Risk of stillbirth following in vitro methods of conception: a systematic review and meta-analysis

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Abstract

Objective

The purpose of this systematic review and meta-analysis is to determine if there is an increased risk of stillbirth among singleton gestations following in vitro methods of conception (including in vitro fertilization and intracytoplasmic sperm injection) compared with non-in vitro methods of conception (including spontaneous conception, intrauterine insemination, or ovarian stimulation).

Methods

Medline, EMBASE, CINAHL, and Cochrane Library databases were searched from inception to June 2019. Reference lists of included studies and obstetric guidelines were also reviewed. Meta-analysis was undertaken using a random effects model and inverse variance methods to produce a summary odds ratio. Subgroup analyses were completed by type of in vitro or non-in vitro method.

Results

Thirty-three cohort studies, and one case-control study met the inclusion criteria for this systematic review. There was an increased odds of stillbirth associated with in vitro methods of conception, (OR 1.43, 95% CI 1.23-1.67). A subgroup analysis demonstrated no increased risk when comparing in vitro methods to those conceiving with a history of infertility.

Conclusion

Compared to non-in vitro methods of conception, in vitro methods are associated with an increased risk of stillbirth. There is insufficient evidence to determine whether this risk is due to the treatment modality or underlying infertility.

General Summary

Purpose

The purpose of this study is to determine if invasive methods of conceiving a pregnancy, such as in vitro fertilization and related techniques, are associated with an increased risk of stillbirth, in comparison to less invasive methods of conception.

Method

The literature was comprehensively searched for all studies related to the research question. Data from eligible studies were combined to estimate the overall effect on stillbirth of conceiving using invasive methods.

Results

We found an increased risk of stillbirth associated with invasive methods of conception compared to non-invasive methods. However, when comparing invasive methods to pregnancies conceived non-invasively following a documented history of difficulty getting pregnant, there was no longer an increased risk.

Conclusion

There is an increased risk of stillbirth following invasive methods of conception, such as in vitro fertilization, but it is unclear whether this is due to the treatment or the condition of infertility itself.

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A significantly abbreviated version of this thesis, based on the same data and including some of the same analyses, has been submitted for publication with the American Journal of Obstetrics & Gynecology, and is pending peer review.

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List of Abbreviations

ACOG, *American College of Obstetricians and Gynecologists*

ART, *assisted reproductive technology*

BMI, *body mass index*

BPP, *biophysical profile*

BW, *birth weight*

CARTR, *Canadian assisted reproductive technology register*

CI, *confidence interval*

ET, *embryo transfer*

FET, *frozen embryo transfer*

FSH, *follicle stimulating hormone*

GA, *gestational age*

GIFT, *gamete intrafallopian transfer*

GnRH, *gonadotropin releasing hormone*

GRADE, *grading of recommendations, assessment, development, and evaluation*

HELLP, *hemolysis, elevated liver enzymes, low platelets*

ICSI, *intracytoplasmic sperm injection*

IUI, *intrauterine insemination*

IVF, *in vitro fertilization*

IUFD, *intrauterine fetal demise*

IUGR, *intrauterine growth restriction*

LBW, *low birth weight*

LGA, *large for gestational age*

LH, *luteinizing hormone*

NICU, *neonatal intensive care unit*

NND, *neonatal death*

NOS, *Newcastle-Ottawa Scale*

NST, *non-stress test*

(a)OR, *(adjusted) odds ratio*

OS, *ovarian stimulation*

PCOS, *polycystic ovarian syndrome*

PPROM, *preterm prelabour rupture of membranes*

PROSPERO*, international prospective register of systematic reviews*

(s)PTB, *(spontaneous) preterm birth*

SC, *spontaneous conception*

SET, *single embryo transfer*

SGA, *small for gestational age*

WHO, *World Health Organization*

ZIFT, *zygote intrafallopian transfer*

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Publication of this work

A significantly abbreviated version of this text has been submitted for publication in a medical journal, but not yet accepted. The journal editors have been informed that the work is also the basis of this thesis. Text from the submitted manuscript has been re-written for this work, although the same figures and tables appear in both. I designed the study protocol, and all tables and figures (with the exception of Figure 2a and 2b, designed by Dr. Kaitlyn Carson); conducted the literature search and data analysis; and am the writer of both the submitted manuscript and this thesis.

Chapter 1: Introduction

Treatment of infertility has increased over the past several decades[1], and although most children conceived following infertility treatment are healthy[2], there remains the possibility of increased risk of certain rare adverse perinatal outcomes, including stillbirth. Unfortunately, such rare outcomes are difficult to study, since large sample sizes are required to show a difference in risk. Systematic review and meta-analysis is a study design that has been used to demonstrate an increased risk of small for gestational age (SGA) infants, intrauterine growth restriction (IUGR), and preterm labour following assisted reproduction, but no meta-analysis to date has specifically reviewed the risk of stillbirth following infertility treatment in singleton gestations. The objective of this work is to use a systematic review and meta-analysis to determine if there is indeed an increased risk of stillbirth following specific types of infertility treatment compared to all other types of conceptions, in singleton gestations. Furthermore, subgroup analyses will explore whether any increased risk is related to the specific method of treatment, or a history of infertility itself. While an increased risk of stillbirth associated with infertility treatment is unlikely to deter couples from treatment, it may be a reason to change management of pregnancies conceived in this way.

In order to better understand this work, it is important to first have a rudimentary understanding of normal fertility and the treatment of infertility, and then to detail what is meant by the term "stillbirth" and the challenges of studying this outcome.

Infertility

Infertility, defined clinically as the lack of pregnancy after 12 months of unprotected intercourse[3], affects 11.5-15.7% of Canadians.[4] The diagnosis has increased over the past three decades, with an associated increase in use of assisted reproduction.[1,4] A closely related concept,

and sometimes interchangeable term is *subfertility*, which implies that a couple will ultimately conceive given time and/or intervention, while *infertility* could be understood as the complete inability to conceive.[5] The terms will be used interchangeably in this text. Couples are generally referred for fertility evaluation if they fail to conceive after 12 months of unprotected, regular intercourse; or 6 months if the woman is older than 35 years of age.[1,6] Age impacts the timing of referral because age-related fertility decline is a significant contributor to infertility, making investigations and treatment time-sensitive.[1]

Hormonal requirements for conception

In females, conception requires an appropriate hormonal milieu for development of a dominant ovarian follicle containing an oocyte, and preparation of the uterus for implantation of the fertilized oocyte (zygote).[5] In the brain, the hypothalamus produces gonadotropin-releasing hormone (GnRH), which acts within the nearby pituitary gland to induce the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH acts in the ovary to recruit primordial follicles to develop.[5] Growing follicles secrete estrogen, which causes the inner lining of the uterus, the endometrium, to proliferate.[5] Release of FSH and LH peak just before the midpoint of the menstrual cycle, causing the most developed follicle to rupture and release its egg, an event termed ovulation.[5] The ruptured follicle then becomes the corpus luteum, an endocrine structure that releases progesterone.[5] Progesterone causes the endometrium to develop glands, becoming "secretory endometrium".[5] This prepares the endometrium for implantation of the zygote.[5] If pregnancy occurs, the placenta takes over production of required hormones, and the corpus luteum regresses.[5] If no implantation occurs, when the corpus luteum regresses, progesterone levels drop, which induces shedding of the endometrium for menses.[5]

In males, GnRH acts in the same way on the pituitary gland to cause release of FSH and LH.[1] FSH acts in the testes to control production of sperm, while LH acts to produce testosterone, which supports sperm production.[1]

Anatomic requirements for conception

In females, after release from its follicle, the oocyte is swept into the fallopian tube by finger-like projections on the end of the tube known as fimbriae.[7] The fallopian tube is the typical site of fertilization.[7] The resultant zygote, is then transported through the remainder of the tube to the uterine body, where implantation occurs in the prepared, secretory endometrium.[7]

In males, sperm are produced in the seminiferous tubules of the testes, and are then transported to the epididymis, where they continue to develop and mature.[1] They travel through the vas deferens, then the urethra, at which time they are diluted by secretions from the seminal vesicles and prostate.[1] Sperm must be deposited in the female's vagina, and then travel through the cervix and uterine body to reach the fallopian tubes, which are the typical sites of fertilization.

Causes of infertility

The cause of infertility has been described as female-factor or male-factor.[6] Female factors include: ovulatory dysfunction, tubal factors, uterine factors, cervical factors, or ovarian factors; while male factors include ejaculatory dysfunction, oligozoospermia (few sperm) or azoospermia (complete lack of sperm).[6] Both male and female factors may be implicated for a couple, and unexplained infertility (i.e. not explained by the above male and female factors) is not uncommon.[6]

Treatment of infertility

Treatment of infertility varies by cause, and may start with such non-invasive recommendations as weight loss and lifestyle changes, but for many couples, also involves assisted reproduction.[8]

Assisted reproduction

The terms "assisted reproduction" or "assisted reproductive technology" (ART) generally include in vitro methods of conception[8,9], but some authors or clinicians also include medications taken orally or parenterally to increase ovulation. Medications taken orally by women to induce ovulation or cause super-physiologic ovulation (henceforth termed "ovarian stimulation") include clomiphene citrate, a selective estrogen receptor modulator; and letrozole, an aromatase inhibitor.[8] This is often used in conjunction with timed intercourse or intrauterine insemination (IUI), a procedure in which sperm is injected directly into the uterus via the cervix to increase the chance of pregnancy. Women may also use injected medications for ovarian stimulation, including synthetic and equine-derived follicle stimulating hormone (FSH) or "menotropins" (a mixture of FSH and LH). Injected medications are rarely used alone for ovarian stimulation—they typically precede either IUI, or in vitro methods, in which sperm and oocyte are combined outside of the body.[8] "In vitro fertilization" is at times used as a general term for all in vitro methods, but when used to describe a specific method, is the process of mixing sperm with an oocyte outside of the body, resulting in fertilization.[8] In contrast, intracytoplasmic sperm injection (ICSI) is an in vitro method in which an individual sperm is directly injected into an oocyte.[8] Once an embryo is created following an in vitro method, there are a variety of subsequent techniques before transfer into the woman and pregnancy can take place. The embryo is cultured in a specialized medium for a specified amount of time: either 3 days resulting in

"cleavage stage" embryos or 5 days for "blastocyst stage" embryos.[8] Following this, the embryo may either be transferred "fresh", meaning the embryo is transferred in the same menstrual cycle it was created; or can be frozen for transfer during a later cycle.[8] Methods of freezing embryos have evolved over the years, from a "slow freeze" method to the current rapid vitrification.[8] Fresh or frozen embryos are therefore always created using some in vitro method (IVF or ICSI), and the terms do not describe a specific technique of conception. Other techniques that are currently rarely used in Canada include gamete intrafallopian transfer (GIFT), in which sperm and oocytes are extracted and transferred into the fallopian tube of the woman during laparoscopic surgery; and zygote intrafallopian transfer (ZIFT), in which a zygote is created by the combination of egg and sperm in vitro, and transferred directly into the fallopian tube either trans-cervically or laparoscopically.[10]

The Canadian ART Register (CARTR) defines ART as in vitro methods of conception such as IVF, ICSI, frozen embryo transfer (FET), and GIFT.[9,10] Comparing reports over 18 years demonstrates that the use of ART in Canada is increasing.[9,10] There are likely multiple reasons for this increase, which may include improved and more accessible technology on the one hand, and delayed childbearing on the other.[1] Indeed, the number of pregnant women over age 30 has increased in Canada between 2014 – 2018[11], likely reflecting both of these factors.

Outcomes following ART

With this increase in use of ART, comes the question of safety of these procedures. Many of the early reports of increased obstetrical morbidity and perinatal mortality associated with ART attributed an elevated risk to multiple gestation, which is more common following ART due to the development of multiple dominant follicles following OS; or the transfer of multiple embryos following in vitro methods.[12] It is well described in the literature that multiple

gestations are at a higher risk of neonatal complications such as preterm birth and SGA; fetal complications such as IUGR or congenital anomalies; maternal complications such as gestational hypertension, preeclampsia, gestational diabetes, intrahepatic cholestasis of pregnancy, and anemia; and obstetrical complications such as antepartum hemorrhage, placenta previa, and need for Caesarian delivery.[13]

More recently, several meta-analyses have been undertaken in singleton gestations, which have identified increased rates of preterm birth (<37 weeks' gestation), low birth weight (<2500 g), congenital malformations, and perinatal mortality associated with ART.[14–18]

These outcomes may be interrelated. Infants born preterm may have low birth weight if they are extremely preterm (e.g. <34 weeks) as they have not achieved the growth of a term infant. Fetuses with congenital malformations may be more likely to be born preterm and/or with low birth weight.[19] Finally, infants born extremely preterm may be more likely to experience perinatal mortality since they are likely to experience neonatal complications such as respiratory distress syndrome and intracranial hemorrhage.[20]

Stillbirth

Stillbirth is also an important potential pregnancy outcome that may be related to other perinatal outcomes. Stillbirths are generally reported in Canada for births without signs of life at a gestational age greater than or equal to 20 weeks, or weighing greater than or equal to 500 g[21]; however provinces may have slightly different reporting criteria.[22] International reporting requirements of stillbirth vary widely: in the United States, a gestational age ≥20 weeks or birth weight ≥350 g is used[23]; while the WHO recommends using a gestational age ≥28 weeks.[24]

Stillbirth reporting criteria are often related to fetal viability since it is essentially the converse of livebirth.[25] Thus, the gestational age at which countries will require reporting of

livebirth is the same required for stillbirth. Births before the gestational age cut-off are therefore considered spontaneous abortions (colloquially, miscarriages) since they are not viable. Countries also differ in reporting of elective termination of pregnancy after the gestational age cut-off for livebirth/stillbirth: in Canada and Australia, elective termination of pregnancy by induction of labour between 20-24 weeks are counted among stillbirths.[25] Again, regional reporting requirements within a country may differ. Thus, while stillbirth itself is not a challenging concept to understand, the variety of reporting requirements make global studies quite heterogeneous.

"Intrauterine fetal death" (IUFD) is another related, but subtly different concept, although the term is occasionally used synonymously with "stillbirth". IUFD refers to the death of the fetus (i.e. after 9 weeks gestational age).[26] Thus, some IUFDs may be considered stillbirths while others may be considered spontaneous abortion, depending on the gestational age that products of conception are expelled. Complicating definitions and reporting further is the fact that the actual time of death of a fetus may precede expulsion of the products of conception by several weeks.[25] Theoretically, an IUFD may occur at less than 20 weeks (i.e. be a spontaneous abortion), but the fetus not be born until after 20 weeks, and therefore be reported as a stillbirth.

Risk factors for stillbirth vary by region, but in high income countries have been reported to include: obesity, smoking, increasing maternal age over 35 years, primiparity, illicit drug use, low education, pre-existing and gestational hypertension, pre-existing diabetes mellitus, post-term gestations, being small for gestational age (<10% percentile), previous stillbirth, and the use of assisted reproductive technology.[27] Of note, these are largely antepartum risk factors, in contrast to stillbirths in low income countries, which often occur intrapartum.[24] Pregnancies

known to be at high risk for stillbirth are often monitored more closely antepartum using ultrasound, and induction of labour may be recommended to decrease the risk of fetal death.[28–30]

The meta-analyses of perinatal outcomes following ART have largely assessed perinatal mortality instead of stillbirth.[14–17] Perinatal mortality encompasses both stillbirth and early neonatal death (within 7 days of delivery).[31] Both are clinically relevant outcomes for patients, though perinatal mortality reflects early neonatal care in addition to intrapartum events and antepartum risk; while stillbirth reflects the latter two alone. Recommendations for antepartum surveillance and induction of labour have been based on the risk of stillbirth (i.e. reflecting antepartum risk) rather than perinatal mortality[28,30], and thus the specific risk of stillbirth following ART is relevant in determining recommended antepartum management.

Antepartum fetal surveillance

There is debate in the literature regarding the clinical usefulness of antepartum fetal surveillance. No methods of antepartum fetal surveillance have been shown to improve fetal survival, except for umbilical artery doppler ultrasound for growth-restricted fetuses.[29] In particular, increased antepartum surveillance in the form of the non-stress test (NST) or biophysical profile (BPP) in pregnancies at high risk for stillbirth have not been demonstrated to reduce the risk of stillbirth.[29] However, in many centers, high risk pregnancies are monitored more closely using these methods given that there are few other tools at the clinicians' disposal.

National societies have developed some recommendations for pregnancies following ART given the potential increased risks. The American College of Obstetricians and Gynecologists (ACOG) in a 2016 Committee Opinion recommends ultrasound surveillance in ART pregnancies for structural abnormalities, possibly including fetal echocardiography given the increased risk for congenital malformations.[32] The Society of Obstetricians and

Gynaecologists Canada identifies preterm birth and low birth weight as the primary adverse perinatal outcomes in singleton gestations following ART, but notes that most of these pregnancies are uncomplicated, and result in the birth of healthy children.[2] The 2018 guidelines for antepartum fetal surveillance do recommend additional surveillance for pregnancies following ART, including fetal movement counting and non-stress tests, but does not specify the timing or frequency of the latter.[29]

Purpose

The purpose of this thesis is to conduct a systematic review and meta-analysis of the existing literature to determine whether there is an increased risk of stillbirth following in vitro methods of conception (comprising IVF, ICSI, FET, and GIFT) among singleton gestations, in comparison to non-in vitro methods (including spontaneous conceptions, intrauterine insemination or ovarian stimulation without IVF/ICSI). Given the challenges in reporting and defining stillbirth described above, particular attention will be paid to the definition of stillbirth used by authors of included studies. An increased risk of stillbirth related to in vitro procedures that is independent of other risk factors for stillbirth, such as age or obstetrical complications would warrant increased antepartum surveillance, and potentially induction of labour.

Chapter 2: Literature Review

An understanding of the risk of stillbirth following in vitro methods of conception first requires an understanding of stillbirth itself, and the challenges inherent in researching this outcome. Secondly, there are a number of other interrelated risks associated with in vitro methods of conception, which may also impact the risk of stillbirth. Namely, these include the risks of multiple gestation, growth restriction, and preterm delivery. Finally, there is the question of whether risk stems from the *condition* of infertility or the *treatment* of infertility. This literature review provides context for risks related to stillbirth and in vitro methods of conception, and describes some of the existing research in this area.

Studying stillbirth

There are many reasons why stillbirth is a difficult outcome to assess in the literature. First, stillbirth is a rare perinatal outcome, with a reported incidence in the general Canadian population of 7.91/1000.[21] Therefore, any single study would require a large sample size to be powered to find a difference. For example, using a stillbirth incidence of 7.91/1000, in order to see a reduction of 2/1000, with 80% power and two-sided alpha 0.05, the required sample size would be 26930 total births. Second, it is challenging to pool international or even regional data given differences in reporting criteria. For example, although the national reporting criterion for stillbirth in Canada is birth of an infant with no signs of life at ≥20 weeks' gestational age or ≥500g birth weight,[21] the criterion in the province of Quebec is simply a birthweight ≥500g.[22]

Although quality of evidence is heavily influenced by study design, randomized control trials are unethical and not feasible in the assessment of stillbirth. This leaves the researcher with large observational (i.e. cohort or case-control) studies. These may or may not exist depending on regional reporting and resources. Meta-analysis may be an ideal methodology for assessment

of stillbirth in terms of achieving sufficient power, but the definitions of stillbirth used in the included studies must be clear.

Risk factors for stillbirth

The rate of stillbirth reflects maternal access to health care, among other factors, and therefore varies by region. McClure (2009) reviewed differences in stillbirth rates in developing and developed countries, and reported that stillbirth rates in developing countries are ten times higher than in developed countries, and typically occur at term (≥37 weeks) or intrapartum.[33] In developing countries, fetal asphyxia and infection associated with prolonged labour; pre-existing infection such as malaria and syphilis; and poor nutritional status are more common.[33] Pre-eclampsia and eclampsia occur in both developed and developing countries, but due to a lack of resources for blood pressure and urine protein screening, induction of labour, and Caesarian section, fetuses die more frequently as a result of the hypoxia occurring secondary to severe pre-eclampsia or eclamptic seizures.[33]

In high income countries, a 2011 meta-analysis identified risk factors for stillbirth, including: maternal factors (previous stillbirth, age >35, primiparity, overweight/obesity, smoking, illicit drug use, low level of education, and lack of antenatal care); obstetrical factors (placental abruption, pre-existing and gestational hypertension, pre-existing diabetes); and fetal factors (post term, and SGA).[27] Multiple gestation and congenital anomalies were largely excluded from studies included in the meta-analysis. High income countries included USA, Sweden, Canada, Australia, UK, Denmark, Belgium, Norway, Italy, Germany, Scotland, New Zealand, and Spain. Most studies were from the USA. Interestingly, this study also identified "ART singletons" as having an OR for stillbirth of 2.7 (95% CI 1.6-4.7). It is unclear what procedures are included in the definition of ART. However, in supplementary materials, they

report that no meta-analysis was undertaken due to differences in types of ART and populations. They conclude that pregnancies following ART are likely to be at increased risk of adverse outcomes, but further research is required.

