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Role of Ovum in Reproductive Outcomes

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This journey would not have been possible without sacrifices by my wife Vasudha and daughter Aarna and son Arjun. Finally, I express my profound gratitude to my beloved parents for their love and continuous support.

Submission declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by me and has not been submitted in any previous application for any degree.

Word count: 10410

Table of publications submitted

Statements of the candidate's contribution to the published work

No.	Paper and Contribution	Authorship
1	The combined use of antimullerian hormone and age to predict the ovarian response to controlled ovarian hyperstimulation in poor responders: A novel approach <i>J Hum Reprod Sci.</i> 2013 Oct;6(4):259-62.	Jeve YB
2	Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis <i>J Hum Reprod Sci.</i> 2016 Apr-Jun;9(2):70-81 Yadava contributed significantly in this manuscript. It was his idea to complete meta-analysis on this topic. He developed study design, literature search, quality analysis, data extraction as first author. He analysed data and interpreted the results. He drafted the manuscript and incorporating my comments and suggestions.	Jeve YB, Bhandari HM.
3	Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies <i>Int J Gynaecol Obstet.</i> 2016 May; 133(2):156-8. Yadava Jeve as a first author, contributed to this manuscript significantly. He developed study design, data extraction sheet and he collected data with help of co-authors. He analysed data and interpreted the results. He drafted the manuscript and incorporating co-authors' comments and feedbacks. He then revised the manuscript following the reviewer's comments.	Jeve YB, Potdar N, Opoku A, Khare M.
4	Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis <i>BJOG.</i> 2016 Aug;123(9):1471-80	Jeve YB, Potdar N, Opoku A, Khare M.

	<p>Yadava Jeve as a first author, contributed to this manuscript substantially. He performed the literature search, reviews, data extraction and meta-analysis. He prepared the manuscript and addressed all comments and feedbacks by co-authors. He revised the manuscript incorporating co-authors' reviewer's comments.</p>	
5	<p>Strategies to improve fertilisation rates with assisted conception: a systematic review <i>Hum Fertil (Camb)</i>. 2017 May 26:1-19</p> <p>Yadava Jeve as a first author, contributed to this manuscript significantly. He developed study design and searched for literature. He, along with other authors, included studies and analysed quality of evidence. He drafted the manuscript and incorporating co-authors' comments and suggestions. He later revised the manuscript following the reviewer's and editorial comments.</p>	<p>Jeve YB, Potdar N, Blower JA, Gelbaya T.</p>
6	<p>Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review <i>Ultrasound Obstet Gynecol</i>. 2011 Nov;38(5):489-96</p> <p>Yadava Jeve as a first author, contributed to this manuscript significantly. He developed study design, data extraction sheet and he collected data with help of co-authors. He analysed data and interpreted the results with other authors. He drafted the manuscript and incorporating co-authors' comments and feedbacks. He later revised the manuscript following the reviewer's and editorial comments.</p>	<p>Jeve Y, Rana R, Bhide A, Thangaratinam S.</p>
7	<p>Evidence-based management of recurrent miscarriages <i>J Hum Reprod Sci</i>. 2014 Jul;7(3):159-69. (co-author is deceased)</p>	<p>Jeve YB, Davies W.</p>

Copies of these statements of contribution signed by all co-authors are in Appendix A

Abstract

The ovary is one of the most dynamic organs in the human body. Ovaries have a finite pool of follicles during fetal life. On-going follicular atresia significantly contributes to a quantitative and qualitative decline in ovarian reserve. Only a tiny number of follicles are released between puberty and menopause in the form of ovulation. Reproductive outcome following assisted conception significantly depends upon the quality and quantity of oocytes. Ovarian reserve is a significant predictor of ovarian response during the assisted conception treatment cycle. It not only determines the number of oocytes to be collected but also controls the pregnancy outcome. The first two publications evaluate the prediction of ovarian reserve and management of the poor ovarian reserve. The third and fourth publications analyse use of donor oocyte as a treatment option and the pregnancy complications associated with donor oocyte. The fifth publication examines the factors affecting fertilisation rates in assisted conception and strategies to improve the fertilisation process. Poor quality oocytes and the resultant poor-quality embryo is one of the primary causes for miscarriage in natural and assisted conception. The sixth and seventh publications discuss the diagnosis and management of miscarriages. These publications have significant clinical and research implications which are discussed in results and discussion chapter. Especially sixth publication contributed to changing the national guideline on the diagnosis of missed miscarriage. Recent research updates that occurred after our research period are discussed in a separate chapter. Further research is required to manage the reproductive outcomes related to the poor quantity and quality of oocytes.

