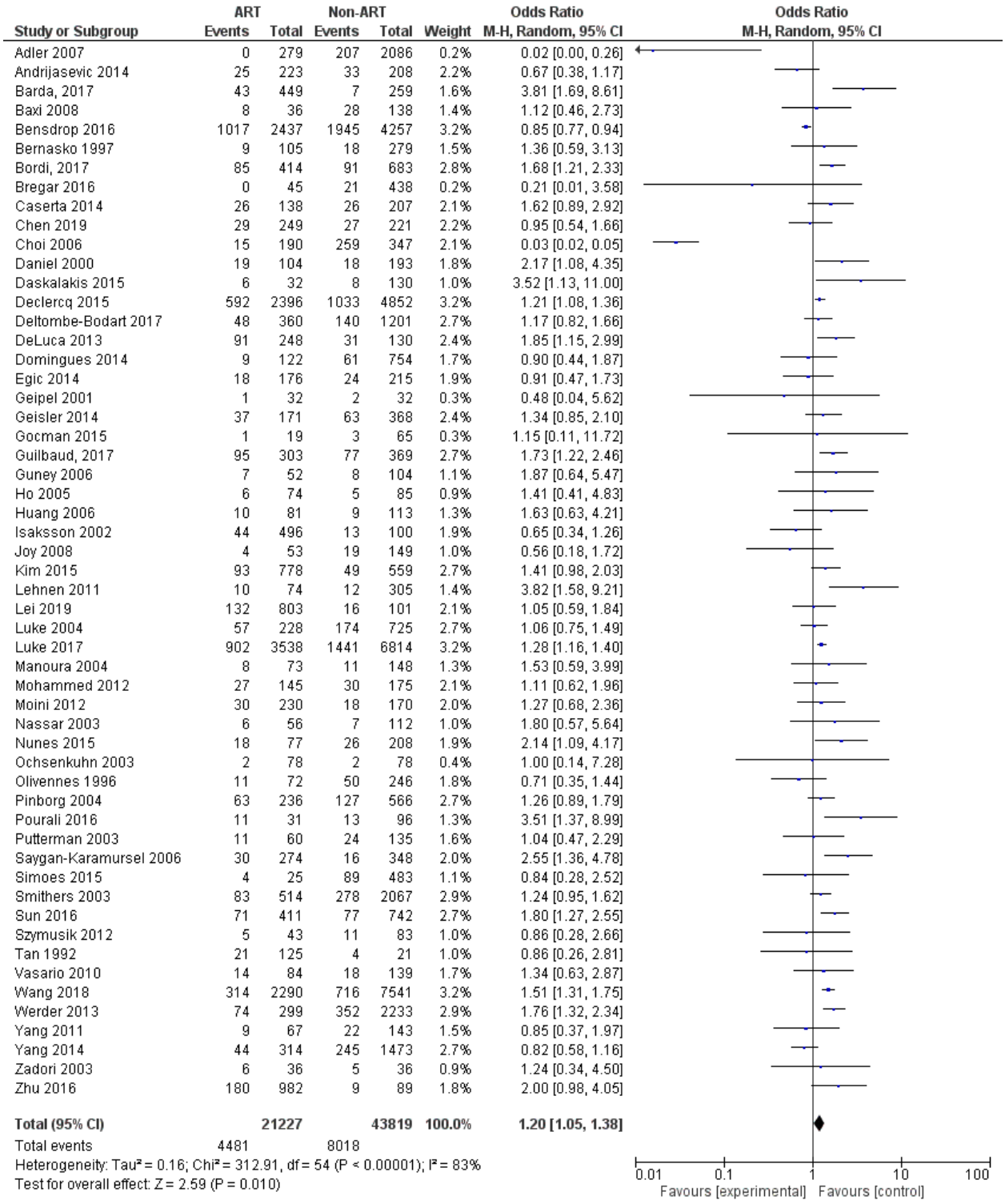
2. Preterm birth <37 weeks



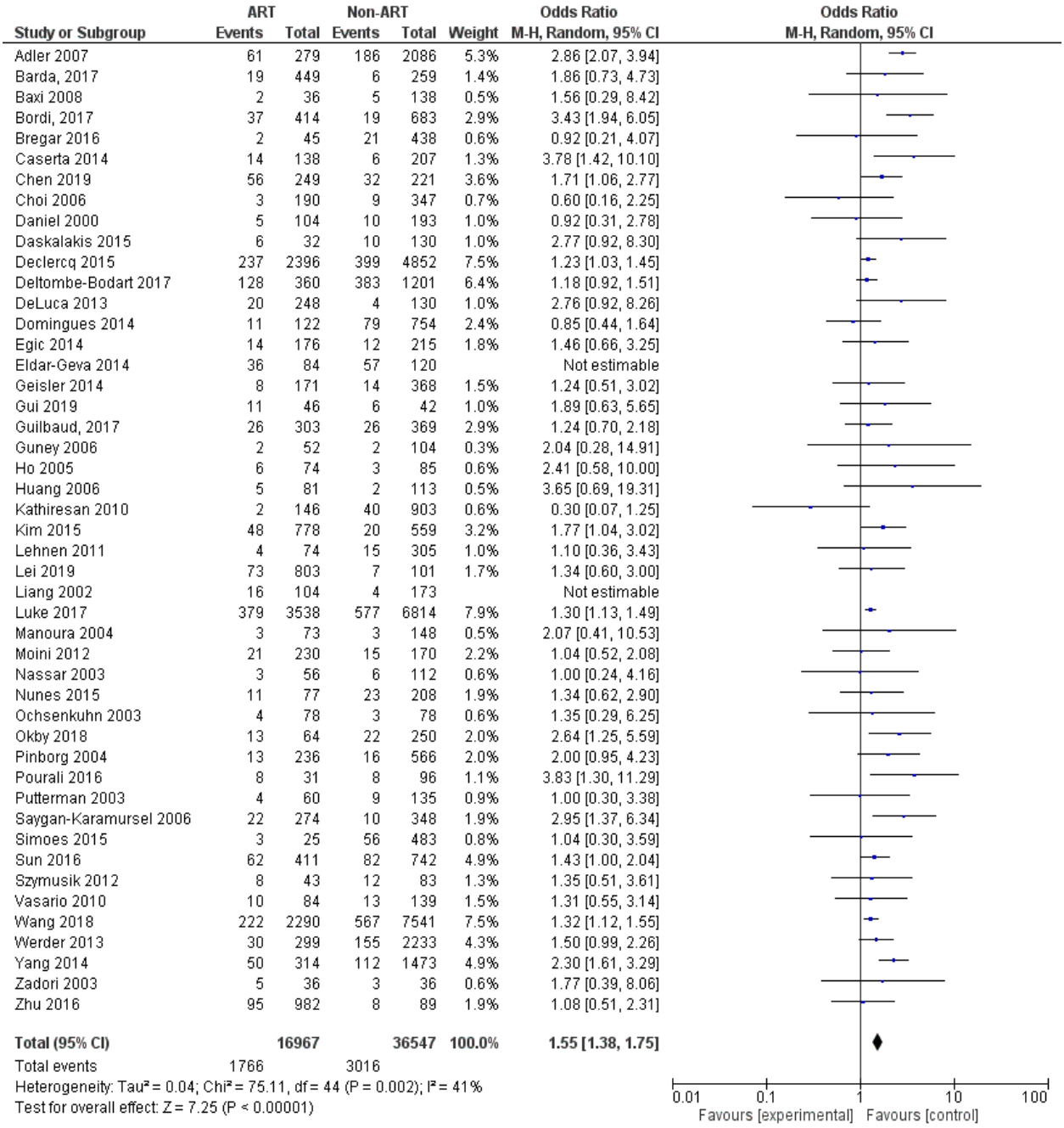
Study or Subgroup	ART Events	ART Total	Non-ART Events	Non-ART Total	Weight	Odds Ratio M-H, Random, 95% CI
1.4.1 ART vs Non-ART						
Adler 2007	234	279	1380	2086	1.2%	2.66 [1.91, 3.71]
Almonte 2012	60	185	61	161	0.9%	0.79 [0.51, 1.23]
Antsaklis 2013	381	500	916	1459	1.5%	1.90 [1.51, 2.39]
Barda, 2017	265	449	123	259	1.2%	1.59 [1.17, 2.17]
Baxi 2008	32	36	80	138	0.3%	5.80 [1.94, 17.30]
Bensdrop 2016	1246	2437	2197	4257	1.8%	0.98 [0.89, 1.08]
Bernasko 1997	24	105	82	279	0.8%	0.71 [0.42, 1.20]
Boulet 2008	801	1446	1408	2729	1.7%	1.17 [1.03, 1.32]
Bregar 2016	14	45	230	438	0.6%	0.41 [0.21, 0.79]
Caserta 2014	100	138	121	207	0.9%	1.87 [1.18, 2.98]
Choi 2006	157	190	260	347	0.9%	1.59 [1.02, 2.49]
Daskalakis 2015	16	32	61	130	0.4%	1.13 [0.52, 2.45]
Declercq 2015	1284	2396	2567	4852	1.8%	1.03 [0.93, 1.13]
Deltombe-Bodart 2017	231	360	733	1201	1.4%	1.14 [0.90, 1.46]
Deltombe-Bodart 2017	0	0	0	0		Not estimable
Dhont 1997	60	115	49	115	0.8%	1.47 [0.87, 2.47]
Eldar-Geva 2014	38	84	49	120	0.7%	1.20 [0.68, 2.10]
Erez 2008	240	286	1516	2315	1.2%	2.75 [1.98, 3.81]
Fedder 2013	2049	4905	5670	15001	1.8%	1.18 [1.11, 1.26]
Feng 2018	49	51	76	91	0.1%	4.84 [1.06, 22.08]
Geipel 2001	19	32	16	32	0.3%	1.46 [0.54, 3.93]
Geisler 2014	84	171	185	368	1.1%	0.96 [0.66, 1.37]
Gui 2019	29	46	25	42	0.4%	1.16 [0.49, 2.74]
Guilbaud, 2017	142	303	191	369	1.3%	0.82 [0.61, 1.11]
Guney 2006	29	52	49	104	0.6%	1.42 [0.72, 2.76]
Haghighi 2013	129	159	382	519	0.9%	1.54 [0.99, 2.40]
Hansen 2009	225	350	1085	2048	1.5%	1.60 [1.26, 2.02]
Henningsen 2018	13513	28920	56247	131741		Not estimable
Huang 2006	35	81	43	113	0.7%	1.24 [0.69, 2.21]
Isaksson 2002	81	496	41	100	0.9%	0.28 [0.18, 0.45]
Kallen 2010	828	1544	4149	8659	1.8%	1.26 [1.13, 1.40]
Kathiresan 2010	138	146	747	903	0.5%	3.60 [1.73, 7.50]
Koivurova 2002	45	103	45	103	0.7%	1.00 [0.58, 1.73]
Koudstaal 2000	49	96	40	96	0.7%	1.46 [0.83, 2.58]
Lehnen 2011	37	74	147	305	0.8%	1.07 [0.65, 1.79]
Lei 2019	610	803	73	101	0.9%	1.21 [0.76, 1.93]
Liang 2002	70	104	78	173	0.8%	2.51 [1.51, 4.17]
Luke 2017	1903	3538	3625	6814	1.8%	1.02 [0.94, 1.11]
Manoura 2004	55	73	91	148	0.6%	1.91 [1.02, 3.58]
Michaluk 2013	14	31	295	545	0.5%	0.70 [0.34, 1.44]
Mohammed 2012	66	145	82	175	0.9%	0.95 [0.61, 1.47]
Moini 2012	118	230	85	170	1.0%	1.05 [0.71, 1.57]
Moise 1998	12	20	8	40	0.2%	6.00 [1.84, 19.59]
Nassar 2003	38	56	46	112	0.5%	3.03 [1.54, 5.95]
Ochsenkuhn 2003	38	78	28	78	0.6%	1.70 [0.89, 3.22]
Okby 2018	49	64	151	250	0.6%	2.14 [1.14, 4.03]
Olivennes 1996	28	72	102	246	0.7%	0.90 [0.52, 1.54]
Ombelet 2005	0	0	0	0		Not estimable
Ombelet 2016	0	0	0	0		Not estimable
Petersen 1995	3	16	6	16	0.1%	0.38 [0.08, 1.93]
Pinborg 2004	140	269	369	748	1.3%	1.11 [0.84, 1.47]
Pinborg 2014	2019	4829	5727	15112	1.8%	1.18 [1.10, 1.26]
Pourali 2016	29	31	74	96	0.1%	4.31 [0.95, 19.51]
Putterman 2003	32	60	81	135	0.6%	0.76 [0.41, 1.41]
Rhode 2012	34	83	62	135	0.7%	0.82 [0.47, 1.42]
Sauber-Schatz 2012	0	0	0	0		Not estimable
Saygan-Karamursel 2006	210	274	223	348	1.1%	1.84 [1.29, 2.62]
Saygan-Karamursel 2006	0	0	0	0		Not estimable
Simoes 2015	17	25	244	483	0.4%	2.08 [0.88, 4.91]
Skrypchenko 2016	18	23	11	28	0.2%	5.56 [1.60, 19.38]
Smithers 2003	277	514	928	2067	1.6%	1.43 [1.18, 1.74]
Szymusik 2012	29	43	54	83	0.4%	1.11 [0.51, 2.43]
Tan 1992	73	125	11	21	0.3%	1.28 [0.50, 3.23]
Vasario 2010	62	84	94	139	0.6%	1.35 [0.74, 2.46]
Verstraelen 2005	441	743	1699	3625	1.7%	1.66 [1.41, 1.94]
Wang 2018	1409	2290	4293	7541	1.8%	1.21 [1.10, 1.33]
Wennerholm 1998	42	98	15	49	0.5%	1.70 [0.82, 3.52]
Wennerholm 1998	0	0	0	0		Not estimable
Werder 2013	198	299	1471	2233	1.4%	1.02 [0.79, 1.31]
Westergaad 1999	176	427	184	427	1.3%	0.93 [0.71, 1.22]
Yang 2011	38	67	80	143	0.7%	1.03 [0.57, 1.85]
Yang 2014	180	314	733	1473	1.4%	1.36 [1.06, 1.73]
Zhang 2008	0	0	0	0		Not estimable
Zhang 2008	20	43	15	75	0.4%	3.48 [1.53, 7.93]
Zhu 2016	733	982	67	89	0.8%	0.97 [0.58, 1.60]
Subtotal (95% CI)		**34545**		**93864**	**60.6%**	**1.28 [1.18, 1.37]**
Total events	18332		45829			

Heterogeneity: Tau² = 0.05; Chi² = 290.86, df = 66 (P < 0.00001); I² = 77%
Test for overall effect: Z = 6.43 (P < 0.00001)