De Graaf (2017) assessed risk factors for stillbirth, including IVF, utilizing a case-control study design in the northern suburbs of Adelaide, Australia.[34] Only singleton stillbirths were included, identified through the South Australian Health Pregnancy Outcome Unit, and defined as fetal death at ≥20 weeks' gestation or birthweight ≥400 g. Elective termination of pregnancy was excluded. Two controls were selected per case, matched for parity. One hundred-thirty stillbirths, and 260 livebirths were analysed in both univariate and multivariate analysis. Risk factors identified in univariate analysis included: higher BMI category; non-Caucasian (i.e. ethnic minority, primarily Indigenous) ethnicity; social issues (including domestic violence, social isolation, and financial or housing problems); diabetes; polycystic ovarian syndrome (PCOS), and conception by IVF. Multivariate analysis identified BMI >40 kg/m^2; indigenous ethnicity; and social issues. IVF was no longer a risk factor in multivariate analysis. This suggests that IVF in this population was related to BMI >40 kg/m^2, indigenous ethnicity, or social issues; the most likely of these is that IVF was related to BMI >40 kg/m^2. Notably, this population may not be highly reflective of the typical IVF patient. In most regions, IVF is an expensive procedure, and patients who undergo this method of conception typically have a higher socioeconomic status. In contrast, the population of this study was acknowledged to be of a lower socio-economic status.

Risks following in vitro methods due to multiple gestation

Shortly after the introduction of IVF as a viable technology for the treatment of infertility, it was recognized that it was associated with increased rates of multiple birth.[12,35] It

has been estimated that ART accounts for 10-24% of twin gestations, and 22-59% of triplets.[35] This increase in multiple gestation is associated with an increase in preterm delivery, low birth weight, stillbirth, perinatal mortality, and infant mortality.[12] In Canada, USA, and France, 10-19% of preterm births are attributable to twin gestation.[35] The higher the number of fetuses in the pregnancy, the higher the risks[35], which is particularly concerning given that IVF is the primary reason for increased rates of higher order multiple gestation.[36] Thus, in vitro methods are associated with increased risks of poor perinatal outcomes, including stillbirth, due to the associated increase in multiple gestations.

Others have questioned whether ART twin pregnancies are at increased risk compared to spontaneously conceived (SC) twins. Some have reported more complications in ART-conceived twins[37–39], potentially secondary to increased abnormal placentation and antepartum hemorrhage.[39] One study found an increased risk of intrauterine fetal demise in ART pregnancies compared to SC pregnancies.[38] However, this same study found that this risk was no longer significant after multivariate regression analysis controlling for maternal age and parity.[38]

Thus, due to the increase in multiple gestations, particularly higher order multiple gestation associated with ART, the risk of stillbirth is also higher. It is unclear if the ART itself increases risk of stillbirth, even in multiple gestation.

The case of singleton gestations

In singleton pregnancies, more recent data has demonstrated increased risks for singleton gestations following in vitro methods of conception.

An early report comparing perinatal outcomes in singleton pregnancies following IVF to those following SC was published in 1992 by Tan et al.[40] They compared IVF pregnancies in a group from the Bourn Hall and Hallam infertility clinics in the UK, with a group conceiving

spontaneously, matched for maternal age and delivering in the UK. Data points were collected from clinic records and questionnaires answered by consultant obstetricians at delivering hospitals. A total of 961 IVF pregnancies were included, and 978 singleton spontaneous pregnancies. Despite matching controls by age, the mean age of the IVF group was older than that of the SC group. They report a stillbirth rate of 5.07/1000 births in the IVF group, and state this was not different from *national rates*. There was no comparison made for stillbirth to the control group in the study; this would have been the more rigorous comparison to report. It is not ideal to compare to a national rate, because IVF pregnancies would be included in the national data. It does not appear that the assessment of stillbirth was the primary objective of this study, although no primary outcome is explicitly stated. Other obstetric outcomes assessed included vaginal bleeding, Caesarian section, hypertension requiring hospitalization, IUGR, placenta previa, and preterm delivery. All of these were increased in the IVF group. Given the increased risk of these other perinatal outcomes, it is possible that there was in fact an increased risk of stillbirth, but an insufficient sample size to identify this.

Henningsen (2014) conducted a large cohort study combining databases from Denmark, Sweden, Finland and Norway from 1982-2007.[41] They matched singletons following ART (including IVF, ICSI, FET) with four controls from the same country, matching for parity and year of birth. However, they did not match for gestation plurality, and subsequently excluded controls who were twins. Controls could include those who had undergone IUI or OS. Stillbirth was assessed as the primary outcome, with other perinatal outcomes as secondary outcomes. Multivariate logistic regression adjusting for parity, year of birth, country, maternal age, and fetal sex was used to assess the risk of stillbirth at specific gestational age ranges. They were able to include a total of 425,283 singleton gestations, but used a "fetus at risk approach" since the

analysis was done by gestational age. This means that delivered fetuses from one cohort were excluded from the next gestational age cohort. In the gestational age group 22-27+6 weeks, including 29,736 fetuses following ART and 177,412 fetuses following SC, an increased risk of stillbirth (RR 2.08, 95% CI 1.55-2.78) was found. However, in groups beyond 28 weeks' gestation, they reported no increased risk of stillbirth following ART. This suggests that the increased risk of stillbirth exists for gestations in the late second and early third trimester, and pregnancies progressing further are not subject to increased risk. While pregnancies can be monitored in the late second and early third trimester, it may be difficult to decide on clinical action at this gestational age. Evidence for fetal compromise would need to be overwhelming and certain in order to recommend delivery at such early gestational ages. Morbidity and neonatal mortality due to such preterm delivery would be significant. More research on the risk profile of stillbirth through gestation is therefore necessary. A notable challenge from such a large multi-national database study is standardization of definitions. The study assessed stillbirth beginning at 22 weeks' gestational age; however, Denmark and Sweden only reported stillbirth after 28 weeks' gestational age until 2004 and 2008 respectively. Thus, the data regarding increased risk of stillbirth <28 weeks exclusively reflects stillbirths from Finland and Norway.

Chughtai (2018) also assessed gestational age specific rates of perinatal mortality following ART.[42] This study included 407,368 births from five states/territories in Australia. They compared pregnancies following ART to all other pregnancies. However, it is unclear whether ART includes IUI or OS, in addition to IVF and ICSI. Stillbirth, neonatal death, and perinatal death were all analysed in multivariate models adjusting for maternal age, parity, BMI, Indigenous status, smoking in pregnancy, and insurance status. Singletons were analysed separately from multiple gestations. Stillbirth rate in ART singletons is reported as 7.93/1000

total births, compared to 7.73/1000 total births in the non-ART group. These do not appear to be materially different, but no p-value or OR were reported. However, it is reported that perinatal mortality is increased following ART, with adjusted OR 1.45 (1.26-1.68). Thus, although this study did assess stillbirth as an outcome, the focus appears to have been on perinatal mortality. Overall rate of perinatal mortality in the singleton ART group was 11.57/1000 total births, compared to non-ART 10.43/1000 total births. Neonatal mortality in the ART group is higher following ART (3.66/1000), compared to non-ART (2.75/1000). Therefore, the increase in perinatal mortality in the ART group may have been driven by a difference in neonatal death rather than stillbirth. However, the authors do not report on this specifically. The significance would be that an increase in neonatal mortality but not stillbirth suggests that fetuses following ART are compromised during delivery (intrapartum) or in the immediate postpartum period, rather than antepartum.

More recently, Bay et al (2019) conducted a retrospective cohort study utilizing data from the Danish Medical Birth Register linked with the Danish IVF Register, comparing the risk of stillbirth between those conceiving after IVF/ICSI to those conceiving after IUI or SC.[43] This is the largest study of stillbirth following IVF/ICSI to date, with 425,732 pregnancies included. The gestational age criterion for reporting stillbirths in Denmark is ≥22 weeks. In an attempt to distinguish stillbirths secondary to IVF/ICSI from stillbirths secondary to other conditions, they applied extensive exclusion criteria, including: preterm birth, multiple pregnancy, maternal age ≥40 years, BMI ≥35 kg/m^2, induction of labour for any reason other than stillbirth, pre-existing or gestational hypertension, preeclampsia, eclampsia, HELLP syndrome (a severe obstetrical complication characterized by hemolysis, elevated liver enzymes, low platelets), pre-existing or gestational diabetes, intrahepatic cholestasis of pregnancy, and alloimmunization. The authors

performed a multiple logistic regression analysis to control for maternal age, parity, smoking, and fetal sex, and found an increased risk of stillbirth following IVF/ICSI (OR 2.1, 95% CI 1.4-3.1). The authors also performed a Cox regression analysis to assess the risk of stillbirth by gestational age and found that the risk increased between 37 weeks (OR 1.6) to 42 weeks (OR 6.8). Interestingly, in subgroup analysis they found that the risk was primarily associated with ICSI alone (OR 2.2, 95% CI 1.2-3.1), and the risk of IVF alone was no different than the risk following SC (OR 1.7, 95% CI 0.9-3.1). When assessing fresh and frozen embryo transfers, they found there was increased risk associated with fresh embryo transfer (ET), but not frozen. This is consistent with a recent meta-analysis (see below) that found that other poor perinatal outcomes were increased following fresh ET compared to frozen ET.[44] This study highlights some of the strengths and weaknesses of a study design utilizing a large database. In terms of strengths, the sample size is certainly sufficiently large. The authors provide a sample size calculation based on the baseline risk of stillbirth in the study population of 0.1%. In order to detect a doubling of risk (i.e. to 0.2%), with alpha 0.5 and beta 0.2, both exposed and non-exposed groups would require 2,500 pregnancies. This study achieved this, with 10,235 IVF/ICSI pregnancies and 410,976 SC pregnancies included. The baseline risk of stillbirth reported in this study is low, which likely reflects the multiple exclusions, which were all themselves risk factors for stillbirth (or reflective of increased risk of stillbirth, as in the case of induction of labour). The data captured in a national database is rigorous, since livebirths and stillbirths are required to be reported as vital statistics; and in Denmark, all IVF cycles must also be reported. Conversely, details of the pregnancies, including whether SC had a history of infertility, and the precise treatment protocols for IVF/ICSI pregnancies were not reported by the authors.

Other perinatal outcomes following in vitro methods of conception

As previously described, there have been meta-analyses comparing perinatal outcomes between those conceiving using in vitro methods with those conceiving using non-in vitro methods (typically spontaneous conception). The perinatal outcomes examined typically include preterm birth (various definitions, including <37 weeks and <34 weeks), small for gestational age, low birth weight (<2500g) and related parameters (e.g. very low birth weight, <1500g), antepartum hemorrhage, type of delivery, and rate or perinatal mortality. Stillbirth is rarely examined as an outcome, possibly due to the rare nature of this outcome, or perhaps the inconsistent reporting criteria internationally.

Jackson et al conducted a meta-analysis in 2004 comparing perinatal outcomes in those conceiving after IVF (only, attempting to exclude ICSI, FET, and GIFT) to those conceiving spontaneously in singleton gestations, and reported on studies published between 1978-2002.[16] The primary outcome was perinatal mortality (including both stillbirth and neonatal death), and used the study author's definition of stillbirth when assessing this outcome. In calculation of the summary OR the authors used adjusted OR (ideally with adjustment for age, parity, and delivery date) where available. The authors report low statistical heterogeneity for the perinatal mortality outcome, although clinical heterogeneity may have been expected given the large number of countries represented in the review; the authors appear to have anticipated this, reporting that a random effects model was used for meta-analysis.

This group reported an increased risk of perinatal mortality associated with IVF, with OR 2.19 (1.61-2.98), as well as an increased odds of stillbirth, with OR 2.55 (1.78-3.64), but cautioned that an explicit search for stillbirth as an outcome was not completed. In addition, this analysis found increased odds of preterm delivery, gestational diabetes, preeclampsia, placenta

previa, induction of labour, Caesarian delivery (elective and emergent), neonatal death, and NICU admission associated with IVF conception. Thus, while this meta-analysis was able to confirm some increased risk in singleton gestations following IVF, particularly with regard to perinatal mortality, there was limited information provided about the risk of stillbirth.

The following year, the systematic review published by McDonald et al compared singleton gestations following IVF/ICSI with spontaneous conceptions, reporting on perinatal mortality as the primary outcome and preterm birth as a secondary outcome.[14] Both cohort and case-control studies were considered. The authors analysed studies they classified as "cohort" and "case-control" separately. However, what is classified as "case-control" in this systematic review might otherwise be called "matched cohort". That is, the studies classified as "case-control" used an exposure group (i.e. IVF, called "cases") matched for certain demographic variables (e.g. age, parity) to a non-exposed group (i.e. spontaneous conception, called "controls"). In contrast, others might consider a case-control study to assess exposure retrospectively from an outcome. For example, all stillbirths would be identified as cases, with patient histories reviewed for risk factors; and all livebirths identified as controls. Instead of "stillbirth", the authors were only able to report on intrauterine fetal death. Two studies were found reporting on intrauterine fetal death: one reported IUFD as early as 15 weeks. IUFD at 15-20 week would generally not be reported as stillbirths in any country. This study was not used in the meta-analysis for the IUFD outcome. Thus, the authors report on odds of IUFD from one study, which had not found an increased risk following IVF, with OR 1.56 (0.67 – 3.62).

Helmerhorst et al. (2004) also conducted a systematic review assessing perinatal outcomes following assisted conception, without specifying what was included in the definition of "assisted conception".[15] Included studies were published between 1985-2002, and separate

meta-analyses were performed for studies that performed any matching; and for studies that did not match. There was no requirement for what variables were included in matching. The authors reported an increased risk of perinatal mortality (OR 1.68, 95% CI 1.11-2.55) in singletons when including studies that performed matching but did not assess stillbirth alone. Furthermore, the authors report that the increased risk of perinatal mortality associated with the assisted conception group was entirely driven by the risk reported in a single study. When this study was removed in a sensitivity analysis, there was no difference in risk of perinatal mortality between groups. Consistent with other literature, this review also found increased risk of preterm birth, low birth weight (LBW), SGA, Caesarian delivery, and admission to the neonatal intensive care unit (NICU).

A more recent meta-analysis by Pandey (2012) noted differences between the aforementioned 2004 meta-analyses and later publications, speculating that this was due to differences in particular in vitro techniques, such as the increasing use of blastocyst stage transfer and FET.[17] Their aim was to quantify the risks associated with IVF or ICSI in singleton pregnancies in comparison with SC pregnancies, with subgroup analyses for different procedures. The meta-analyses were initially performed using a fixed effects model. They did find an increased risk of a number of poor perinatal outcomes associated with IVF/ICSI, including: antepartum hemorrhage, major and minor malformations, any hypertensive disorder or pregnancy, preterm prelabour rupture of membranes (PPROM), elective and emergent Caesarian delivery, LBW, SGA, preterm delivery, NICU admission, gestational diabetes, and induction of labour. In particular, this study reported an increased risk of perinatal mortality in IVF/ICSI pregnancies compared to spontaneous conceptions (OR 1.87, 95% CI 1.48 – 2.37), but this did not persist when utilizing a random effects model for meta-analysis, which may have been

warranted given an I^2 for heterogeneity of 73%. In addition, the use of a fixed effects model may not have been ideal given that the authors expected clinics to have changed the specific procedures and technologies used (i.e. type of transfer) over time.

In addition to rarely assessing stillbirth as an outcome, these meta-analyses were unable to include a comparison to conceptions with a history of infertility. This is an important comparison in considering the cause of increased perinatal risk, since a history of infertility may be a significant confounder.

The treatment vs. the condition of infertility

Wisborg et al. (2010) used data from the Aarhus Birth Cohort in a cohort study to compare the risk of stillbirth in singleton gestations following IVF/ICSI, non-IVF ART (i.e. OS, IUI), and SC with and without a history of infertility.[45] Women were considered to be fertile with SC if their time to conception was <12 months, and sub-fertile with SC if their time to conception was ≥12 months. The Aarhus Birth Cohort comprised pregnant women delivering at Aarhus University Hospital in Denmark and agreeing to participate in a series of questionnaires. There were 20,166 singleton pregnancies included in total from this cohort, of which 742 were IVF/ICSI. In multivariate regression analysis adjusting for maternal age, BMI, education, smoking status, alcohol use, and coffee consumption, they found that there was an increased risk of stillbirth following IVF/ICSI compared to fertile couples with SC (OR 4.08, 95% CI 2.11-7.93). When comparing non-IVF ART to fertile SC, there was no difference (OR 0.53, 95% CI 0.13-2.18). Similarly, when comparing sub-fertile SC to fertile SC, there was no increased risk (OR 1.33, 95% CI 0.70-2.56). These results suggest that the risk is due to the method of in vitro conception, rather than the condition of infertility. One limitation of this study is the reliance on

patient recall and understanding of their medical procedures in determining the exposure. For example, couples may not precisely recall the time to conception.

Merritt et al. (2014) were also able to contribute data about the risk of stillbirth following ART in comparison to those with a history of infertility, but conceiving spontaneously.[46] In this study, ART included artificial insemination i.e. IUI. This was a retrospective study utilizing data from patient discharge files following admission to all non-federal hospitals in California, USA. The stated purpose of this study was to explore the relative costs of ART pregnancies however, and not an assessment of perinatal outcomes. The data is therefore presented as number of stillbirths per group in each year, and no baseline comparison is provided between groups. Using the raw numbers provided in the text, the rate of stillbirth following ART over the three years of the study was 25.6/1000 total births; in those with a history of infertility but conceiving spontaneously it was 15.2/1000 total births; and in those without a history of infertility and conceiving spontaneously it was 5.5/1000 total births. The relative risk for stillbirth following ART compared to natural conceptions without a history of infertility is 4.65; the RR for ART vs. natural conceptions *with* a history of infertility is 1.68. Thus, data from this study suggests that there is a graded increase in risk with increasing severity of infertility or degree of intervention. The data is clearly limited in that it was not able to match or adjust for common confounders such as maternal age, parity, or comorbidities. This study neither adjusted for, nor identified these confounders in the sample population.

Using infertility clinic records, Marino et al. (2014) were able to specifically assess the risk of a number of perinatal outcomes, including stillbirth in the context of infertility history and method of conception, while adjusting for common confounders.[47] In this study, assisted reproduction included both in vitro methods (IVF/ICSI) and non-in vitro methods (IUI, OS),

although they did perform subgroup analyses by type of assisted reproduction. This study linked infertility records from two clinics in the state of South Australia to the South Australian Perinatal Statistics Collection database, which records all livebirths and stillbirths in the state. They performed multiple logistic regression analysis, adjusting for maternal age, parity, and infant sex. Both singleton and twin pregnancies were included but analysed separately. Using the group conceiving spontaneously with no history of infertility as the reference group ("spontaneous conception, fertile"), they reported an increased risk of stillbirth associated with a diagnosis of infertility, but *no* assisted conception treatment from a specialist clinic ("spontaneous conception, if DX"; OR 4.11, 95% CI 2.33-7.27), IVF with fresh embryo transfer (OR 2.35, 95% CI 1.34-4.11), ICSI with fresh embryo transfer (OR 2.46, 95% CI 1.29-4.68), and "any assisted conception" (OR 1.82, 95% CI 1.34-2.48). Interestingly, there was no increased risk found for IVF with frozen embryo transfer (OR 2.31, 95% CI 0.997-5.37), ICSI with frozen embryo transfer (OR 0.74, 95% CI 0.15-3.70), OS alone (OR 0.52, 95% CI 0.07-4.18), or IUI (OR 1.21, 95% CI 0.44-3.33). While this initially seems to support the theory that a history of infertility itself is associated with increased risk independent of in vitro treatment, the results of the IUI and OS analyses are not consistent with the remainder of the findings. One would expect that if a history of infertility increased the risk of stillbirth, then this would remain true for those conceiving using IUI or OS. This finding would only be true if IUI and OS were protective against stillbirth, or if the majority of OS and IUI were performed for reasons unrelated to infertility. It is possible that IUI is performed for reasons unrelated to infertility (i.e. for same-sex couples), but this seems unlikely for OS alone. Additionally, while the group is called "spontaneous conception, if DX", the inclusion criterion for this classification is "births to women who had a recorded diagnosis of infertility but not assisted conception treatment from a

specialist clinic", which is not necessarily a spontaneous conception. This group may have included those conceiving via assisted reproduction, but not in the two clinics where records were obtained. For instance, the couple may have travelled out of state or country to obtain assisted reproduction. In addition, some OS using oral medications can be prescribed by general obstetricians/gynecologists, meaning this group could be contaminated with those receiving ART. All of these factors have the potential to bias the results towards an increased effect size. The question of whether there is an increased risk of stillbirth following assisted reproduction or in vitro methods is still therefore unanswered by this study.

Pinborg et al. (2013) conducted a meta-analysis for adverse perinatal outcomes in ART singletons, and included pregnancies following a history of subfertility without treatment.[18] Reported outcomes to be assessed included preterm birth, LBW, SGA, perinatal mortality and stillbirth (study author's definition). The authors conducted a number of subgroup comparisons for specific procedure used. Unfortunately, the only reported outcome was preterm birth. Interestingly, there was an increased risk of preterm birth (OR 1.35, 95% CI 1.22-1.50) when comparing SC known to be sub-fertile to SC known to be fertile (i.e. time to pregnancy <1 year). This implies that preterm birth at least may be secondary to the condition of infertility. However, when comparing IVF/ICSI pregnancies to SC sub-fertile pregnancies, there was also an increased risk of preterm birth (OR 1.55, 95% CI 1.30-1.85). Thus, it would appear that even if infertility increased the risk of preterm birth independently, the risk is further increased by treatment using in vitro methods. One important caveat for the findings of this study is that the authors did not differentiate between iatrogenic and spontaneous preterm birth. Thus, it is unclear if clinicians treat patients with a history of infertility differently (i.e. more cautiously), or if there is in fact a biologic difference that increases the risk of preterm birth.