List of abbreviations and acronyms

Abbreviation	Meaning
AMH	Anti-Müllerian hormone
CI	Confidence interval
CRL	Crown-rump length
DHEA	Dehydroepiandrosterone
DO	Donor oocyte
FSH	Follicle stimulating hormone
GH	Growth hormone
ICSI	Intra-cytoplasmic sperm injection
IMSI	Intracytoplasmic morphologically selected sperm injection
IVF	In-vitro fertilization
LH	Luteinising hormone
LMWH	Low molecular weight heparin
MSD	Mean sac diameter
NGF	Non-growing follicle
OR	Odds Ratio
PICSI	Physiological Intra-cytoplasmic sperm injection

Chapter 1: Introduction

“Formulation of a problem is often more essential than its solution, which may be merely a matter of mathematical or experimental skills. To raise new questions, new possibilities, to regard old problems from a new angle requires creative imagination and marks real advances in science.”

Albert Einstein (Einstein and Infeld, *Evolution of Physics*, 1938, page 92).

This year is the 40th birth anniversary of the first human born following in-vitro fertilisation (IVF), a milestone in human history and the landmark achievement for Reproductive Science. It has opened the path for new scientific breakthroughs and clinical success, enabling the creation of new families and treatment for subfertility. More than 8 million babies have been born using IVF since 1978 (Niederberger et al., 2018). Although the advancements in Reproductive Science have helped many patients, IVF still is unsuccessful for more than half of patients undergoing treatment. Each failed approach to improve the success rate of the treatment resulted in the seeds of further research and technological advances. Reproductive Science continues to build on failures. Each stage in the IVF pathway, from obtaining good quality gametes to the birth of a healthy child, can result in challenges for health-professionals and patients. Finding solutions to overcome these challenges through rigorous and robust research is essential. In the words of Albert Einstein, the underlying problems require robust analysis before searching for solutions. The current work presented here is a systematic analysis of some of the critical challenges in Reproductive Medicine with the central theme of the ovum.

1.1: Human ovarian physiology and physiology of conception

Ovaries are dynamic structures that are composed of multiple compartments. Primordial germ cells originate in the endoderm of the yolk sac, hindgut, and allantois. The maximum number of oocytes, around 6-7 million in both ovaries (Baker, 1963), is reached at 16-20 weeks of gestation. The ovaries undergo a regular monthly cycle consisting of follicular growth, ovulation, and formation of corpus luteum. If pregnancy does not occur then, the corpus luteum is lysed resulting in the onset of in the onset of menses. Follicular recruitment and subsequent growth are a complex process that is coordinated by multiple hormones. At puberty, follicular recruitment

begins at the beginning of monthly menstrual cycles. Every month the ovaries produced a single egg, which is ready to be fertilised by sperm. The female body is adapted for conception and implantation of fertilised egg in the form of an embryo by coordinated secretion of hormones. If fertilisation fails, then luteolysis begins and the cycle is terminated. The average time for ovulation and next luteolysis with the onset of another period is average of 28 days. The entire cycle consists of three distinct phases that include the selection of the dominant follicle, establishment of the ovulatory phase and corpus luteum maintenance or its lysis (Gougeon, 1986).

Follicular development is usually seen in both the ovaries. The time required from a primary follicle to ovulation is approximately 85 days (Oktay et al., 1998). Most of time needed during this development is gonadotropin independent. Many small follicles start developing in response to gonadotrophic hormones. All these small follicles become mitotically active and start growing at the same time. The number of small follicles that mature is dependent on the amount of follicle stimulating hormone FSH available and the sensitivity of the follicles to the gonadotropins. In healthy women who are cycling naturally, only one of the follicles is destined to release the oocyte. This single follicle is known as the dominant follicle (Goodman and Hodgen, 1983). The intraovarian environment governs the choice of dominant follicle. Hormones secreted during each phase play a distinct role in the whole process. Follicle stimulating hormones is the hormone responsible for the development of multiple follicles at the same time. FSH levels fall after choice of the dominant follicle because of negative feedback mechanism. Granulosa cells start releasing oestrogen. Oestrogen produces cyclic changes in the uterine endometrium and vaginal epithelium. It prepares the endometrium for implantation. Oestrogen production from the developing follicle leads to the luteinizing hormone (LH) surge that results in ovulation. Luteinising hormone plays an important role and serves as the essential maker for ovulation. Once LH levels show a surge, then ovulation happens within 36-38hrs of LH peak. If fertilisation does not occur, then the corpus luteum disappears. Fall in oestrogen and progesterone levels result in the initiation of menses (McNatty et al., 1979, McNatty, 1979).