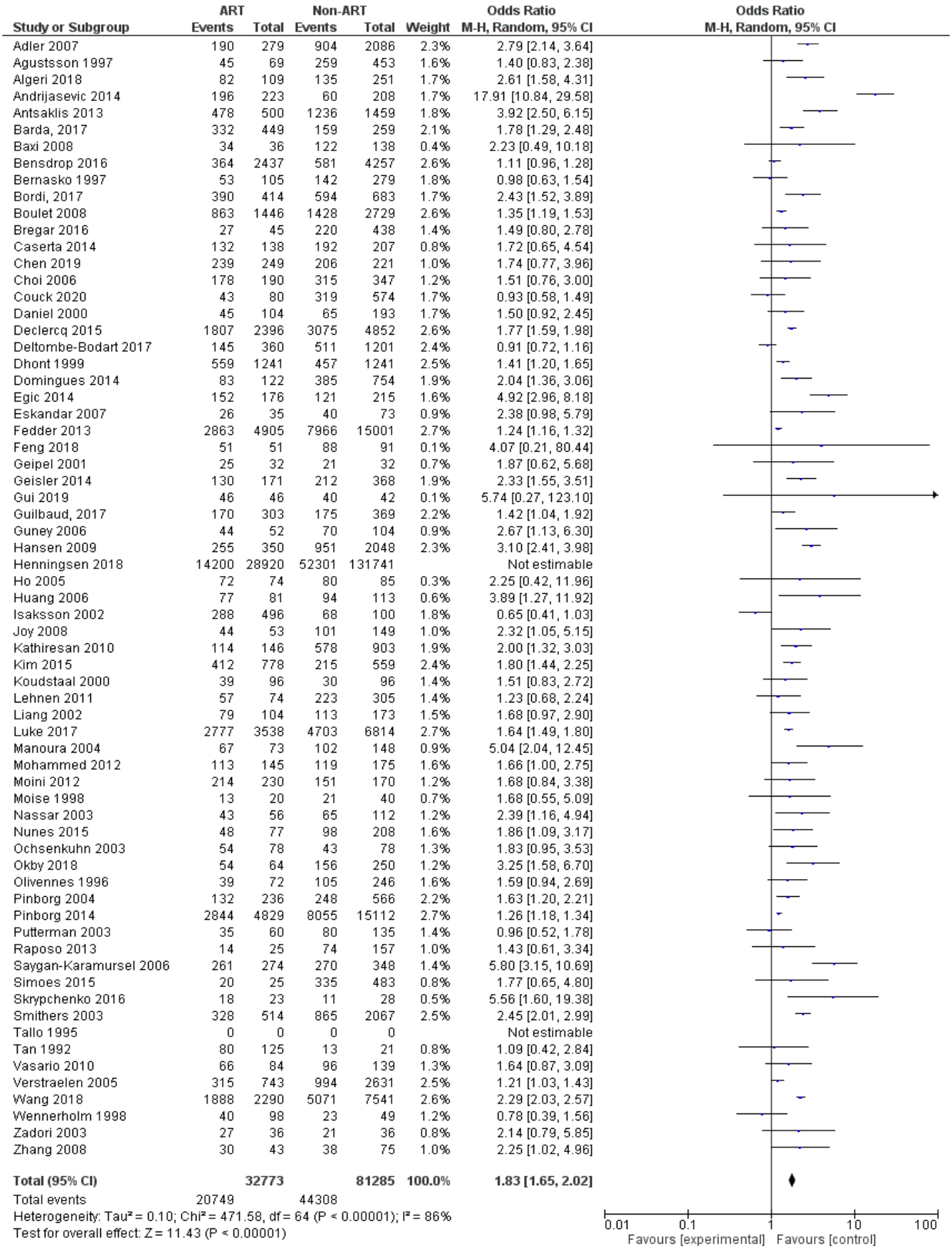
3. Hypertensive disorders in pregnancy



4. Gestational diabetes mellitus

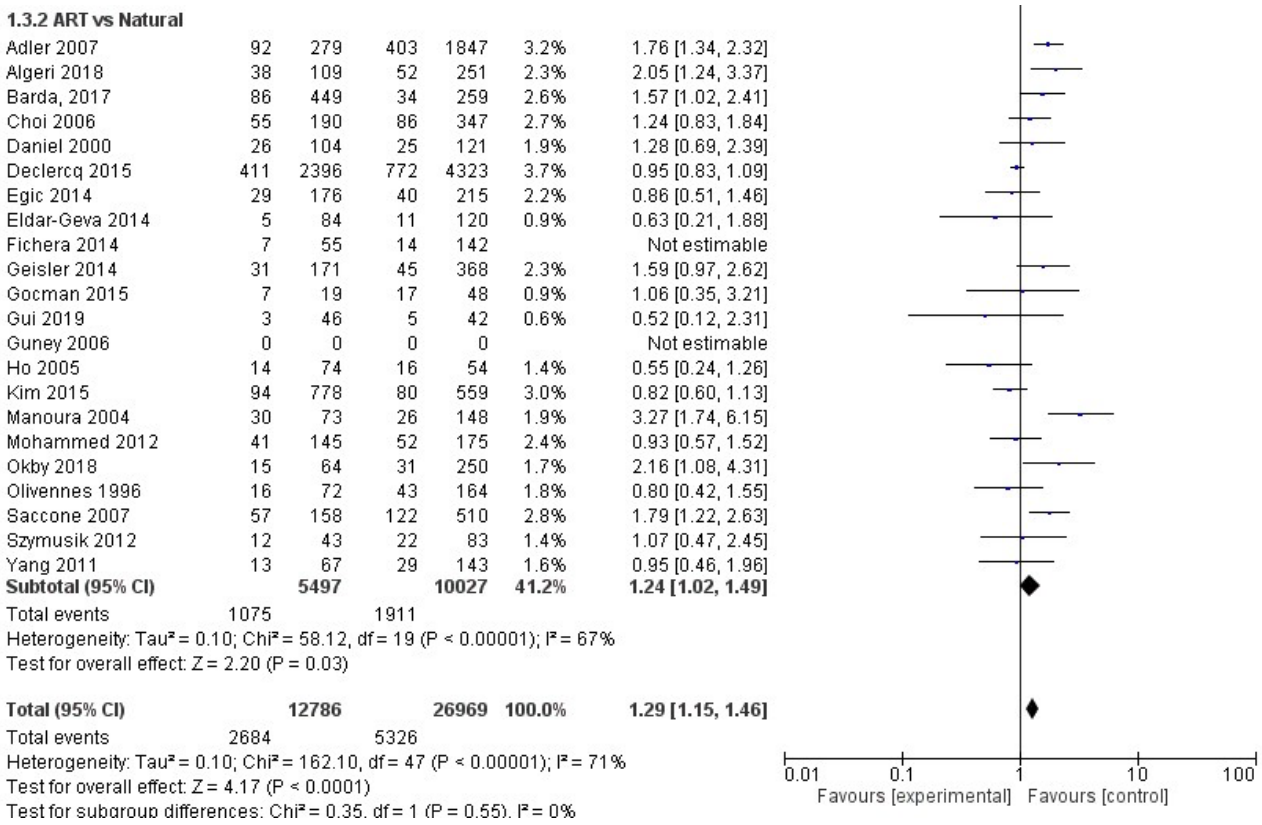


5. Caesarean delivery



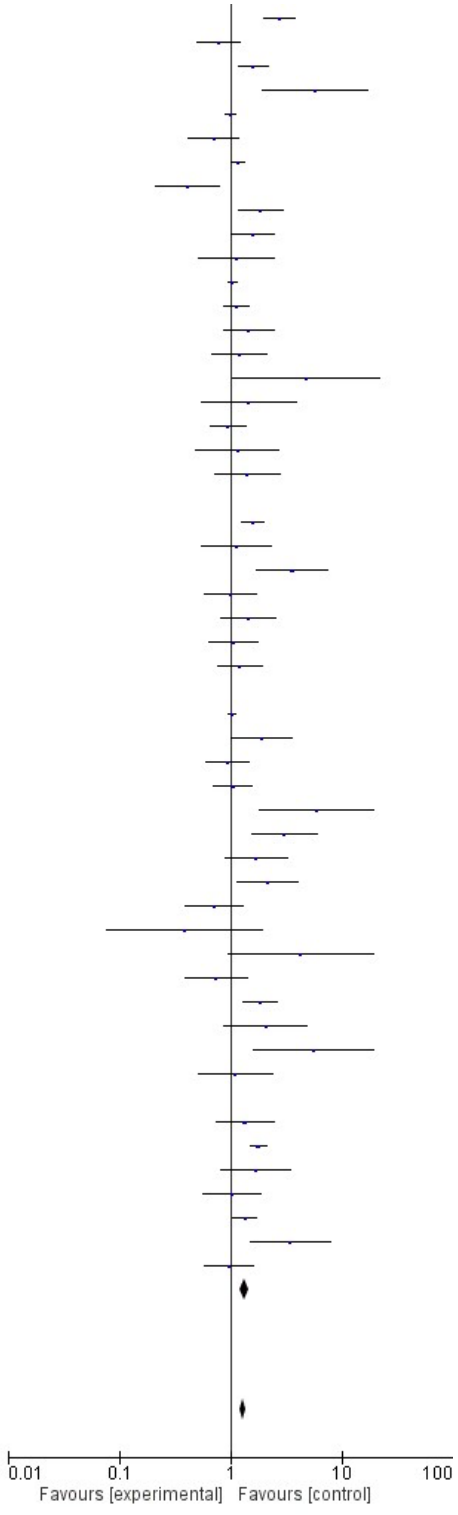
ART vs Natural

1. Preterm birth <34 weeks



2. Preterm birth <37 weeks

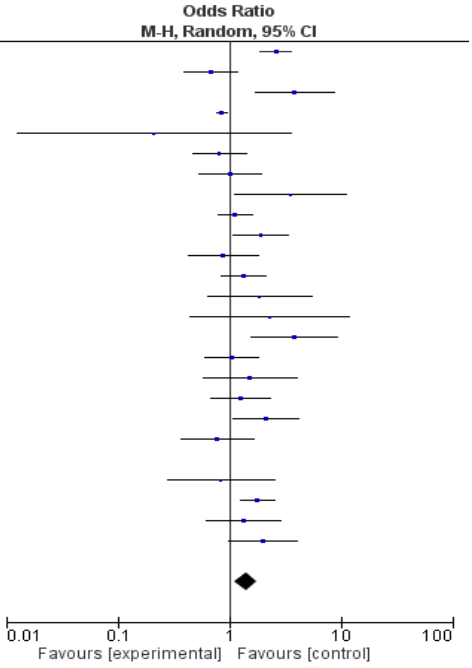
Adler 2007	234	279	1206	1847	1.2%	2.76 [1.98, 3.86]
Almonte 2012	60	185	61	161	0.9%	0.79 [0.51, 1.23]
Barda, 2017	265	449	123	259	1.2%	1.59 [1.17, 2.17]
Baxi 2008	32	36	80	138	0.3%	5.80 [1.94, 17.30]
Bensdrop 2016	1246	2437	1667	3276	1.8%	1.01 [0.91, 1.12]
Bernasko 1997	24	105	82	279	0.8%	0.71 [0.42, 1.20]
Boulet 2008	801	1446	1408	2729	1.7%	1.17 [1.03, 1.32]
Bregar 2016	14	45	230	438	0.6%	0.41 [0.21, 0.79]
Caserta 2014	100	138	121	207	0.9%	1.87 [1.18, 2.98]
Choi 2006	157	190	260	347	0.9%	1.59 [1.02, 2.49]
Daskalakis 2015	16	32	61	130	0.4%	1.13 [0.52, 2.45]
Declercq 2015	1284	2396	2271	4323	1.8%	1.04 [0.94, 1.15]
Deltombe-Bodart 2017	231	360	605	986	1.4%	1.13 [0.88, 1.45]
Dhont 1997	60	115	49	115	0.8%	1.47 [0.87, 2.47]
Eldar-Geva 2014	38	84	49	120	0.7%	1.20 [0.68, 2.10]
Feng 2018	49	51	76	91	0.1%	4.84 [1.06, 22.08]
Geipel 2001	19	32	16	32	0.3%	1.46 [0.54, 3.93]
Geisler 2014	84	171	185	368	1.1%	0.96 [0.66, 1.37]
Gui 2019	29	46	25	42	0.4%	1.16 [0.49, 2.74]
Guney 2006	29	52	49	104	0.6%	1.42 [0.72, 2.76]
Haghighi 2013	129	159	382	519		Not estimable
Hansen 2009	225	350	1085	2048	1.5%	1.60 [1.26, 2.02]
Huang 2006	35	81	20	50	0.5%	1.14 [0.56, 2.34]
Kathiresan 2010	138	146	747	903	0.5%	3.60 [1.73, 7.50]
Koivurova 2002	45	103	45	103	0.7%	1.00 [0.58, 1.73]
Koudstaal 2000	49	96	40	96	0.7%	1.46 [0.83, 2.58]
Lehnen 2011	37	74	147	305	0.8%	1.07 [0.65, 1.79]
Lei 2019	610	803	73	101	0.9%	1.21 [0.76, 1.93]
Liang 2002	70	104	78	173		Not estimable
Luke 2017	1903	3538	3228	6090	1.8%	1.03 [0.95, 1.12]
Manoura 2004	55	73	91	148	0.6%	1.91 [1.02, 3.58]
Mohammed 2012	66	145	82	175	0.9%	0.95 [0.61, 1.47]
Moini 2012	118	230	85	170	1.0%	1.05 [0.71, 1.57]
Moise 1998	12	20	8	40	0.2%	6.00 [1.84, 19.59]
Nassar 2003	38	56	46	112	0.5%	3.03 [1.54, 5.95]
Ochsenkuhn 2003	38	78	28	78	0.6%	1.70 [0.89, 3.22]
Okby 2018	49	64	151	250	0.6%	2.14 [1.14, 4.03]
Olivennes 1996	23	72	65	164	0.7%	0.71 [0.40, 1.28]
Petersen 1995	3	16	6	16	0.1%	0.38 [0.08, 1.93]
Pourali 2016	29	31	74	96	0.1%	4.31 [0.95, 19.51]
Putterman 2003	32	60	61	101	0.6%	0.75 [0.39, 1.43]
Saygan-Karamursel 2006	210	274	223	348	1.1%	1.84 [1.29, 2.62]
Simoes 2015	17	25	244	483	0.4%	2.08 [0.88, 4.91]
Skrypchenko 2016	18	23	11	28	0.2%	5.56 [1.60, 19.38]
Szymusik 2012	29	43	54	83	0.4%	1.11 [0.51, 2.43]
Tan 1992	73	125	11	21		Not estimable
Vasario 2010	62	84	94	139	0.6%	1.35 [0.74, 2.46]
Verstraelen 2005	441	743	1314	2915	1.6%	1.78 [1.51, 2.10]
Wennerholm 1998	42	98	15	49	0.5%	1.70 [0.82, 3.52]
Yang 2011	38	67	80	143	0.7%	1.03 [0.57, 1.85]
Yang 2014	180	314	733	1473	1.4%	1.36 [1.06, 1.73]
Zhang 2008	20	43	15	75	0.4%	3.48 [1.53, 7.93]
Zhu 2016	733	982	67	89	0.8%	0.97 [0.58, 1.60]
Subtotal (95% CI)		**17381**		**32863**	**39.4%**	**1.34 [1.21, 1.48]**
Total events	10067		17556			
Heterogeneity: Tau² = 0.07; Chi² = 185.78, df = 49 (P < 0.00001); I² = 74%						
Test for overall effect: Z = 5.56 (P < 0.00001)						
Total (95% CI)		**51926**		**126727**	**100.0%**	**1.30 [1.22, 1.38]**
Total events	28399		63385			
Heterogeneity: Tau² = 0.05; Chi² = 476.70, df = 116 (P < 0.00001); I² = 76%						
Test for overall effect: Z = 8.62 (P < 0.00001)						
Test for subgroup differences: Chi² = 0.55, df = 1 (P = 0.46), I² = 0%						



3. Hypertensive disorders in pregnancy

Study or Subgroup	ART Events	ART Total	Natural Events	Natural Total	Weight	Odds Ratio M-H, Random, 95% CI
Adler 2007	60	279	175	1847	6.1%	2.62 [1.89, 3.62]
Andrijasevic 2014	25	223	31	198	4.9%	0.68 [0.39, 1.20]
Barda, 2017	43	449	7	259	3.7%	3.81 [1.69, 8.61]
Bensdrop 2016	1017	2437	1498	3276	6.9%	0.85 [0.76, 0.94]
Bregar 2016	0	45	21	438	0.6%	0.21 [0.01, 3.58]
Chen 2019	29	249	27	194	4.9%	0.82 [0.47, 1.43]
Choi 2006	15	190	27	347	4.4%	1.02 [0.53, 1.96]
Daskalakis 2015	6	32	8	130	2.6%	3.52 [1.13, 11.00]
Deltombe-Bodart 2017	48	360	118	986	5.9%	1.13 [0.79, 1.62]
DeLuca 2013	91	248	19	82	4.8%	1.92 [1.08, 3.41]
Domingues 2014	9	122	58	698	4.1%	0.88 [0.42, 1.82]
Geisler 2014	37	171	63	368	5.5%	1.34 [0.85, 2.10]
Guney 2006	7	52	8	104	2.8%	1.87 [0.64, 5.47]
Ho 2005	6	74	2	54	1.5%	2.29 [0.44, 11.83]
Lehnen 2011	10	74	12	305	3.4%	3.82 [1.58, 9.21]
Lei 2019	132	803	16	101	4.9%	1.05 [0.59, 1.84]
Manoura 2004	8	73	11	148	3.1%	1.53 [0.59, 3.99]
Moini 2012	30	230	18	170	4.6%	1.27 [0.68, 2.36]
Nunes 2015	18	77	26	208	4.4%	2.14 [1.09, 4.17]
Olivennes 1996	11	72	31	164	4.0%	0.77 [0.36, 1.64]
Saygan-Karamursel 2006	30	274	16	348		Not estimable
Simoes 2015	4	25	89	483	2.7%	0.84 [0.28, 2.52]
Sun 2016	71	411	77	742	6.0%	1.80 [1.27, 2.55]
Vasario 2010	14	84	18	139	4.0%	1.34 [0.63, 2.87]
Zhu 2016	180	982	9	89	4.2%	2.00 [0.98, 4.05]
Total (95% CI)		**7762**		**11530**	**100.0%**	**1.40 [1.11, 1.76]**
Total events	1871		2369			

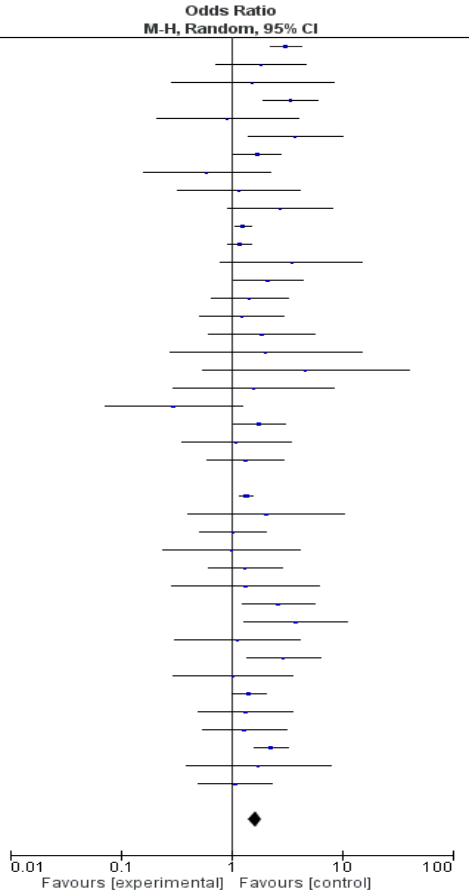
Heterogeneity: Tau² = 0.20; Chi² = 98.34, df = 23 (P < 0.00001); I² = 77%
Test for overall effect: Z = 2.89 (P = 0.004)



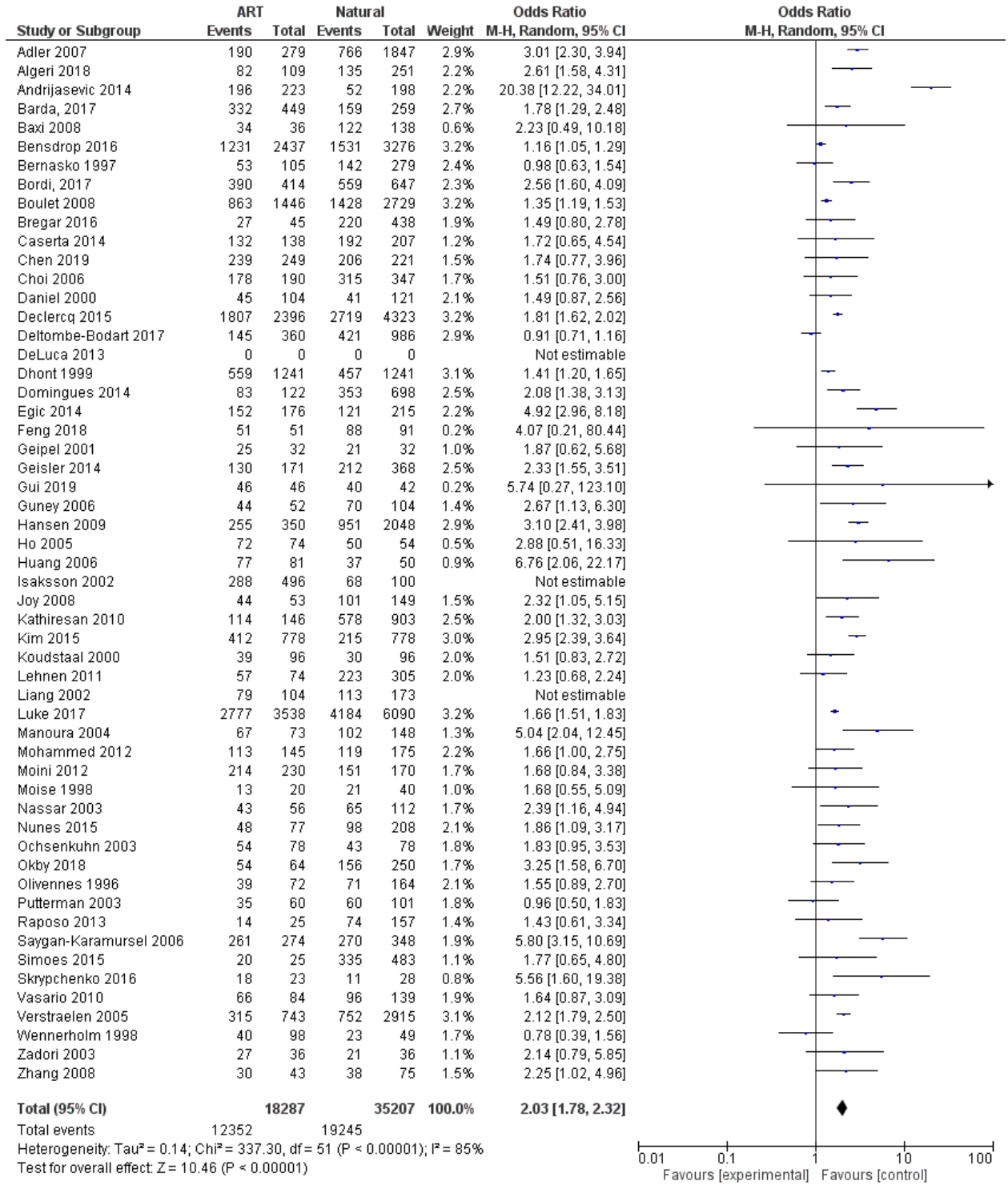
4. Gestational diabetes mellitus

Study or Subgroup	ART Events	ART Total	Natural Events	Natural Total	Weight	Odds Ratio M-H, Random, 95% CI
Adler 2007	61	279	153	1847	6.2%	3.10 [2.23, 4.30]
Barda, 2017	19	449	6	259	1.8%	1.86 [0.73, 4.73]
Baxi 2008	2	36	5	138	0.6%	1.56 [0.29, 8.42]
Bordi, 2017	37	414	18	647	3.6%	3.43 [1.92, 6.11]
Bregar 2016	2	45	21	438	0.8%	0.92 [0.21, 4.07]
Caserta 2014	14	138	6	207	1.6%	3.78 [1.42, 10.10]
Chen 2019	56	249	32	221	4.5%	1.71 [1.06, 2.77]
Choi 2006	3	190	9	347	1.0%	0.60 [0.16, 2.25]
Daniel 2000	5	104	5	121	1.1%	1.17 [0.33, 4.17]
Daskalakis 2015	6	32	10	130	1.4%	2.77 [0.92, 8.30]
Declercq 2015	237	2396	342	4323	8.2%	1.28 [1.07, 1.52]
Deltombe-Bodart 2017	128	360	312	986	7.2%	1.19 [0.92, 1.54]
DeLuca 2013	20	248	2	82	0.8%	3.51 [0.80, 15.35]
Domingues 2014	11	122	31	698	2.7%	2.13 [1.04, 4.37]
Egic 2014	14	176	12	215	2.3%	1.46 [0.66, 3.25]
Geisler 2014	8	171	14	368	1.9%	1.24 [0.51, 3.02]
Gui 2019	11	46	6	42	1.4%	1.89 [0.63, 5.65]
Guney 2006	2	52	2	104	0.5%	2.04 [0.28, 14.91]
Ho 2005	6	74	1	54	0.4%	4.68 [0.55, 40.04]
Huang 2006	5	81	2	50	0.6%	1.58 [0.29, 8.46]
Kathiresan 2010	2	146	40	903	0.9%	0.30 [0.07, 1.25]
Kim 2015	48	778	20	559	3.9%	1.77 [1.04, 3.02]
Lehnen 2011	4	74	15	305	1.3%	1.10 [0.36, 3.43]
Lei 2019	73	803	7	101	2.3%	1.34 [0.60, 3.00]
Liang 2002	16	104	4	173		Not estimable
Luke 2017	379	3538	493	6090	8.6%	1.36 [1.18, 1.57]
Manoura 2004	3	73	3	148	0.7%	2.07 [0.41, 10.53]
Moini 2012	21	230	15	170	2.8%	1.04 [0.52, 2.08]
Nassar 2003	3	56	6	112	0.9%	1.00 [0.24, 4.16]
Nunes 2015	11	77	23	208	2.4%	1.34 [0.62, 2.90]
Ochsenkuhn 2003	4	78	3	78	0.8%	1.35 [0.29, 6.25]
Okby 2018	13	64	22	250	2.5%	2.64 [1.25, 5.59]
Pourali 2016	8	31	8	96	1.4%	3.83 [1.30, 11.29]
Putterman 2003	4	60	6	101	1.0%	1.13 [0.31, 4.18]
Saygan-Karamursel 2006	22	274	10	348	2.4%	2.95 [1.37, 6.34]
Simoes 2015	3	25	56	483	1.1%	1.04 [0.30, 3.59]
Sun 2016	62	411	82	742	5.8%	1.43 [1.00, 2.04]
Szymusik 2012	8	43	12	83	1.6%	1.35 [0.51, 3.61]
Vasario 2010	10	84	13	139	2.0%	1.31 [0.55, 3.14]
Yang 2014	50	314	112	1473	5.8%	2.30 [1.61, 3.29]
Zadori 2003	5	36	3	36	0.8%	1.77 [0.39, 8.06]
Zhu 2016	95	982	8	89	2.5%	1.08 [0.51, 2.31]
Total (95% CI)		**13839**		**23791**	**100.0%**	**1.65 [1.44, 1.89]**
Total events	1475		1946			

Heterogeneity: Tau² = 0.05; Chi² = 68.35, df = 40 (P = 0.003); I² = 41%
Test for overall effect: Z = 7.07 (P < 0.00001)



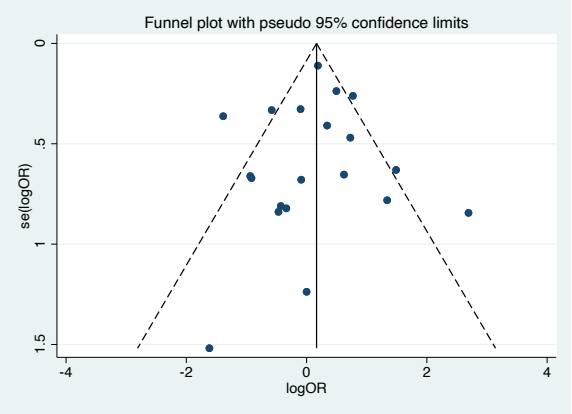
5. Caesarean delivery



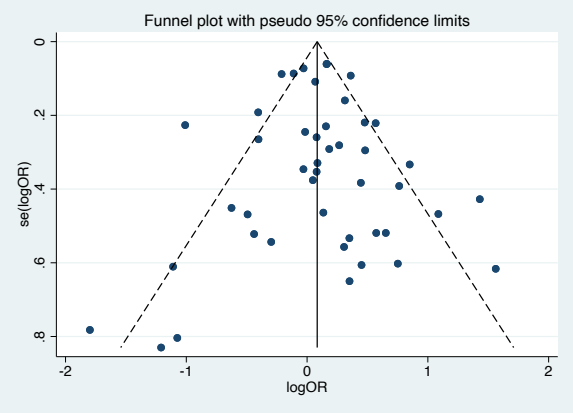
APPENDIX 22: Funnel plots for meta-analyses with more than 10 included studies in the systemic review of maternal outcomes in twin pregnancies following assisted reproduction.

ART vs Non-ART

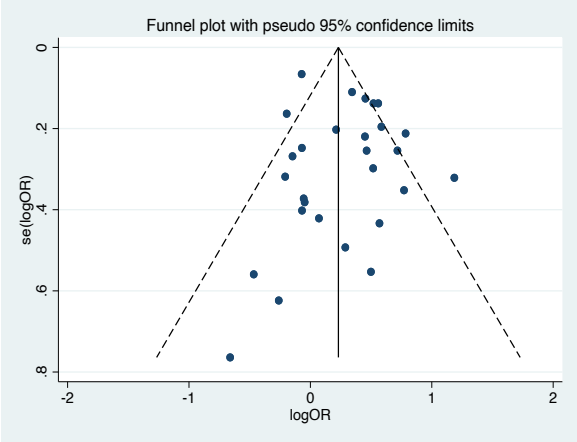
Preterm birth <28 weeks (p = 0.753)



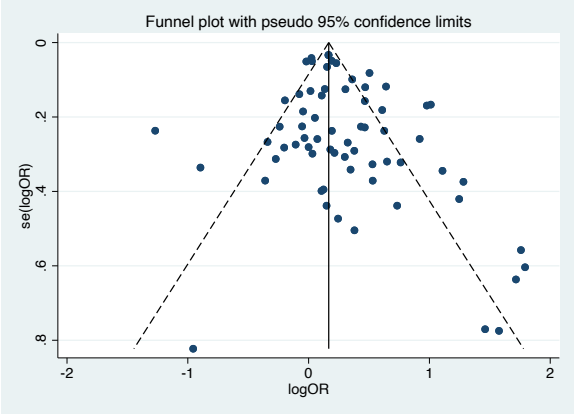
Preterm birth <32 weeks (p = 0.775)



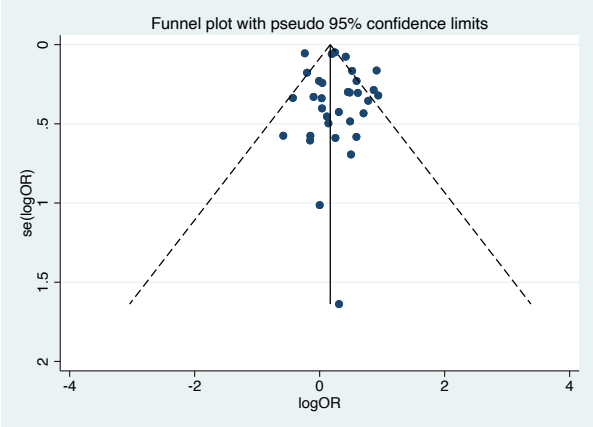
Preterm birth <34 weeks (p = 0.315)



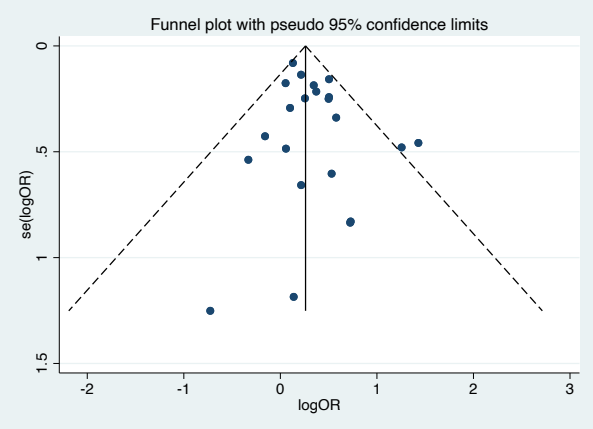
Preterm birth <37 weeks (p = 0.033)



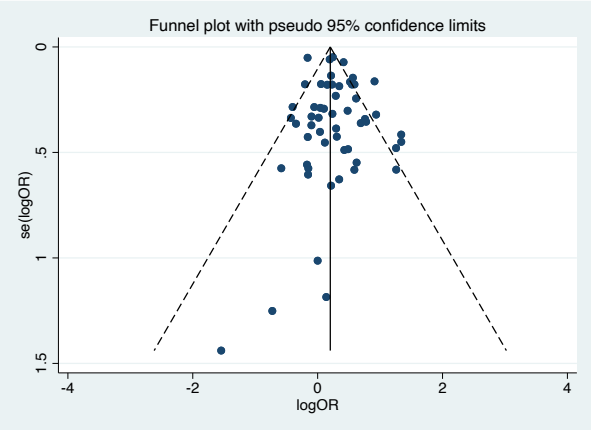
Gestational hypertension (p = 0.229)



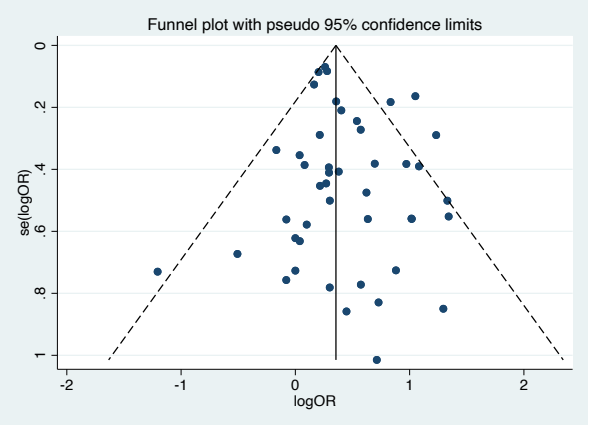
Pre-eclampsia (p = 0.152)



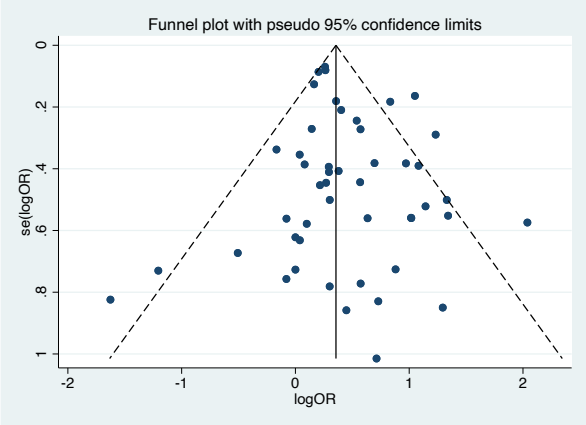
Hypertensive disorders in pregnancy (p = 0.150)



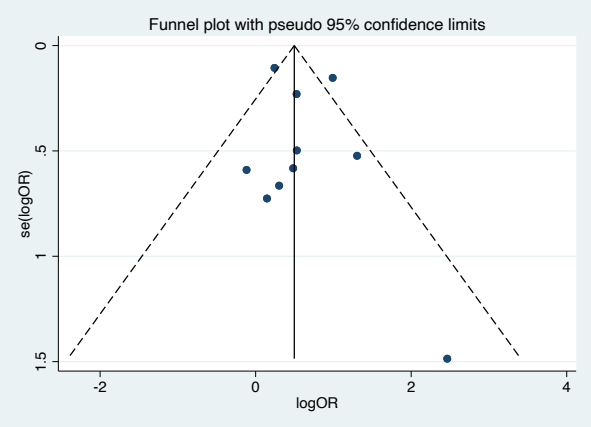
Gestational diabetes mellitus (p = 0.116)