Technique-specific risk

The question of whether risk is increased by the condition of infertility or the treatment of infertility is complicated by the many techniques and technologies that are used, which may themselves have differing risk profiles.

In the meta-analysis by Pinborg et al. (2013) described above, the authors were not able to identify an increased risk of preterm birth associated with: FET (vs. SC); blastocyst vs. cleavage stage transfer; or single embryo transfer vs. double embryo transfer.[18] Thus, although they found the risk of stillbirth increased with the use of infertility treatment, it is unclear what part of the treatment may be contributing. Techniques continue to evolve in the field, which may also change the risk associated with treatment.

Due to the risks associated with multiple gestation following multiple embryo transfer, single embryo transfer (SET), in which one high-quality embryo is transferred into the uterus at a time has become increasingly popular[48]; and in fact is the official recommendation in some regions.[49] De Neuborg et al. (2006) conducted a retrospective cohort study comparing perinatal outcomes between single ET gestations following in vitro methods, to singleton gestations conceived spontaneously.[50] Data about SET was collected from a single fertility clinic, with outcomes assessed using questionnaires sent to delivering obstetricians and patients. These were then compared with the reported outcomes in the Belgian Study Centre for Perinatal Epidemiology (SPE), the registry for all deliveries in Flanders, Belgium. They reported a higher incidence of preterm birth, and hypertensive disorders of pregnancy in the single ET group. They reported a similar rate of stillbirth in both the single ET and SC singleton group, which is certainly reassuring. Single ET pregnancies may be advantaged over pregnancies following multiple ET. As described earlier, the embryo selected for transfer is the highest quality;

additionally, there is a greatly reduced risk of vanishing twins with single ET compared to multiple ET. Both of these may explain why single ET specifically is not associated with an increased risk of stillbirth. However, the number of in vitro pregnancies included in this study was somewhat small (251), which may limit the power of the study.

Maheshwari et al. updated a meta-analysis in 2018 comparing specific in vitro procedures and techniques.[44] The authors were particularly interested in differences in perinatal outcomes following frozen or fresh embryo transfer. They found that frozen embryo transfer was associated with decreased risk of SGA, LBW, and preterm birth; conversely there was also an increased risk of large for gestational age and hypertensive disorders of pregnancy. Perinatal mortality (study author's definition) was assessed, and no difference was found. The authors conducted meta-analyses with both fixed and random effects models, reporting with only the random effects model due to significant expected clinical heterogeneity. In particular, the authors described differences in embryo stage of freezing (i.e. cleavage vs. blastocyst stage), and method of freezing (i.e. vitrification or slow freezing). Thus, this meta-analysis suggests that while there are differences between fresh and frozen embryo transfer, this does not necessarily translate to a difference in perinatal mortality. Similar to other analyses reporting only perinatal mortality, the difference in risk of stillbirth remains unclear.

Stillbirth is a difficult outcome to study due to varying reporting requirements, and its rare nature. Thus, a common problem when studies describe stillbirth as an outcome is that they are frequently under-powered to find a difference between groups. Large database studies and meta-analyses help to overcome this issue. But while many systematic reviews and meta-analyses have been conducted assessing perinatal outcomes following in vitro methods of conception, none have explicitly included the risk of stillbirth. Furthermore, because none have

explicitly assessed stillbirth, there have been no detailed analyses by type of method (e.g. IVF vs. ICSI), specifics of the procedure used (e.g. fresh vs. frozen transfer), or patient history (i.e. a comparison with a history of infertility) that help us to understand the etiology of this risk. A search of the PROSPERO database of systematic review and meta-analysis protocols does not reveal any forthcoming reviews on this topic. This thesis addresses the risk of stillbirth following in vitro methods in detail.

Chapter 3: Method

The possibility of increased risk of stillbirth following in vitro methods of conception is suggested by the increased risk of other adverse perinatal outcomes such as perinatal mortality, intrauterine growth restriction (IUGR), and preterm delivery. The latter adverse outcomes have been shown to be more frequent following in vitro methods of conception in meta-analyses; however, no meta-analysis to date has specifically assessed stillbirth as an outcome. This may be because stillbirth is such a rare outcome, and therefore difficult to study without large numbers. An additional challenge lies in defining stillbirth, since reporting criteria are different across the globe, and even sometimes within the same country. This systematic review was conducted to assess the risk of stillbirth following in vitro methods of conception in singleton gestations.

Research question

In singleton pregnancies, are in vitro methods of conception (i.e. in vitro fertilization, intracytoplasmic sperm injection) associated with an increased risk of stillbirth compared with non-in vitro methods (i.e. spontaneous conceptions, intrauterine insemination, ovarian stimulation)?

Objectives

The objectives of the study were:

1) To determine if an increased risk of stillbirth exists

2) To estimate when this risk might be greatest, and

3) To explore the possible etiology of any increased risk (i.e. whether it is secondary to the procedure or the condition of infertility).

Method development

The Cochrane Handbook version 5 (2011) was used in the initial development of this systematic review and meta-analysis.[51] An updated version of the Cochrane Handbook (2019) was released after the protocol had been developed, and was used to inform outstanding decisions.[52] The protocol was written and registered with the International Prospective Register of Systematic Reviews (PROSPERO) in June 2019 (# CRD42019134414), see Appendix 1.

Information sources and search strategy

A search strategy within PubMed was developed in collaboration with a health sciences librarian. The search strategy was not peer reviewed. Search strategies for other databases were developed by the author based on the PubMed strategy, and reviewed by the librarian. Searches were conducted in PubMed, EMBASE, CINAHL, and Cochrane Library databases from inception to June 2019 for search terms related to "stillbirth", "intrauterine fetal death", "in vitro fertilization", and "assisted reproduction" (see Appendix 2: Search Strategy). No filters or language restrictions were applied.

Reference lists of included studies, and national obstetric guidelines related to assisted reproduction were also reviewed for additional eligible studies.

Selection criteria

Eligible studies included randomized controlled trials and observational studies (i.e. with a cohort or case-control design) comparing those conceiving through in vitro methods (IVF, ICSI, or GIFT, including donor oocytes or embryos and fresh or frozen embryo transfer) with those conceiving through any other method (including spontaneous conception, IUI, or OS). Randomized controlled trials were not expected, as studies with this design would be unethical and unfeasible, but if found, they would have been included. The primary study design of interest

was therefore the observational study. The specific type of observational study is often mis-labelled in the literature. For the purposes of this systematic review, a cohort study was defined as an observational study design in which individuals exposed to in vitro methods of conception were compared to individuals who were not exposed to in vitro methods, for the outcome of stillbirth. The non-exposed (control) group may have been matched to the exposed group on a number of variables. On the other hand, a case-control study was defined as an observational study design in which individuals with stillbirths were compared to individuals without stillbirths (i.e. livebirths) for the odds of exposure to in vitro methods. Although cohort studies are considered superior evidence of causality compared with case-control studies, both types were included to increase the sensitivity of the review.

The studies must have reported stillbirth or intrauterine fetal death as an outcome, distinct from perinatal mortality. As previously described, reporting criteria for stillbirth vary globally. Therefore, the study authors' definition of stillbirth was used, but must have been explicit, and must have been after 20 weeks' gestation.

Singleton gestations were the population under analysis; studies including multiple gestation must have presented separate data for singletons. One complicating issue was the vanishing twin phenomenon. This is a type of pregnancy that began as a twin pregnancy, but spontaneously reduced to a singleton pregnancy. Thus, they are singletons at delivery, which would allow their inclusion in this systematic review; however, this phenomenon is also a significant known confounder. Vanishing twins seem to be more common after in vitro methods, and are also associated with increased incidence of SGA and preterm birth.[53] These two poor outcomes may also be associated with stillbirth through similar mechanisms. However, the true incidence of vanishing twins following spontaneous conception is unknown, since early

ultrasounds that would be able to assess this are not routinely performed. Since the phenomenon is likely a confounder, studies that exclusively assessed outcomes following vanishing twins were not included.

Studies were excluded if general population statistics were used as the comparator, since in vitro conceptions would be included in general statistics; or if non-in vitro methods could not be distinguished from in vitro methods (i.e. IUI pooled with IVF/ICSI).

Missing information

Study authors were contacted for missing information. If this information was relevant for eligibility and authors did not respond, attempts were made to determine the information through other means. For example, if the missing information was the definition of stillbirth used, national definitions were sought for the timeframe in question. If this information was not available from the authors or other means, the study was excluded.

When authors did not respond for requests for missing or unreported data, the study was included in narrative synthesis but not in the meta-analysis.

Data extraction

Initial title and abstract screening, and subsequent full text review were completed separately by two reviewers (author and collaborator) utilizing Covidence software (Veritas Health Innovation Ltd, Melbourne, Australia). A Title & Abstract Screening Guide (see Appendix 3) was used to support consistency between reviewers. Discrepancies were resolved through discussion, with a third reviewer resolving remaining conflicts. Data was extracted separately by two reviewers into pre-planned data tables, which were then compared for consistency. Data tables were piloted using one case-control and one cohort study. Tables included country and year(s) of study, study data sources, confounders assessed, raw numbers for

stillbirths and livebirths, as well as any adjusted odds ratio for odds of stillbirth from regression analyses.

Clinical variables considered confounding factors included:

- Maternal demographics: age
- Maternal medical history: parity, smoking, previous stillbirth, pre-existing medical conditions
- Obstetrical risk factors: hypertensive disorders of pregnancy, gestational diabetes, intrahepatic cholestasis of pregnancy, selective fetal reduction in current pregnancy (iatrogenic reduction)

Risk of bias assessment

Risk of bias of included studies was independently assessed by two review authors using the Newcastle-Ottawa Quality Assessment Scale (NOS)[51,54], adapted for this review (see Appendix 4). The NOS, modified for the review question, has been recommended in the Cochrane Handbook for assessment of non-randomized studies of interventions.[51] In the updated version of the Cochrane Handbook, it is recommended to perform risk of bias assessment using the new ROBINS-I tool.[52] This tool has been specifically developed by the Cochrane group for the assessment of risk of bias in non-randomized studies of interventions. Using this tool, the Cochrane Handbook suggests that assessment of certainty of evidence can then begin at a baseline high certainty of evidence, rather than the low certainty of evidence previously used for all non-randomized studies.[52] However, the ROBINS-I tool currently only exists for assessment of non-randomized studies using a follow-up design[52], and a case-control design cannot be assessed using this tool. Therefore, the reviewers proceeded with use of the NOS for risk of bias

assessment and made appropriate adjustments to the assessment of certainty of evidence (see Certainty of Evidence, below).

The NOS comprises separate scales for case-control and cohort studies, assessing each type of study on three domains: selection of study groups, comparability of groups, and ascertainment of exposure (for case-control) or outcome (for cohort). A list of risk factors for stillbirth was used to assess Comparability criterion 1a (study controls for obstetrical risk factors for stillbirth). Studies controlling for ≥50% of listed risk factors were deemed to satisfy this criterion. There are no accepted ranges for scores that constitute low, moderate or high risk of bias using the NOS. For the purposes of this review, the authors have deemed a score of 7-9 would constitute a low risk of bias; 4-6 a moderate risk of bias; and 0-3 a high risk of bias. This is consistent with other systematic reviews in this area.[55] The main meta-analysis could include studies with any risk of bias score; sensitivity analysis was undertaken for studies at low risk of bias (see below).

Data synthesis and statistical analysis

RevMan (Review Manager Version 5, The Cochrane Collaboration, London, UK) was used for the statistical analysis. Separate analyses were planned for randomized controlled trials, cohort studies, and case-control studies since such starkly differing trial designs would contribute to significant heterogeneity and would likely be inappropriate to combine statistically.

Due to varying definitions of stillbirth and clinical practice across the globe, it was expected that there was sufficient clinical heterogeneity to cause underlying risk differences between studies; therefore, a random effects model was used for meta-analysis. A random effects model assumes that studies are estimating different, normally distributed effects. The summary

statistic from a random effects meta-analysis estimates the mean of the effect sizes from all studies in the analysis.[52]

Meta-analyses were completed using the inverse variance method of DerSimonian and Laird (1986).[56] In this model, the observed effect size in a study is the sum of the true effect size and the sampling error from that study. The sampling error itself has a variance, the inverse of which is used in the estimation of the mean treatment effect from the population of all studies included in the analysis.[56]

Odds ratios (ORs) with 95% confidence intervals (CIs) were the target estimate of effect to be extracted from studies. Where risk ratios or raw numbers for stillbirths and livebirths were provided, these were extracted and converted to ORs with 95% CIs. These were then used to calculate the standard error of the OR, and the natural log of the OR (lnOR), which were then inputted into RevMan.

Reviewers independently assessed clinical heterogeneity and decided data was suitable to be pooled statistically. Statistical heterogeneity was assessed using the I^2 value calculated through RevMan. It has been suggested that I^2 values be reported with their 95% CIs, as I^2 alone may be misleading as a single measure of heterogeneity.[57] Therefore, 95% CI for I^2 values were calculated using Higgins' *test based* method, as described in Thorlund, 2012.

For studies in which multiple effect estimates were presented due to multiple models with different confounders, the model accounting for the largest number of relevant confounders was used. For studies in which multiple subgroup analyses provided multiple *adjusted* effect estimates, the analysis with the largest number of pregnancies was included. For example, if separate analyses were presented for IVF vs. SC and ICSI vs. SC, the analysis that included the highest number of pregnancies was used. For studies that presented multiple comparisons, but

did *not* include an adjusted analysis, in vitro groups were pooled together, and non-in vitro groups were pooled together.

Studies may have originated from the same country or region, and therefore included the same pregnancies. For studies originating in the same region from the same or overlapping time period, the estimate from the study including the largest number of pregnancies was included. An exception was for studies compiling national databases from several countries (e.g. Denmark, Finland, Norway). Separate meta-analyses were completed with studies from individual countries and studies pooling those same countries.

The issue of no events (also known as zero-value cells) has been debated in the literature.[58–60] When no events occur in either the exposed or non-exposed group, the odds ratio cannot be calculated, as this results in division by zero. Although a constant correction factor—that is, the addition of some small constant number such as 0.5 to all cells is commonly used, it has been demonstrated that this method of continuity correction can introduce bias, particularly when groups are unbalanced.[59] It has been recommended to use instead the reciprocal of the opposite treatment arm size or an empirical continuity correction based on the pooled OR of the non-zero event studies.[59] A constant continuity correction was used for baseline analyses, and compared with the reciprocal correction factor in subsequent sensitivity analyses. When no events occur in both groups, the study was excluded from the analysis, since no information is added from this data.

Subgroup and sensitivity analyses

In order to better understand the nuances of the effects of method of conception and gestational age on stillbirth, subgroup analyses by type of conception were planned. These included:

a. by type of in vitro method e.g. IVF vs. non-in vitro methods; ICSI vs. non-in vitro methods; and
b. by type of non-in vitro methods e.g. in vitro methods vs. IUI; in vitro methods vs. SC.
c. by gestational age definition of stillbirth; and
d. studies including only term pregnancies.

Lastly, sensitivity analyses were completed to explore the effect of study quality and method of managing zero-value cells. Study quality sensitivity analyses included studies with

a. low risk of bias only and
b. the lowest risk of bias (i.e. highest score on risk of bias scale).

While data management sensitivity analyses performed analyses using

a. constant continuity correction (i.e. adding 0.5 to all cells when one group had zero events)
b. reciprocal of the opposite "treatment" arm size correction (i.e. adding the reciprocal of the opposite arm sample size to all cells when one group had zero events).

Assessment of certainty of evidence

Overall certainty of evidence was assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach. Each comparison (i.e. main analysis and subgroup analyses) were assessed separately.

Criteria for upgrading or downgrading certainty of evidence were decided by consensus between two reviewers. Since this review examined only observational studies, the baseline certainty of evidence was low. It had been planned that the certainty of evidence could be upgraded if the effect size was large (e.g. OR ≥5) or if there were no other important sources of bias contributing to the effect estimate.

Certainty of evidence was further downgraded based on the following domains:

- Methodological quality: ≥25% of pregnancies are from studies rated as having a high risk of bias (Newcastle Ottawa Scale 0-3).
- Inconsistency of results: ≥25% of studies had treatment effects in a different direction, I^2 ≥75% (considerable heterogeneity), p-value for heterogeneity <0.05, or unable to draw a straight line through the Forest plot
- Indirectness of evidence: more than 50% of patients were outside of the target group
- Imprecision of evidence: fewer than 400 total stillbirths were included in the comparison. With ≥400 total events, and a relative risk increase of ≥25% (based on sample size calculation above using risk difference of 2/1000, with baseline event rate 7.91/1000) the threshold for optimal information size will always be met.[61]

Certainty of evidence for the outcome of stillbirth was reduced by one level for each domain, according to the rules above.

Reporting bias was assessed by construction of funnel plots for analyses including ≥10 studies.

Chapter 4: Results

Description of included studies

The literature search identified 1593 records, and review of reference lists yielded an additional 16 records. After de-duplication, 1590 records underwent title and abstract screening for initial eligibility, and 222 full-text articles were reviewed for final eligibility. At the title and abstract screening stage, 5 studies were excluded for exclusively assessing the vanishing twin phenomenon. There were 34 studies meeting inclusion criteria.[34,41,67–76,43,77–86,45,87–89,47,62–66] Of these 29 were cohort studies that provided sufficient data for meta-analyses. There was one case-control study, and no randomized controlled trials meeting eligibility criteria. See Figure 1: Prisma Flow Diagram and Appendix 5: List of excluded studies in full text review, with reasons.

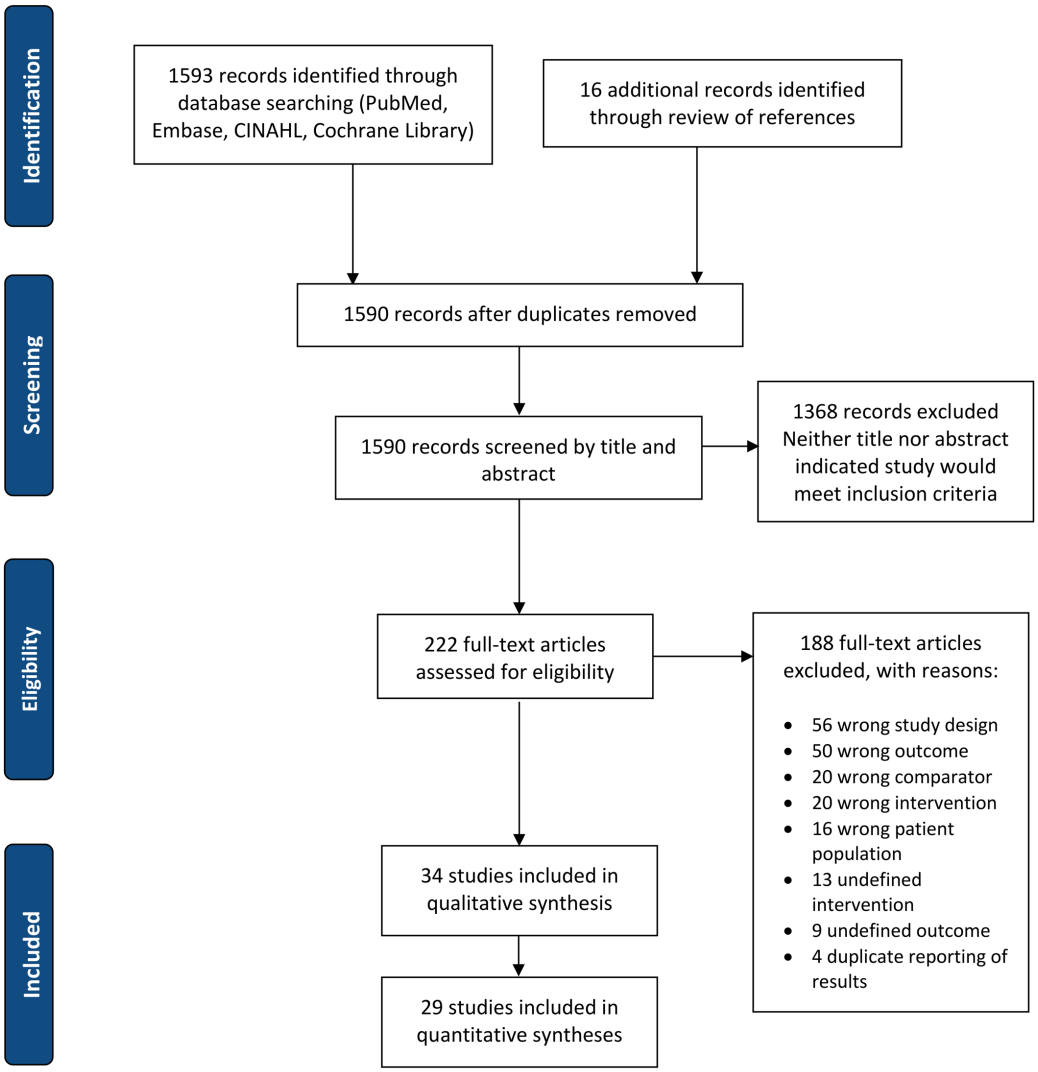

Figure 1: PRISMA flow diagram.