To summarise the events, initial follicular development occurs independent of hormone influence, FSH stimulation drives follicles to the preantral stage, FSH induced aromatisation of androgen in the granulosa results in the production of

oestrogen, and FSH and oestrogen both together increases the number of FSH receptor of the follicle. The next step is a choice of a dominant follicle. Dominant follicle is established by day 5-7. This selection process is the result of oestrogen action within the maturing follicle, which is a positive influence of FSH action and negative feedback at the hypothalamic-pituitary level. LH levels rise steadily during the late follicular phase stimulating androgen production in the theca. FSH induces LH receptors. LH initiate luteinisation and progesterone production in the granulosa layer. LH surge stimulates luteinisation, synthesis of progesterone and prostaglandins within the follicles (McNatty, 1979). Progesterone enhances the activity of proteolytic enzymes and with prostaglandins digestion and rupture of follicle wall.

If the released oocyte is fertilised by sperm, early pregnancy human chorionic gonadotropin HCG rescues the corpus luteum. It continues to maintain luteal function until placental steroidogenesis is well established. Spermatozoa require approximately 72 days for production and maturation. Only a few hundred sperm reach the oocyte. They are stored at the cervix for up to 72 hours (Bedford, 1994). After ovulation, oocyte starts to travel through the fallopian tubes. Tubal transport depends on smooth muscle contraction and ciliary action. Sperm penetration depends on acrosomal proteinase and binding of sperm receptors to zona ligands (Katz et al., 1989). The capacitation and following acrosome reaction results in activated sperm that discharges the nucleus into the oocyte cytoplasm. The fertilization triggers cell division that result in the formation of the embryo. The early embryo enters the uterine cavity as a morula. It develops further to blastocyst stage before implantation. The implantation window is when the endometrium is receptive for an embryo. A complex activity of cytokines, lipids and growth factors modulated by progesterone progresses endometrium to the receptive stage. Adhesion molecules integrins and cytokines play a crucial role in implantation. Transient expression of the leukaemia-inhibitory factor in mice is essential for implantation (Stewart et al., 1992). Other vital cytokines are colony stimulating factor-1 and interleukin-1 (Simon et al., 1996).

Thus, if the fertilisation and implantation are successful, it results in maintained levels of progesterone for gestation. Human chorionic gonadotropin secretion is ensured to continue the relevant process.

1.2: The quantitative and qualitative decline in oocyte with age and reproductive implications

Age alone has a significant impact on fertility. The average age of first-time mothers has increased worldwide; this delay in childbearing contributes to an increasing incidence of subfertility. A primordial follicle is a non-growing follicle (NGF). These follicles continue to have growth and atresia under all circumstances continuously. Therefore, the number continues to decline with age. The rate of decline is proportional to the total number present. Most rapid drop occurs before birth. Approximately 2 million oocytes present at birth and 300,000 at puberty as compared to 6-7 million at 16-20 weeks. Out of all these follicles, 400 will ovulate throughout reproductive years. Figure 1 shows a model of non-growing follicle numbers based upon several histological studies of the human ovary (Wallace and Kelsey, 2010).

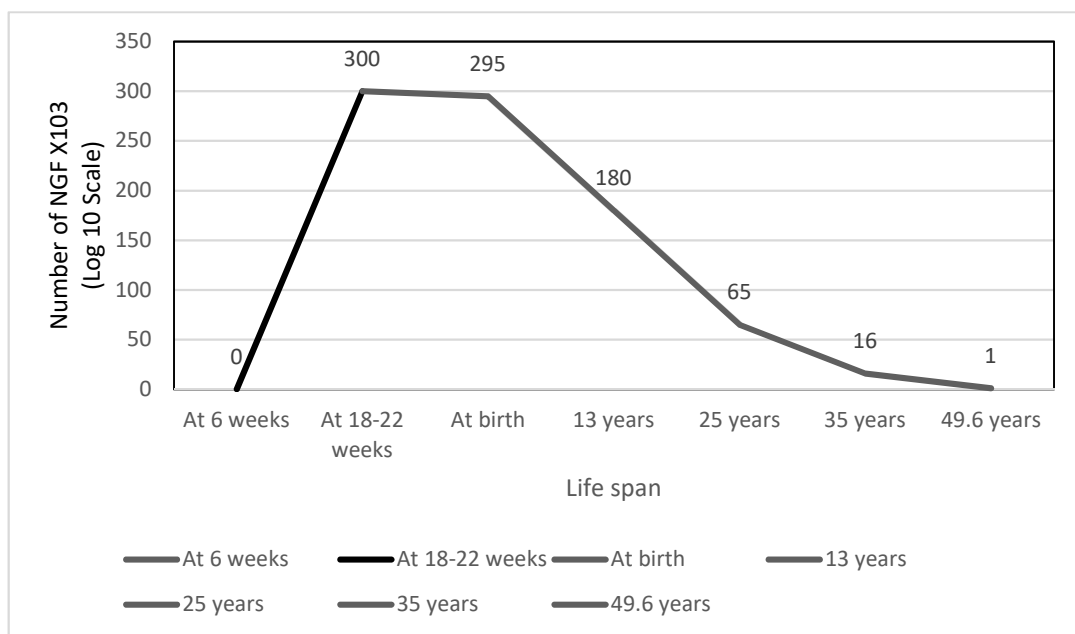


Figure 1: The age-related decline in NGF

The gradual decrease in oocyte number is associated with an increase in circulating levels of follicle stimulating hormones and increased atresia of follicles. The early growth of follicles takes several months. This growth is not dependent on the hormone. The cohort of follicles reaches a stage when if it is not recruited by FSH action, it undergoes atresia.