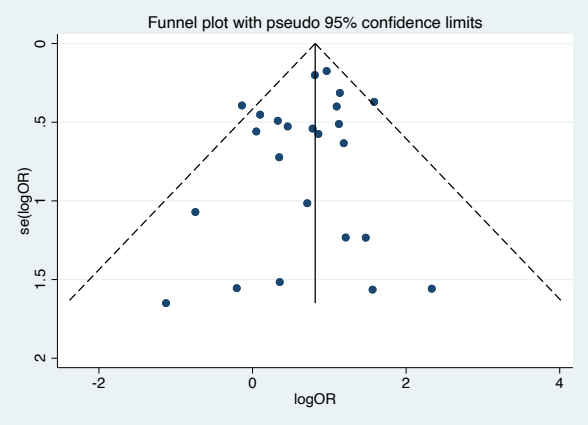
Diabetes in pregnancy (p = 0.108)



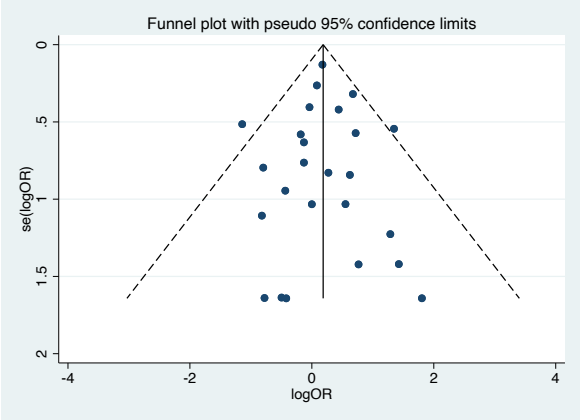
Antepartum haemorrhage (p = 0.555)



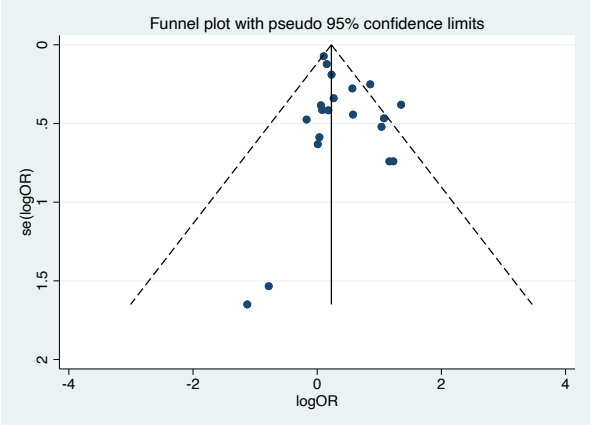
Placenta previa (p = 0.270)



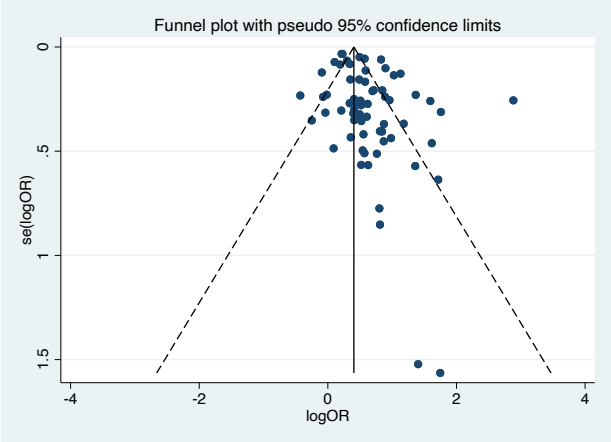
Placental abruption (p = 0.958)



Postpartum haemorrhage (p = 0.061)

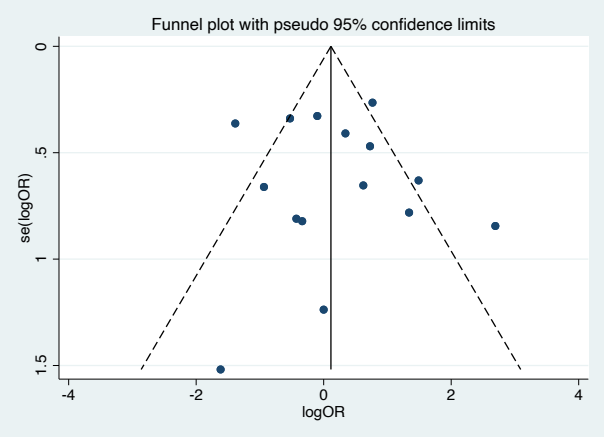


Caesarean section (p = 0.002)

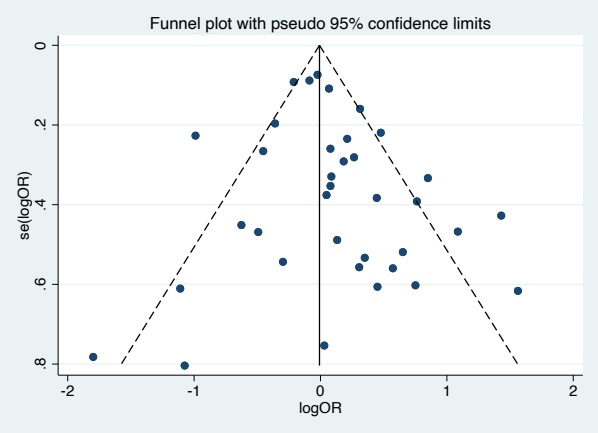


ART vs Natural

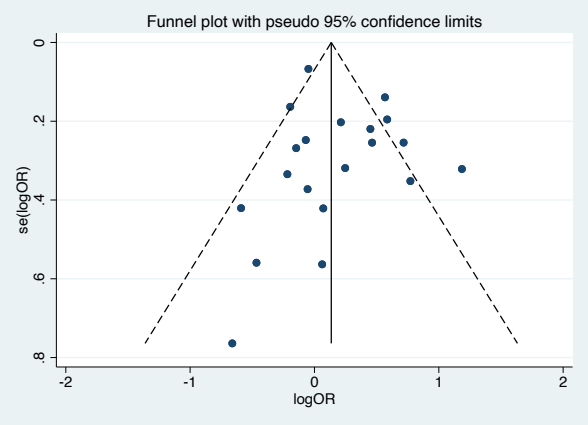
Preterm birth <28 weeks (p = 0.725)



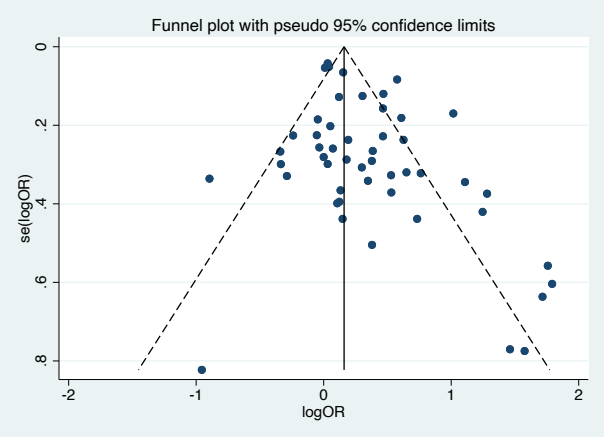
Preterm birth <32 weeks (p = 0.160)



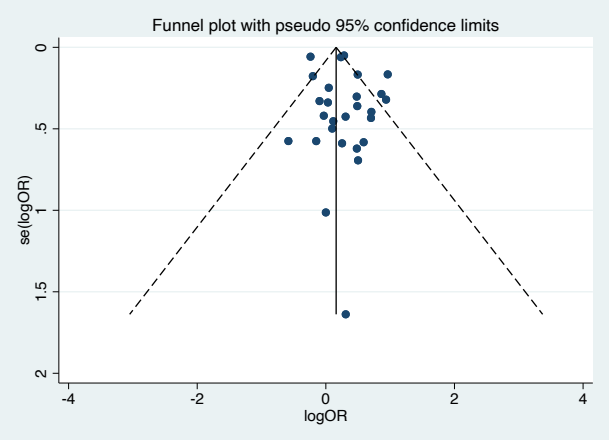
Preterm birth <34 weeks (p = 0.396)



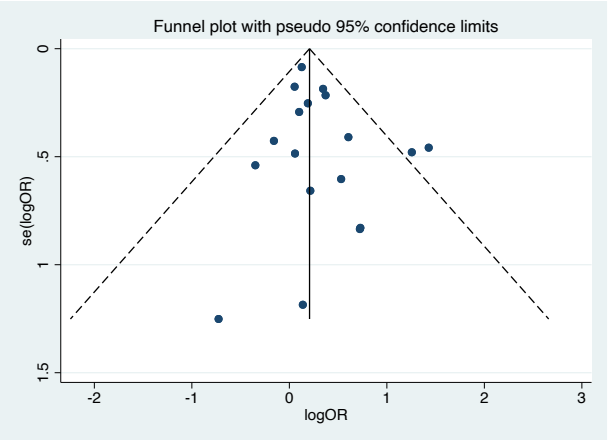
Preterm birth <37 weeks (p = 0.004)



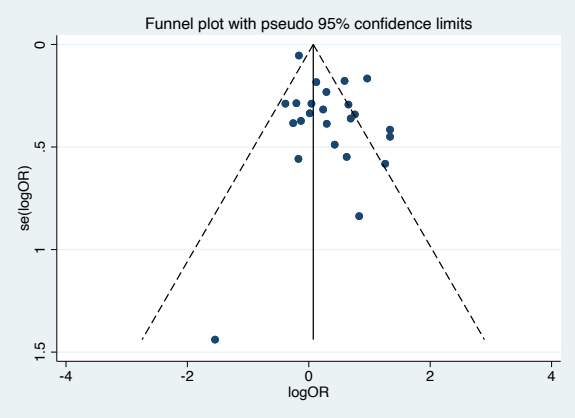
Gestational hypertension (p = 0.265)



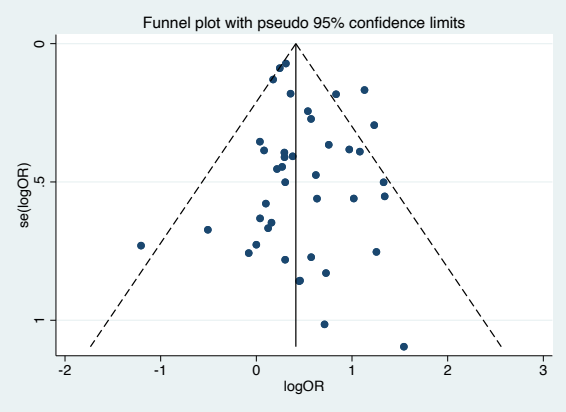
Pre-eclampsia (p = 0.187)



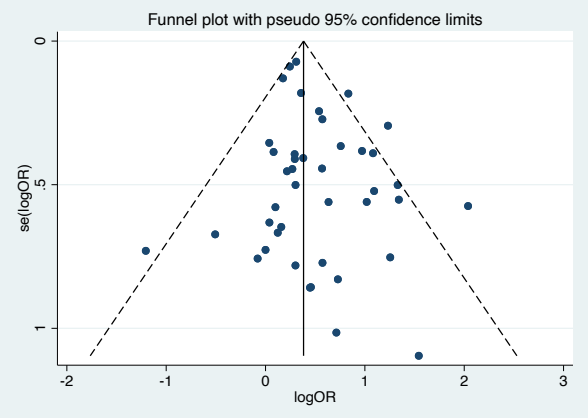
Hypertensive disorders in pregnancy (p = 0.012)



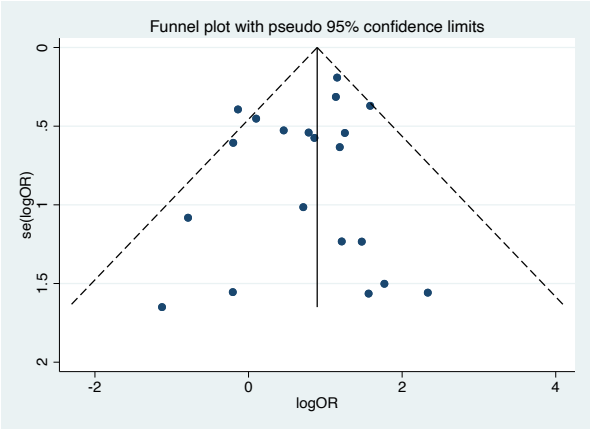
Gestational diabetes mellitus (p = 0.208)



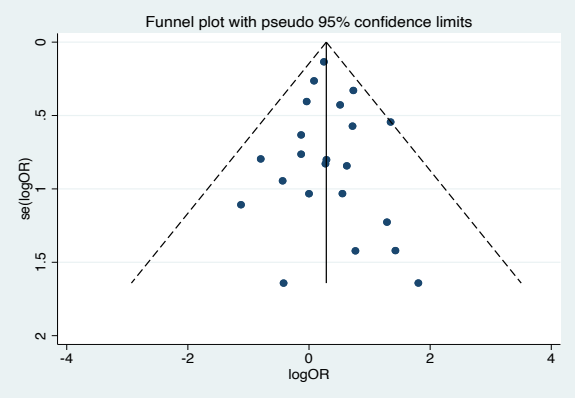
Diabetes in pregnancy (p = 0.047)



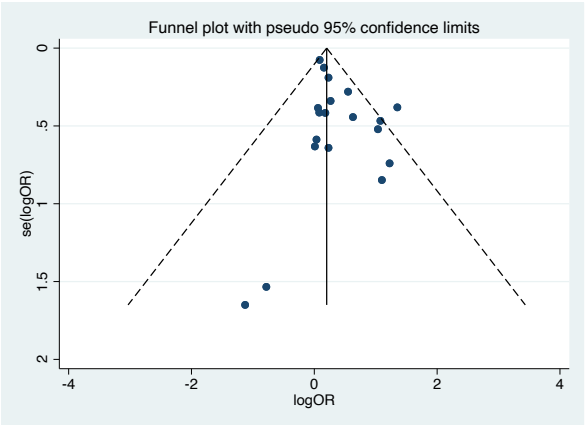
Placenta previa (p = 0.298)



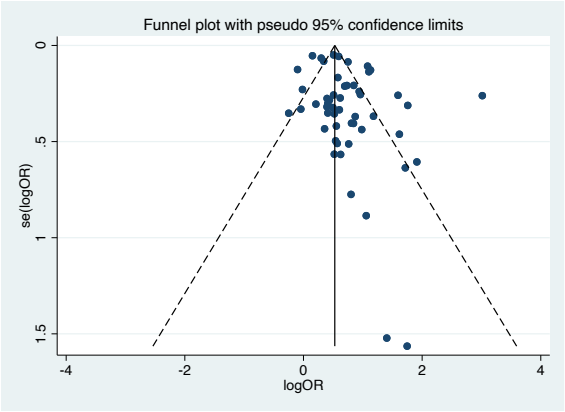
Placental abruption (p = 0.723)



Postpartum haemorrhage (p = 0.045)

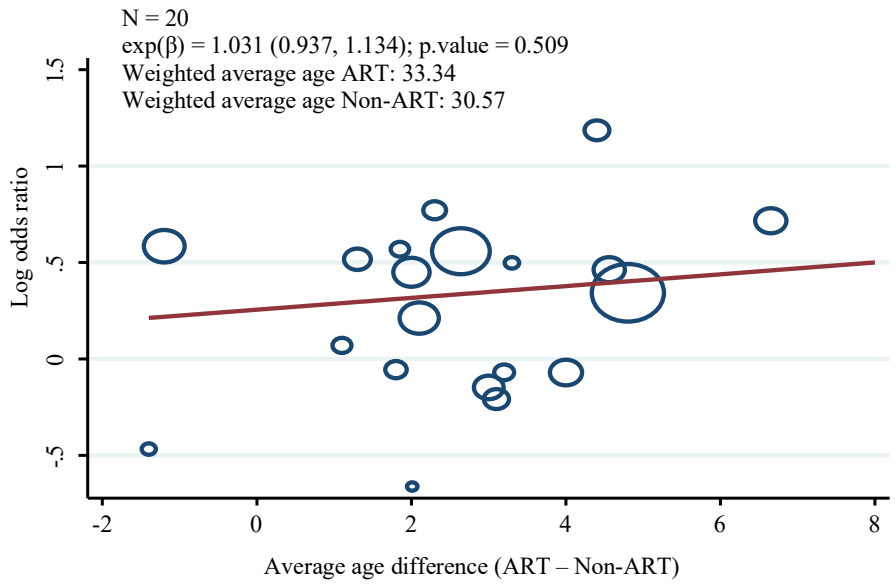


Caesarean section (p = 0.018)

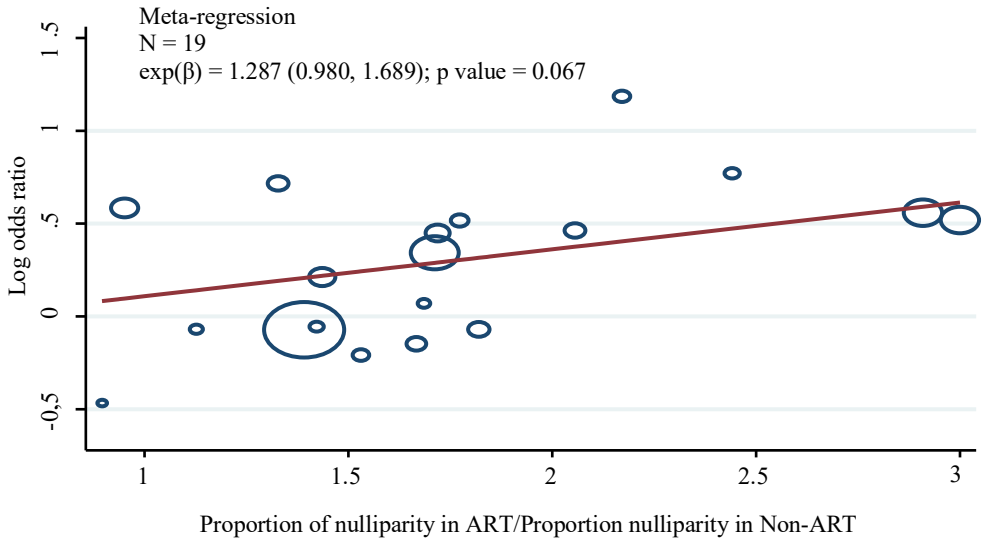


APPENDIX 23: Meta-regression analysis on certain maternal outcomes to adjust for maternal age and parity (ART vs Non-ART) in the systematic review of maternal outcomes in twin pregnancies following assisted reproduction.

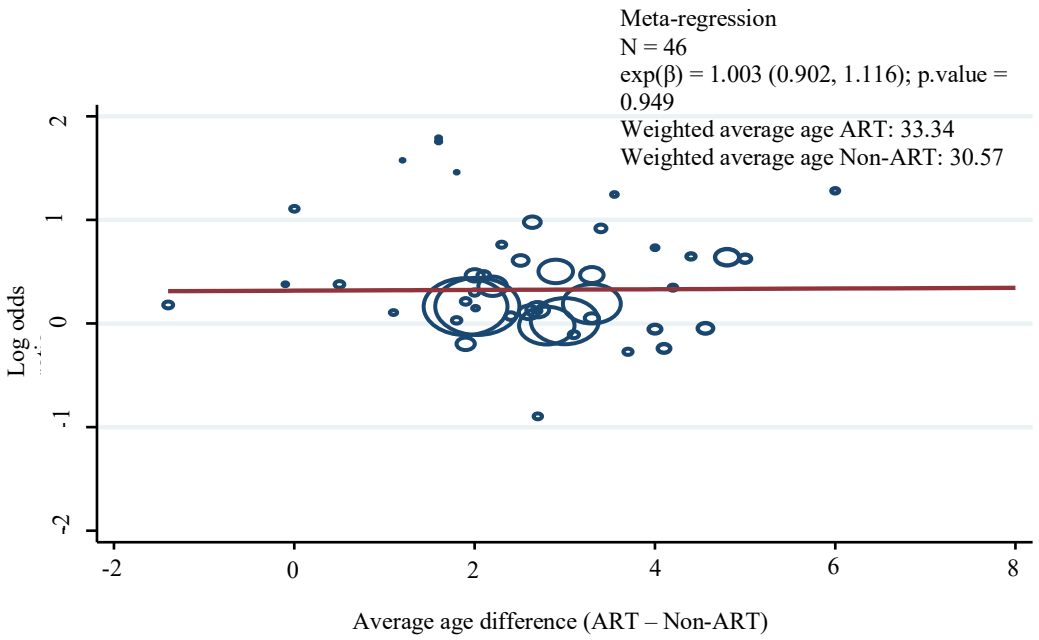
1. Preterm birth <34 weeks and maternal age



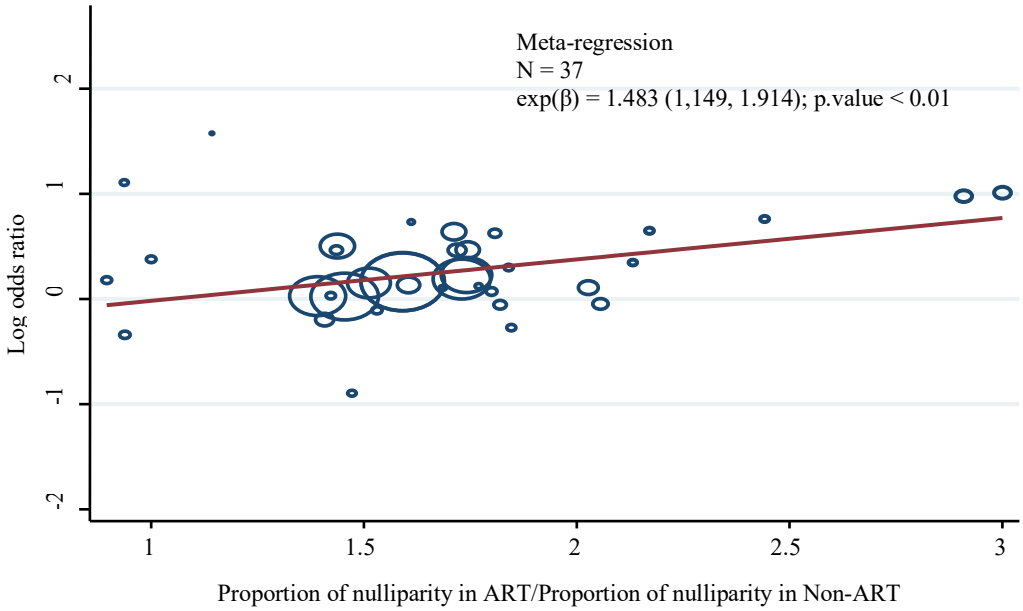
2. Preterm birth <34 weeks and parity



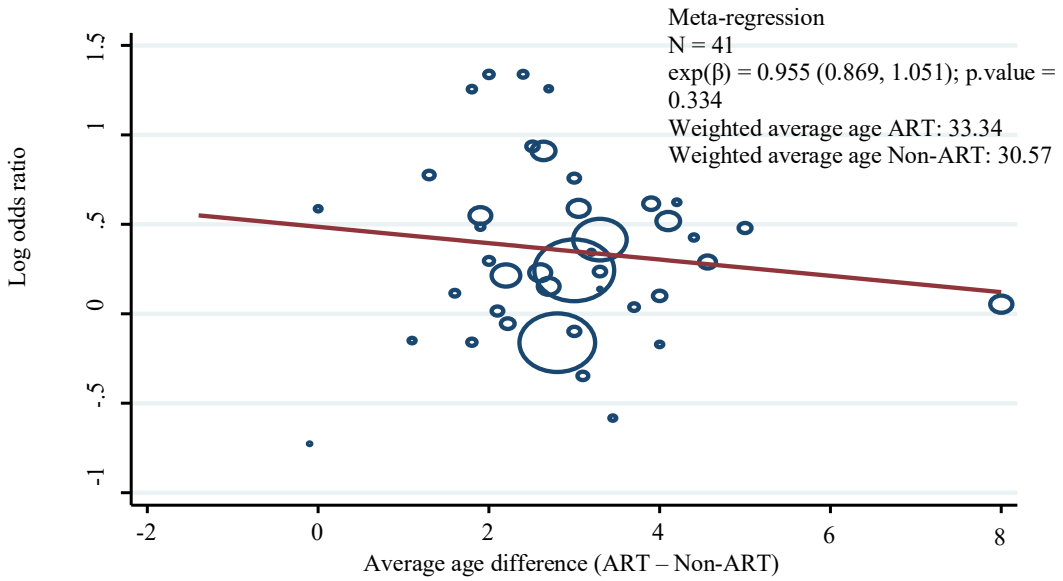
3. Preterm birth <37 weeks and maternal age