The case-control study meeting inclusion criteria was conducted in Australia, spanning 2002-2012, and has been described earlier in this text.[34] It utilized a regional pregnancy outcome database to identify stillbirths of singleton, structurally normal fetuses; and reviewed their medical records to determine risk factors, including IVF. Cases were matched to two livebirth controls based on parity and time of birth i.e. the livebirths with matching parity immediately before and after the stillbirth. Univariate analysis identified that IVF was associated with an increased odds of stillbirth compared to spontaneous conception, adjusted OR 7.27 (95% CI 0.69-76.85). After multivariate analysis, IVF was no longer associated with stillbirth.

Table I describes characteristics of the included cohort studies. Thirty-three cohort studies were identified in total, of which four did not have usable data due to stillbirth numbers not being reported or zero total stillbirths.[70,72,79,88] Therefore, 29 studies provided data for meta-analyses. Studies were international in scope, though only 2 studies were identified from Asia (Japan[84], India[75]), and 14 studies were identified from Nordic countries (Finland, Iceland, Norway, Denmark, Sweden).[41,43,81,87–89,45,66,69,72,74,76–78] Two studies pooled data from multiple countries.[41,87] Most studies used regional or national birth databases for identification of either the in vitro or control group. The definition of stillbirth reported by authors ranged from gestational age 20 weeks to 28 weeks, with some providing only a birth weight definition (≥400g or ≥500g). Two studies included only pregnancies reaching term (≥37 weeks).[43,65]

Table 1: Characteristics of included cohort studies

Study, year	Country (region)	Years included	Identification of method of conception	Reporting of stillbirth	Other perinatal outcomes assessed*
Apantaku, 2009[14]	UK (West England)	1999-2004	Single clinic records	≥24 weeks or 500g	Apgar scores, BW, congenital anomalies, GA at delivery, LBW, NICU admission, PTB
Bay, 2019[15]	Denmark	2003-2013	National register	At term (≥37 weeks)	None
Chaveeva, 2011[16]	UK (London and Kent)	Not reported	Patient questionnaire	≥24 weeks	LGA, PTB, SGA, SPTB
Dayan, 2016[17]	Canada (Ontario)	2006-2012	Regional register	≥20 weeks	NND, PTB, SGA
Dhont, 1999[19]	Belgium (Flanders)	1992-1997	Regional register	≥500g	Congenital anomalies, early NND, LBW, NICU admission, perinatal death
Ensing, 2015[20]	Netherlands	1999-2010	National register	At term (≥37 weeks)	Apgar scores, congenital anomalies, NICU admission, perinatal death, SGA
Fedder, 2013[21]	Denmark	1995-2009	National register	≥22 weeks	BW, congenital anomalies, LBW, NND, perinatal mortality, PTB
Hansen, 2002[22]	Australia (West)	1993-1997	Regional register	≥20 weeks	Congenital anomalies, LBW
Hansen, 2012[23]	Australia (West)	1994-2002	Regional register	≥20 weeks or ≥400g	Congenital anomalies
Henningsen, 2011[24]	Denmark	1994-2006	National register	≥22 weeks	BW, GA at delivery, LBW, perinatal death, PTB
Henningsen, 2014[25]	Denmark, Finland, Norway, Sweden	1982-2007	National registers of each country	≥28 weeks†	Early NND, infant death, LBW, LGA, perinatal death, PTB, SGA
Hill, 1990[26]	USA (Tennessee)	1982-1988	Single clinic records	≥24 weeks	PTB

Study, year	Country (region)	Years included	Identification of method of conception	Reporting of stillbirth	Other perinatal outcomes assessed*
Lucovnik, 2018[27]	Slovenia	2002-2015	National register	≥22 weeks or 500g	GA at delivery, LBW, LGA, NND, PTB, SGA
Marino, 2014[28]	Australia (South)	1986-2002	Multiple clinic records	≥20 weeks or ≥400g	Apgar scores, BW, LBW, LGA, NND, PTB, SGA
Norrman, 2015[29]	Sweden	1973-2012	National register	≥28 weeks (until 2008)/≥22 weeks (after 2008)	BW, congenital anomalies, early NND, GA at delivery, infant death, LBW, NICU admission, NND, perinatal death, PTB, SGA
Ombelet, 2005[30]	Belgium (Flanders)	1997-2003	Regional register	≥500g	Apgar scores, BW, congenital anomalies, GA at delivery, NICU admission, perinatal death
Pelkonen, 2010[31]	Finland	1995-2006	Multiple clinic records	≥22 weeks or ≥500g	Apgar scores, BW, early NND, GA at delivery, infant death, LGA, NICU admission, perinatal death, SGA
Pochiraju, 2014[32]	India	2012	Clinic records	≥24 weeks	LBW, NND, PTB, SGA
Poikkeus, 2006[33]	Finland	1999	Multiple clinic records	≥22 weeks	Apgar scores, BW, LBW, NICU admission, perinatal death, PTB
Poikkeus, 2007[34]	Finland	1997-2003	Single clinic records	≥22 weeks or ≥500g	Apgar scores, BW, GA at delivery, LBW, NICU admission, NND, PTB, SGA
Raisanen, 2013[35]	Finland	2006-2010	National register	≥22 weeks or ≥500g	Apgar scores, congenital anomalies, LBW, NICU admission, PTB, SGA
Reubinoff, 1997[36]	Israel	1983-1993	Single clinic records	≥25 weeks or ≥500g	BW, GA at delivery, LBW, PTB, SGA, SPTB
Ricciarelli, 2013[37]	Spain	2008-2009	Clinic records	>20 weeks	Congenital anomalies, PTB
Romundstad, 2008[38]	Norway	1984-2006	National register	≥22 weeks	BW, GA at delivery, LBW, perinatal death, PTB
Shevell, 2005[39]	USA	1999-2002	Previous study database	≥24 weeks	Congenital anomalies, GA at delivery, LBW, PTB

Study, year	Country (region)	Years included	Identification of method of conception	Reporting of stillbirth	Other perinatal outcomes assessed*
Sun, 2009[40]	Canada (Ontario)	2004-2007	Regional register	≥20 weeks	SGA
Tsutsumi, 2012[41]	Japan	2000-2008	Single clinic records	≥22 weeks	LBW, PTB
Verlaenen, 1995[42]	Belgium	1988-1994	No description	≥20 weeks	Apgar scores, BW, congenital anomalies, GA at delivery, NICU admission, perinatal death, PTB
Wen, 2010[43]	Canada (Ottawa)	1996-2005	Single clinic records	≥20 weeks or ≥500g	Apgar scores, congenital anomalies, PTB, SGA
Wennerholm, 1997[44]	Sweden	1990-1995	Multiple clinic records	≥28 weeks	BW, congenital anomalies, perinatal death, PTB, SGA
Wennerholm, 2013[45]	Denmark, Norway, Sweden	1982-2007	National registers of each country	≥28 weeks or ≥22 weeks‡	BW, GA at delivery, infant death, LBW, LGA, NND, perinatal death, PTB, SGA
Westergaard, 1999[46]	Denmark	1994-1996	National register	≥28 weeks	BW, LBW, NND, PTB
Wisborg, 2010[47]	Denmark	1989-2006	Single centre questionnaire	≥28 weeks (until 2004)/ ≥22 weeks (after 2004)	None

* Perinatal outcomes listed can include: BW (birth weight), GA (gestational age), LBW (low birth weight), LGA (large for gestational age), NICU (neonatal intensive care unit), NND (neonatal death), PTB (preterm birth), SGA (small for gestational age), SPTB (spontaneous preterm birth)
† Definitions varied by country and over time. Only rates ≥28 weeks were extractable.
‡ Definitions varied by country and over time. Authors reported on outcomes ≥28 weeks and ≥22 weeks. Data for ≥22 weeks were extracted.

Of the studies that did not include usable data for meta-analysis, two[69,75] reported "similar rates" of stillbirth between groups, but did not provide raw numbers; and two studies reported zero stillbirths in both.[72,88]

Most studies (20/34) were assessed to be at low risk of bias on the NOS. There were 13 studies at moderate risk of bias, including the only case-control study. One study was assessed as being at a high risk of bias. The domain most often scored low leading to increased risk of bias was Comparability, particularly the criteria assessing comparability of other risk factors for stillbirth (see Figure 2a Risk of Bias of Included Studies, by domain and Figure 2b Risk of Bias of Included Studies, by study).

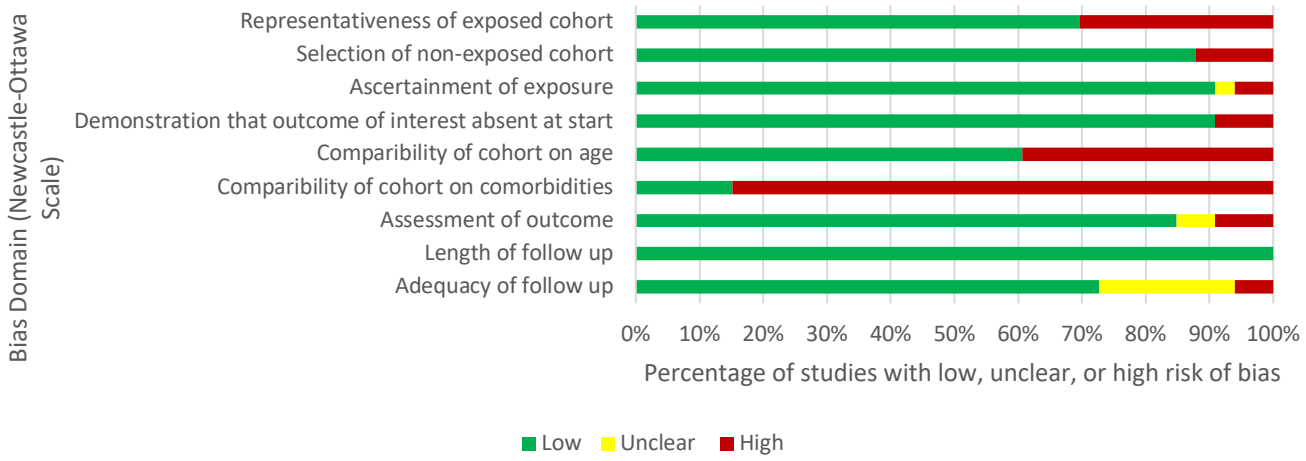

Figure 2a: Percentage of all studies with low, unclear, or high risk of bias, by domain.

Figure 2b: Classification of low or high risk of bias for each domain of bias, by individual studies. *Blank cells represent uncertain risk of bias (insufficient information provided in text to determine risk of bias).*

Qualitative analysis

Few studies focused on the risk of stillbirth. Of the four studies that explicitly focused on stillbirth as a primary outcome, all found an increased risk of stillbirth in in vitro conceptions compared with general spontaneous conceptions.[41,43,45,47] There were slight differences in the results of subgroup analysis. For example, Bay (2019) did not find an increased risk of stillbirth for the subgroup of IVF alone compared to SC, but Marino (2014) did find an increased risk for both procedures when embryos were transferred fresh. Henningsen (2014) was able to break down risk by gestational age, and found that there was only an increased risk before 28 weeks, in contrast with Bay (2019), which found this risk was elevated even among term pregnancies.

Among those finding an increased risk following in vitro methods, conclusions were mixed with regard to whether the risk was due to the procedure itself or the condition of infertility. Four studies made explicit comparisons to pregnancies with a history of infertility, but not conceiving with in vitro methods.[45,47,81,84] Romundstad (2008), Marino (2010), and Tsutsumi (2012) all suggested that the increased risk of stillbirth was more related to a history of subfertility, while Wisborg (2010) concluded that the risk was related to the treatment rather than the history of infertility.

There were mixed findings from studies that did not explicitly look to assess the risk of stillbirth. Most included stillbirth within broader "perinatal outcomes" or "perinatal mortality" and reported very few stillbirths. They were likely underpowered to find a significant difference.

Thus, studies that are powered to find a difference in risk of stillbirth, do find there is an increased risk following in vitro methods, although the specifics regarding which procedures are inconsistent. There is also inconsistent data on whether the risk is more attributable to a history of infertility or the in vitro procedures themselves.

Summary of findings

Tables 2-8 summarize the findings of the meta-analyses for select comparisons. They include illustrative comparative risks based on pooled data. See subsequent sections for details on certainty of evidence evaluation.

Table 2: Summary of findings for risk of stillbirth following in vitro methods of conception vs. non-in vitro methods

Risk of stillbirth following in vitro methods of fertilization compared to all non-in vitro methods						
Population: singleton pregnancies Setting: general pregnant population Intervention: in vitro methods (IVF, ICSI, FET, GIFT, donor oocytes) Comparison: non-in vitro methods (spontaneous conceptions, IUI, ovarian stimulation)						
Outcome	Illustrative comparative risks (number of stillbirths per total births)		Relative Effect (Odds Ratio, 95% CI)	No. of participants (studies)	Certainty of Evidence (GRADE)	Comments
	Non-in vitro	In vitro methods				
Stillbirth	4.54/1000*	6.49/1000 (5.58/1000 to 7.58/1000)	1.43 (1.23 – 1.67)	3,582,239 (19)	Low ⊕⊕OO	Repeated analysis for studies that pooled Nordic countries: 1.29 (1.11 – 1.50) Including studies that used adjusted OR only: 1.63 (1.34-1.97)

Footnotes
* based on average rate of stillbirth in all included studies

Table 3: Summary of findings for risk of stillbirth following in vitro methods of conception vs. pregnancies following a history of infertility

Risk of stillbirth in pregnant women following in vitro methods compared to pregnant women with a history of infertility						
Population: singleton pregnancies Setting: individuals with a history of subfertility Intervention: in vitro methods (IVF, ICSI, FET, GIFT, donor oocytes) Comparison: pregnancies in women with a history of infertility, but conceiving by non-in vitro methods (IUI, OS, or natural conception)						
Outcome	Illustrative comparative risks (number of stillbirths per total births)		Relative Effect (Odds Ratio, 95% CI)	No. of participants (studies)	Certainty of Evidence (GRADE)	Comments
	Non-in vitro	In vitro methods				
Stillbirth	7.27/1000*	8.36/1000 (6.18/1000 – 11.34/1000)	1.15 (0.85-1.56)	30,884 (6)	Very Low ⊕OOO	

Footnotes
* based on average rate of stillbirth in all included studies
Downgraded for imprecision, as only 208 events (stillbirths) occurred

Table 4: Summary of findings for risk of stillbirth following in vitro fertilization vs. all non-in vitro methods of conception

Risk of stillbirth in pregnant women following in vitro fertilization compared to all non-in vitro methods						
Population: singleton pregnancies Setting: general pregnant population Intervention: in vitro fertilization (IVF, without intracytoplasmic sperm injection) Comparison: all non-in vitro methods, including intrauterine insemination, ovarian stimulation, or spontaneous conceptions						
Outcome	Illustrative comparative risks (number of stillbirths per total births)		Relative Effect (Odds Ratio, 95% CI)	No. of participants (studies)	Certainty of Evidence (GRADE)	Comments
	Non-in vitro	In vitro methods				
Stillbirth	2.15/1000*	3.87/1000 (2.41/1000 – 6.21/1000)	1.80 (1.12-2.89)	715,025 (5)	Low ⊕⊕OO	

Footnotes
* based on average rate of stillbirth in all included studies

Table 5: Summary of findings for risk of stillbirth following intracytoplasmic sperm injection vs. non-in vitro methods

Risk of stillbirth in pregnant women following intracytoplasmic sperm injection compared to all non-in vitro methods						
Population: singleton pregnancies Setting: general pregnant population Intervention: intracytoplasmic sperm injection only Comparison: all non-in vitro methods, including intrauterine insemination, ovarian stimulation, or spontaneous conceptions						
Outcome	Illustrative comparative risks (number of stillbirths per total births)		Relative Effect (Odds Ratio, 95% CI)	No. of participants (studies)	Certainty of Evidence (GRADE)	Comments
	Non-in vitro	In vitro methods				
Stillbirth	2.93/1000*	5.13/1000 (3.31/1000 – 7.97/1000)	1.75 (1.13-2.72)	721,597 (5)	Low ⊕⊕OO	

Footnotes
* based on average rate of stillbirth in all included studies

Table 6: Summary of findings for risk of stillbirth following frozen embryo transfer vs. non-in vitro methods

Risk of stillbirth in pregnant women following frozen embryo transfer compared to all non-in vitro methods						
Population: singleton pregnancies Setting: general pregnant population Intervention: frozen embryo transfers only Comparison: all non-in vitro methods, including intrauterine insemination, ovarian stimulation, or spontaneous conceptions						
Outcome	Illustrative comparative risks (number of stillbirths per total births)		Relative Effect (Odds Ratio, 95% CI)	No. of participants (studies)	Certainty of Evidence (GRADE)	Comments
	Non-in vitro	In vitro methods				
Stillbirth	2.94/1000*	3.70/1000 (2.21/1000 – 6.17/1000)	1.26 (0.75 – 2.10)	739,124 (4)	Low ⊕⊕OO	Repeated analysis for studies that pooled Nordic countries: 1.16 (0.83 – 1.64)

Footnotes
* based on average rate of stillbirth in all included studies

Table 7: Summary of findings for risk of stillbirth following fresh embryo transfer vs. non-in vitro methods

Risk of stillbirth in pregnant women following fresh embryo transfer compared to all non-in vitro methods						
Population: singleton pregnancies Setting: general pregnant population Intervention: fresh embryo transfers only Comparison: all non-in vitro methods, including intrauterine insemination, ovarian stimulation, or spontaneous conceptions						
Outcome	Illustrative comparative risks (number of stillbirths per total births)		Relative Effect (Odds Ratio, 95% CI)	No. of participants (studies)	Certainty of Evidence (GRADE)	Comments
	Non-in vitro	In vitro methods				
Stillbirth	2.18/1000*	3.11/1000 (1.81/1000 – 5.35/1000)	1.43 (0.83 – 2.46)	748,934 (4)	Very Low ⊕OOO	Repeated analysis for studies that pooled Nordic countries: 1.30 (0.88-1.91)

Footnotes
* based on average rate of stillbirth in all included studies
Downgraded for inconsistency due to statistically significant heterogeneity, as indicated by p value (0.03) for chi-square test

Table 8: Summary of findings for risk of stillbirth following in vitro methods of conception vs. intrauterine insemination

Risk of stillbirth in pregnant women following in vitro methods compared with intrauterine insemination						
Population: singleton pregnancies Setting: individuals with a history of subfertility requiring treatment Intervention: in vitro methods, including IVF, ICSI, frozen or fresh embryo transfer, GIFT Comparison: intrauterine insemination						
Outcome	Illustrative comparative risks (number of stillbirths per total births)		Relative Effect (Odds Ratio, 95% CI)	No. of participants (studies)	Certainty of Evidence (GRADE)	Comments
	Non-in vitro	In vitro methods				
Stillbirth	6.33/1000*	7.41/1000 (4.94/1000 – 11.08/1000)	1.17 (0.78-1.75)	28,429 (5)	Very Low ⊕OOO	

Footnotes
* based on average rate of stillbirth in all included studies
Downgraded for imprecision due to low number of events (179 stillbirths).

Missing information

A study by Chughtai et al (2018) reported that perinatal mortality rates following assisted reproductive technology births, but the study description was unclear about what constituted "assisted reproductive technology" i.e. whether ovarian stimulation or intrauterine insemination were included.[42] An email requesting further clarification was sent to corresponding author, Dr. Alex Wang, in January 2020, and again in February 2020. There was no reply.

The study by Pochiraju et al. (2014) reported on an adjusted odds ratio for risk of stillbirth following assisted reproduction (comprising IVF, ICSI, IUI, and OS), and performed subgroup analysis by type of assisted conception.[75] However, although they report that IVF was not associated with stillborn babies, with p=0.92, no raw numbers or odds ratio are reported. Corresponding author Dr. Praveen Nirmalan was contacted by email in May 2020 to request additional data for inclusion in the meta-analysis. There was no reply.

Meta-analysis of in vitro methods vs. all non-in vitro methods

Although 29 studies were included in any quantitative analysis, no single analysis included all of these studies. The main analysis of this review compared in vitro methods (IVF, ICSI) to non-in vitro methods of conception (SC, IUI, OS) using 19 studies, and found an increased odds of stillbirth. Figure 3a presents the forest plot for this analysis. Statistical heterogeneity is low, as demonstrated by I^2=26%, although assessment of the 95% confidence interval ranges from 0 (no clinical heterogeneity) to 58% (moderate heterogeneity). Clinical heterogeneity is likely high given that international clinics over decades of practice will have different ovarian stimulation protocols, culture media, embryo freezing/thawing techniques, and embryo transfer methods. Therefore, a random effects model was decided to be suitable for

meta-analysis. The odds ratio for stillbirth following in vitro conception compared to non-in vitro conception was 1.43 (95% CI 1.23-1.67).

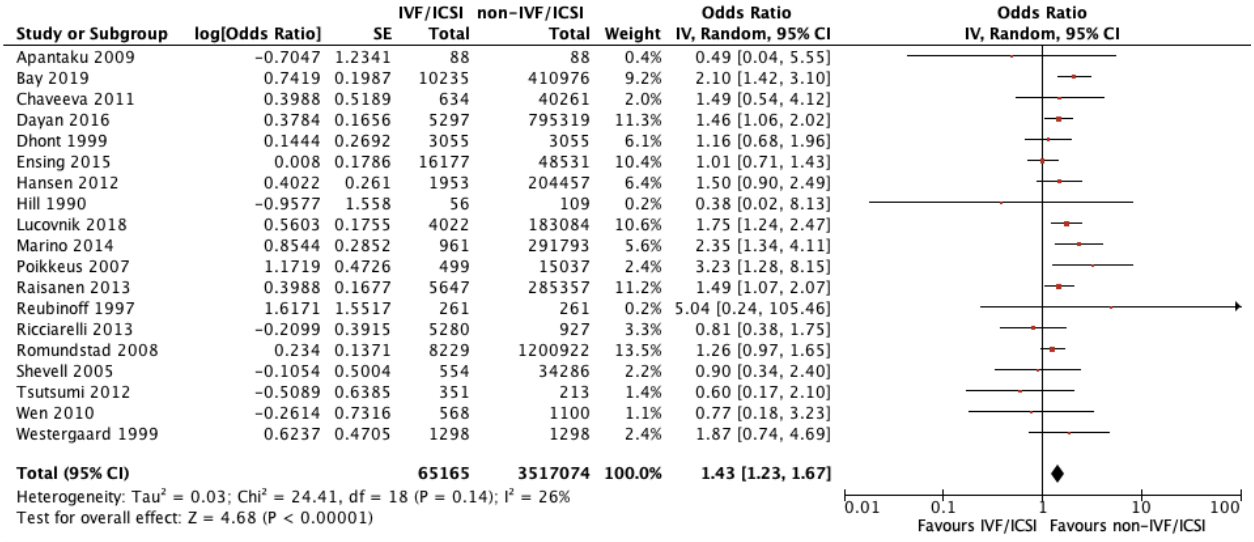

Figure 3a: Stillbirth following in vitro methods vs. all non-in vitro methods, analysis using Nordic countries separately.

Repeating the analysis, substituting all Nordic countries for the largest study that pooled the Nordic countries did not change the direction or significance of effect, with OR 1.29 (1.11-1.50; 16 studies, see Figure 3b).

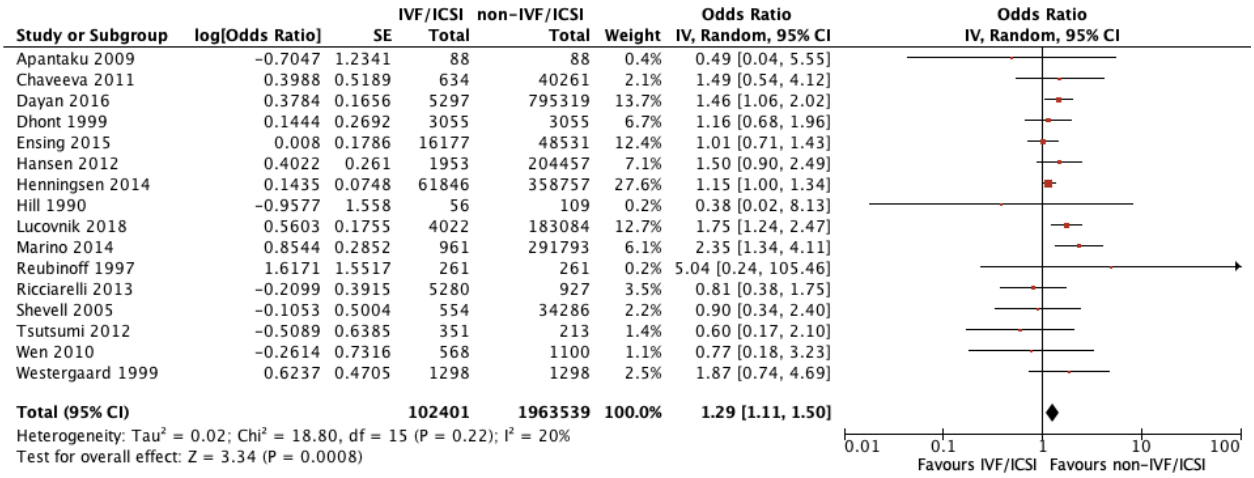

Figure 3b: Stillbirth following in vitro methods vs. all non-in vitro methods, analysis pooling Nordic countries.

Subsequent subgroup analyses did not demonstrate a difference in direction of effect, or the significance of the effect when studies including Nordic country were analysed separately or substituted for the largest study that pooled Nordic data. Therefore, analyses using studies from separate Nordic studies will be presented here.

Subgroup analyses

Table 9 summarizes all subgroup analyses conducted.

Subgroup analyses by type of in vitro or non-in vitro method used

The odds of stillbirth following IVF alone compared with non-in vitro methods was 1.80 (1.12-2.89; 5 studies); see Figure 4. Similarly, the odds of stillbirth following ICSI alone compared to all non-in vitro methods was 1.75 (1.13 – 2.72; 5 studies); see Figure 5. When considering frozen and fresh embryo transfer separately, there was no significant increased odds of stillbirth, with OR 1.26 (0.75-2.10; 4 studies) for FET and OR 1.43 (0.83-2.46; 4 studies) for fresh embryo transfer; see Figures 6 and 7. When comparing in vitro methods to ovarian stimulation with or without IUI, there was again no increased odds of stillbirth (OR 1.17, 95% CI 0.78-1.75; 5 studies), as illustrated in Figure 8. However, Figure 9 demonstrates there was an increased odds of stillbirth when comparing in vitro methods to SC (OR 1.32, 95% CI 1.07-1.63; 13 studies). An illuminating subgroup analysis compared those conceiving with in vitro methods to all those using non-in vitro methods but with a known history of infertility (including spontaneous conceptions with a known history of subfertility, IUI and OS). There was no increased odds of stillbirth (OR 1.15, 95% CI 0.85-1.56; 6 studies); see Figure 10.

Table 9: Subgroup analyses by type of in vitro or non-in vitro method of conception

Exposed Group	Control group	Number of studies	Number of pregnancies exposed group	Number of pregnancies non-exposed group	Odds ratio (95% CI)	Heterogeneity, I^2 (95% CI)	Direction of effect	Certainty of evidence (GRADE)
All in vitro	All non-in vitro	19	65,165	3,517,074	1.43 (1.23-1.67)	26% (0-58%)	↑	Low ⊕⊕OO
IVF	All non-in vitro	5	7,162	707,863	1.80 (1.12-2.89)	27% (0-71%)	↑	Low ⊕⊕OO
ICSI	All non-in vitro	5	10,716	710,881	1.75 (1.13-2.72)	36% (0-76%)	↑	Low ⊕⊕OO
FET only	All non-in vitro	4	4,185	734,939	1.26 (0.75-2.10)	5% (0-86%)	↔	Low ⊕⊕OO
Fresh embryo transfer	All non-in vitro	4	13,995	734,939	1.43 (0.83-2.46)	67% (4-89%)	↔	Very low ⊕OOO
All in vitro	OS, IUI	5	19,594	8835	1.17 (0.78-1.75)	18% (0-83%)	↔	Very low ⊕OOO
All in vitro	SC	13	50,887	2,857,878	1.32 (1.07-1.63)	37% (0-68%)	↑	Low ⊕⊕OO
All in vitro	History of infertility (including OS, IUI, no treatment)	6	19,945	10,939	1.15 (0.85-1.56)	0% (0-74%)	↔	Very low ⊕OOO

CI, confidence interval; *IVF*, in vitro fertilization; *ICSI,* intracytoplasmic sperm injection; *FET,* frozen embryo transfer; *SC*, spontaneous conception; *OS*, ovarian stimulation or superovulation; *IUI*, intrauterine insemination

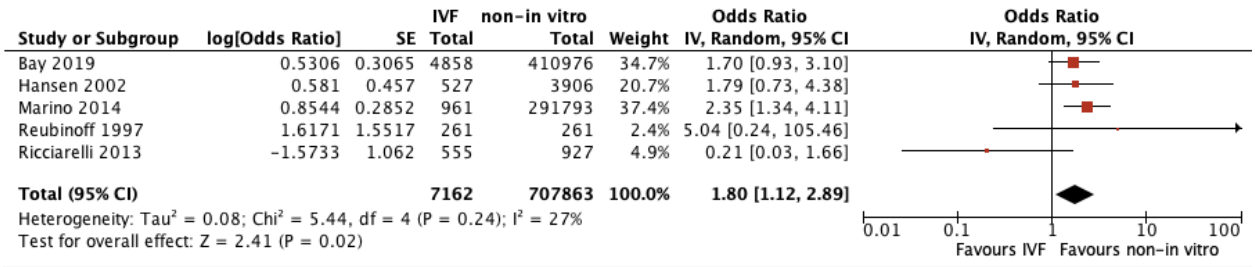

Figure 4: Stillbirth following in vitro fertilization (IVF) only vs. all non-in vitro methods.

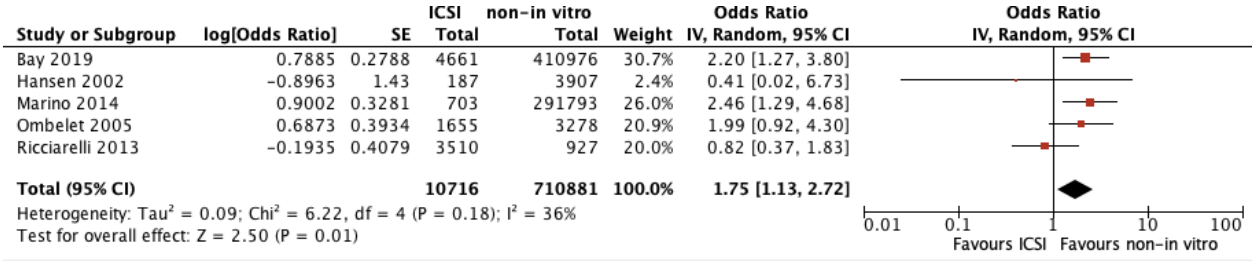

Figure 5: Stillbirth following intracytoplasmic sperm injection (ICSI) vs. all non-in vitro methods.

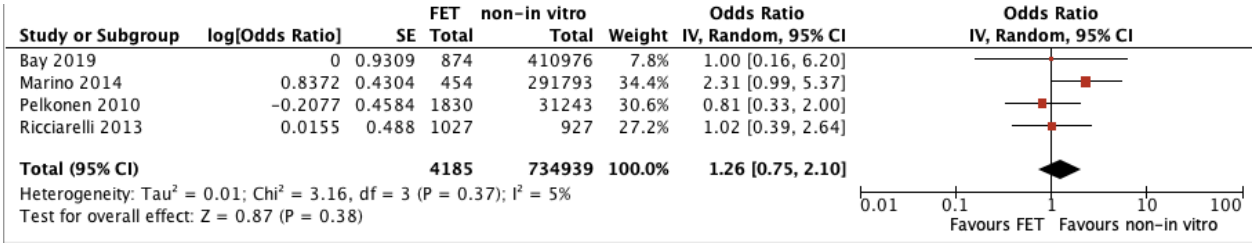

Figure 6: Stillbirth following frozen embryo transfer (FET) vs. all non-in vitro methods.

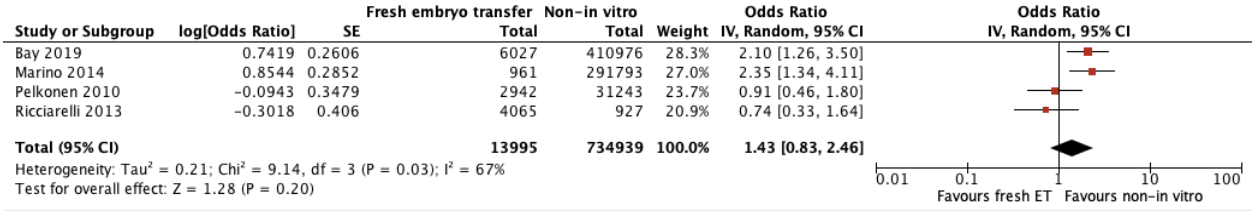

Figure 7: Stillbirth following fresh embryo transfer vs. all non-in vitro methods.

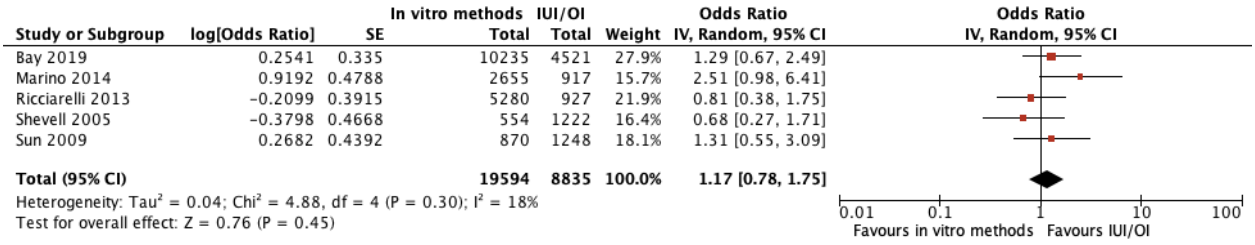

Figure 8: Stillbirth following in vitro methods vs. IUI/OS.

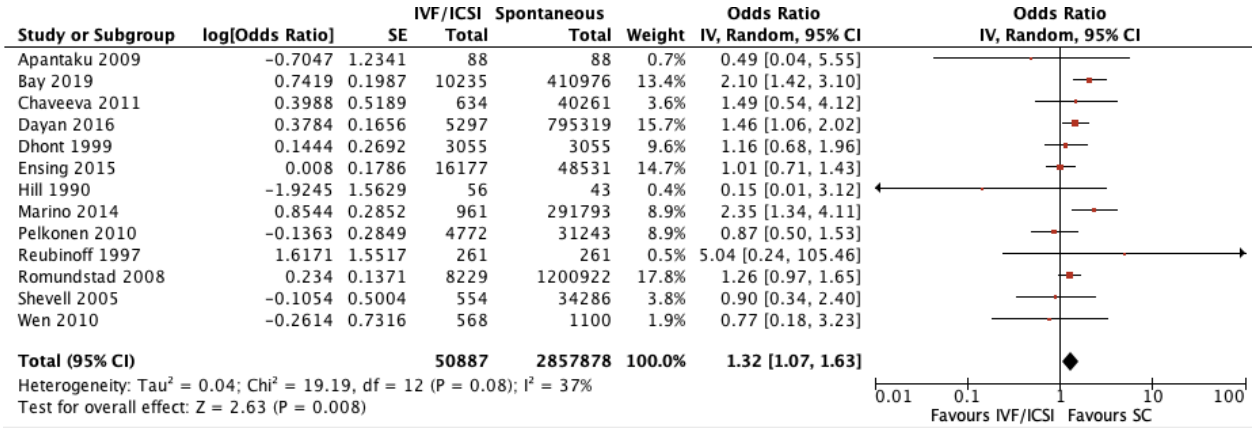

Figure 9: Stillbirth following in vitro methods vs. spontaneous conceptions (SC).

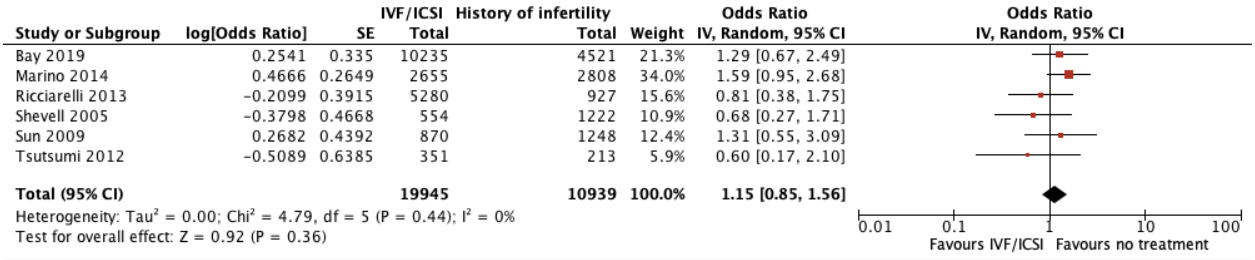

Figure 10: Stillbirths following in vitro methods vs. conceptions in those with a history of infertility (including OS/IUI, no treatment with history of infertility).

Subgroup analyses by gestational age definition of stillbirth

Further subgroup analysis by gestational age definition of stillbirth demonstrated an increased odds of stillbirth for definitions ≥20 weeks or ≥22 weeks (see Figures 11a and 11b). There was no increased odds when using a definition ≥24 weeks; or when restricting analysis to term gestations (Figures 11c and 12, Table 3).

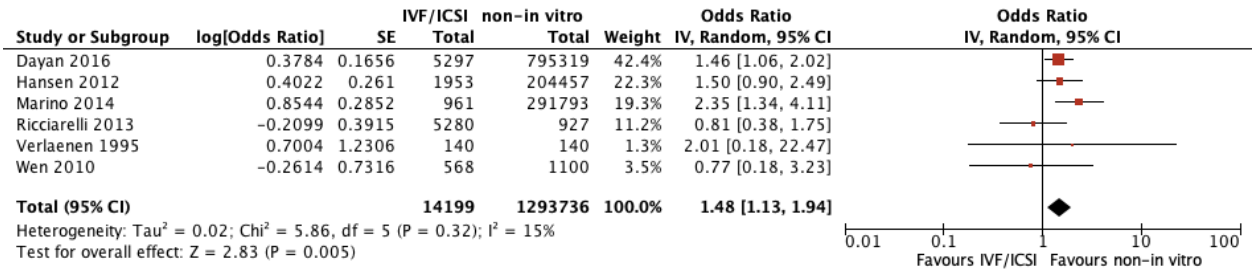

Figure 11a: Stillbirth ≥20 weeks following in vitro methods vs. non-in vitro methods.

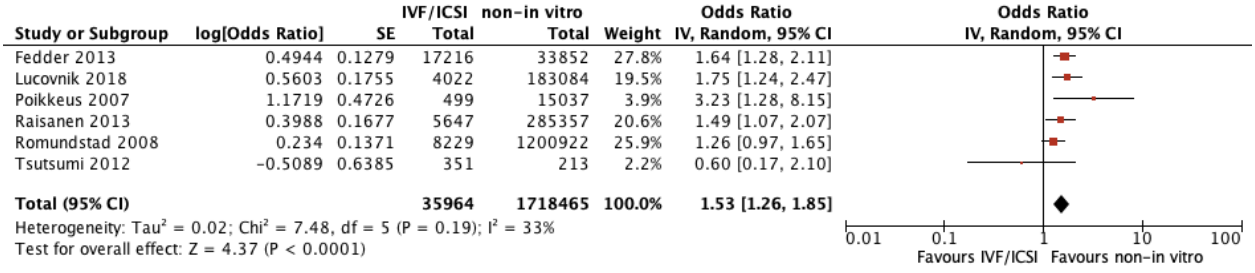

Figure 11b: Stillbirth ≥22 weeks following in vitro methods vs. non-in vitro methods.

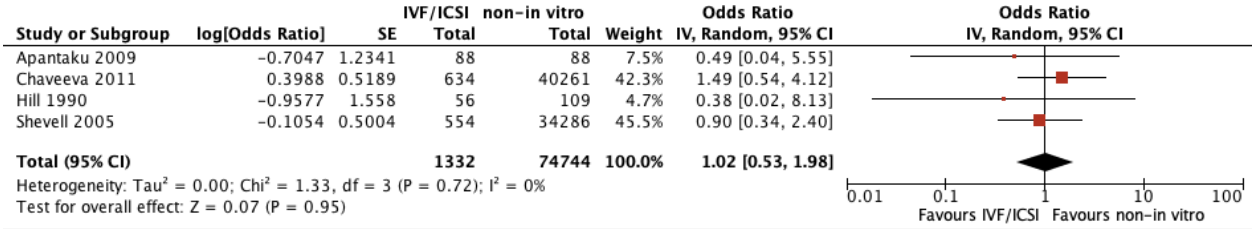

Figure 11c: Stillbirth ≥24 weeks following in vitro methods vs. non-in vitro methods.

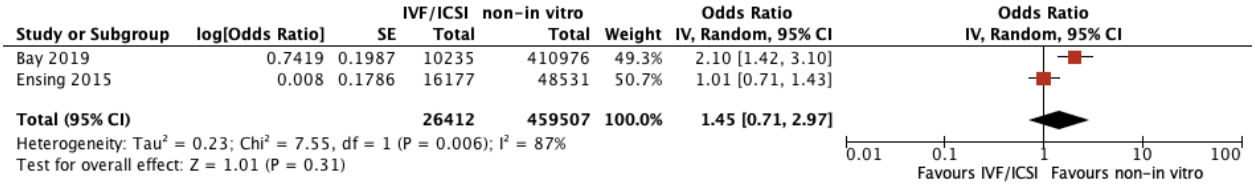

Figure 12: Stillbirth (any definition) at term following in vitro methods vs. non-in vitro methods.