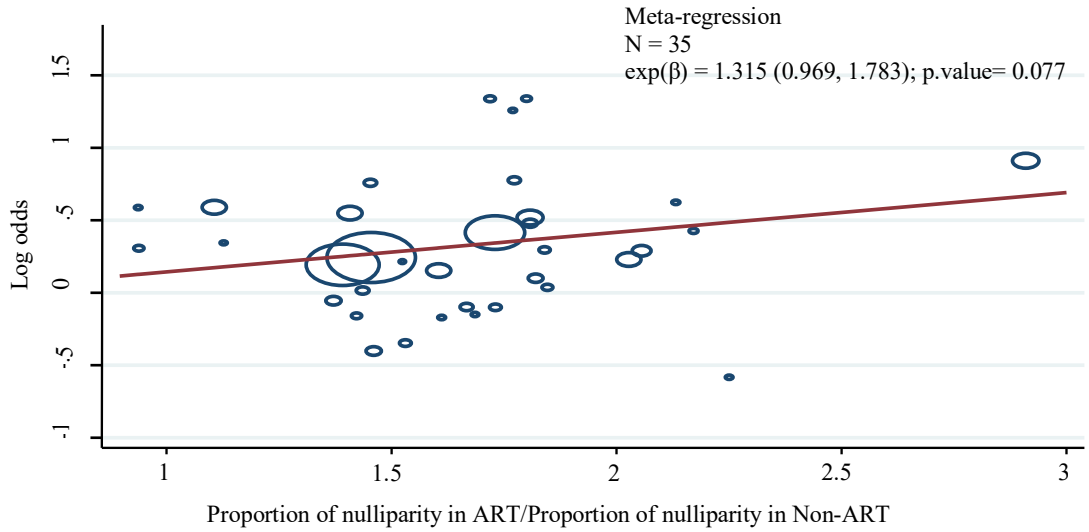
4. Preterm birth <37 weeks and parity



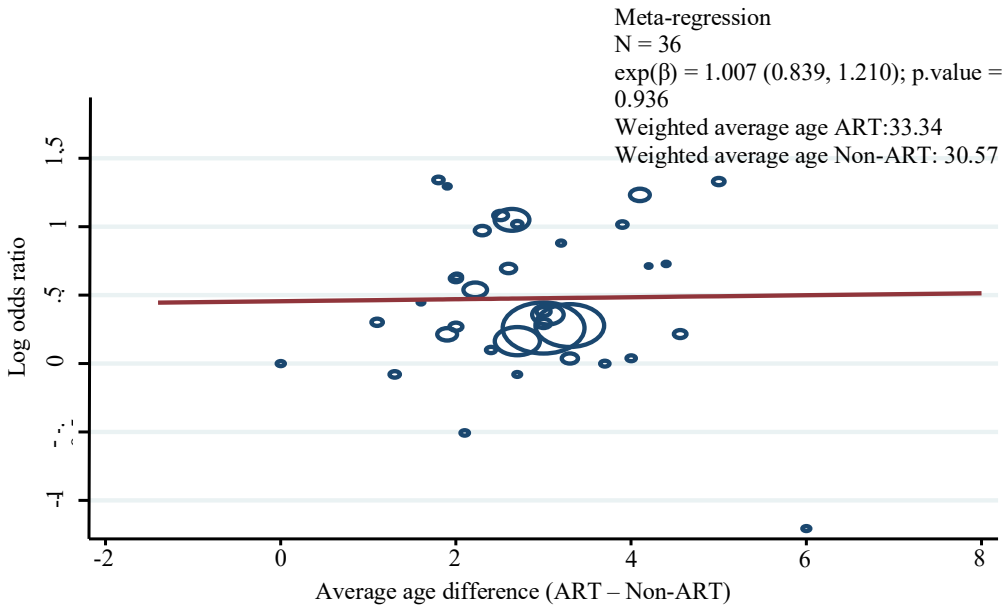
5. Hypertensive disorders in pregnancy and maternal age



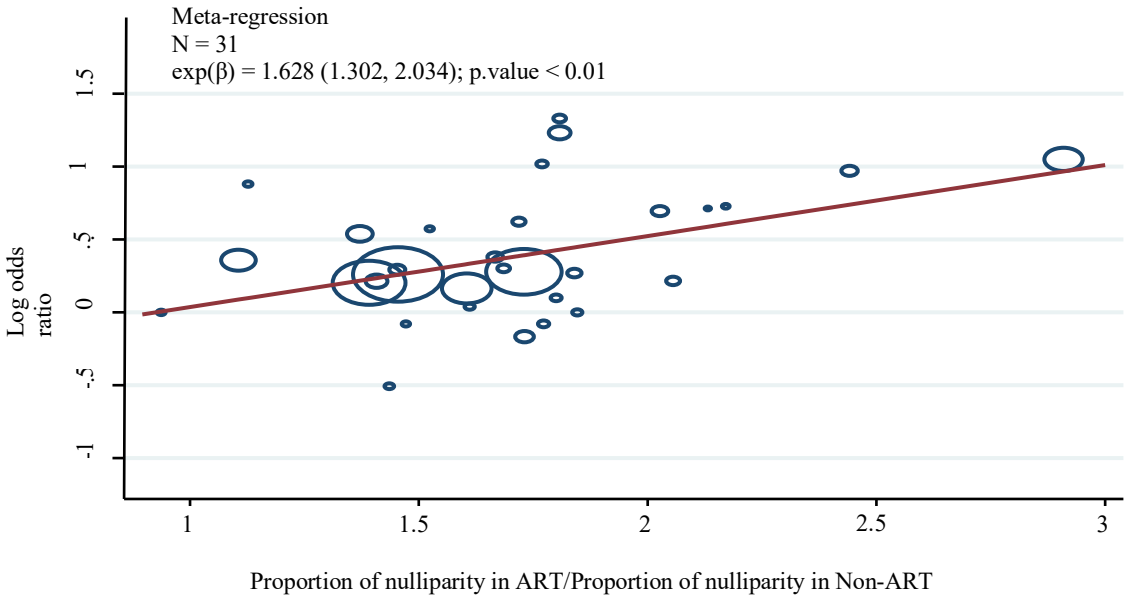
6. Hypertensive disorders in pregnancy and parity



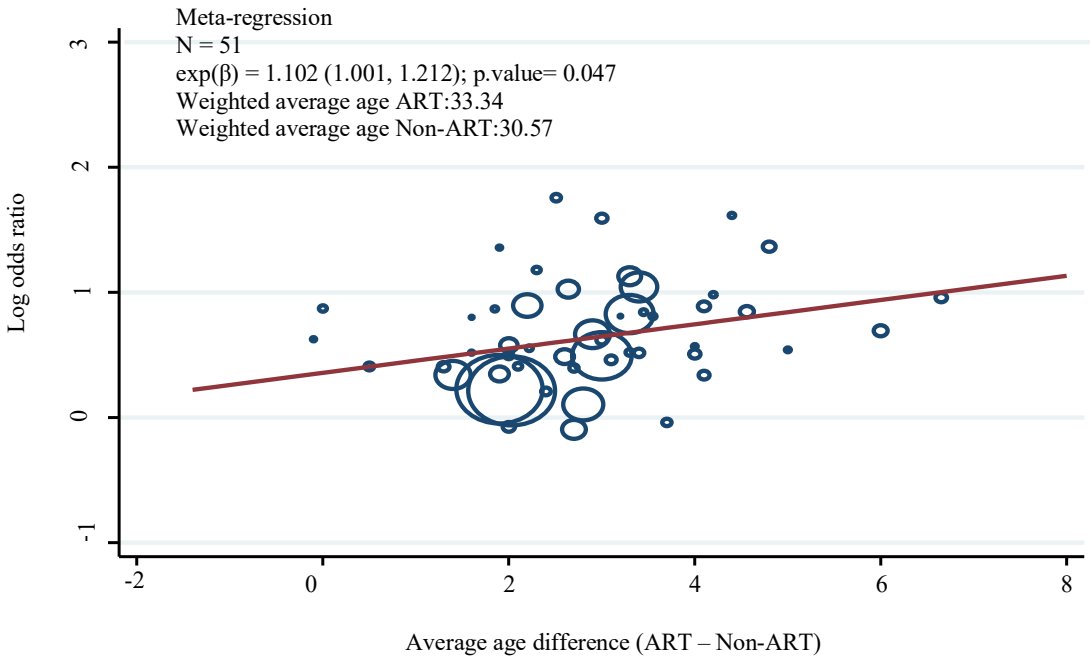
7. Gestational diabetes mellitus and maternal age



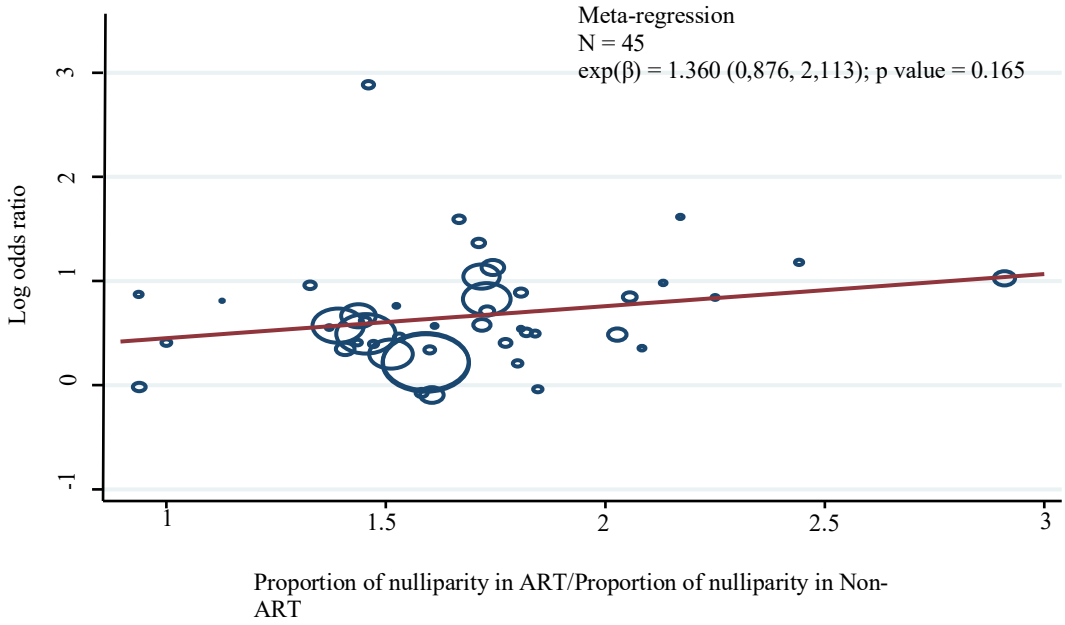
8. Gestational diabetes mellitus and parity



9. Caesarean section and maternal age



10. Caesarean section and parity



APPENDIX 24: Search strategy used in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

1. exp Twins/ or exp Twins, Monozygotic/ or exp Twins, Dizygotic/ or twins.mp.
2. twin$.mp.
3. multiple pregnan$.mp.
4. multiple pregnancy.mp. or exp Pregnancy, Multiple/ or exp Multiple Birth Offspring/
5. (multipl$ gestation or multifetal pregnancy or multifetal gestation).mp.
6. 1 or 2 or 3 or 4 or 5
7. Assisted reproductive techniques.mp. or exp Reproductive Techniques, Assisted/ or assisted reproductive technology.mp.
8. ART.mp.
9. Method of conception.mp. or exp Insemination, Artificial/ or infertility treatment$.mp.
10. infertility therap$.mp.
11. Intracytoplasmic Sperm Injection.mp. or exp Sperm Injections, Intracytoplasmic/
12. ICSI.mp.
13. Intracytoplasmic morphologically selected sperm injection.mp.
14. IMSI.mp.
15. (Gamete intrafallopian transfer or GIFT).mp.
16. exp Ovulation Induction/ or ovulation induction.mp.
17. in vitro fertilization.mp. or exp Fertilization in Vitro/ or IVF.mp.
18. ZIFT.mp. or exp Zygote Intrafallopian Transfer/
19. exp Embryo Culture Techniques/ or frozen embryo transfer.mp.
20. (spontaneous conception or Self-Fertilization).mp.
21. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20
22. 6 and 21
23. exp Maternal Death/ or exp Maternal Health/ or exp Maternal Mortality/
24. (maternal morbidity or maternal complications or maternal outcomes or maternal mortality).mp.
25. (Prenatal outcomes or Prenatal morbidity or Prenatal mortality or Prenatal complications).mp.
26. exp Prenatal Care/ or exp Ultrasonography, Prenatal/ or exp Prenatal Diagnosis/ or exp Prenatal Injuries/
27. Antenatal complications.mp.
28. Postpartum complications.mp.
29. exp Obstetrics/
30. (Obstetric outcomes or Obstetric morbidity or Obstetric mortality).mp.
31. antepartum h$emorrhage.mp.
32. placenta previa.mp. or exp Placenta Previa/
33. placental abruption.mp. or exp Abruptio Placentae/
34. PROM.mp. or exp Fetal Membranes, Premature Rupture/
35. exp Fetal Membranes, Premature Rupture/ or pPROM.mp. or exp Obstetric Labor, Premature/
36. oligohydramnios.mp. or exp Oligohydramnios/
37. polyhydramnios.mp. or exp Polyhydramnios/

38. exp Cesarean Section/ or c$esarian section.mp. or C section.mp.
39. exp Postpartum Hemorrhage/ or PPH.mp. or postpartum haemorrhage.mp.
40. exp Fetal Growth Retardation/ or IUGR.mp. or fetal growth retardation.mp.
41. anemia in pregnancy.mp. or exp Pregnancy Complications, Hematologic/
42. (anaemia or iron deficiency).mp.
43. obstetric cholestasis.mp.
44. preterm birth.mp. or exp Premature Birth/
45. exp Hypertension, Pregnancy-Induced/ or PIH.mp. or hypertensive disorder.mp.
46. preeclampsia.mp. or exp Pre-Eclampsia/
47. HELLP syndrome.mp. or exp HELLP Syndrome/
48. exp Diabetes, Gestational/ or GDM.mp. or hyperglycemia in pregnancy.mp.
49. APH.mp.
50. 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49
51. exp Neonatal Abstinence Syndrome/ or exp Intensive Care, Neonatal/ or exp Neonatal Brachial Plexus Palsy/ or exp Neonatal Screening/ or exp Hyperbilirubinemia, Neonatal/ or exp Thrombocytopenia, Neonatal Alloimmune/ or exp Neonatal Nursing/ or exp Epilepsy, Benign Neonatal/ or exp Intensive Care Units, Neonatal/ or exp Anemia, Neonatal/ or exp Neonatal Sepsis/ or exp Jaundice, Neonatal/
52. (Neonatal outcomes or Neonatal morbidity or Neonatal mortality or Neonatal complications or neonatal death or offspring outcomes or offspring complications).mp.
53. (Perinatal outcomes or Perinatal morbidity or Perinatal mortality or Perinatal complications).mp.
54. exp Perinatal Death/ or exp Perinatal Care/ or exp Perinatal Mortality/
55. exp Child Health/ or exp Child/ or Child health outcomes.mp. or exp Infant, Newborn/
56. (Newborn morbidity or Newborn mortality).mp.
57. postnatal.mp. or exp Postnatal Care/
58. exp Pregnancy Outcome/ or exp Infant Mortality/ or stillbirth.mp. or exp Fetal Death/ or exp Stillbirth/
59. exp Infant, Small for Gestational Age/ or small for gestational age.mp.
60. intrauterine growth restriction.mp.
61. congenital malformation$.mp. or exp Congenital Abnormalities/
62. apgar score.mp. or exp Apgar Score/
63. Hypoxia-Ischemia, Brain/ or HIE.mp. or hypoxic ischemic encephalopathy.mp.
64. birth weight.mp. or exp Birth Weight/
65. gestational age.mp. or exp Gestational Age/
66. delivery.mp. or exp Delivery, Obstetric/
67. cord gas.mp. or Acidosis/
68. (pregnancy outcome or fetal outcome).mp.
69. exp Respiratory Distress Syndrome, Newborn/ or RDS.mp.
70. exp Respiration, Artificial/
71. exp Sepsis/ or Sepsis.mp. or exp Neonatal Sepsis/ or neonatal sepsis.mp.
72. intraventricular h$emorrhage.mp.
73. exp Cerebral Intraventricular Hemorrhage/ or IVH.mp.
74. exp Enterocolitis, Necrotizing/ or NEC.mp.
75. exp Hyperbilirubinemia, Neonatal/ or hyperbilirubinemia.mp. or Hyperbilirubinemia/

76. hypoglycemia.mp. or exp Hypoglycemia/
77. neurolog$ complications.mp.
78. exp Fetofetal Transfusion/ or Fetofetal Transfusion.mp.
79. twin to twin transfusion syndrome.mp.
80. transient tachypnoea of newborn.mp.
81. composite morbidity.mp.
82. 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81
83. Premature labor.mp. or exp Obstetric Labor, Premature/
84. premature birth.mp. or exp Premature Birth/
85. exp Infant, Premature/
86. (Premat* or Preterm*).mp.
87. 83 or 84 or 85 or 86
88. limit 22 to yr="1990 -Current"
89. 50 or 82 or 87
90. 88 and 89

APPENDIX 25: Study characteristics in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

No	Author, Year, Country	Study design	Type of ART	Inclusion period (months)	Inclusion criteria	Exclusion criteria	Pregnancies	Neonates	Outcomes
01	**Adler-Levy, 2007** Israel	Retrospective cohort	IVF, ICSI	168	All twin deliveries achieved by IVF, ovulation induction and spontaneously conceived twins, deliveries > 24 weeks of gestation	Vanishing twins	2365	4730	Stillbirth, neonatal death, SGA <10th centile, twin birth weight discordance >25%, any congenital malformations, APGAR< 7 at 5 minutes
02	**Agustsson, 1997** Iceland and Scotland	Retrospective cohort	IVF (+ 1 case of IUI+ 1 case of GIFT)	48	All twin pregnancies, where delivery occurred after 16 completed weeks of pregnancy (≥112 days)		522	1044	Stillbirth, NICU admission

03	**Algeri, 2018** Italy	Retrospective cohort	Autologous ART; IVF with or without ICSI. Heterologous ART; egg/ embryo donation	98	Nulliparous and pluriparous diamniotic twin pregnancies managed and delivered at the study centre during the study period	Triplets, twin pregnancies resulted from selected fetal reductions/ termination of pregnancy, monoamnionicity, lack of data on conception method, use of conception techniques including IUI, induction of ovulation, evidence of pregestational chronic disease at the first antenatal visit and any maternal pregnancy-related complications such as gestational hypertension pre-eclampsia, cholestasis, or GDM	360	720	Stillbirth, SGA <10th centile, any congenital malformations, NICU admission, neonatal jaundice, neonatal hypoglycaemia, umbilical cord pH <7.2

04	**Barda, 2017** Israel	Retrospective cohort	IVF	77	All consecutive dichorionic diamniotic twins delivered after gestational age >20 weeks in a single centre	Monochorionic twins	708	1416	Stillbirth, APGAR <7 at 5 minutes, RDS, NEC, neonatal sepsis, IVH, neonatal jaundice, neonatal hypoglycaemia, umbilical cord pH <7.2, mechanical ventilation
05	**Bensdorp, 2016** Netherland	Retrospective cohort	IVF, ICSI	156	All primiparous women with twin offspring of the opposite sex, offspring each weighing at least 500g after 22 weeks of gestation	Same-sex twins	6694	13388	Perinatal mortality, SGA <10th centile, any congenital malformations, APGAR <7 at 5 minutes, NICU admission
06	**Bordi, 2017** Italy	Retrospective cohort	IVF, ICSI, egg or embryo donation	186	All ART and spontaneously conceived pregnancies ending by spontaneous delivery by 24 weeks of gestation	Spontaneous gestation with a discrepancy in menstrual and USS gestational age more than 7 days, triplet pregnancies reduced to twins, insufficient clinical data	1097	2194	Stillbirth, neonatal death, SGA <10th centile, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS, NEC, neonatal sepsis, neonatal jaundice, neonatal hypoglycaemia, neurological complications, TTTS

07	**Bregar, 2016** Slovenia	Retrospective cohort	Type of ART not mentioned	132	Monochorionic diamniotic twin pregnancies and dichorionic twins conceived by ART delivered at >22 weeks of gestation or when the foetus weighs >500g during the study period	Monoamniotic twins	483	966	Stillbirth, any congenital malformations, major congenital malformations, TTTS, APGAR <7 at 5 minutes
08	**Caserta, 2014** Italy	Retrospective cohort	IVF, ICSI	54	Dichorionic diamniotic twins conceived via conventional IVF and ICSI	Monochorionic twins, twin pregnancies reduced to singleton births, pregnancies conceived via OI, IUI, egg donation. Spontaneous pregnancies with discrepancies in menstrual and ultrasound gestational age estimates, history of hypertension or diabetes mellitus before pregnancy	345	680	Stillbirth, neonatal death, SGA <10th centile, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS, NEC, neonatal sepsis, neonatal jaundice, neonatal hypoglycaemia, neurological complications

09	**Chen, 2019** China	Retrospective cohort	IVF, ICSI	10	Dizygotic twin pregnancies delivered after 28 weeks gestation and only those who conceived following IVF/ICSI treatment at the study hospital	Those conceived by other forms of ART than IVF/ICSI. Twin gestations obtained after natural abortion or fetal reduction in multiple pregnancies	470	940	Stillbirth, any congenital malformations
10	**Choi, 2006** South Korea	Retrospective cohort	IVF	100	All twin pregnancies >24 weeks gestation following IVF and spontaneous fertilisation		537	1074	Neonatal death, any congenital malformations, APGAR <7 at 5 minutes, TTTS, NICU admission, RDS, NEC, neonatal sepsis, IVH
11	**Couck, 2020** Belgium	Retrospective cohort	IVF, ICSI, Egg donation	200	Ongoing MCDA twin pregnancies in the first trimester during the study period	Women referred for invasive testing because of an anomaly, pregnancies resulting from ovulation stimulation	654	1308	Stillbirth, neonatal death, any congenital malformations, major congenital malformations, TTTS
12	**Daniel, 2000** Israel	Retrospective cohort	IVF, ICSI	24	All twin pregnancies delivered at >24 weeks gestation	Higher-order multiple pregnancies with or without intra uterine fetal demise, early vanishing twin pregnancies, twins reduced to singletons	297	692	Neonatal death, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, neonatal jaundice, neonatal hypoglycaemia, respiratory disorders

13	**Daskalakis, 2015** Greece	Retrospective cohort	Type of ART not mentioned	324	Dichorionic twin pregnancies conceived after ART or spontaneous conception which underwent chorionic villous sampling during the study period at the study hospital	Women having undergone embryo reduction, selective feticide; with fetal anatomic or chromosomal anomalies, with the demise of one twin at the time of the procedure, monochorionic twin pregnancy, cases that underwent a repeat invasive procedure due to culture failure, incomplete data	162	318	Stillbirth, neonatal death, NICU admission
14	**Declercq, 2015** USA	Prospective cohort	Type of ART not mentioned	54	Twins with complete data in Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS) and Pregnancy to Early Life Longitudinal (PELL) data systems		7248	14493	Stillbirth, Perinatal mortality, SGA $<10^{th}$ centile
15	**DeLuca, 2013** USA	Retrospective cohort	IVF	72	All infants of twin pregnancies >20 weeks gestation who were born at the study hospital and delivered by a single maternal-fetal medicine practice	Incomplete data	378	756	SGA $<10^{th}$ centile, any congenital malformations, NICU admission, RDS, NEC, neonatal sepsis, mechanical ventilation

16	**Dhont, 1999** Belgium	Retrospective cohort	IVF, ICSI	72	All twin pregnancies resulting in babies weighing >500g conceived using ART (IVF/ICSI), natural conception matched for age, parity, fetal sex, order of gestation, the multiplicity of birth	Pregnancies resulting from OI, gestations of a higher order than twins	2482	4964	Stillbirth, perinatal mortality, any congenital malformations, NICU admission,
17	**Domingues, 2014** Portugal	Prospective cohort	IVF, ICSI	192	All twin pregnancies conceived spontaneously and those following an induction method (OI, IVF, ICSI)	Triplets and higher orders, monoamniotic multiple gestations	876		TTTS
18	**Eskandar, 2007** Saudi Arabia	Prospective cohort	ICSI	24	ICSI conceived twins and spontaneously conceived twin pregnancies		108	216	RDS, neonatal sepsis, neonatal jaundice

19	**Fedder, 2013** Denmark	Retrospective cohort	Conventional IVF. ICSI with epididymal, testicular or ejaculated sperm	180	Study - All Danish children born after ICSI with testicular or epididymal sperm. Control group - children conceived by ICSI with ejaculated sperm, IVF and natural conception.	Children born after transfer of frozen-thawed embryos	19906	39811	Stillbirth, neonatal death, Perinatal mortality, any congenital malformations
20	**Feng, 2018** China	Retrospective cohort	IVF-ET	109	Twin pregnancies complicated with Intrahepatic cholestasis of pregnancy who were conceived natural or by IVF	Infertility factors except for tubal factor infertility, pregnancies after OI or artificial insemination, any of the causes of liver dysfunction: viral or autoimmune hepatitis, acute fatty liver of pregnancy, primary biliary cirrhosis, pre-eclampsia, haemolysis, elevated liver enzymes and low platelets (HELLP) syndrome, other hepatic imaging abnormalities	142	284	SGA $<10^{th}$ centile

21	**Geisler, 2014** Ireland	Retrospective cohort	IVF, ICSI, Oocyte donation	48	All viable DCDA twin pregnancies around 12 weeks gestation	Twin pregnancy complicated by early fetal loss (<12 weeks gestation), 2nd-trimester loss of both twins, women conceived using IUI /OI, monochorionic twins	539	1078	Stillbirth, neonatal death, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS, neonatal hypoglycaemia
22	**Guilbaud, 2017** France	Retrospective cohort	IVF with autologous and donor oocytes	58	All women with twin pregnancies who gave birth after 24 weeks of gestation	Monochorionic, monoamniotic pregnancy, women transferred during pregnancy from another maternity unit due to maternal or fetal disease	672	1344	Stillbirth, neonatal death, SGA <5th centile
23	**Hansen, 2009** Australia	Retrospective cohort	IVF, ICSI, GIFT	84	Children born as twins up to the age of 3 years following ART or spontaneous conception	Aboriginal children	2398	4797	Stillbirth, neonatal death, any congenital malformations, major congenital malformations
24	**Hansen, 2012** Australia	Retrospective cohort	IVF, ICSI-partial zona dissection, subzonal inseminatio n	108	All births from 20 weeks of gestation or birth weight of 400g or more, including stillbirths	Data relating to Aboriginal children, births after GIFT, triplets and higher-order multiples	3159	6295	Stillbirth, any congenital malformations, major congenital malformations

25	**Henningsen, 2018** Denmark, Finland, Sweden, Norway	Retrospective cohort	IVF, ICSI, frozen embryo transfer	300	Children born after a gestational age of 22+0 weeks or more conceived by ART. Controls: Spontaneously conceived twins born within the study period		Number of neonates only: 161974	160661	SGA <5th centile, any congenital malformations, major congenital malformations
26	**Ho, 2005** Taiwan	Retrospective cohort	IVF-ET, tubal embryo transfer	24	All twin pregnancies delivered at 23 or more weeks of gestation, including higher-order multiples reduced to twin		159	318	Stillbirth, Perinatal mortality, SGA <10th centile, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS, neonatal sepsis, IVH, neonatal hypoglycaemia, neonatal jaundice
27	**Huang, 2006** Taiwan	Retrospective cohort	IVF, ICSI-ET	120	Twin births delivered at Taipei Medical University Hospital during 1992-2001	All patients with any history of hypertension, diabetes mellitus, abortions, foetuses with a birth age of <24 gestational weeks, higher-order multiples, cases with incomplete data	194	388	Stillbirth, neonatal death, APGAR <7 at 5 minutes
28	**Isaksson, 2002** Finland	Retrospective cohort	IVF, ICSI, Frozen Embryo Transfer	75	Pregnancies achieved after IVF/ICSI in women with unexplained infertility ending with birth. Comparison group 1: women with non-assisted pregnancy, group 2: all women	Spontaneous abortion, triplet pregnancies	596	1192	Perinatal mortality, SGA <5th centile, any congenital malformations, major congenital malformations, NICU admission, umbilical cord pH <7.2, HIE

					delivering after IVF, ICSI, frozen ET				
29	**Joy, 2008** Ireland	Retrospective cohort	IVF, ICSI	24	All twin births with available records	Births conceived after ovulation induction	202	404	Stillbirth, neonatal death, Perinatal mortality, twin birth weight discordance >25%, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, umbilical cord pH <7.2
30	**Kallen, 2010** Sweden	Retrospective cohort	IVF, ICSI	276	Cases: Complete dizygotic twin pairs born after IVF/ICSI. Controls: Dizygotic twins where no information on the presence of IVF existed	Twins with incomplete data	10220	20440	Neonatal jaundice, respiratory complications
31	**Katalinic, 2004** Germany	Prospective cohort	ICSI, fresh ET	36	Pregnancies conceived after ICSI/fresh ET recruited before 16th week of gestation	Unresponsive participants		1310	Any congenital malformations, major congenital malformations
32	**Kim, 2015** South Korea	Retrospective cohort	IVF	120	Women with IVF and natural conception	Selective fetal reduction, MCMA twins, delayed interval delivery, no available data on conception method or chorionicity	1337	2274	Neonatal death, SGA <10th centile, APGAR <7 at 5 minutes, NICU admission

33	**Koudstaal, 2000** Netherland	Retrospective cohort	IVF	Not mentioned	Ongoing twin pregnancies >16 weeks Pregnancy before the end of 1992 and obstetric care provided by the hospital where the IVF care was provided. Spontaneous twin pregnancies from the same hospital as the cases, maternal age maximum 3 years apart from IVF mothers	IVF pregnancy after transfer of frozen embryos, embryo reduction	192	384	Perinatal mortality, SGA <10th centile, any congenital malformations, APGAR <7 at 5 minutes
34	**Kuwata, 2004** Japan	Prospective cohort	IVF, ICSI, GIFT	138	Mothers with DC twin pregnancies who received continuous antenatal care from <20 weeks of gestation and gave birth to infants after >24 weeks of gestation	Women who were referred after >20 weeks of gestation, suspicion of fetal morphological abnormalities, mothers who became pregnant after receiving frozen embryo transfer	406	812	Any congenital malformations

35	**Lehnen, 2011** Germany	Retrospective cohort	IVF, ICSI	111	Twins conceived by spontaneous conception and ART that have been delivered in the ICU of the study hospital		379		TTTS
36	**Lei, 2019** China	Retrospective cohort	IVF, ICSI, fresh or frozen Embryo transfer	36	Live newborns after 28th week of gestation	Donor oocytes/ sperm, embryo recipients, ovulation induction, women applied preimplantation genetic diagnosis	904	1808	APGAR <7 at 5 minutes
37	**Luke, 2017** USA	Retrospective cohort	IVF, ICSI	78	All live twin births of >22 weeks gestation and >350g birth weight of residents of Massachusetts		10352	20704	Neonatal death, SGA <10th centile, any congenital malformations

38	**Malchau, 2013** Denmark	Retrospective cohort	IVF, ICSI, oocyte donation	192	All children born after oocyte donation in Denmark from 1995-2010 and children born after IVF/ICSI, spontaneous conception matched by date and year of birth	Women who delivered in Denmark after an oocyte donation procedure performed in another country	19114	38228	Stillbirth, SGA <10th centile
39	**Manoura, 2004** Greece	Retrospective cohort	IVF	103	Twin pregnancies conceived by IVF and spontaneous conception	Higher-order multiples, pregnancies conceived after OI, twin pregnancies reduced to a singleton at 10th week, early loss of one twin (12th week), uncontrolled diabetes mellitus type 1, SLE	221	442	Neonatal death, perinatal mortality, SGA <10th centile, twin birth weight discordance >25%, any congenital malformations, APGAR <7 at 5 minutes, NICU admission
40	**Marino, 2014** Australia	Retrospective cohort	Oocyte donation, GIFT, IVF, ICSI	204	All live births and stillbirths of at least 20 weeks gestation or 400g birth weight	Pregnancies among mothers under 20 years of age, births of higher-order multiples, births and terminations of indeterminate sex and where the sex was unknown		9006	Stillbirth, neonatal death, SGA <10th centile, APGAR <7 at 5 minutes

41	**Mohammed, 2012** Doha Qatar	Retrospective cohort	IVF	120	All eligible cases of DCDA twin pregnancies	IUFD, BW <500g, <24 weeks at delivery, higher-order multiples, monochorionic twins, singletons complicated by early vanishing fetus, twins reduced to singletons, triplets reduced to twins	320	640	Perinatal mortality, SGA <10th centile, twin birth weight discordance >25%, any congenital malformations, APGAR <7 at 5 minutes, NICU admission
42	**Moini, 2012** Iran	Prospective cohort	IVF, ICSI	33	All DCDA twin pregnancies to nulliparous women referred <14 weeks and delivering >22 weeks.	Twin pregnancies following infertility treatment due to PCOS and uterine factor, those who had experienced OHSS during controlled ovarian hyperstimulation protocols, history of medical diseases and surgery on pelvic organs, height <150 cm, smokers, non-Iranian race, pregnancies conceived by OI, IUI and selective fetal reduction, pregnancies with vanishing embryos	400	800	Stillbirth, perinatal mortality, twin birth weight discordance >25%, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS

43	**Moise, 1998** Israel	Retrospective cohort	IVF	66	All IVF twins, dizygotic pairs of twins		60	120	Neonatal death, SGA <10th centile, NICU admission, mechanical ventilation
44	**Nassar, 2003** Lebanon	Retrospective cohort	IVF	72	All twin pregnancies that were delivered at ≥25 weeks of gestation	Women who underwent OI only, multifetal pregnancy reduction, underlying maternal disease (HTN, pre GDM, renal disease)	168	226	Perinatal mortality, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS, NEC, neonatal sepsis, IVH, mechanical ventilation
45	**Ochsenkuhn, 2003** Germany	Retrospective cohort	IVF, GIFT	60	All twin and singleton pregnancies after GIFT/ IVF with live-born infants at least 24 weeks with more than 499g birth weight. Next respective pregnancy with a live birth after spontaneous conception who were matched for gestational age, maternal age and parity		156	312	Perinatal mortality, NICU admission

46	**Okby, 2018** Israel	Retrospective cohort	IVF	276	Diagnosis of pre-eclampsia in twin pregnancies conceived via IVF and spontaneous conception during the study period	Women suffering from chronic hypertension or gestational hypertension, pregnancies conceived after ovulation induction	314	628	Perinatal mortality, APGAR <7 at 5 minutes, neonatal death, stillbirth
47	**Olivennes, 1996** France	Retrospective cohort	IVF-ET	66	Deliveries that occurred after 28 weeks of gestation following IVF	Twin pregnancies resulting from selective fetal reduction	318	636	Perinatal mortality, SGA <10th centile
48	**Ombelet, 2005** Belgium	Retrospective cohort	ICSI	84	ICSI pregnancies births ≥21 weeks and ≥500g at birth, – Control - Natural conception, matched for the multiplicity of birth, the same place of birth, maternal age not more than two years apart from the study group, same parity, date of delivery no more than 1 year apart, same fetal sex	Higher-order gestations	1633	3265	Stillbirth, neonatal death, Perinatal mortality, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS, mechanical ventilation
49	**Ombelet, 2016** Belgium	Retrospective cohort	IVF, ICSI	216	Infants born more than 21 weeks gestation or >500g birth weight following ART		19521	39041	Stillbirth, neonatal death, Perinatal mortality, NICU admission, mechanical ventilation

50	**Pinborg, 2004** Denmark	Retrospective cohort	IVF, ICSI	12	All twin pregnancies born after 24 weeks completed		1017	2034	Stillbirth, neonatal death, perinatal mortality, TTTS
51	**Pourali, 2016** Iran	Prospective cohort	long protocol	60	Women with DCDA twin pregnancies	History of underlying disease before gestation such as overt diabetes mellitus, chronic hypertension, autoimmune diseases, monochorionic twins, incomplete data	127	254	Stillbirth, any congenital malformations, APGAR <7 at 5 minutes, NICU admission, RDS, neonatal jaundice, neonatal death
52	**Putterman, 2003** USA	Retrospective cohort	IVF	24	All twin gestations where two live neonates were delivered after 20 weeks	Higher-order gestations reduced to twins or twins that were delivered in a single live birth	195	390	SGA <10th centile, NICU admission
53	**Sagot, 2012** France	Retrospective cohort	IVF, ICSI, Frozen Embryo Transfer	102	All deliveries live or not with gestational age >22 weeks and/or the birth weight >500g			2208	Any congenital malformations, major congenital malformations

54	**Simoes, 2015** Portugal	Retrospective cohort	IVF, ET	244	Monochorionic twin pregnancies followed up and delivered ≥24 weeks gestation to mothers conceived by ART and spontaneous conception	Twin gestations delivered but not followed up at the hospital of study, monoamniotic pregnancies	508	1016	Stillbirth, neonatal death, any congenital malformation, APGAR <7 at 5 minutes, TTTS
55	**Skrypchenko, 2016** Ukraine	Retrospective cohort	Type of ART not mentioned	12	Twin pregnancies conceived by ART and natural conception		51		TTTS
56	**Sun, 2016** China	Retrospective cohort	IVF, ICSI	60	All twin pregnancies undergoing serial US examinations at the study hospital	Structural anomalies, fetal reduction, feticide or termination	1153	2306	Perinatal mortality, SGA<10th centile, NICU admission
57	**Szymusik, 2012** Poland	Retrospective cohort	IVF	60	Cases - IVF twin pregnancies from 2005-2009. Controls: Spontaneous conception DCDA from 2005-2009	Monochorionic twins, TOP <22 weeks	126	246	Stillbirth, neonatal death, any congenital malformations, NICU admission, neonatal sepsis, neonatal jaundice, IVH

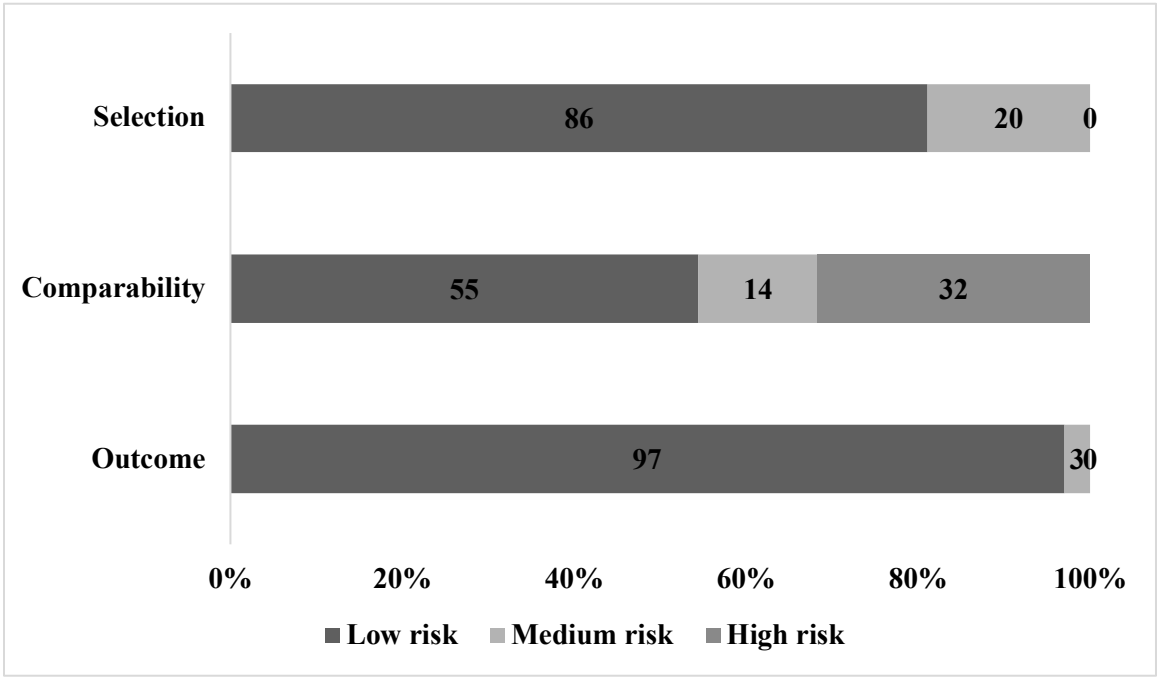
58	**Tallo, 1995** USA	Retrospective cohort	IVF	59	IVF pregnancies that progressed beyond the 20th week of gestation. Pregnancies conceived naturally, matched by age, race, type of insurance, order of gestation		72	144	Perinatal mortality, SGA <10th centile, RDS, neonatal sepsis
59	**Vasario, 2010** Italy	Prospective cohort	IVF, ICSI	48	Patients referred to study centre <14 weeks and delivered >22 weeks, DCDA twins	Triplets, monochorionic twins, twin pregnancies deriving from heterologous IVF or ART other than IVF, pregnancies referred >14 weeks, delivery <22 weeks, twin pregnancies obtained after fetocides undertaken in triplet or multiple pregnancies	223	446	Perinatal mortality, SGA <10th centile, birth weight discordance >25%, any congenital malformations, NICU admission, RDS
60	**Wang, 2018** Australia	Retrospective cohort	Type of ART not mentioned	48	Twins born >20 weeks gestation or >400g birth weight		9831	19662	Stillbirth, neonatal death, APGAR <7 at 5 minutes, NICU admission

61	**Wen, 2010** Canada	Retrospective cohort	IVF, ICSI	120	Cases: IVF, ICSI patients with a viable pregnancy (>20 weeks of gestation. Controls: 2 naturally conceived mothers for each IVF/ICSI patient matched for age and plurality		643	1286	Stillbirth, any congenital malformations, APGAR <7 at 5 minutes, neonatal sepsis, IVH, mechanical ventilation
62	**Wennerholm, 1997** Sweden	Retrospective cohort	IVF, Cryopreserved fresh embryo transfer	61	Cases: Births conceived after IVF with cryopreserved-thawed embryos, all liveborn and stillborn infants after more than 28 weeks gestation. Controls: Group 1: Births after IVF with fresh embryos Group 2: Spontaneous pregnancies; Controls were matched according to maternal age, =/-5 years, parity, plurality and date of delivery	Parents who declined to participate	147	294	SGA <5th centile
63	**Westergaard, 1999** Denmark	Retrospective cohort	IVF, ICSI	24	Cases: Births after IVF/ICSI, registered in Danish IVF registry. Controls: Births following non-ART conception matched by maternal age, child age, parity and multiplicity		854	1708	Stillbirth, neonatal death

64	**Yang, 2011** South Korea	Retrospective cohort	IVF	168	All dichorionic twin births after IVF or natural conception	IUFD, neonates with birth weight <500g, or <24 weeks gestation at delivery, higher order multiple pregnancies, singleton deliveries complicated by early vanishing foetuses, twin pregnancies reduced to singleton, triplet pregnancies reduced to twins	210	420	Neonatal death, SGA <10th centile, birth weight discordance >25%, any congenital malformations, APGAR <7 at 5 minutes, NICU admission
65	**Zadori, 2003** Hungary	Retrospective cohort	IVF-ET	86	All pregnancies conceived spontaneously and by IVF-ET		72	144	SGA <10th centile
66	**Zhu, 2016** China	Retrospective cohort	IVF, ICSI	108	Live newborns after 28th week of gestation following ART and natural conception	Donor oocytes/sperms or embryo recipients, ovulation induction or women applied pre-implantation genetic diagnosis, chronic hypertension, diabetes, or heart disease, fetal anomalies	1071	2142	SGA <10th centile, APGAR <7 at 5 minutes

ART – Assisted Reproductive Techniques, IVF – In Vitro Fertilization, ICSI – Intra Cytoplasmic Sperm Injection, GIFT – Gamete Intra Fallopian Transfer, ET – Embryo Transfer, OI – Ovulation Induction, IUI – Intra Uterine Insemination, SGA- Small for Gestational Age, TTTS – Twin-Twin Transfusion Syndrome, NICU – Neonatal Intensive Care Unit, RDS – Respiratory Distress Syndrome, NEC – Necrotizing Enterocolitis, GDM- Gestational diabetes mellitus, IVH – Intra Ventricular Haemorrhage, HIE – Hypoxic Ischemic Encephalopathy, IUFD – Intra Uterine Fetal Demise, DCDA- Dichorionic Diamniotic, MCDA – Monochorionic Monoamniotic, PELL – Pregnancy to Early Life Longitudinal, SART CORS – Society of Assisted Reproductive Technology Clinical Outcomes Reporting System, TOP – Termination of Pregnancy, USS- Ultrasonographic

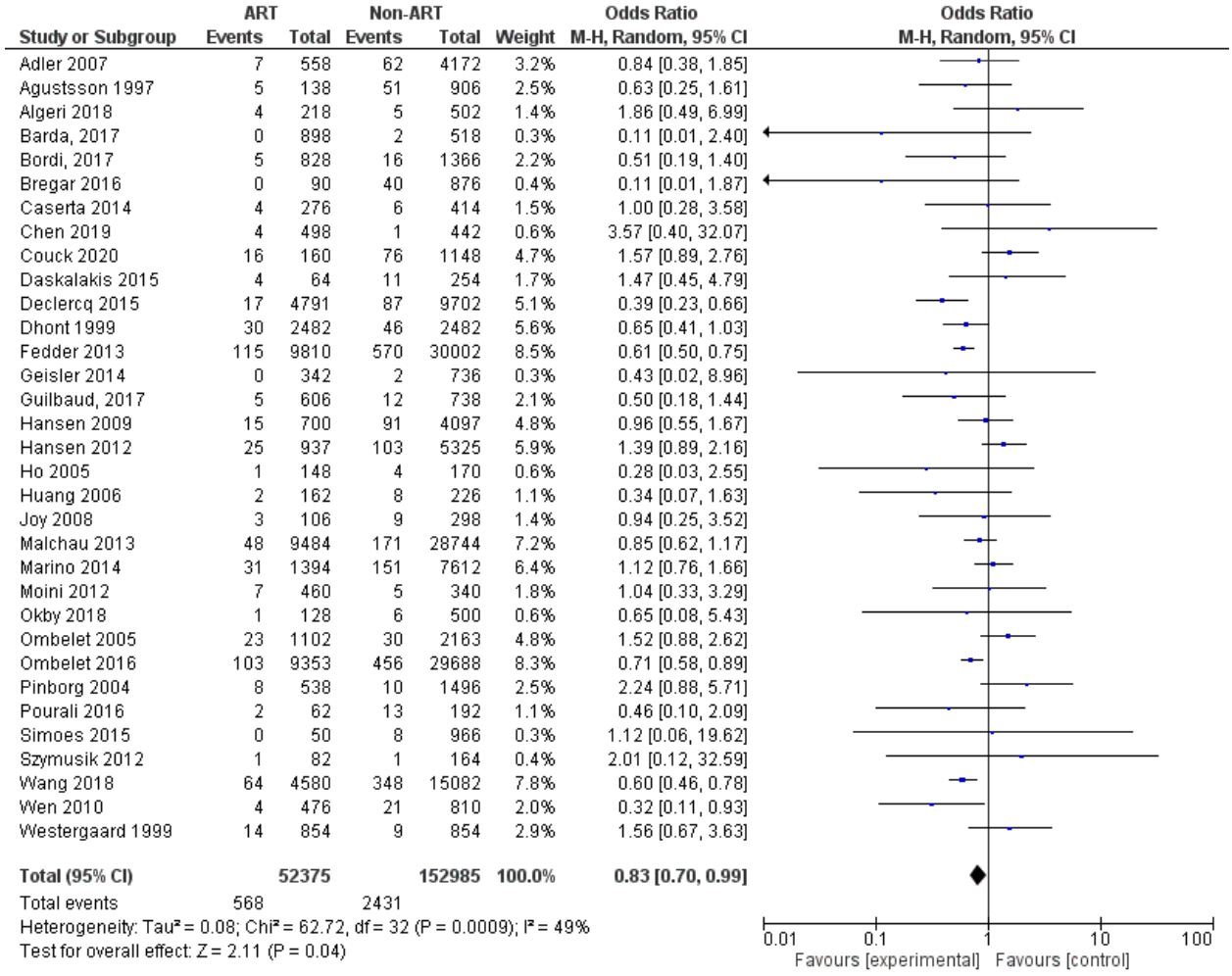
APPENDIX 26: Quality assessment using the Newcastle Ottawa Scale in the systemic review of perinatal outcomes in twin pregnancies following assisted reproduction.



APPENDIX 27: Forest plots of pooled odds ratios (OR) for certain perinatal outcomes comparing ART vs non-ART and ART vs natural conception in the systematic review of perinatal outcomes in twin pregnancies following assisted reproduction.