Table 10: Subgroup analyses by gestational age of stillbirth

Gestational age of stillbirth	Number of studies in analysis	Number of pregnancies in in vitro group	Number of pregnancies in non-in vitro group	Heterogeneity, I^2 (95% CI)	Odds ratio (95% CI)	Direction of effect	Certainty of evidence (GRADE)
≥20 weeks	6	14,199	1,293,736	15% (0-79%)	1.48 (1.13-1.94)	↑	Low ⊕⊕OO
≥22 weeks	6	35,964	1,718,465	33% (0-73%)	1.53 (1.26-1.85)	↑	Low ⊕⊕OO
≥24 weeks	4	1332	74,744	0% (0-67%)	1.02 (0.53-1.98)	↔	Very low ⊕OOO
≥37 weeks	2	26,412	459,507	87% (48-97%)	1.45 (0.71-2.97)	↔	Very low ⊕OOO

CI, confidence interval.

Sensitivity analyses

Sensitivity analysis for study quality was completed, restricting meta-analyses to studies that were assessed as being at low risk of bias; and studies with the lowest risk of bias (NOS score 9). When including only the 11 studies that were assessed as being at low risk of bias for the main comparison, the OR for stillbirth remained significant, at 1.47 (1.23-1.76). There were similar results when restricting analyses to the 4 studies at the lowest risk of bias (OR 1.74, 95% CI 1.37-2.21). See Figures 13 and 14. Table 11 summarizes the results of these sensitivity analyses.

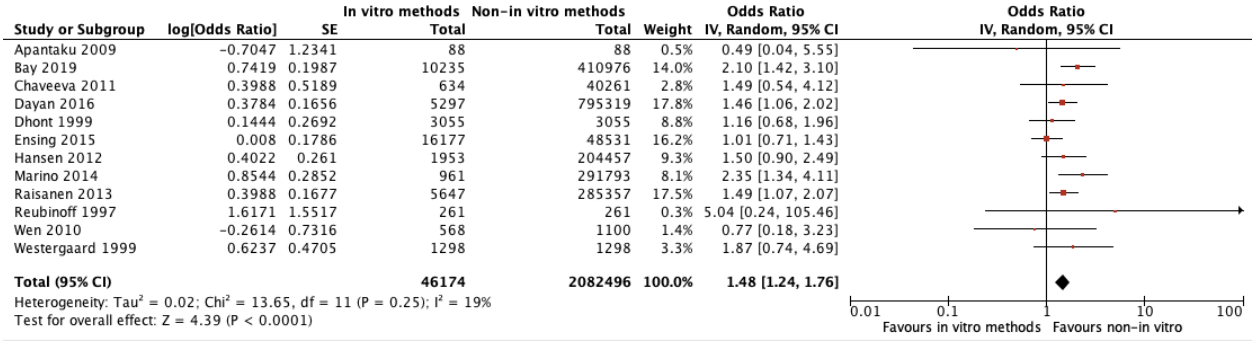

Figure 13: Stillbirth following in vitro methods vs. non-in vitro methods, sensitivity analysis (low risk of bias only).

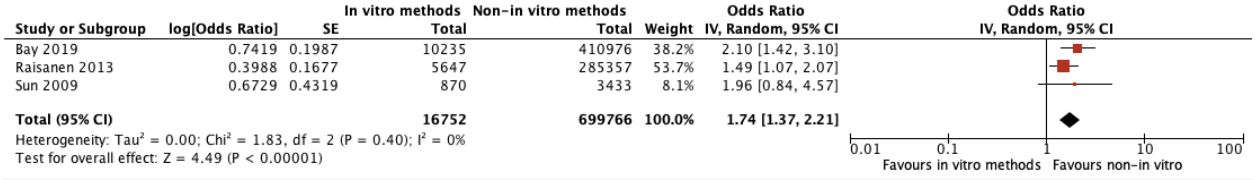

Figure 14: Stillbirth following in vitro methods vs. non-in vitro methods, NOS 9 only.

As described above, when it was likely that studies reported on the same pregnancies (i.e. due to overlapping time period and region/country), the study with the highest number of pregnancies was selected, thereby maximizing the total number of pregnancies in the analysis. When the analysis was repeated maximizing the number of studies (without overlapping

time/region), the results were again similar (OR 1.47, 95% CI 1.27-1.71; 20 studies, Figure 15). Finally, when including only studies that reported an adjusted OR i.e. controlled for at least maternal age, the OR was 1.45 (1.20-1.76; 12 studies); see Figure 16.

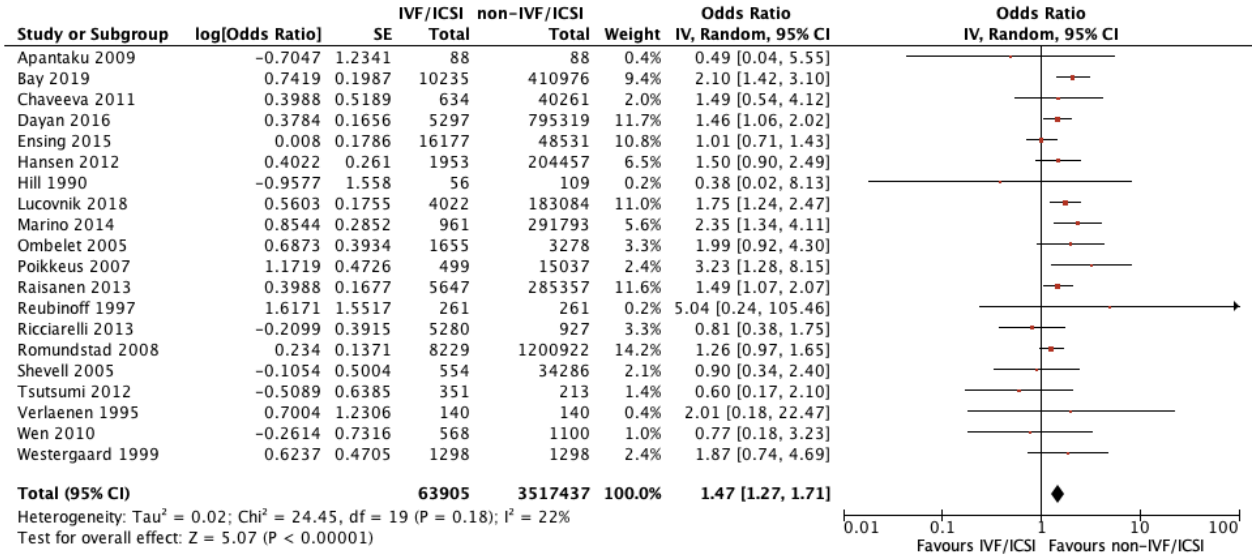

Figure 15: Stillbirth following in vitro methods vs. all non-in vitro methods, maximizing number of studies.

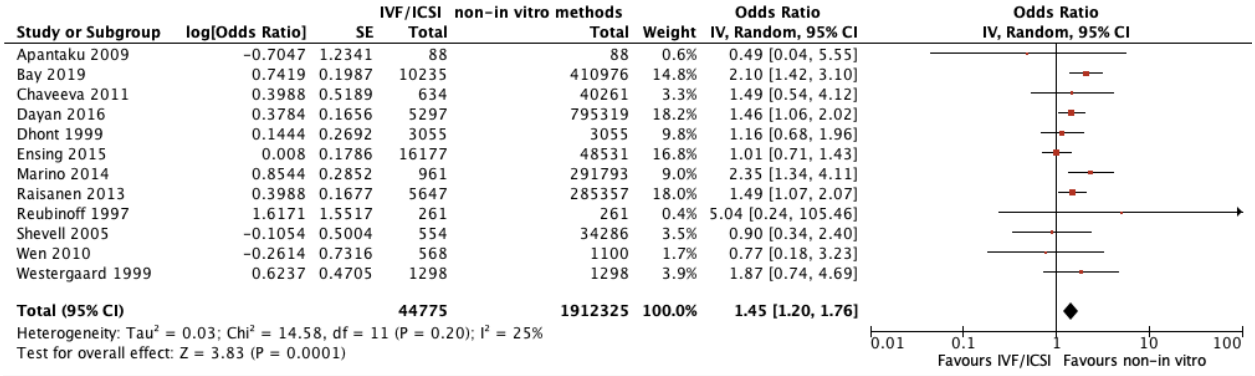

Figure 16: Stillbirth following in vitro methods vs. all non-in vitro methods, in studies controlling for age.

Table 11: Sensitivity analyses by number and quality of studies

Comparison	**Number of studies included**	**Number of pregnancies in exposed group**	**Number of pregnancies in control group**	**Odds ratio (95% confidence interval)**	**Heterogeneity, I^2 (95% CI)**	**Direction of Effect**
Maximal number of pregnancies (main analysis)	19	65165	3517074	1.43 (1.23, 1.67)	26% (0-58%)	↑
Maximal number of studies	20	63905	3517437	1.47 (1.27,1.71)	22% (0-55%)	↑
Studies controlling for age	12	44775	1912325	1.45 (1.20, 1.76)	25% (0-62%)	↑
Low risk of bias only (NOS 7-9)	12	46174	2082496	1.48 (1.24,1.76)	19% (0-58%)	↑
Lowest risk of bias (NOS=9)	3	16752	699766	1.74 (1.37,2.21)	0% (0-92%)	↑

A separate sensitivity analysis for data management was completed for the method of correcting for zero-value cells. The main analysis applied a constant 0.5 correction to all cells when any cell was zero. The sensitivity analysis applied a variable correction factor that was the reciprocal of the opposite group size when any cell was zero. Not all analyses included studies with zero-value cells. The results of this analysis did not differ significantly from the main analysis (see Table 12) in value or direction of effect.

Table 12: Odds ratios by method of handling zero-cells

Comparison	**0.5 Correction Factor**	**Reciprocal of Opposite Group Size Correction Factor**
All in vitro vs. all non-in vitro, main analysis	1.43 (1.23-1.67)	1.44 (1.24-1.66)
All in vitro vs. all non-in vitro, studies controlling for age	1.45 (1.20-1.76)	1.45 (1.20-1.75)
All in vitro vs. all non-in vitro, maximizing number of studies	1.47 (1.27-1.71)	1.47 (1.27-1.70)
IVF vs all non-in vitro	1.80 (1.12-2.89)	1.77 (1.11-2.81)
ICSI vs. all non-in vitro	1.75 (1.13-2.72)	1.84 (1.24-2.730
All in vitro vs. SC	1.32 (1.07-1.63)	1.33 (1.09-1.61)
All in vitro vs. all non-in vitro, stillbirth ≥24 weeks GA	1.02 (0.53-1.98)	1.07 (0.54-2.10)
All in vitro vs. all non-in vitro, Low risk of bias only	1.48 (1.24-1.76)	1.47 (1.24-1.74)

Certainty of evidence

Certainty of evidence was assessed using the GRADE method. No comparison met the criteria to be upgraded.

With regard to stillbirth (19 studies, n=3582239), we found low-certainty evidence that in vitro methods are associated with an increased odds of stillbirth compared to non-in vitro methods (see Table 2, above). Since all identified studies were observational in nature (cohort or

case-control), the baseline risk of bias was low. We were unable to upgrade the certainty of evidence due to the moderate effect size (OR 1.43), and because neither the individual studies nor the analysis could account for all confounders.

When subgrouping by type of in vitro method, the comparison using IVF, ICSI, and frozen embryo transfer (vs. all non-in vitro methods), the certainty of evidence was likewise low. In the comparison of fresh embryo transfer to all non-in vitro methods, the certainty of evidence was very low due to inconsistency, since the statistical heterogeneity as described by the chi-square test was significant ($p=0.03$). The I^2 value did correspond to a significant amount of heterogeneity (67%, 95% CI 4-89%) as well.

When subgrouping by type of non-in vitro method, there was lower certainty of evidence. For the subgroup comparisons in vitro methods compared to a history of infertility; and in vitro methods compared to IUI, the certainty of evidence was very low (see Tables 3-8, above). Certainty of evidence was downgraded for the history of infertility comparison due to imprecision, as less than 400 stillbirths occurred. Similarly, for the comparison of all in vitro methods to intrauterine insemination, certainty of evidence was downgraded for imprecision, since the number of stillbirths could not be definitively determined due to data not reported.

Funnel plots were constructed to assess for publication bias (see Figures 17-20) in meta-analyses that included more than 10 studies. These plots are symmetric, indicating that there is a low risk that small studies or non-significant results were not published.

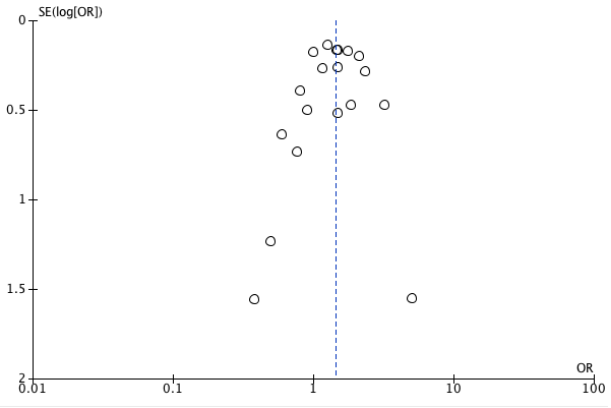

Figure 17: Funnel plot for comparison of all in vitro methods vs. all non-in vitro methods of conception.

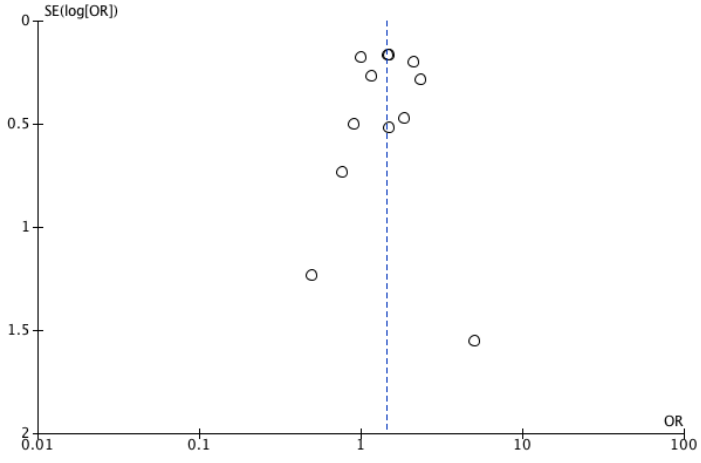

Figure 18: Funnel plot for comparison of all in vitro methods to all non-in vitro methods, studies controlling for age only.

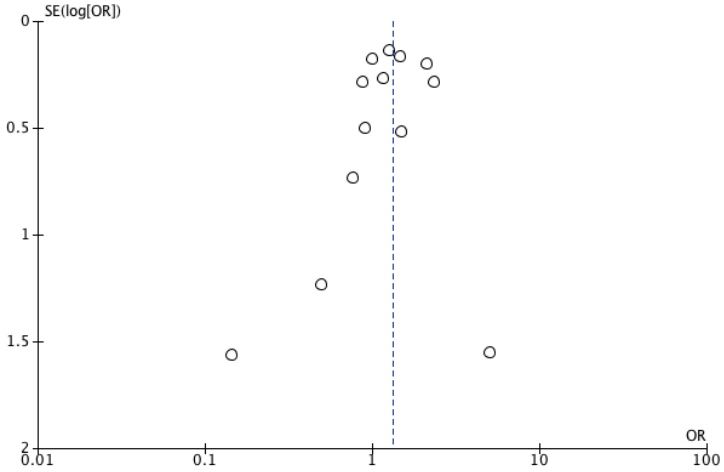

Figure 19: Funnel plot for comparison of all in vitro methods vs. spontaneous conceptions.

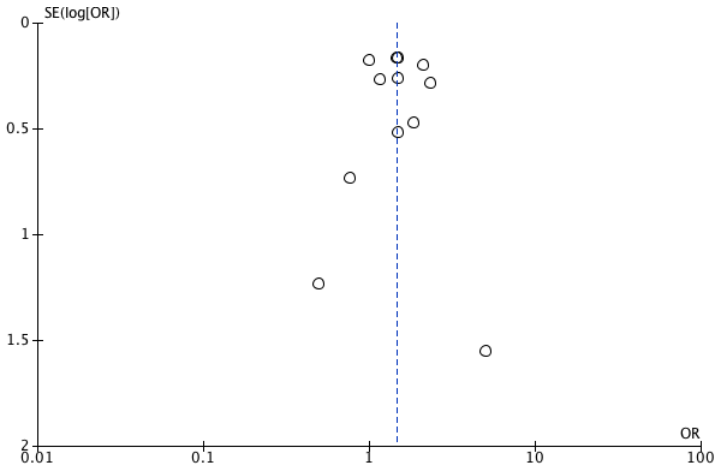

Figure 20: Funnel plot for comparison of all in vitro methods vs. all non-in vitro methods, studies with low risk of bias only.

Chapter 5: Discussion

Summary of main results

This systematic review and meta-analysis showed an increased odds of stillbirth in pregnancies conceived after in vitro methods (including IVF and ICSI, with fresh or frozen ET) compared with pregnancies not conceived using these methods (such as SC, IUI, OS), with an increased OR 1.43 (1.23-1.67). Even after controlling for confounders, the OR remained elevated: 1.63 (1.34-1.97). This increased odds of stillbirth is significant for subgroup analyses restricted to IVF alone compared to all non-in vitro methods, and ICSI alone compared to all non-in vitro methods. This implies that pregnancies conceived with either IVF or ICSI are at increased risk and may therefore warrant increased antenatal monitoring. The fact that the risk persists with both of the main in vitro techniques suggests that the manipulation of sperm and ovum involved in ICSI is not a causative factor.

Subgroup analysis: type of in vitro method

The question of whether technique matters is complicated by our subgroup findings for fresh and frozen embryo transfer. Neither of these analyses demonstrated an increased risk of stillbirth. The certainty of evidence related to fresh embryo transfer is very low quality due to significant heterogeneity in included studies. In addition, there are fewer pregnancies included in this analysis (n=662,715), which may be insufficient to demonstrate a difference in stillbirth risk. Therefore, it is difficult to draw an appropriate conclusion from the existing data.

With regards to frozen embryo transfer, other literature for perinatal outcomes following FET may provide an explanation for why there may be no increased odds of stillbirth. Maheshwari's meta-analysis in 2018 demonstrated that there was decreased risk of small for gestational age, low birth weight, or preterm birth following FET compared with fresh embryo transfer.[44] The

meta-analysis did not find a difference in perinatal mortality between the two methods, but did not assess stillbirth alone as an outcome.[44]

One may have expected to find an increased risk of stillbirth following fresh embryo transfer, consistent with the findings for IVF and ICSI. Many of the IVF/ICSI pregnancies included in the main analysis would likely have been fresh embryo transfers, but there was insufficient information included in database studies to conclude this with enough certainty to include them in the subgroup analysis of fresh embryo transfer. Thus, while the data seems inconsistent, this is likely an artefact of the type of data available.

Subgroup analysis: type of non-in vitro method

When subgrouping by non-in vitro method, the increased odds of stillbirth persisted for the comparison between all in vitro methods and SC, but not for all in vitro methods compared with IUI/OS or a history of infertility. Notably, the number of pregnancies in the analysis fell dramatically for all, except for the analysis of SC. There are two possible explanations for these findings. The first is that, given the lower number of pregnancies, we are unable to discern a change in the odds of stillbirth. The second is that there is in fact no difference in stillbirth risk between in vitro methods and a history of infertility, because the risk is associated with a history of infertility itself. This latter explanation appears to be consistent with the conclusions of other authors who conducted studies to specifically assess risks following ART in comparison to pregnancies in couples with a history of infertility. [47,81,84] Unfortunately it was not within the scope of this thesis to compare IUI/OS pregnancies to SC pregnancies (with or without a history of infertility), which might further contribute to answering this question.

Pandey (2012) speculated that newer studies do not support increased perinatal risks for pregnancies, in contrast to older studies, due to changes in technique and technology.[17] In the

case of this meta-analysis, this may superficially seem to be true: the primary comparison includes studies spanning publication years 1999-2019; while the FET comparison includes studies spanning 2005-2019. One might conclude that the newer techniques used for in vitro methods in recent years may result in a risk of stillbirth more similar to spontaneous conceptions. However, on closer inspection, most comparisons, whether they found an increased odds of stillbirth or not, included Marino 2010, which analyzed births from as early as 1986. The main comparison includes pregnancies from only marginally earlier years (i.e. from 1984 in Romundstad 2008). Thus, improved technology does not satisfactorily explain these findings.

However, the findings from subgroup analyses do suggest interesting hypotheses for why there may be an increased odds of stillbirth associated with in vitro methods of conception.

First, as briefly discussed above, there is the possibility that the condition of infertility itself leads to an increased risk of stillbirth. Previous studies have demonstrated that women with a history of subfertility have poorer pregnancy outcomes.[90–93] Specifically, Axmon and Hagmar (2005) reviewed three previous studies that were able to report on time to pregnancy, and found that an extended time to pregnancy was related to spontaneous abortion and extra-uterine pregnancy.[93] However, there was no difference in time to pregnancy for pregnancies ending in stillbirth compared to live birth.[93] Jaques (2010) and Basso (2003, 2005) both demonstrated worse perinatal outcomes, including: preterm delivery, low birth weight, and perinatal death with extended time to pregnancy.[90–92] Worse perinatal outcomes in general may suggest a common root cause that is itself the result of subfertility, although previous studies have not specifically demonstrated an increased risk of stillbirth. It would appear that both male and female infertility could be contributory, given that subgroup analyses for IVF (conducted for a number of reasons, including female infertility) and ICSI (largely conducted for male factor infertility) respectively

demonstrate an increased odds of stillbirth. Most included studies, however, did not report cause of infertility or reason for treatment.

Other studies have suggested that specific techniques of in vitro methods could account for the poorer perinatal outcomes. For instance, Kallen et al. (2010) suggested that there may be an increase in preterm birth and congenital malformations in embryos transferred at the blastocyst stage compared with the cleavage stage.[94] Dumoulin et al. (2010) found differences in mean birthweight between groups of IVF/ICSI pregnancies that had been cultured in different media.[95] Thus, different perinatal outcomes in different regions and/or times may be accounted for by differences in technique; although this has not been shown for stillbirth specifically.

The phenomenon of vanishing twins may have an effect on perinatal outcome. Vanishing twins refers to the presence of twin pregnancy (i.e. two gestational sacs or detected fetal heart beats) in early pregnancy, with spontaneous reduction of one twin, and a resultant singleton delivery.[53] Pinborg et al. (2005) retrospectively assessed perinatal outcomes in a group of pregnancies with a vanished twin ("survivor group") compared with a group of pregnancies confirmed to be singleton with early ultrasound ("singleton group").[53] They demonstrated that the surviving fetus following a spontaneously reduced (i.e. vanished) twin had worse outcomes than fetuses who began as singleton gestations. Mean birth weight was lower, and the incidence of preterm birth was higher in the survivor group compared to the singleton group. In addition, child death was higher in the survivor group, although stillbirth was not specifically assessed.

Finally, others have speculated that there is a difference in placentation in ART pregnancies[96], which may be the result of the above factors. In a comparison of the pathological examination of singleton placentas following ART compared to SC, Daniel et al. (1999) demonstrated that ART placentas had a higher incidence of abnormal cord insertion, were

thicker, and weighed more compared to the fetus.[96] They speculate that this could predispose to fetal anomalies, and generally lead to or reflect abnormal placentation.[96]

Subgroup analysis: gestational age

When subgrouping by gestational age definition of stillbirth, the elevated risk associated with in vitro methods was only apparent when defining stillbirth as fetal death greater than 20 weeks or greater than 22 weeks. There was no statistically significant difference in stillbirth risk when defining stillbirth as death greater than 24 weeks. There was low certainty of evidence for the first two definitions, and very low certainty of evidence for the latter. This reflects the fewer pregnancies included using the 24 weeks definition. Another possible explanation is that the risk of stillbirth peaks around 20-22 weeks, and decreases after 24 weeks. The comparison restricted to pregnancies at term (>37 weeks) supports this explanation, since no increased risk was found for these pregnancies in this analysis. This is relevant when considering the role of antepartum fetal surveillance, which is only useful later in pregnancy, when delivery would be a reasonable management option.

Interestingly, although it is the definition used by the WHO[24], there was an insufficient number of studies to perform a subgroup analysis for defining stillbirth as >28 weeks. This suggests that most studies exploring this risk used earlier definitions of stillbirth, likely reflecting settings with improved neonatal care capabilities such that fetal viability can occur at a lower gestational age.

Overall completeness and applicability of the evidence

This systematic review was quite sensitive, as studies were included even if they assessed stillbirth as a secondary outcome. However, some studies that have been included in other systematic reviews of perinatal outcomes following in vitro methods of conception were

excluded from this review due to study design. A large emphasis was placed on studies that approximated an ideal randomized control trial as closely as possible, with strict definitions required for exposed and non-exposed groups, and the outcome definition.

An important factor that may limit the applicability of the evidence is the assessment of stillbirth as an outcome. When considering the international evidence, there is clearly the issue of differing gestational age and/or birth weight criteria for reporting of stillbirth. The subgroup analysis by gestational age demonstrated an increased odds of stillbirth when it was defined as ≥20 weeks and ≥22 weeks, but not when it was defined as ≥24 weeks. Therefore, in areas where stillbirth is only reported after 24 weeks gestational age, the results may not be applicable.

Furthermore, countries will variably include medical termination of pregnancy in reports of fetal death.[25] In vitro conceptions may have an increased incidence of medical termination of pregnancy past 20 weeks since they may be associated with congenital malformations.[68] Thus, although they are highly desired pregnancies, couples may choose to medically terminate a pregnancy once a mid-trimester (i.e. at 20 weeks' gestation) reveals anatomic abnormalities. Counting termination of pregnancy after 20 weeks among stillbirths would therefore inflate the rate of stillbirth. Studies included in this meta-analysis did not report whether medical termination of pregnancy was counted among stillbirths.

Certainty of the evidence

This systematic review and meta-analysis did not identify any randomized control trials. The main comparison included cohort studies, which are observational in nature, and therefore the baseline certainty of evidence is low. The effect size was not large for any comparison (all OR <2), and most studies were unable to account for important confounders for stillbirth,

including maternal age and parity. Therefore, the true effect of in vitro methods of conception on stillbirth risk may be different from the estimate reported here.

The effect of heterogeneity

Confidence intervals for I^2 for each analysis are shown in Tables 9-11. These wide confidence intervals demonstrate the uncertainty around the estimate of statistical heterogeneity, which may be due, in part, to the relatively small number of studies included in the analyses. Thorlund et al. (2012) found that at least 11 studies needed to be included in a given meta-analysis to provide a stable confidence interval, and most subgroup analyses described in this thesis included fewer studies. This may affect the grading of certainty of evidence since the I^2 value (as a single point estimate) is part of the assessment of inconsistency. With wide confidence intervals that span no heterogeneity to high heterogeneity (i.e. in the subgroup analyses by type of in vitro or non-in vitro method), this may be further reason to interpret these results with caution.

Potential biases in the review process

A limitation of this study is the difficulty of measuring a rare event. As discussed above, studies assessing the risk of stillbirth require large sample sizes. Evidence regarding this risk is difficult to attain from small studies; and therefore, most of the studies included for meta-analyses were large population-level databases. However, funnel plots for comparisons including ≥10 studies are symmetric, indicating that there were some smaller studies represented.

Large databases also have the issue of not being able to provide data for some important variables. For example, the studies based on regional databases or national registers were largely unable to report on protocol for ovarian stimulation, embryo transfer day, culture medium, or the incidence of vanishing twins. As alluded to in the discussion of fresh and frozen embryo

transfers, many database studies also did not report on fresh or frozen embryo transfer. Although one could assume that cycles not explicitly reported as frozen were fresh, using this assumption in meta-analysis was felt to be inappropriate. Thus, while important information is provided from these large database studies, we are unable to determine the potential impact of certain factors.

Agreements and disagreements with other reviews and studies

Other meta-analyses either do not perform an explicit search for stillbirth as an outcome[16], or report only on perinatal mortality.[14,15,17] However, the increased odds of stillbirth found in our meta-analysis is consistent with the reports of increased perinatal mortality from these other meta-analyses. This suggests that the increase in perinatal mortality reported previously is not simply due to an increase in neonatal death, which might be expected given an increased risk of preterm delivery.[15,18]

As demonstrated in this meta-analysis, some studies do not report an increased risk of stillbirth. Smaller studies may not be able to demonstrate an increased risk of stillbirth due to an insufficient sample size for this rare outcome. As seen in Figure 3a, most studies that report no increased risk of stillbirth include fewer than 100 000 total pregnancies.

Chapter 6: Conclusions

Most literature regarding perinatal outcomes following ART has focused on intrauterine growth restriction, low birth weight, preterm delivery, and perinatal mortality. Few studies have specifically evaluated stillbirth as an outcome, and therefore few studies are adequately powered to comment on the risk of stillbirth following ART. Furthermore, stillbirth can be a difficult outcome to assess due to different gestational age reporting criteria across the globe. This systematic review and meta-analysis pooled together international data from both large and small cohort studies, and demonstrated an increased risk of stillbirth following in vitro methods of conception (including IVF, ICSI, or GIFT with fresh or frozen embryo transfer). Further subgroup analyses by type of in vitro method demonstrated that the risk was apparent for IVF and ICSI considered separately. However, there was no increased risk when comparing in vitro methods to those conceiving after IUI or OS, or with a history of infertility. This latter finding may be due to the lower sample size in those analyses, or one may theorize that a history of infertility itself increases the risk of stillbirth, rather than in vitro procedures.

Implications for practice

The implication of an increased odds of stillbirth following in vitro methods of conception is significant. Like pre-existing and gestational diabetes, gestational hypertension, advanced maternal age, and post-term pregnancy, the higher risk of stillbirth may necessitate increased antepartum fetal surveillance.[29,97] The goal of such surveillance is to identify fetuses at risk of decompensation and demise, and might encompass a combination of fetal heart rate monitoring (i.e. a non-stress test), ultrasounds for growth, ultrasounds for fetal well-being (i.e. the biophysical profile); and ultrasound doppler assessment of blood flow through the umbilical artery. As an example, and extrapolating from what is done for gestational diabetes, ultrasounds

for growth might begin at 28 weeks, occurring every 2-4 weeks; biophysical profile with non-stress test would begin at 32 weeks, occurring weekly.[28] In high-risk conditions, induction of labour at term has also been recommended[28,30], since the risk of continuing pregnancy is deemed higher than the risk of delivery.

The potential benefit of fetal surveillance would be the ability to identify fetuses who have or will shortly decompensate, and to deliver them before their demise. On the other hand, a risk of this approach is premature delivery, which can lead to such neonatal complications as respiratory distress syndrome, intraventricular hemorrhage, necrotizing enterocolitis, and cerebral palsy.[98] These risks are increased at earlier gestations, with decreased risk close to or at term. Therefore, the ultimate decision to deliver a fetus based on antepartum surveillance would include a careful weighing of the risks of prematurity against the potential for in utero demise.

Fertility specialists may choose to discuss an increased risk of stillbirth following in vitro conceptions with patient seeking these procedures. It would be important to highlight the uncertainty regarding whether the increased risk is secondary to the procedure or the condition of infertility itself. There are many reasons a couple may seek an in vitro method of conception, and this knowledge is relevant if the couple is a in a position where both non-in vitro (e.g. OS, IUI) and in vitro methods (e.g. IVF, ICSI) are available to them. Arguably, for most couples seeking in vitro methods of conception secondary to severe and/or unexplained infertility, the alternative to an in vitro method of conception is *no conception*. However, full informed consent would certainly include this discussion.

Implications for research

If induction of labour were to be considered for in vitro conceptions, more evidence would be required demonstrating the risk of stillbirth by gestational age, since induction would be useful

only for elevated stillbirth risk at or near term. The results of Bay et al. (2019) suggests that the risk may persist at term[43], although the results of the present meta-analysis do not. Further large studies from different regions may help clarify the risk.

This study was unable to demonstrate an increased risk when in vitro methods of conception were compared to those conceiving with non-in vitro methods, but with a history of infertility. It is unclear if there is actually no difference in risk between these groups, or if there were too few pregnancies in the history of infertility group to detect a difference.

Additional research comparing these groups would help to clarify this question. It may be difficult to identify those with a history of infertility but conceiving without in vitro methods using database studies. A dedicated review of patient history would be required, or databases would need to be rigorous in their classification of infertility compared to infertility treatment, and the type of treatment. Those with subfertility might be defined as those with a time to conception greater than one year, or conceiving with OS or IUI. One would need to specify that IUI pregnancies not include same-sex couples in order to capture the target population. This "sub-fertile" group could then be compared to the in vitro methods group. Alternatively, a future systematic review could compare those conceiving with IUI/OS only to those conceiving spontaneously using dedicated search terms and excluding IVF/ICSI in order to test the hypothesis that those with a history of infertility (i.e. requiring IUI/OS) are also at higher risk.

Prospective studies could include an assessment of risk of stillbirth based on cause of infertility. If it were indeed the case that the condition of infertility increases the risk of stillbirth, it would also be relevant to determine if certain types of infertility were particularly implicated.

Other research might control for the role of vanishing twins. Vanishing twins are more commonly diagnosed after ART, but this may be due to surveillance bias, that is, ART

pregnancies undergo more frequent ultrasounds in the first trimester. Thus, future research might be able to compare pregnancies known to be singleton based on first trimester ultrasound and conceived spontaneously, to pregnancies confirmed to be singleton on first trimester ultrasound following in vitro methods. It is now recommended in Canada that first trimester ultrasound be used to accurately date a pregnancy[99]; this may lead to more early ultrasounds on which to base this type of research.

This systematic review and meta-analysis demonstrated an increased odds of stillbirth following in vitro methods of conception (such as IVF and ICSI, including fresh or frozen ET) compared with spontaneous conceptions, with a low certainty of evidence. This is the case when only including data that controlled for other risk factors for stillbirth as well, suggesting that the effect is independent of other obstetrical complications. The increased odds of stillbirth may be explained by the condition of subfertility itself, as suggested by our subgroup analysis that demonstrated no difference in those conceived by in vitro methods compared with those with a history of subfertility. However, the technology itself or specific procedures utilized (e.g. number of embryos transferred) may also play a roll. Whatever the etiology of the increased risk, if in vitro methods of conception independently increase the odds of stillbirth, this likely warrants increased antepartum surveillance.

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Appendix 1: Protocol

Stillbirth following in-vitro fertilization or intracytoplasmic sperm injection: a systematic review and meta-analysis

Timeframe: start date June 1, 2019

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Introduction

Rationale

In vitro methods of conception, including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), can be expected to become more common with advances in assisted reproductive technologies, public funding for these methods, and a trend toward delayed childbearing.

Of methods for assisted reproduction, IVF and ICSI are considered more "invasive", in that oocytes are extracted following medically-induced supraphysiologic ovulation and combined with sperm outside the body. The resulting zygote is then transferred into the uterus. This may occur in the same menstrual cycle, considered a "fresh" transfer, or a subsequent cycle after vitrification of the embryo ("frozen" transfer). Embryos may be frozen or transferred 3 or 5 days following fertilization (1). This is in contrast to other methods of assisted reproduction, such as ovulation induction or intrauterine insemination, in which there is no oocyte extraction and fertilization occurs *in vivo*, but which may or may not include supraphysiologic ovulation.

Some studies have suggested that there may be an increased risk of stillbirth associated with in vitro methods (2,3,4,5). On the other hand, meta-analyses examining stillbirth as a secondary outcome have been unable to identify an increased risk (6,7). This apparent discordance is likely explained by stillbirth not being analysed as the primary outcome. These meta-analyses did not include many studies that examined stillbirth, or that examined stillbirth independent of neonatal mortality (i.e. reported on overall perinatal mortality only). To date, there have been no systematic reviews or meta-analyses that have analysed stillbirth as the primary outcome.

Analyses of stillbirth is further complicated by inconsistencies in defining stillbirth across the globe. Even within Canada, the definition of stillbirth may vary by province. In general, the Canadian definition is consistent with the American one, and birth at ≥20 weeks gestational age or with a birthweight ≥500 g with no signs of life (8). In marked contrast, the World Health Organization definition is birth at ≥ 28 weeks gestational age with no signs of life (9).

However stillbirth is defined, when pregnancies are thought to be at an increased risk of stillbirth, obstetrical care providers offer increased antenatal surveillance and induction of labour (10). Increased antenatal surveillance includes methods such as biweekly ultrasounds for fetal well-being and growth, fetal heart rate assessment (i.e. non-stress test), and/or assessment of amniotic fluid volume (11). Induction of labour is the process of initiating uterine contractions in

a women who is otherwise not in labour in order to help her achieve vaginal birth. Due to the uncertainty regarding stillbirth risk, there are no current guidelines regarding increased fetal monitoring for pregnancies resulting from in vitro methods of conception. Such preventative strategies would be recommended should rigorous evidence arise of an increased risk of stillbirth following in vitro methods of conception.

Objectives

Research Question

In singleton pregnancies, are in vitro methods of conception (i.e. in vitro fertilization, intracytoplasmic sperm injection) associated with an increased risk of stillbirth compared with non-in vitro methods (i.e. spontaneous conceptions, intrauterine insemination, ovulation induction, ovarian hyperstimulation)?

The objectives of the study are:

1) To determine if an increased risk of stillbirth exists because this may warrant increased antenatal surveillance
2) To estimate when this risk might be greatest, and
3) To explore the possible etiology of any increased risk (i.e. whether it is secondary to the procedure or the condition of infertility).

Methods/Data Collection and Analysis Plan

Eligibility criteria

Studies will be eligible if they are randomized control trials or observational studies (both cohort and case-control studies) comparing the outcome of stillbirth in those who used any in vitro methods to achieve pregnancy, compared with those not using in vitro methods. Non-in vitro methods include: spontaneous conception, intrauterine insemination (IUI), ovulation induction with oral or injectable medications, or fertility awareness methods.

Cohort and case-control studies will be selected, as it would be both unethical and unfeasible to conduct a randomized control trial for in vitro methods compared with non-in vitro methods of conception. Although cohort studies provide superior evidence of causality compared with case-control studies, both types of studies will be included to increase the sensitivity of the review. Data will be analyzed separately for cohort and case-control studies.

With regard to the population under analysis, only singleton pregnancies will be included. Multiple pregnancy (i.e. twin and higher order gestations) are known to be at an increased risk of most obstetrical outcomes, including stillbirth. It is not expected that randomized control trials will be found. However, if they are found, they will be included in a narrative review.

The outcome of stillbirth must be included in eligible studies, but need not be the primary outcome. It must be possible to differentiate stillbirth from perinatal mortality (which includes both antepartum and postpartum demise). The definition of stillbirth should be provided, with regards to gestational age and/or birth weight criteria, as definitions vary globally.

With regards to study groups, patients with certain non-in vitro methods (i.e. ovulation induction, intrauterine insemination) may be included together with in vitro methods, provided enough detail is provided to isolate the data from the patients with in vitro methods. If it is not possible to distinguish patients who conceived by in vitro methods from those using intrauterine insemination or ovulation induction, that study would not meet inclusion criteria.
Studies in all languages will be included. Studies from inception of database to present will be included. Only studies with published data (or in press) will be included, to provide data for analysis. See Appendix 4 for Data Extraction From Full Text Studies Form.

Information sources

PubMed, EMBASE, CINAHL, and Cochrane Library databases will be searched using the search terms in Appendix 3 from database inception to present. References of included studies will be reviewed for additional relevant studies. National guidelines from Canada, the United States, and the United Kingdom were reviewed for relevant references as well. No language restrictions will be applied. Searches will not be filtered. See Appendix 3 for full search queries.

Study selection

After duplicates are removed, two reviewers (KW and KC) will independently review all studies obtained from the search strategy for potential eligibility. See Appendix 1 for the reviewer guide for title and abstract screening. Discrepancies will be resolved by consensus following discussion, or where this does not resolve conflict, by a third reviewer (JC). The full text of these studies will be retrieved and independently reviewed by KW and KC for eligibility criteria. Again, discrepancy will be resolved by consensus following discussion, or if conflict

remains unresolved, by JC. All eligible studies will be included in the systematic review, with separate analyses for case-control and cohort studies.

Data extraction and management

Data will be extracted by KW and KC after full text review confirms study eligibility. A data extraction form will be used (see Appendix 4).

Clinical variables that are possible confounding factors include:

- Population characteristics: age, parity, smoking, previous stillbirth
- Obstetrical risk factors present: pre-existing medical conditions, hypertensive disorders of pregnancy, gestational diabetes, intrahepatic cholestasis of pregnancy, fetal reduction in current pregnancy
- Type of in vitro method used: IVF or ICSI
- Type of embryo transfer: fresh, frozen
- Timing of embryo transfer: day 3 post fertilization, day 5 post fertilization
- Non-in vitro method used: spontaneous conception, intrauterine insemination, ovulation induction, fertility awareness
- Confounding outcomes: congenital anomalies, preterm delivery
- Definition of stillbirth: gestational age and/or birthweight criteria
- Population rate of stillbirth for region of study

Where multiple effect estimates are presented in studies, for example due to multiple analyses from models using different confounders, the estimate from the model using the largest number of confounders considered important (see above list) by the review authors will be used.

Authors will be contacted for missing data. Where data is missing with regard to risk of stillbirth or relative risk of stillbirth, and authors are unavailable after contact, the study will not be included in data synthesis.

Assessment of risk of bias in included studies

Two review authors (KW and KC) will independently assess risk of bias in included studies using the Newcastle Ottawa Quality Assessment Scale adapted for this study (Appendix 5), which has been recommended as a useful tool for assessing non-randomized studies in the

Cochrane Handbook (12). This scale judges case-control and cohort studies across three domains: selection of study groups, comparability of groups, and ascertainment of exposure (case-control) or outcome (cohort). The Cochrane Handbook recommends that the basic scale be modified for the review question (12).

Specifically, Comparability criteria in both the case-control and cohort versions of the scale require review authors to specify the most important confounding factors for which the study should control. The authors of this review have decided by consensus discussion that the most important confounding factor is having a pre-existing risk factor for stillbirth. To this end, Table 5: Obstetrical Risk Factors Controlled for in Eligible Studies will be used to assess Comparability criterion 1a (study controls for obstetrical risk factors for stillbirth). Studies controlling for ≥50% of listed risk factors will be deemed to satisfy that criterion. The second most important confounding factor was decided to be maternal age. Advanced maternal age is both a risk factor for stillbirth, and a common reason that women may seek assisted reproduction techniques, and therefore this is likely to be a common confounder. The cohort version of the Newcastle Ottawa scale includes Outcome criteria, including adequacy of follow-up. A follow-up rate of 85% was decided to be adequate through consensus discussion.