ART vs Non-ART

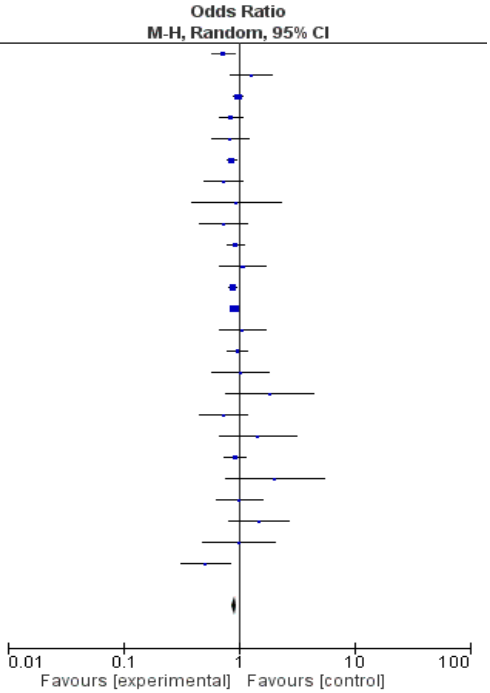
1. Stillbirth



2. SGA <10th centile

Study or Subgroup	ART Events	ART Total	Non-ART Events	Non-ART Total	Weight	Odds Ratio M-H, Random, 95% CI
Adler 2007	93	558	896	4172	3.6%	0.73 [0.58, 0.92]
Algeri 2018	42	218	79	502	1.3%	1.28 [0.85, 1.93]
Bensdrop 2016	1068	4874	1884	8514	14.6%	0.99 [0.91, 1.08]
Bordi, 2017	131	828	247	1366	3.7%	0.85 [0.68, 1.07]
Caserta 2014	56	276	96	414	1.6%	0.84 [0.58, 1.22]
Declercq 2015	855	4791	1942	9702	13.9%	0.87 [0.79, 0.95]
DeLuca 2013	82	496	55	260	1.5%	0.74 [0.50, 1.08]
Feng 2018	8	102	15	182	0.3%	0.95 [0.39, 2.32]
Ho 2005	42	148	59	170	1.0%	0.75 [0.46, 1.20]
Kim 2015	445	1556	334	1118	6.2%	0.94 [0.79, 1.11]
Koudstaal 2000	49	192	46	192	1.0%	1.09 [0.68, 1.73]
Luke 2017	1245	7076	2638	13628	16.3%	0.89 [0.83, 0.96]
Malchau 2013	1974	9484	6399	28744	19.5%	0.92 [0.87, 0.97]
Manoura 2004	35	146	67	296	1.0%	1.08 [0.68, 1.72]
Marino 2014	127	1394	710	7612	4.8%	0.97 [0.80, 1.19]
Mohammed 2012	24	290	28	350	0.7%	1.04 [0.59, 1.83]
Moise 1998	12	40	15	80	0.3%	1.86 [0.77, 4.47]
Olivennes 1996	26	144	113	492	1.0%	0.74 [0.46, 1.19]
Putterman 2003	12	120	19	270	0.4%	1.47 [0.69, 3.13]
Sun 2016	153	822	292	1484	4.1%	0.93 [0.75, 1.16]
Tallo 1995	13	72	7	72	0.2%	2.05 [0.76, 5.47]
Vasario 2010	41	168	67	278	1.1%	1.02 [0.65, 1.59]
Yang 2011	20	134	30	286	0.6%	1.50 [0.82, 2.75]
Zadori 2003	22	72	22	72	0.4%	1.00 [0.49, 2.03]
Zhu 2016	120	1964	20	178	0.9%	0.51 [0.31, 0.85]
Total (95% CI)		**35965**		**80434**	**100.0%**	**0.91 [0.87, 0.96]**
Total events	6695		16080			

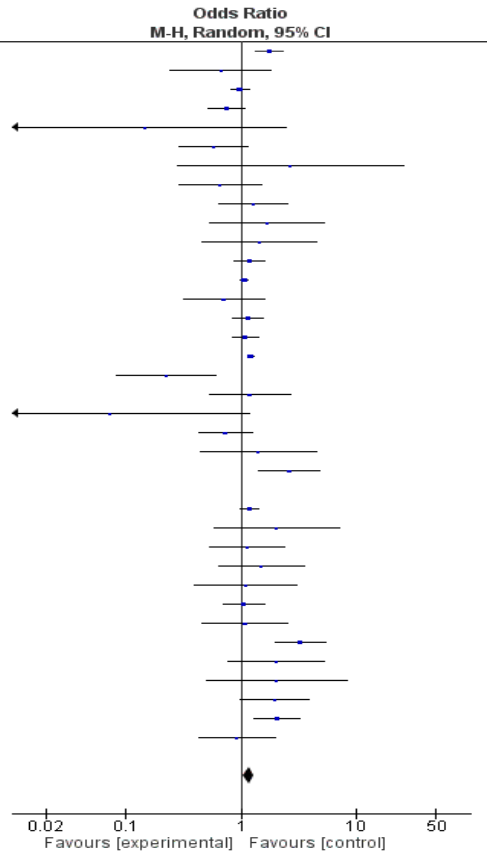
Heterogeneity: Tau² = 0.00; Chi² = 30.45, df = 24 (P = 0.17); I² = 21%
Test for overall effect: Z = 3.64 (P = 0.0003)



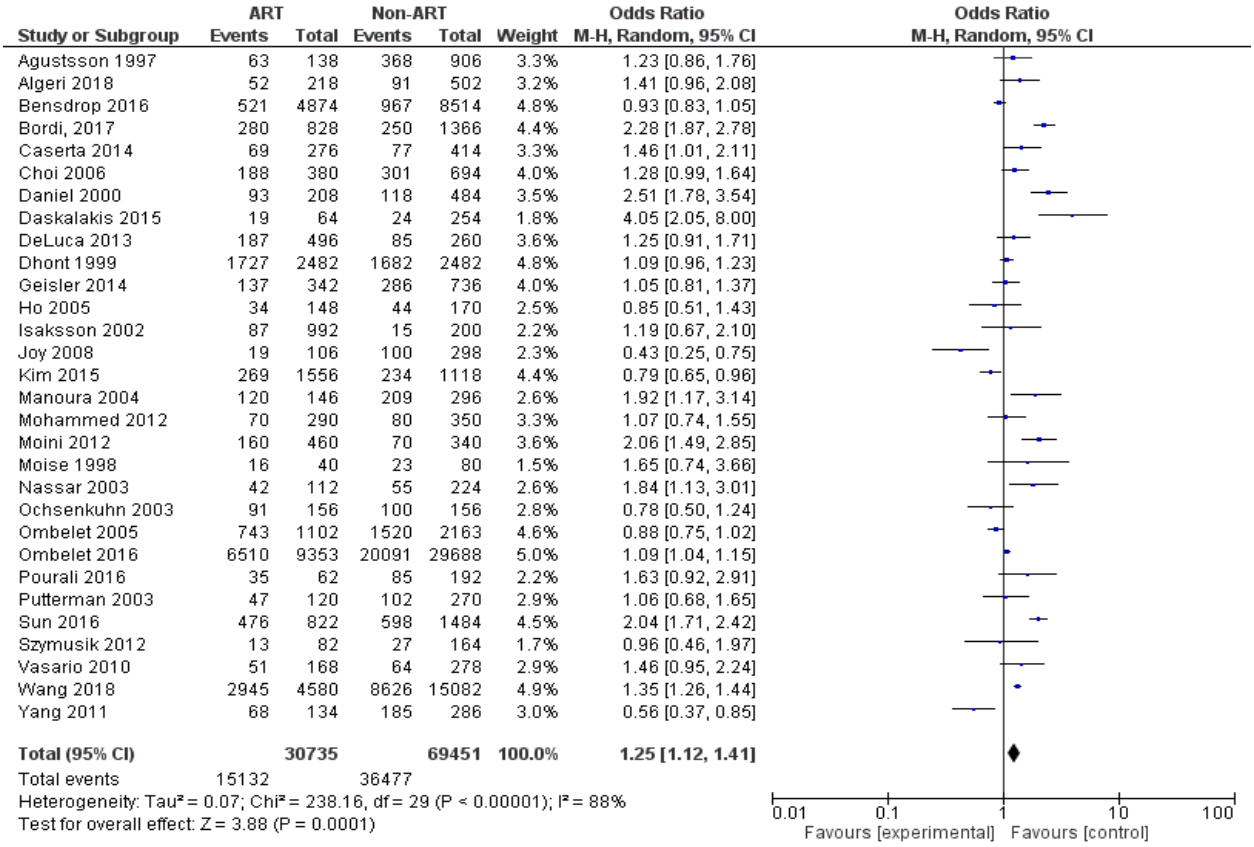
3. Congenital malformation

Study or Subgroup	ART Events	ART Total	Non-ART Events	Non-ART Total	Weight	Odds Ratio M-H, Random, 95% CI
Adler 2007	67	558	298	4172	5.8%	1.77 [1.34, 2.35]
Algeri 2018	5	218	17	502	1.0%	0.67 [0.24, 1.84]
Bensdrop 2016	176	4874	314	8514	7.4%	0.98 [0.81, 1.18]
Bordi, 2017	41	828	89	1366	4.4%	0.75 [0.51, 1.09]
Bregar 2016	0	90	31	876	0.1%	0.15 [0.01, 2.44]
Caserta 2014	12	276	30	414	1.9%	0.58 [0.29, 1.16]
Chen 2019	3	498	1	442	0.2%	2.67 [0.28, 25.79]
Choi 2006	8	380	22	694	1.5%	0.66 [0.29, 1.49]
Couck 2020	10	160	57	1148	1.9%	1.28 [0.64, 2.55]
Daniel 2000	5	208	7	484	0.8%	1.68 [0.53, 5.35]
DeLuca 2013	11	496	4	260	0.8%	1.45 [0.46, 4.60]
Dhont 1999	86	2482	73	2482	5.2%	1.18 [0.86, 1.63]
Fedder 2013	1104	9810	3188	30002	9.2%	1.07 [0.99, 1.15]
Geisler 2014	8	342	24	736	1.5%	0.71 [0.32, 1.60]
Hansen 2009	48	700	247	4097	5.2%	1.15 [0.83, 1.58]
Hansen 2012	67	937	351	5325	5.9%	1.09 [0.83, 1.43]
Henningsen 2018	1528	28920	5822	131741	9.3%	1.21 [1.14, 1.28]
Ho 2005	5	148	23	170	1.0%	0.22 [0.08, 0.60]
Isaksson 2002	41	992	7	200	1.5%	1.19 [0.53, 2.69]
Joy 2008	0	106	18	298	0.1%	0.07 [0.00, 1.19]
Katalinic 2004	98	1158	17	152	2.8%	0.73 [0.43, 1.27]
Koudstaal 2000	7	192	5	192	0.8%	1.42 [0.44, 4.54]
Kuwata 2004	36	398	15	414	2.3%	2.65 [1.42, 4.91]
Lehnen 2011	1	74	0	0		Not estimable
Luke 2017	191	7076	310	13628	7.5%	1.19 [0.99, 1.43]
Manoura 2004	5	146	5	296	0.7%	2.06 [0.59, 7.25]
Mohammed 2012	14	290	15	350	1.7%	1.13 [0.54, 2.39]
Moini 2012	16	460	8	340	1.3%	1.50 [0.63, 3.54]
Nassar 2003	6	112	11	224	1.0%	1.10 [0.39, 3.04]
Ombelet 2005	35	1102	65	2163	3.9%	1.06 [0.70, 1.61]
Pourali 2016	8	62	23	192	1.3%	1.09 [0.46, 2.57]
Sagot 2012	27	362	44	1846	3.2%	3.30 [2.02, 5.40]
Simoes 2015	5	50	50	966	1.1%	2.04 [0.77, 5.35]
Szymusik 2012	4	82	4	164	0.5%	2.05 [0.50, 8.42]
Vasario 2010	19	168	17	278	2.0%	1.96 [0.99, 3.88]
Wen 2010	43	476	37	810	3.5%	2.07 [1.32, 3.27]
Yang 2011	10	134	23	286	1.6%	0.92 [0.43, 2.00]
Total (95% CI)		**65291**		**216224**	**100.0%**	**1.18 [1.06, 1.31]**
Total events	3749		11272			

Heterogeneity: Tau² = 0.03; Chi² = 89.19, df = 35 (P < 0.00001); I² = 61%
Test for overall effect: Z = 3.01 (P = 0.003)

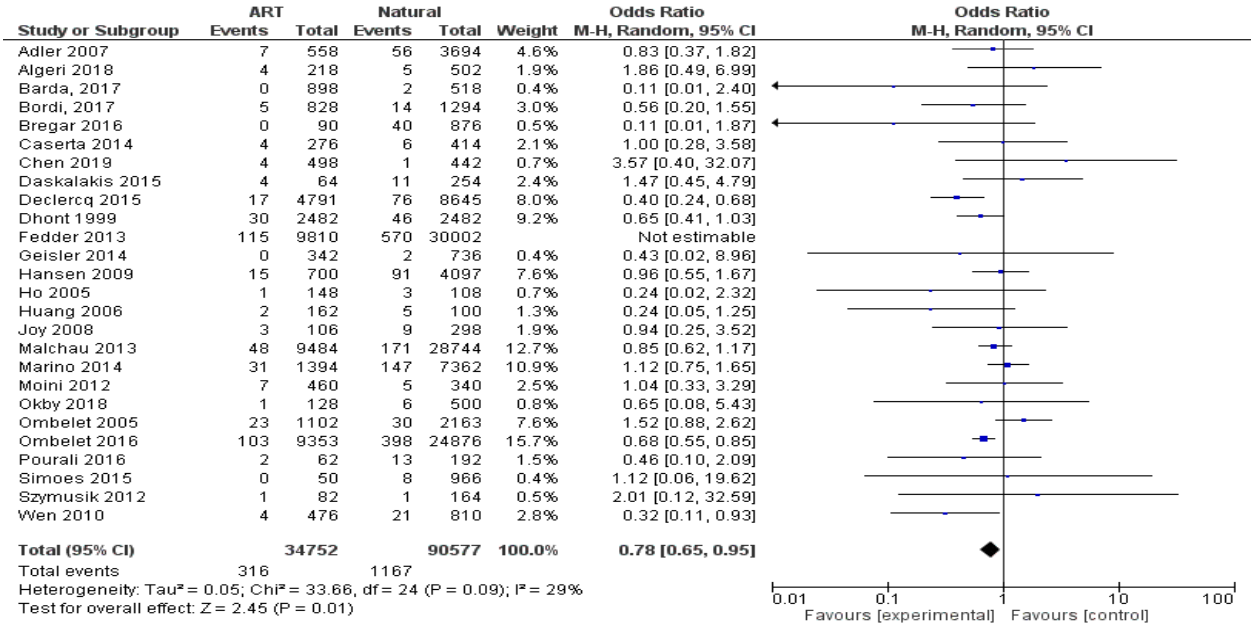


4. NICU admission



ART vs Natural

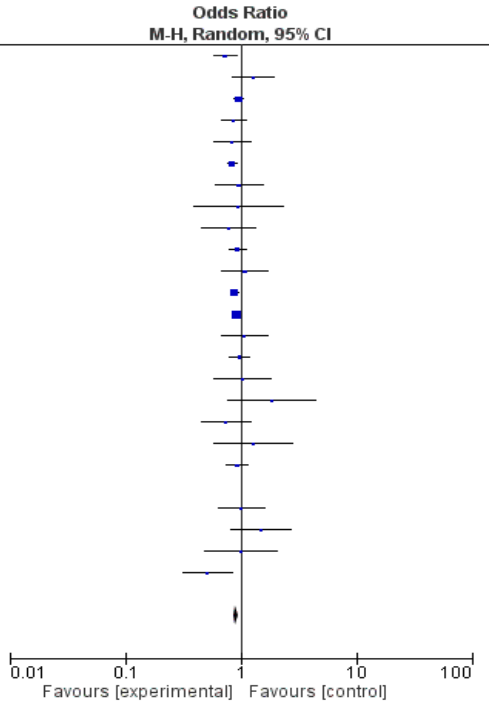
1. Stillbirth



2. SGA <10th centile

Study or Subgroup	ART Events	ART Total	Natural Events	Natural Total	Weight	Odds Ratio M-H, Random, 95% CI
Adler 2007	93	558	794	3694	2.6%	0.73 [0.58, 0.92]
Algeri 2018	42	218	79	502	0.9%	1.28 [0.85, 1.93]
Bensdrop 2016	1068	4874	1481	6552	14.7%	0.96 [0.88, 1.05]
Bordi, 2017	131	828	230	1294	2.6%	0.87 [0.69, 1.10]
Caserta 2014	56	276	96	414	1.1%	0.84 [0.58, 1.22]
Declercq 2015	855	4791	1765	8645	14.4%	0.85 [0.77, 0.93]
DeLuca 2013	82	496	28	164	0.7%	0.96 [0.60, 1.54]
Feng 2018	8	102	15	182	0.2%	0.95 [0.39, 2.32]
Ho 2005	42	148	36	108	0.5%	0.79 [0.46, 1.36]
Kim 2015	445	1556	334	1118	4.9%	0.94 [0.79, 1.11]
Koudstaal 2000	49	192	46	192	0.7%	1.09 [0.68, 1.73]
Luke 2017	1245	7076	2363	12180	18.7%	0.89 [0.82, 0.96]
Malchau 2013	1974	9484	6399	28744	27.1%	0.92 [0.87, 0.97]
Manoura 2004	35	146	67	296	0.7%	1.08 [0.68, 1.72]
Marino 2014	127	1394	683	7362	3.6%	0.98 [0.80, 1.20]
Mohammed 2012	24	290	28	350	0.5%	1.04 [0.59, 1.83]
Moise 1998	12	40	15	80	0.2%	1.86 [0.77, 4.47]
Olivennes 1996	26	144	75	328	0.6%	0.74 [0.45, 1.22]
Putterman 2003	12	120	16	202	0.2%	1.29 [0.59, 2.83]
Sun 2016	153	822	292	1484	3.1%	0.93 [0.75, 1.16]
Tallo 1995	13	72	7	72		Not estimable
Vasario 2010	41	168	67	278	0.8%	1.02 [0.65, 1.59]
Yang 2011	20	134	30	286	0.4%	1.50 [0.82, 2.75]
Zadori 2003	22	72	22	72	0.3%	1.00 [0.49, 2.03]
Zhu 2016	120	1964	20	178	0.6%	0.51 [0.31, 0.85]
Total (95% CI)		**35893**		**74705**	**100.0%**	**0.91 [0.87, 0.95]**
Total events	6682		14981			

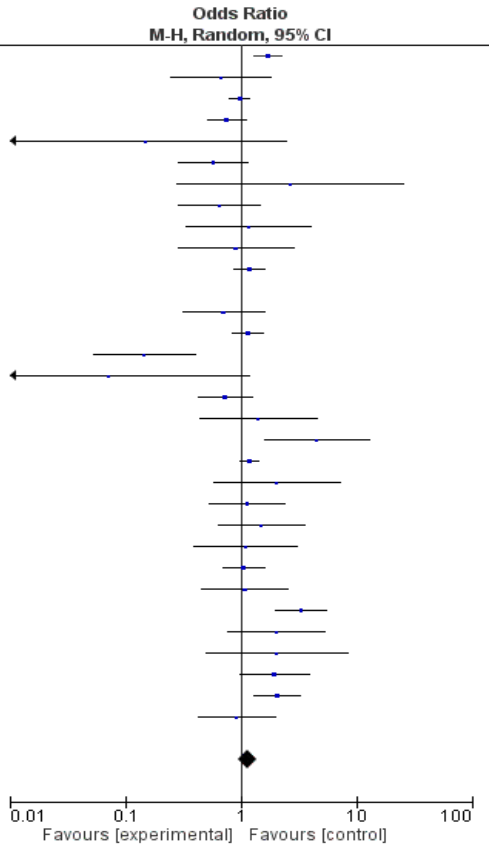
Heterogeneity: Tau² = 0.00; Chi² = 24.74, df = 23 (P = 0.36); I² = 7%
Test for overall effect: Z = 4.79 (P < 0.00001)



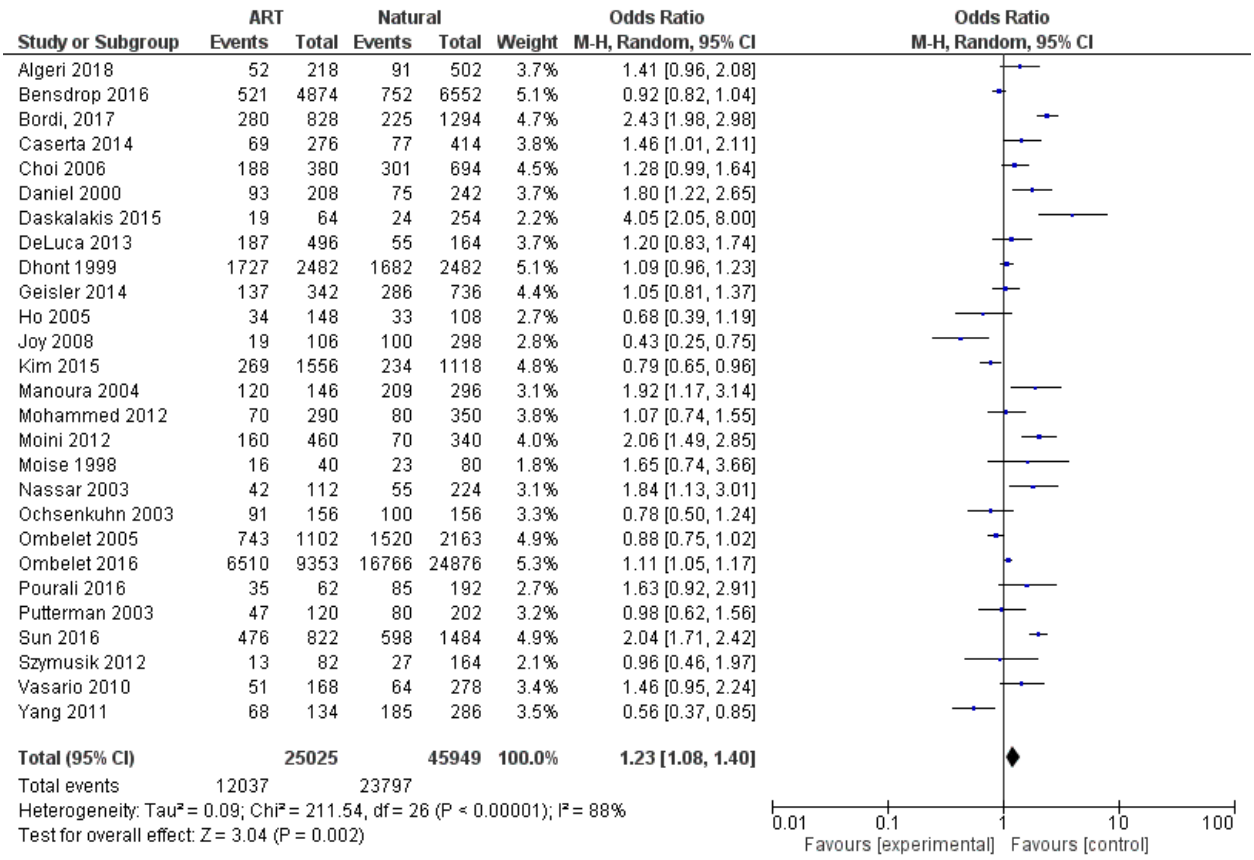
3. Congenital malformation

Study or Subgroup	ART Events	ART Total	Natural Events	Natural Total	Weight	Odds Ratio M-H, Random, 95% CI
Adler 2007	67	558	274	3694	6.0%	1.70 [1.28, 2.26]
Algeri 2018	5	218	17	502	2.2%	0.67 [0.24, 1.84]
Bensdrop 2016	176	4874	241	6552	6.5%	0.98 [0.80, 1.20]
Bordi, 2017	41	828	83	1294	5.4%	0.76 [0.52, 1.12]
Bregar 2016	0	90	31	876	0.4%	0.15 [0.01, 2.44]
Caserta 2014	12	276	30	414	3.5%	0.58 [0.29, 1.16]
Chen 2019	3	498	1	442	0.6%	2.67 [0.28, 25.79]
Choi 2006	8	380	22	694	2.9%	0.66 [0.29, 1.49]
Daniel 2000	5	208	5	242	1.6%	1.17 [0.33, 4.09]
DeLuca 2013	11	496	4	164	1.8%	0.91 [0.28, 2.89]
Dhont 1999	86	2482	73	2482	5.8%	1.18 [0.86, 1.63]
Fedder 2013	1104	9810	3188	30002		Not estimable
Geisler 2014	8	342	24	736	2.9%	0.71 [0.32, 1.60]
Hansen 2009	48	700	247	4097	5.8%	1.15 [0.83, 1.58]
Ho 2005	5	148	21	108	2.2%	0.14 [0.05, 0.40]
Joy 2008	0	106	18	298	0.4%	0.07 [0.00, 1.19]
Katalinic 2004	98	1158	17	152	4.3%	0.73 [0.43, 1.27]
Koudstaal 2000	7	192	5	192	1.8%	1.42 [0.44, 4.54]
Kuwata 2004	36	398	4	188	2.1%	4.57 [1.60, 13.05]
Luke 2017	191	7076	280	12180	6.6%	1.18 [0.98, 1.42]
Manoura 2004	5	146	5	296	1.6%	2.06 [0.59, 7.25]
Mohammed 2012	14	290	15	350	3.2%	1.13 [0.54, 2.39]
Moini 2012	16	460	8	340	2.7%	1.50 [0.63, 3.54]
Nassar 2003	6	112	11	224	2.2%	1.10 [0.39, 3.04]
Ombelet 2005	35	1102	65	2163	5.1%	1.06 [0.70, 1.61]
Pourali 2016	8	62	23	192	2.7%	1.09 [0.46, 2.57]
Sagot 2012	27	362	39	1656	4.6%	3.34 [2.02, 5.53]
Simoes 2015	5	50	50	966	2.3%	2.04 [0.77, 5.35]
Szymusik 2012	4	82	4	164	1.3%	2.05 [0.50, 8.42]
Vasario 2010	19	168	17	278	3.5%	1.96 [0.99, 3.88]
Wen 2010	43	476	37	810	4.9%	2.07 [1.32, 3.27]
Yang 2011	10	134	23	286	3.1%	0.92 [0.43, 2.00]
Total (95% CI)		**24472**		**43032**	**100.0%**	**1.15 [0.96, 1.37]**
Total events	999		1694			

Heterogeneity: Tau² = 0.12; Chi² = 84.31, df = 30 (P < 0.00001); I² = 64%
Test for overall effect: Z = 1.49 (P = 0.14)



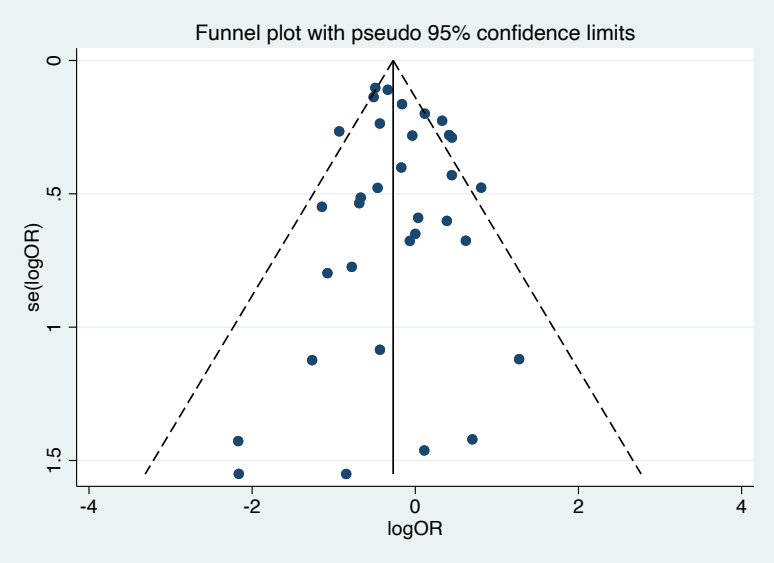
4. NICU admission



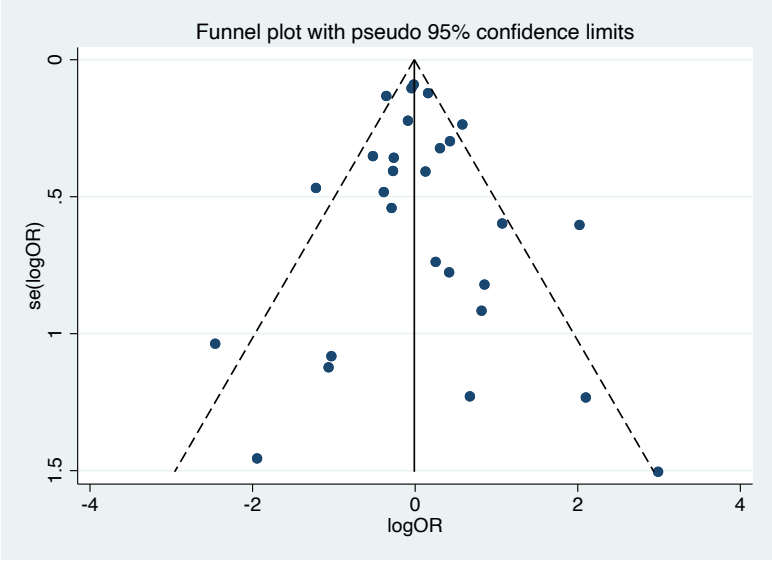
APPENDIX 28: Funnel plots for meta-analyses with more than 10 included studies in the systemic review of perinatal outcomes in twin pregnancies following assisted reproduction.

ART vs Non-ART

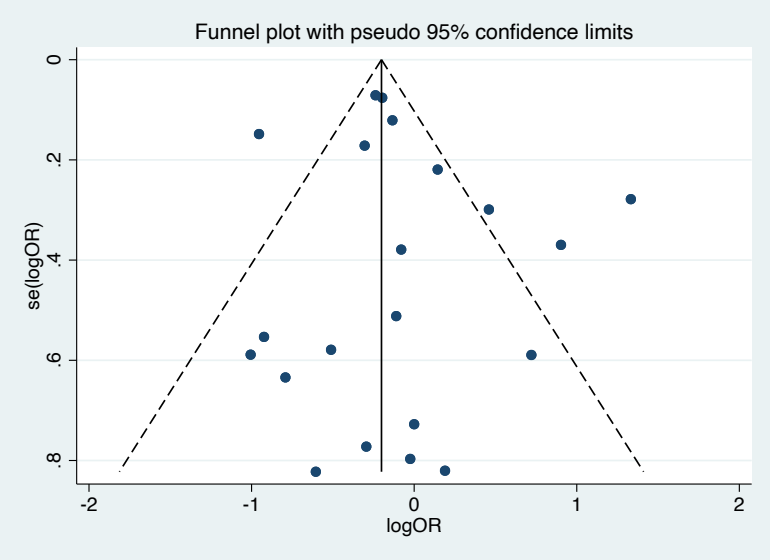
Stillbirth – p = 0.403



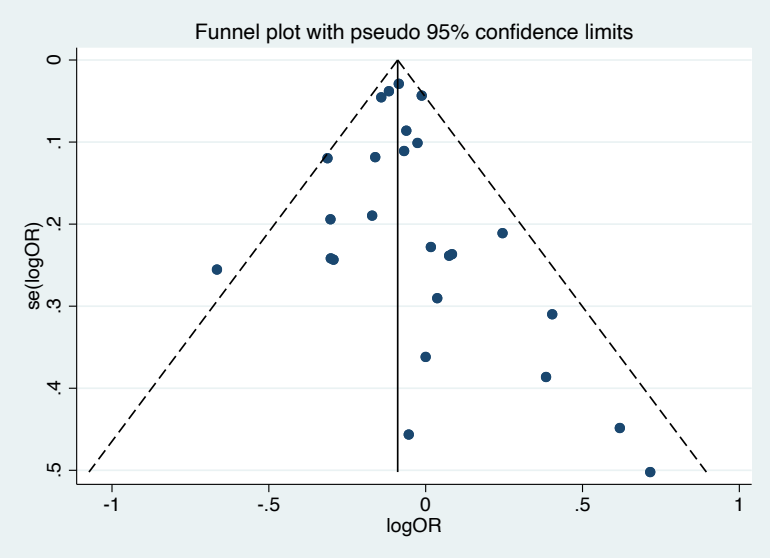
Neonatal death – p = 0.552



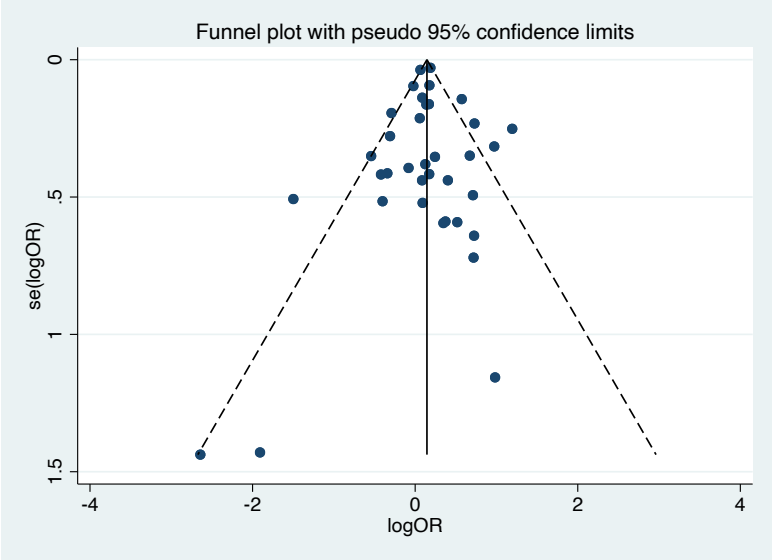
Perinatal mortality – p = 0.448



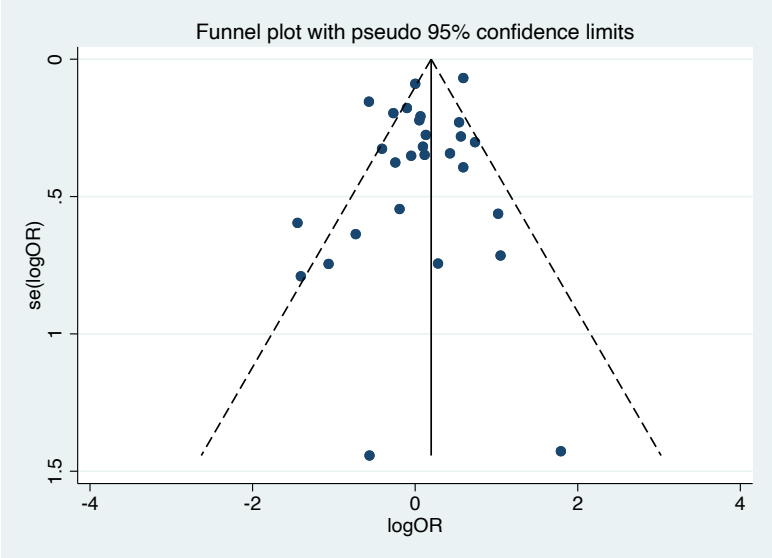
SGA <10th centile – p = 0.410



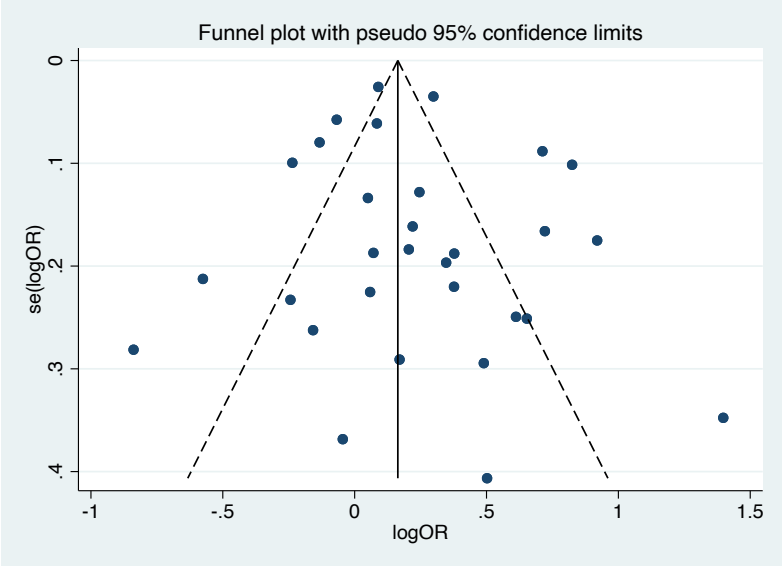
Congenital malformation – p = 0.987



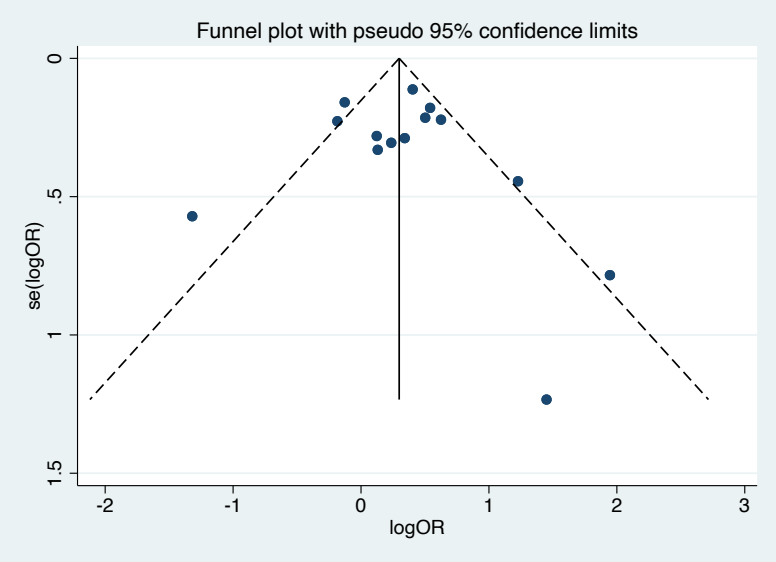
APGAR <7 at 5 minutes – p = 0.132



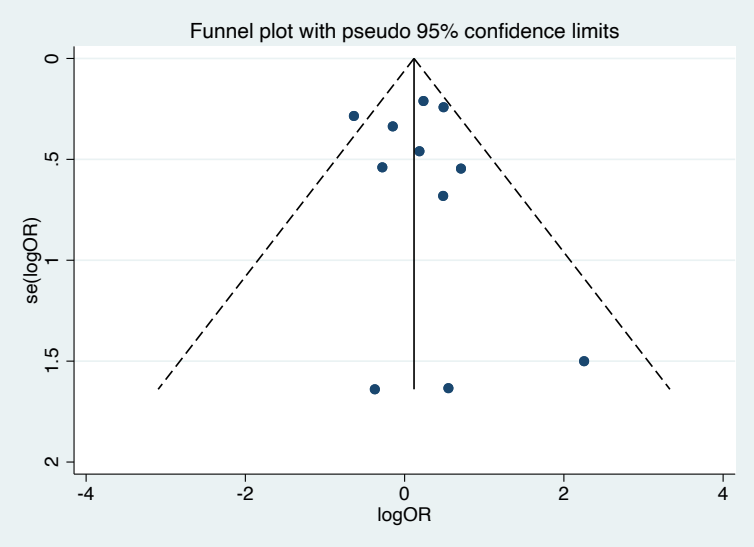
NICU admission – p = 0.394



RDS – p = 0.748

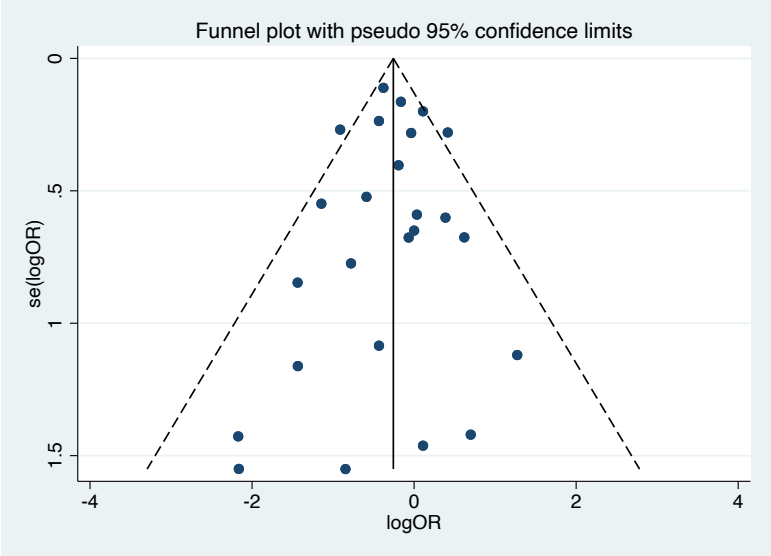


Neonatal sepsis – p = 0.636

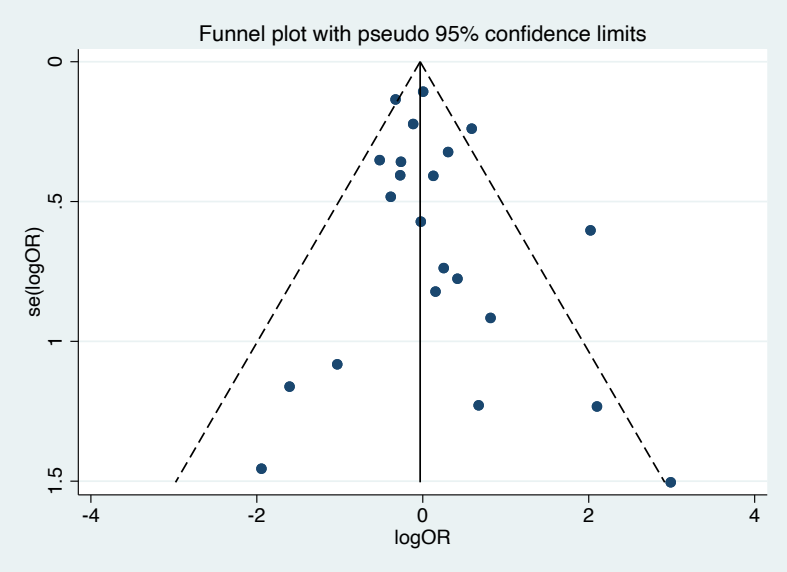


ART vs Natural

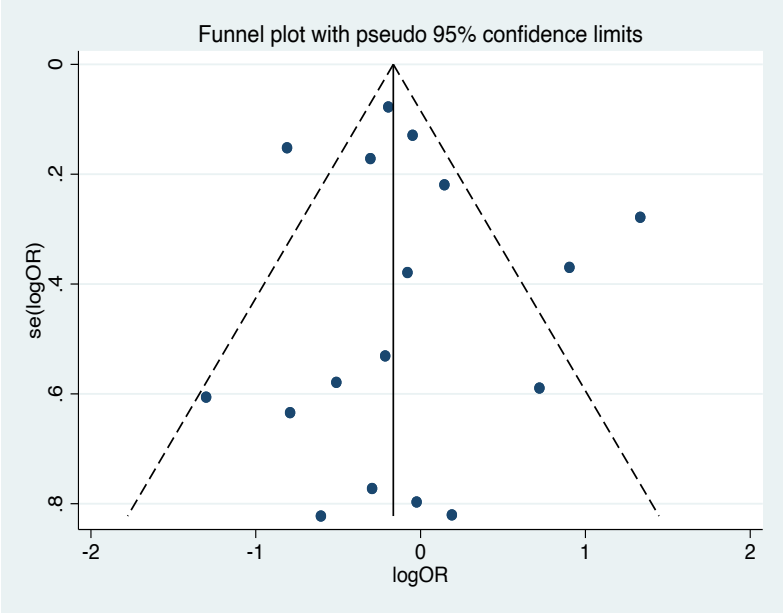
Stillbirth – p = 0.767



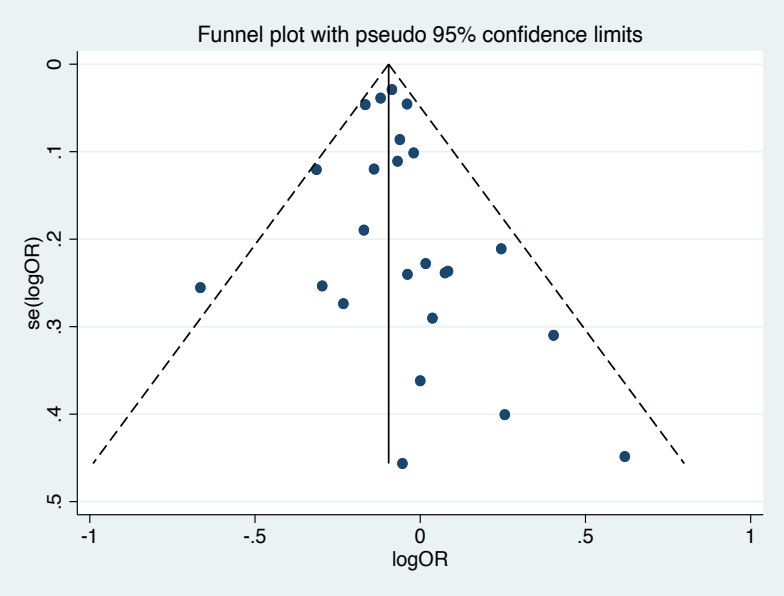
Neonatal death – p = 0.290



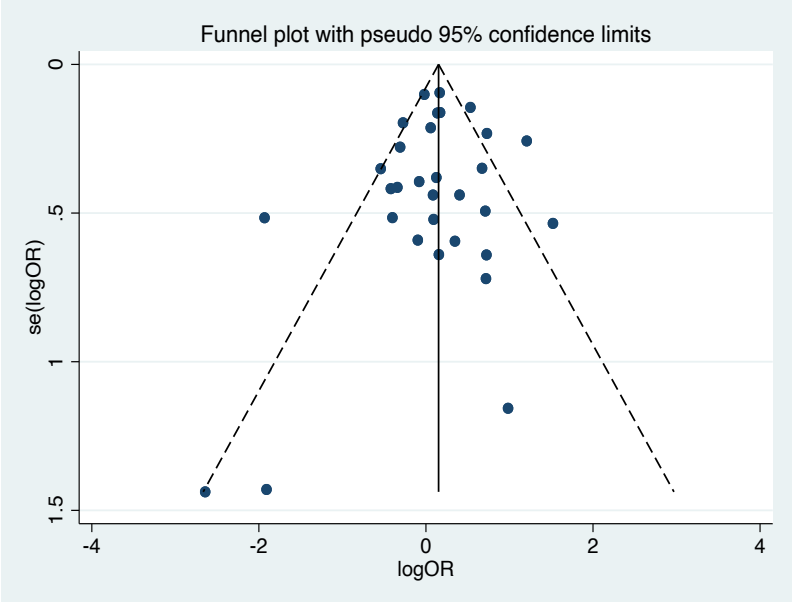
Perinatal mortality – p = 0.576



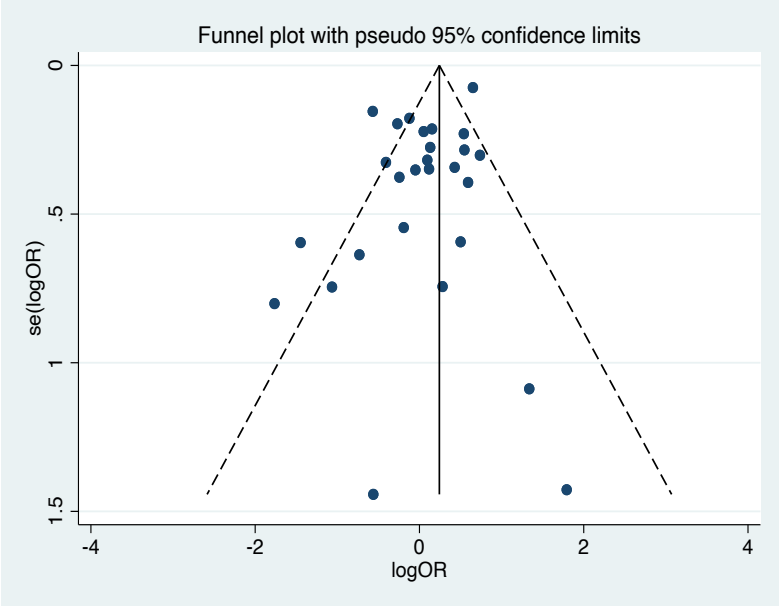
SGA <10th centile – p = 0.351



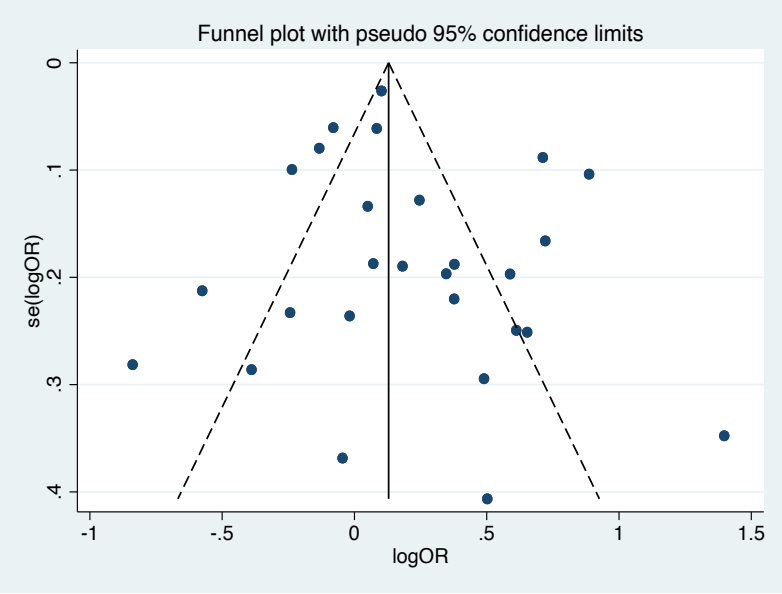
Congenital malformation – p = 0.665



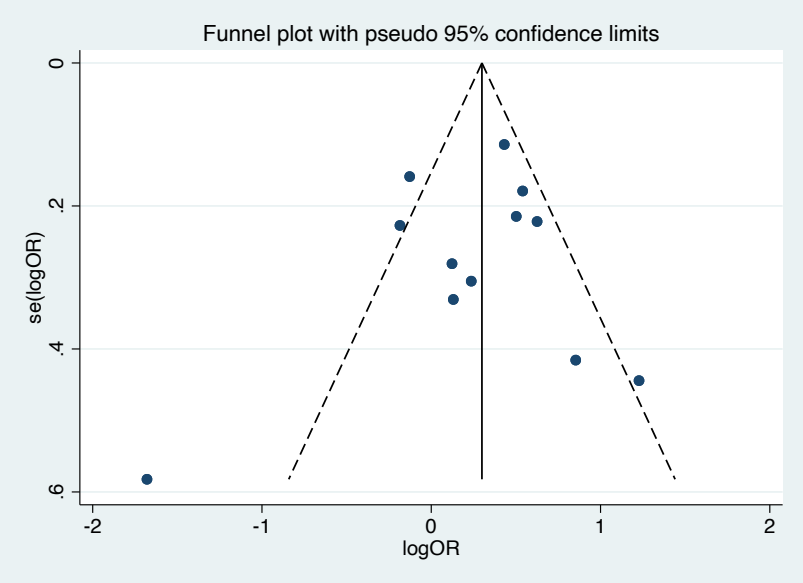
APGAR <7 at 5 minutes – p = 0.040



NICU admission – p = 0.331

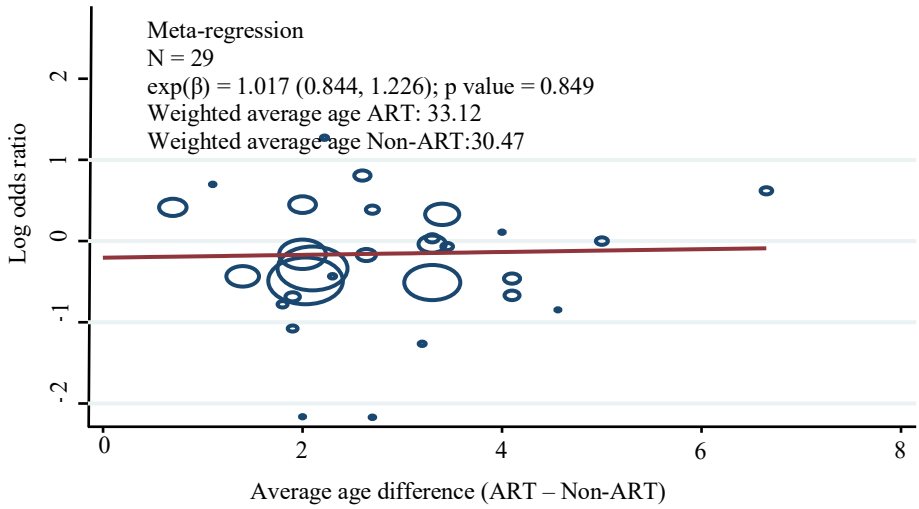


RDS – p = 0.650

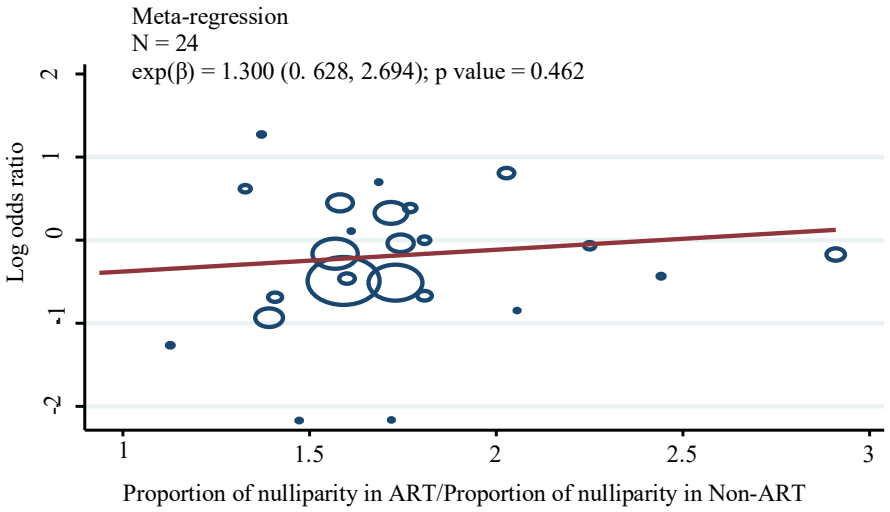

Funnel plot with pseudo 95% confidence limits
se(logOR)
0
.2
.4
.6
-2
-1
0
1
2
logOR

APPENDIX 29: Meta-regression analysis on certain perinatal outcomes to adjust for maternal age and parity (ART vs non-ART) in the systemic review of perinatal outcomes in twin pregnancies following assisted reproduction.