There are no accepted ranges for scores that constitute low, moderate, or high risk of bias using the Newcastle Ottawa Scale. For the purposes of this review, the authors have decided that a score of 7-9 would constitute a low risk of bias, 4-6 a moderate risk of bias, and 0-3 a high risk of bias.

Summary measures of effect

The summary measure of effect from cohort studies will be a risk ratio (RR), with 95% confidence interval.

The summary measure of effect from case-control studies will be an odds ratio (OR), with 95% confidence interval.

Unit of analysis issues

The unit of analysis in the review will be individual pregnancies. The risk of stillbirth may be related, however, to the individual woman (who may have multiple pregnancies over the course of the study) rather than the individual pregnancy circumstances. This will be partially accounted for in studies controlling for history of previous stillbirth.

A further unit of analysis issue could be studies with more than two arms e.g. IVF vs. ICSI vs. spontaneous conception. In this case, data from the two in vitro methods arms will be pooled to avoid double-counting.

Assessment of reporting bias

Where there are ≥ 10 studies in the meta-analysis, reporting bias (publication bias) will be assessed by constructing a funnel plot. The funnel plot will be visually assessed for asymmetry, and therefore reporting bias.

Synthesis of results

Statistical analysis will be carried out using Review Manager software (RevMan 5). Separate analyses will be completed for case-control studies, and cohort studies. Due to varying definitions of stillbirth and clinical practice across the globe, it is expected that there will be sufficient clinical heterogeneity to cause underlying risk differences between studies, and therefore a random effects model is planned for meta-analysis. Reviewers (KW and KC) will independently assess clinical heterogeneity and decide whether data is suitable to be pooled statistically. Statistical heterogeneity will be assessed using the I^2 value calculated through RevMan software.

Should there be sufficient data, planned additional analyses include subgroup analyses by type of in vitro method, type of non-in vitro method, and gestational age at stillbirth. The first subgroup analysis will include IVF compared with non-in vitro methods, and ICSI compared with non-in vitro methods. The second analysis will include in vitro methods compared with IUI; in vitro methods compared with spontaneous conception; and in vitro methods compared with a history of infertility but conceiving spontaneously.

Lastly, in vitro methods will be compared to non in vitro methods, with separate hazard ratios by gestational age. The gestational age subgroups would include:

- 20-21+6 weeks (lowest gestational age at which stillbirth defined)
- 22 – 23+6 (peri-viability)
- 24-27+6 (highest gestational age at which stillbirth defined)
- 28 – 33+6 (early preterm)
- 34 – 36+6 (late preterm)

- 37 – 40+0 (term)
- 40+1 – 42+0 (post-term).

Sensitivity analysis will be conducted to explore the effect of study quality. Only studies at low risk of bias (defined by review authors as Newcastle Ottawa Scale ≥7) will be analysed, and the result compared with the standard analysis.

Overall quality of evidence (GRADE)

The quality of evidence will be assessed using the GRADE (Grading of Recommendations, Assessment, Development, and Evaluation) approach.

Since this review examines only observational study, the baseline grade of evidence is low (13).

Quality will be upgraded if the effect size is large or if there are no other important sources of bias contributing to the effect estimate. Quality will be further downgraded on the following domains if:

- Methodological quality: ≥25% of pregnancies are from studies rated as having a high risk of bias (Newcastle Ottawa Scale 0-3).
- Inconsistency of results: ≥25% of studies had treatment effects in a different direction, I^2 ≥75% (considerable heterogeneity), or unable to draw a straight line through the Forest plot
- Indirectness of evidence: more than 50% of patients were outside of the target group
- Imprecision of evidence: Fewer than 400 total pregnancies were included in the comparison

Quality of evidence for the outcome of stillbirth will be reduced by one level for each domain, according to the rules above. Quality of evidence will be described as:

- High-quality evidence: the effect size is very large (RR ≥5), or the effect size is large (RR ≥2) and all plausible confounders would have biased the evidence in the opposite direction. We are very confident that the true effect lies close to that of effect size estimate
- Moderate-quality evidence: the effect size is large (RR ≥ 2), or all plausible confounders would have biased the evidence in the opposite direction as the effect observed. We are

moderately confident in the effect estimate. The true effect is likely to be close to the estimate of effect, but there is a possibility that it is substantially different.

- Low-quality evidence: the effect size is not large (RR <2) and plausible confounders are consistent with observed effect. Our confidence in the effect is limited. The true effect may be substantially different from the estimate of the effect.
- Very low quality evidence: one of the domains is not met. We have very little confidence in the effect estimate. The true effect is likely to be substantially different from the observed estimate of effect.
- No evidence: no case-control or cohort studies identified that address the stillbirth outcome

Discussion

Strengths and Limitations

One strength of this protocol is its sensitivity. Both case-control and cohort studies are included to identify the highest level of evidence for review. It is more likely that case-control studies have been completed to assess the risk of stillbirth given that it is rare. Furthermore, there will be explicit assessment of important confounders, such as previous stillbirth, and obstetrical risk factors for stillbirth. Subgroup analysis will also attempt to delineate the etiology of any increased risk by assessing risk in those with a history of infertility but conceiving without in vitro methods. Subgroup analysis by gestational age will provide information about when additional clinical interventions might be indicated, such as increased antenatal surveillance or induction of labour.

A limitation of this study is the inability to include randomized trials. Patients undergoing in vitro methods of conception are significantly different from patients conceiving without these methods in that they are likely older, and have anatomic or medical reasons for infertility. These are all factors that also impact the risk of stillbirth. A priori subgroup analyses attempt to account for this, but there is likely to be residual confounding due to unknown factors impacting both fertility and risk of stillbirth. Another limitation of this study resulting from the use of observational study, is the use of the Newcastle Ottawa Scale. Although this is a widely used and recommended scale for assessing risk of bias in observational studies, it is less well-established than risk of bias tools used for randomized trials. Furthermore, the authors have not established scores on the scale that correlate with “high” or “low” risk of bias.

Dissemination

The complete systematic review will be submitted to national journals of obstetrics and gynecology, such as the Journal of Obstetrics and Gynecology Canada (JOGC), Obstetrics & Gynecology ("Green Journal"), the American Journal of Obstetrics & Gynecology ("the Gray Journal"), and BJOG: An International Journal of Obstetrics and Gynaecology.

The abstract will also be submitted for presentation at national conferences, such as the Annual Clinical and Scientific Conference of the Society of Obstetricians and Gynaecologists of Canada, as well as local research day presentations, such as the Discipline of Obstetrics and Gynecology Annual Research Day at Memorial University of Newfoundland.

Key Words

"Stillbirth", "Intrauterine fetal demise"

"In vitro fertilization", "IVF"

"Intracytoplasmic sperm injection", "ICSI"

"Assisted reproductive technologies", "ART", "assisted reproduction"

Appendix 2: Search Strategy

Question: In singleton pregnancies, are in vitro methods of conception (i.e. in vitro fertilization, intracytoplasmic sperm injection) associated with an increased risk of stillbirth compared with non-in vitro methods (i.e. spontaneous conceptions, intrauterine insemination, ovulation induction, ovarian hyperstimulation)?

MEDLINE/PubMed

("in vitro fertilization"[All Fields] OR "in vitro fertilisation"[All Fields] OR "ivf"[All Fields] OR "intracytoplasmic sperm injection"[All Fields] OR "ICSI"[All Fields] OR "PROST"[All Fields] OR "pronuclear stage transfer"[All Fields] OR "ovum donation"[All Fields] OR "zygote intrafallopian transfer"[All Fields] OR "gamete intrafallopian transfer"[All Fields] OR "blastocyst transfer"[All Fields])
OR
(("Reproductive Techniques, Assisted"[Mesh:noexp] OR "Donor Conception"[Mesh]) OR "Embryo Transfer"[Mesh]) OR "Fertilization in Vitro"[Mesh]) OR "Gamete Intrafallopian Transfer"[Mesh]) OR "Oocyte Donation"[Mesh]) OR "Zygote Intrafallopian Transfer"[Mesh]))
AND
(("Stillbirth"[Mesh] OR "Fetal Death"[Mesh:noexp])
OR ("stillborn"[All Fields]) OR "stillbirth"[All Fields]) OR "antepartum fetal demise"[All Fields]) OR "antepartum fetal death"[All Fields]) OR "intrauterine fetal death"[All Fields]) OR "intrauterine fetal demise"[All Fields]) OR "fetal death"[All Fields]) OR "fetal demise"[All Fields])

EMBASE

('in vitro fertilization'/de OR 'embryo transfer'/exp OR 'intracytoplasmic sperm injection'/exp OR 'gamete intrafallopian transfer'/exp OR 'oocyte donation'/exp OR 'zygote intrafallopian transfer'/exp OR 'infertility therapy'/de)
OR
('in vitro fertilization':ab,ti OR 'in vitro fertilisation':ab,ti OR 'ivf':ab,ti OR 'intracytoplasmic sperm injection':ab,ti OR 'icsi':ab,ti OR 'zygote intrafallopian transfer':ab,ti OR 'zift':ab,ti OR 'gamete intrafallopian transfer':ab,ti OR 'gift':ab,ti OR 'oocyte donation':ab,ti OR 'embryo transfer':ab,ti OR 'infertility therapy':ab,ti)
AND
('stillbirth'/exp OR 'fetus death'/de)
OR
('stillbirth':ab,ti OR 'stillborn':ab,ti OR 'antepartum fetal demise':ab,ti OR 'antepartum fetal death':ab,ti OR 'intrauterine fetal demise':ab,ti OR 'intrauterine fetal death':ab,ti OR 'fetus death':ab,ti OR 'fetal death':ab,ti OR 'fetal demise':ab,ti)

CINAHL

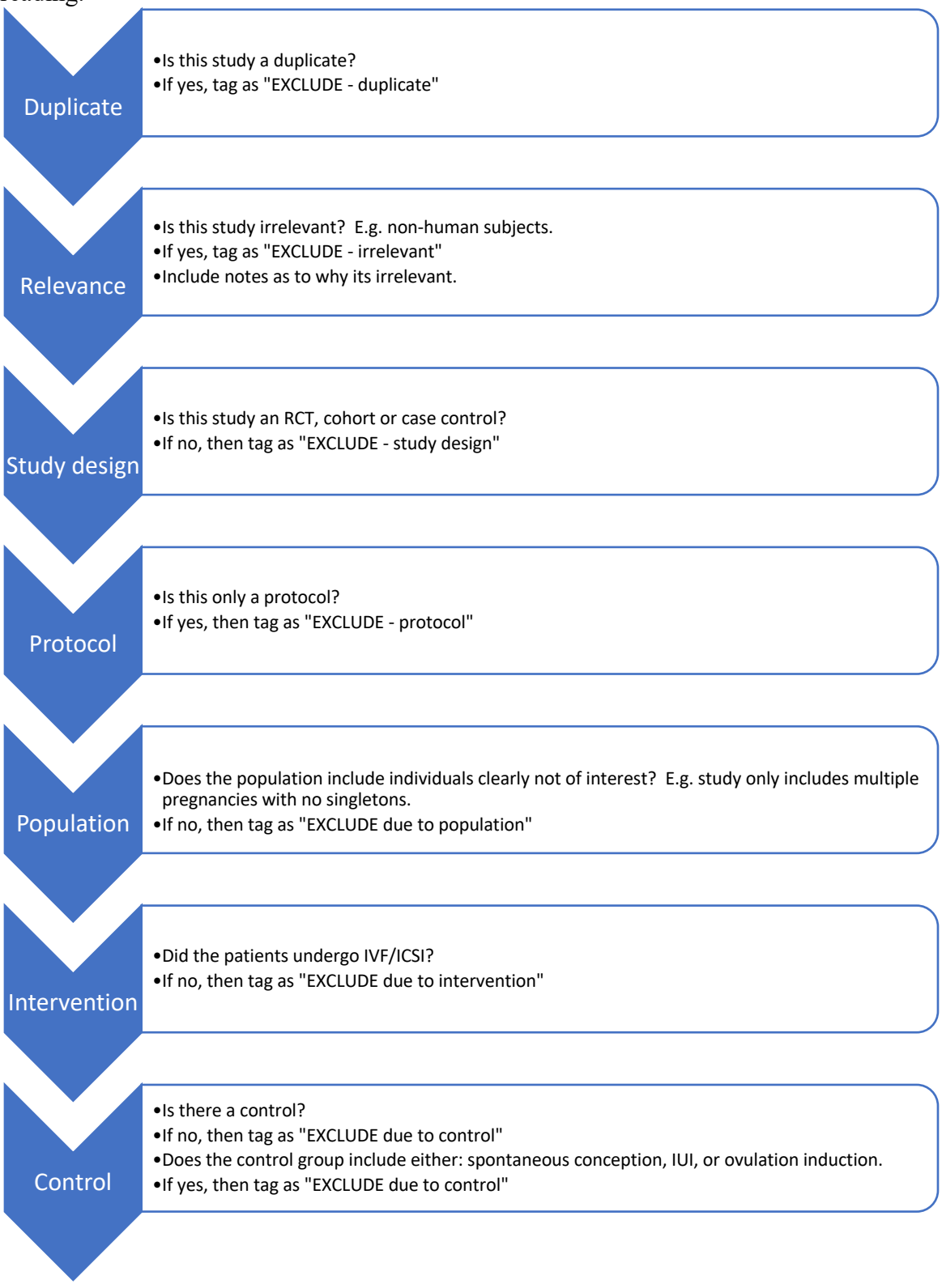
((MH "Fertilization in Vitro") OR (MH "Gamete Intrafallopian Transfer") OR (MH "Oocyte Donation") OR (MH "Embryo Transfer") OR (MH "Sperm Donation") OR (MH "Reproduction Techniques"))
OR
("in vitro fertilization" OR "in vitro fertilisation" OR "intracytoplasmic sperm injection" OR "IVF" or "ICSI" OR "gamete intrafallopian transfer" or "GIFT" OR "zygote intrafallopian transfer" or "ZIFT" OR "PROST" OR "pronuclear stage tubal transfer" or "oocyte donation" or "sperm donation"))
AND
((MH "Perinatal Death"))
OR
("stillbirth" OR "stillborn" OR "antenatal fetal demise" or "antenatal fetal death" or "intrapartum fetal demise" or "intrapartum fetal death")

Cochrane Library

MeSH descriptor: [Reproductive Techniques, Assisted] this term only
OR
MeSH descriptor: [Donor Conception] explode all trees
OR
MeSH descriptor: [Embryo Transfer] explode all trees
OR
MeSH descriptor: [Fertilization in Vitro] explode all trees
OR
MeSH descriptor: [Gamete Intrafallopian Transfer] explode all trees
OR
MeSH descriptor: [Oocyte Donation] explode all trees
OR
MeSH descriptor: [Zygote Intrafallopian Transfer] explode all trees
OR
("in vitro fertilization" OR "IVF" or "in vitro fertilisation"):ti,ab,kw
OR
("intracytoplasmic sperm injection" OR "ICSI"):ti,ab,kw
OR
("gamete intrafallopian transfer" or "GIFT" or "zygote intrafallopian transfer" or "ZIFT" or "PROST" OR "pronuclear stage tubal transfer"):ti,ab,kw
OR
("donor conception" OR "oocyte donation" OR "sperm donation"):ti,ab,kw
AND
MeSH descriptor: [Stillbirth] explode all trees
OR
MeSH descriptor: [Fetal Death] this term only
OR
("stillbirth" or "stillborn" or "antepartum fetal demise" or "antepartum fetal death" or "intrapartum fetal demise" or "intrapartum fetal death"):ti,ab,kw

Appendix 3: Title & Abstract Screening Guide

If any paper seems like it may be relevant for the introduction or discussion, tag as "Future reading."



Appendix 4: Assessment of Risk of Bias

NB: Studies controlling for ≥50% of risk factors in Table 5 will be deemed to satisfy Comparability criterion 1a (study controls for obstetrical risk factors for stillbirth).

Newcastle Ottawa Scale for Case-Control Studies

Note: A study can be awarded a maximum of one star (*) for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
    a. yes, with independent validation *
    b. yes, e.g. record linkage or based on self-reports
    c. no description
2) Representativeness of the cases
    a. consecutive or obviously representative series of cases *
    b. potential for selection biases or not stated
3) Selection of Controls
    a. community controls *
    b. hospital (clinic) controls
    c. no description
4) Definition of Controls
    a. no history of disease (i.e. no stillbirth in index pregnancy) *
    b. no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
    a. study controls for obstetrical risk factors for stillbirth *
    b. study controls for maternal age*

Exposure

1) Ascertainment of exposure
    a. secure record *
    b. structured interview where blind to case/control status *
    c. interview not blinded to case/control status
    d. written self-report or medical record only
    e. no description
2) Same method of ascertainment for cases and controls
    a. yes *
    b. no
3) Non-Response rate
    a. same rate for both groups *
    b. non respondents described
    c. rate different and no designation

Newcastle Ottawa Scale for Cohort Studies

Note: A study can be awarded a maximum of one star (*) for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.

Selection

1) Representativeness of the exposed cohort
    a. truly representative of the average IVF/ICSI pregnancy in the community *
    b. somewhat representative of the IVF/ICSI pregnancy in the community *
    c. selected group of users e.g. nurses, volunteers
    d. no description of the derivation of the cohort
2) Selection of the non-exposed cohort
    a. drawn from the same community as the exposed cohort *
    b. drawn from a different source
    c. no description of the derivation of the non-exposed cohort
3) Ascertainment of exposure
    a. secure record (e.g. surgical records) *
    b. structured interview *
    c. written self-report
    d. no description
4) Demonstration that outcome of interest was not present at start of study (i.e. live fetus at 20 weeks gestation)
    a. yes *
    b. no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
    a. study controls for obstetrical risk factors for stillbirth *
    b. study controls for maternal age *

Outcome

1) Assessment of outcome
    a. independent blind assessment *
    b. record linkage *
    c. self-report
    d. no description
2) Was follow-up long enough for outcomes to occur
    a. yes (length of pregnancy—up to delivery) *
    b. no
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 - > 85% follow up, or description provided of those lost *
    c. follow up rate < 85% (select an adequate %) and no description of those lost
    d. no statement

Obstetrical Risk Factors Controlled For In Eligible Studies

Risk Factor	
Pre-existing hypertension	
Pre-existing diabetes mellitus	
Other pre-existing maternal conditions impacting stillbirth (e.g. chronic kidney disease, systemic lupus erythematosus, thrombophilia)	
Hypertensive disorders of pregnancy	
Gestational diabetes	
Intrahepatic cholestasis of pregnancy	
Alloimmunization	
Smoking in pregnancy	
Obesity	
Parity	
TOTAL (/10)	

Appendix 5: Table of selected excluded studies, with reasons

Study, year	Reason for Exclusion
Buckett, 2007	Population not matched for number of fetuses (multiples matched to singletons in control)
Chughtai, 2018	Unclear interventions ("ART"); no reply to request
De Geyter, 2006	Stillbirth not reported as outcome
Delgadillo, 2006	Stillbirth not reported as outcome
Dhont, 1997	Perinatal death reported instead of stillbirth
Draper, 1999	Perinatal death reported instead of stillbirth
Gissler, 1995	Perinatal death reported instead of stillbirth
Healy, 2010	Stillbirth not reported as outcome
Howe, 1990	"Infant survival" reported instead of stillbirth, which may include neonatal death
Isaksson, 2002	Perinatal death reported instead of stillbirth
Kapiteijn, 2006	Stillbirth not reported as outcome
Katalinic, 2004	Population includes multiple gestations
Koivurova, 2002 (Human Reproduction 17(5): 1391-1398)	Stillbirth not reported as outcome
Koivurova, 2002 (Human Reproduction 17(11): 1897-2903)	Stillbirth not reported as outcome
Koudstaal, 2000	Only included stillbirths are terminations of pregnancy
Nuojua-Huttunen, 1999	Perinatal death reported instead of stillbirth
Ochsenkuhn, 2003	Perinatal death reported instead of stillbirth
Olivennes, 1993	Stillbirth not reported as outcome
Pelinck, 2010	Stillbirth not reported as outcome
Perri, 2001	Stillbirth not reported as outcome
Pinborg, 2010	Unclear definition of stillbirth (stillbirth and perinatal death both reported, but seem to be mutually exclusive; not clear what gestational age for stillbirth is reported)
Raatikainen, 2012	Stillbirth not reported as outcome
Sazonova, 2011	Perinatal death reported instead of stillbirth
Sazonova, 2012	Perinatal death reported instead of stillbirth
Schieve, 2007	Stillbirth not reported as outcome
Tan, 1992	Control group unclear (not explicitly spontaneous conception; may contain some IVF/ICSI)
Tanbo, 1995	Intervention group includes IUI, IVF, GIFT
Thomson, 2005	Intervention group includes IUI, OI, IVF, and ICSI
Von During, 1995	Intrauterine fetal demise from 15 weeks reported instead of stillbirth
Wang, 2002	Stillbirth not reported as outcome
Wennerholm, 1996	No control group
Zadori, 2003	Stillbirth not reported as outcome