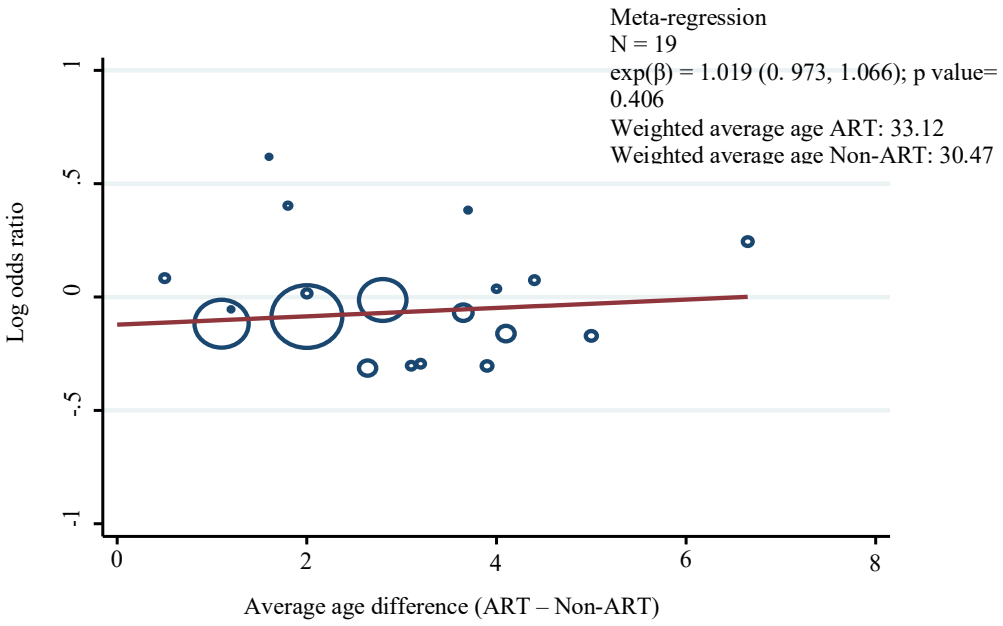
1. Stillbirth and maternal age



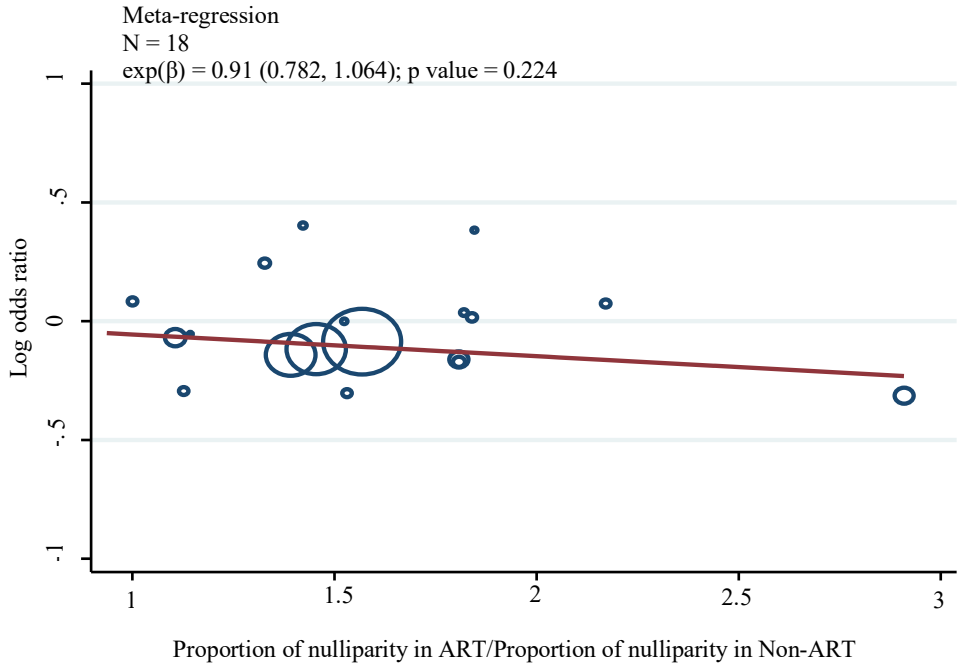
2. Stillbirth and parity



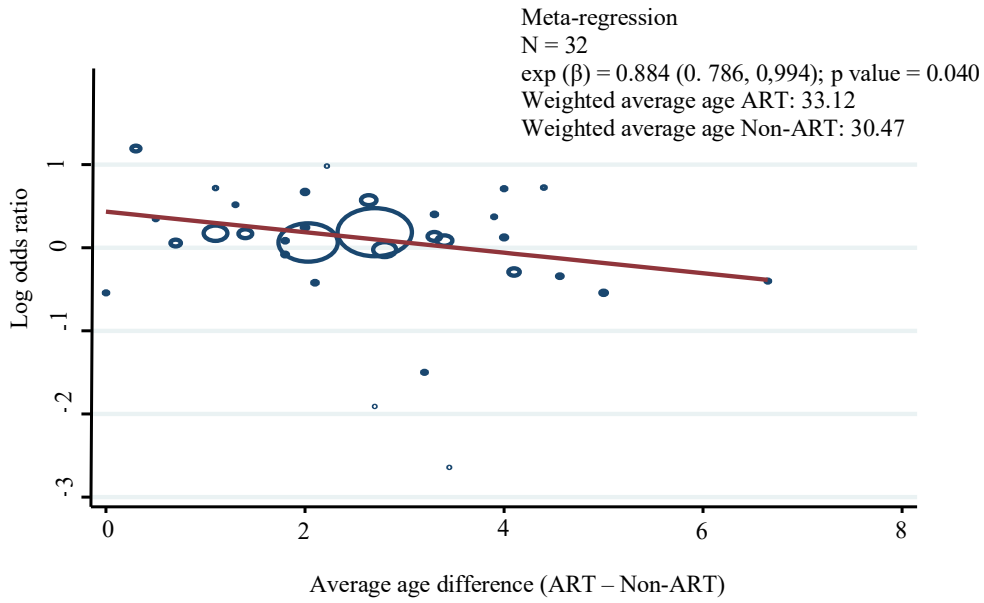
3. SGA <10th centile and maternal age



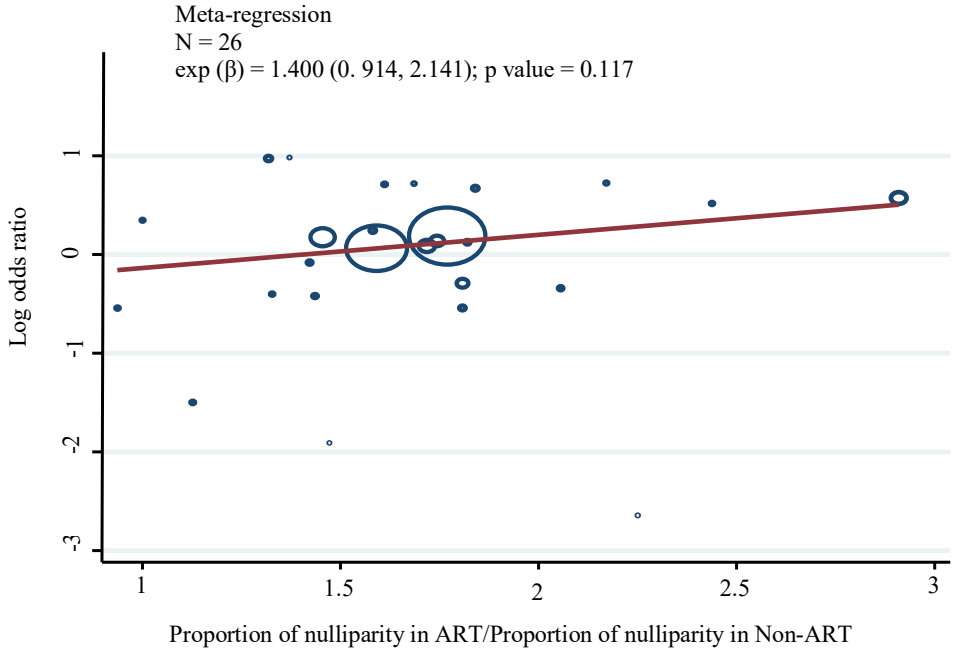
4. SGA <10th centile and parity



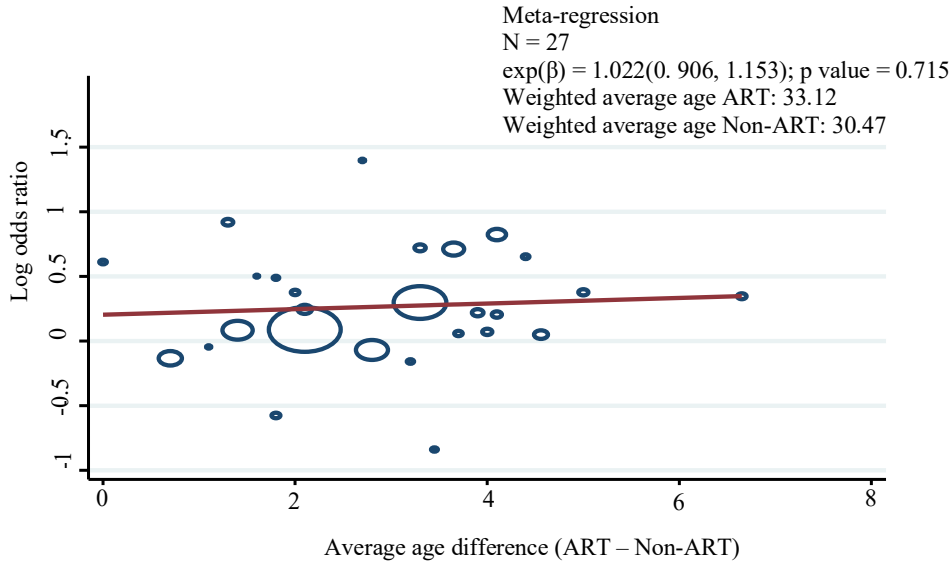
5. Congenital malformation and maternal age



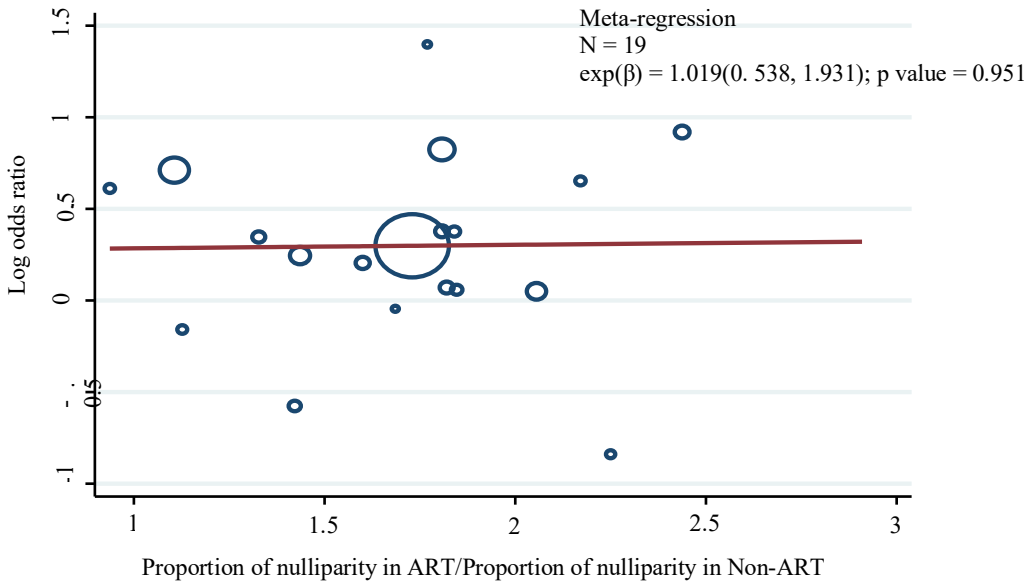
6. Congenital malformation and parity



7. NICU admission and maternal age



8. NICU admission and parity



APPENDIX 30: Published manuscript – Maternal clinical predictors of preterm birth in twin pregnancies.

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Review article

Maternal clinical predictors of preterm birth in twin pregnancies: A systematic review involving 2,930,958 twin pregnancies

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ABSTRACT

In twin pregnancies, which are at high risk of preterm birth, it is not known if maternal clinical characteristics pose additional risks.

We undertook a systematic review to assess the risk of both spontaneous and iatrogenic early (<34 weeks) or late preterm birth (<37 weeks) in twin pregnancies based on maternal clinical predictors. We searched the electronic databases from January 1990 to November 2017 without language restrictions. We included studies on women with monochorionic or dichorionic twin pregnancies that evaluated clinical predictors and preterm births. We reported our findings as odds ratio (OR) with 95% confidence intervals (CI) and pooled the estimates using random-effects meta-analysis for various predictor thresholds.

From 12, 473 citations, we included 59 studies (2,930,958 pregnancies). The risks of early preterm birth in twin pregnancies were significantly increased in women with a previous history of preterm birth (OR 2.67, 95% CI 2.16–3.29, $I^2 = 0\%$), teenagers (OR 1.81, 95% CI 1.68–1.95, $I^2 = 0\%$), BMI > 35 (OR 1.63, 95% CI 1.30–2.05, $I^2 = 52\%$), nulliparous (OR 1.51, 95% CI 1.38–1.65, $I^2 = 73\%$), non-white vs. white (OR 1.31, 95% CI 1.20–1.43, $I^2 = 0\%$), black vs. non-black (OR 1.38, 95% CI 1.07–1.77, $I^2 = 98\%$), diabetes (OR 1.73, 95% CI 1.29–2.33, $I^2 = 0\%$) and smokers (OR 1.30, 95% CI 1.23–1.37, $I^2 = 0\%$). The odds of late preterm birth were also increased in women with history of preterm birth (OR 3.08, 95% CI 2.10–4.51, $I^2 = 73\%$), teenagers (OR 1.36, 95% CI 1.18–1.57, $I^2 = 57\%$), BMI > 35 (OR 1.18, 95% CI 1.02–1.35, $I^2 = 46\%$), nulliparous (OR 1.41, 95% CI 1.23–1.62, $I^2 = 68\%$), diabetes (OR 1.44, 95% CI 1.05–1.98, $I^2 = 55\%$) and hypertension (OR 1.49, CI 1.20–1.86, $I^2 = 52\%$).

The additional risks posed by maternal clinical characteristics for early and late preterm birth should be taken into account while counseling and managing women with twin pregnancies.

Contents

APPENDIX 31: Published manuscript – Biochemical predictors of preterm birth in twin pregnancies.

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Review article

Biochemical predictors of preterm birth in twin pregnancies: A systematic review involving 6077 twin pregnancies

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ABSTRACT

In women with twin pregnancies biomarkers are not used to predict preterm birth in clinical practice. This systematic review assessed the risk of both spontaneous and iatrogenic preterm birth in twin pregnancies based on biochemical predictors.

We searched the electronic databases from January 1990 to June 2019 without language restrictions. All studies on twin pregnancies where biochemical predictors and preterm birth were evaluated were included. We reported our findings as odds ratio (OR) with 95 % confidence intervals (CI) and pooled the estimates using random-effects meta-analysis for various predictor thresholds.

From 12,623 citations, we included 33 studies involving 6077 pregnancies. The odds of preterm birth <28 weeks (OR 12.06, 95 % CI 4.90–29.70, I^2 = 0%), <32 weeks (OR 10.03, 95 % CI 6.11–16.47, I^2 = 0%), <34 weeks (OR 6.26, 95 % CI 3.85–10.17, I^2 = 30 %), <37 weeks (OR 5.34, 95 % CI 3.68–7.76, I^2 = 15 %) and delivery within 14 days of testing (OR 13.95, 95 % CI 4.33–44.98, I^2 = 0%) was increased among women with a positive fetal Fibronectin (fFN) test who were either symptomatic or asymptomatic for preterm birth. Similarly, higher odds of preterm birth was also seen among twin pregnancies asymptomatic for preterm birth with a positive fFN test at gestations <32 weeks (OR 10.54, 95 % CI 5.66–19.64, I^2 = 19 %), < 34 weeks (OR 8.07, 95 % CI 5.28–12.33, I^2 = 0%) and < 37 weeks (OR 6.21, 95 % CI 4.34–8.87, I^2 = 0%). As for other biomarkers, a significantly higher odds of preterm birth <37 weeks was seen among women with elevated maternal serum human Chorionic Gonadotrophin (mshCG) (OR 1.51, 95 % CI 1.07–2.13, I^2 = 0%), 25 Hydroxy Vitamin D level <75 nmol/l (OR 2.59, 95 % CI 1.35–4.95, I^2=NA), positive phosphorylated Insulin-like Growth Factor Binding Protein-1 (phIGFBP-1) (OR 4.23, 95 % CI 1.97–9.09, I^2 = 0%) and in those with elevated Interleukin 8 (IL-8) (OR 3.13, 95 % CI 1.18–8.34, I^2=NA). A higher odds of preterm birth at <34 weeks gestation was seen among women with maternal serum Alpha fetoprotein (AFP)>3.5 MoM (OR 2.35, 95 % CI 1.12–4.96, I^2=NA) while higher odds of preterm birth at <32 weeks was seen among women with 25 Hydroxy Vitamin D level <75 nmol/l (OR 3.01, 95 % CI 1.26–7.19, I^2=NA). Delivery within seven days of testing was significantly increased in women with a positive Matrix Metallo Protein-8 (MMP-8) test (OR 10.59, 95 % CI 3.70–30.29, I^2=NA).

Fetal Fibronectin is strongly associated with predicting preterm birth among women with twin pregnancies who are either asymptomatic or symptomatic for preterm birth as well as in those asymptomatic for preterm birth. Other biomarkers have shown a positive association in the prediction of preterm birth among women with twin pregnancies. Further studies are recommended to evaluate their role.

APPENDIX 32: Published manuscript – Chorionicity and preterm birth in twin pregnancies.

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Systematic review

Association between chorionicity and preterm birth in twin pregnancies: a systematic review involving 29 864 twin pregnancies

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Background The perinatal mortality and morbidity among twins vary by chorionicity. Although it is considered that monochorionicity is associated with an increased risk of preterm birth in twin pregnancies, no systematic review exists evaluating this association.

Objectives This systematic review was undertaken to assess the association between preterm birth and chorionicity in twin pregnancies.

Search strategy We searched the electronic databases from January 1990 to July 2019 without language restrictions.

Selection criteria All studies on twin pregnancies where chorionicity and preterm birth were evaluated were included.

Data collection and analysis Findings are reported as odds ratios with 95% confidence intervals. The estimates are pooled using random-effects meta-analysis.

Main results From 13 156 citations, we included 39 studies (29 864 pregnancies). Monochorionicity was significantly associated with increased risk of preterm birth at ≤28, ≤32, ≤34 and <37 weeks in women asymptomatic and symptomatic for preterm labour (odds ratio [OR] 2.14, 95% CI 1.52–3.02, I^2 = 46%, OR 1.55, 95% CI 1.27–1.89 I^2 = 68%, OR 1.47, 95% CI 1.27–1.69, I^2 = 60%, OR 1.66, 95% CI 1.43–1.93, I^2 = 65%, respectively). Among those asymptomatic for preterm labour, significantly increased odds of preterm birth were seen for monochorionicity at gestations ≤34 weeks (OR 1.85, 95% CI 1.42–2.40, I^2 = 25%) and <37 weeks (OR 1.75, 95% CI 1.22–2.53, I^2 = 61%). Sensitivity analysis showed significantly increased odds of spontaneous preterm birth at ≤34 and <37 weeks for monochorionicity (OR 1.25, 95% CI 1.01–1.55, I^2 = 0% and OR 1.41, 95% CI 1.13–1.78, I^2 = 0%).

Conclusions Monochorionicity is significantly associated with preterm birth at all gestations.

Keywords Chorionicity, multiple pregnancy, preterm birth, preterm labour, twins, ultrasound predictors.

Tweetable abstract In twin pregnancies, monochorionicity is associated with an increased risk of preterm birth at all gestations.

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Introduction

Twin pregnancies are associated with a significantly higher rate of perinatal morbidity and mortality compared with singleton pregnancies.[1] The major contributing factor to this problem is prematurity.[2] A four-fold higher risk of mortality is seen among twins due to preterm birth. The number of twin gestations has increased dramatically over the past few decades due to the expansion of assisted reproductive technology and increase in maternal age at conception.[3] Therefore, tools to predict preterm birth in twin pregnancies are necessary to identify those at risk of preterm birth at an early stage so that necessary preventive measures can be taken.

APPENDIX 33: Published abstract – Maternal and offspring outcomes in twin pregnancies following assisted reproduction.

estimate of the impact of the intervention was used (3 more stillbirths per 10 000 births), the AFFIRM intervention was dominated (i.e. higher costs and more stillbirths) by standard care. When costs associated with Caesarean sections were included, the cost per stillbirth avoided was £344 251.

Conclusions There are costs associated with implementing the AFFIRM intervention, but uncertainty around the impact on stillbirth rates. Although there is not an agreed cost-effectiveness threshold that policy-makers are willing to pay to prevent a stillbirth, these findings will help to inform decision-making for the implementation of interventions to reduce stillbirths.

1623

Maternal and offspring outcomes in twin pregnancies following assisted reproduction

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Objective Artificial reproductive techniques (ART) are associated with higher rates of twin pregnancies. Whether such twin pregnancies conceived following ART are at additional risk of adverse maternal and offspring morbidity needs assessment. Therefore, we undertook this systematic review to evaluate the maternal and offspring outcomes among twin pregnancies conceived following ART and non-ART.

Design Systematic review and meta-analysis

Method We searched electronic databases from January 1990 to November 2020 without language restrictions. All studies comparing ART and non-ART twin pregnancies for the selected maternal and neonatal outcomes were included. Findings are reported as odds ratios with 95% confidence intervals. The estimates are pooled using random-effects meta-analysis.

Results From 4083 citations we included 106 studies (283 213 pregnancies). Among the main maternal outcomes studied, higher odds of preterm birth at < 34 weeks, <37 weeks, gestational hypertension, pre-eclampsia, gestational diabetes mellitus, antepartum haemorrhage, postpartum haemorrhage and caesarean delivery were seen among ART twin pregnancies compared to non-ART twin pregnancies (OR 1.33, 95% CI 1.13–1.57, I^2 = 74%, OR 1.28, 95% CI 1.18–1.37, I^2 = 77%, OR 1.32, 95% CI 1.15–1.53, I^2 = 75%, OR 1.37, 95% CI 1.20–1.57, I^2 = 19, OR 1.55, 95% CI 1.38–1.75, I^2 = 41%, OR 1.77, 95% CI 1.26–2.47, I^2 = 59%, OR 1.45, 95% CI 1.21–1.75, I^2 = 40%, OR 1.83, 95%CI 1.65–2.02, I^2 = 86%).

Of the offspring outcomes studied, the odds of stillbirth, small for gestation age < 10th centile and twin-twin transfusion syndrome were significantly lower among ART twins compared to non-ART twins (OR 0.83, 95% CI 0.70–0.99, I^2 = 49%, OR 0.91, 95% CI 0.87–0.96, I^2 = 21%, OR 0.45, 95% CI 0.25–0.82, I^2 = 25%). However, the odds of birth weight < 2500 g, birth weight discordance > 25%, congenital malformation, respiratory distress syndrome, necrotizing enterocolitis, neurological complications and neonatal intensive care unit admissions were significantly higher for ART twins compared to non-ART twins (OR 1.12, 95% CI 1.06–1.18, I^2 = 81%, OR 1.31, 95% CI 1.05–1.63, I^2 = 0%, OR 1.18, 95% CI 1.06–1.31, I^2 = 61%, OR 1.35, 95% CI 1.07–1.70, I^2 = 64%, OR 1.78, 95% CI 1.06–3.01, I^2 = 0%, OR 1.61, 95% CI 1.04–2.48, I^2 = 0%, OR 1.25, 95% CI 1.12–1.41, I^2 = 88%).

Conclusions Twin pregnancies conceived following ART have significantly higher adverse maternal and offspring outcomes than non-ART twin pregnancies.

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Biochemical predictors of preterm birth in twin pregnancies

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Objective In women with twin pregnancies biomarkers are not used to predict preterm birth (PTB) in clinical practice. This systematic review assessed the risk of both spontaneous and iatrogenic PTB in twin pregnancies based on biochemical predictors.

Design Systematic review and meta-analysis.

Method We searched the electronic databases from January 1990 to June 2019 without language restrictions. All

APPENDIX 34: Poster presentation – Maternal clinical predictors of preterm birth in twin pregnancies.

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APPENDIX 35: Poster presentation – Biochemical predictors of preterm birth in twin pregnancies

APPENDIX 36: Poster presentation – Chorionicity and preterm birth in twin pregnancies

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APPENDIX 37: Oral abstract presentation- Maternal and offspring outcomes in twin pregnancies following assisted reproduction.

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9 - 12 JUNE 2021

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Shemoon Marleen

made an Oral Abstract Presentation entitled

Maternal and offspring outcomes in twin pregnancies following assisted reproduction

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Mr Andrew Sizer FRCOG, Shrewsbury
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