Age not only results in the quantitative decline in oocyte but it also reduces the quality of oocytes. Aged oocytes display increased chromosomal abnormality and dysfunction of cellular organelles, both of which factor into oocyte quality (Igarashi et al., 2015). This age-related decline in fecundity is strongly dependent on oocyte quality, which is critical for fertilization and subsequent embryo development. Aging oocyte negatively influence fertilization and development (Miao et al., 2009).

There is a marked increase in the risk of aneuploidy, miscarriage, and birth defects with increasing maternal age. Although the aetiology of such pathologies is complicated, there is a well-established causative relationship between the age-related decline in oocyte quality and oxidative stress (Mihalas et al., 2017). Chromosomal errors during human conception have an astonishingly high rate, and the most of these errors are derived from the oocyte (Hassold and Hunt, 2001). Aneuploidy rate increases exponentially from 30 to 35 years from baseline 20% of oocytes to on average 80% by 42 years (Capalbo et al., 2017). Thus, the accelerated decline in genetic quality of oocytes starts from 30 years of age. There is a high chance of non-disjunction of spindle poles in oocytes that is associated with higher chances of attrition (Ottolenghi et al., 2004). An age-related increase in meiotic non-disjunction suggested causing chromosomal aneuploidy and early pregnancy loss (te Velde and Pearson, 2002). Women who delay their pregnancies post 30's can have issues with both conceiving and maintenance of pregnancies. Such age-related rapid decline is not seen in men as sperm production and maturation is a continuous process.

1.3: Research gap and evidence synthesis to bridge the gap

Although quantity and quality are interlinked and could be dependent upon each other, ovarian reserve tests predict the amount and not the quality. Antral follicle count (AFC) and anti-Müllerian hormone (AMH) are good predictors of ovarian reserve. A diminished ovarian reserve is not associated with increased aneuploidy or an increase in miscarriage rate among younger women (Bishop et al., 2017). Therefore, clinicians require an appropriate tool to predict both quantitative and qualitative reserve. The most widely used and consistent predictor of the quality of oocytes is maternal age. AMH is a more direct and independent measure of the growing preantral and antral follicular pool. Such prediction is crucial in especially poor responder groups.

Publication 1 attempts to bridge the research gap using a novel approach of combining age and AMH for poor responder group (Jeve, 2013).

Poor ovarian reserve results in poor ovarian response during IVF cycle. Researchers have explored various strategies to treat poor ovarian response. Women with diminished ovarian reserve are one of the most challenging groups to manage in assisted conception. There are various observational studies, randomized controlled trials, and systematic reviews reported (Kyrou et al., 2009, Sunkara et al., 2011a, Polyzos and Devroey, 2011, Narkwichean et al., 2013, Gonzalez-Comadran et al., 2012). However, the published literature has been shown to have qualitative limitations. Many of the published studies are too specific to evaluate one approach (Kyrou et al., 2009, Bosdou et al., 2012) or too inexplicit with cohort and randomised data combined (Narkwichean et al., 2013). There was a need for more comprehensive good quality synthesis of all available evidence at one place.

Publication 2 is a systematic review and meta-analysis of all good quality randomised trials to find out effective treatment protocol for poor responders (Jeve and Bhandari, 2016).

Donor oocyte IVF is an alternative treatment option for women with diminished oocyte quantity and quality. There is a substantial increase in the number of donor oocyte recipients in recent years (Kupka et al., 2014). However, donor oocyte pregnancy is not risk-free. Advanced maternal age in itself can cause pregnancy complications (Michalas et al., 1996, Simchen et al., 2006). Pregnancies achieved by IVF or by intracytoplasmic sperm injection (ICSI) are at a higher risk for pregnancy complications compared with spontaneous pregnancies (Wang et al., 1994, Maman et al., 1998). Therefore, the question is whether increased risks with donor oocyte IVF is due to the biological origin of oocyte or maternal age or IVF technology. Publication 3 is a cohort study, which compares donor oocyte IVF with natural conception and autologous oocyte IVF conception (Jeve et al., 2016b). Publication 4 is a systematic review and meta-analysis of all published evidence on the obstetric outcome of donor oocyte conception (Jeve et al., 2016a).

Poor fertilisation rates and miscarriage rates could be the result of poor quality of gametes (Braude and Rowell, 2003, Hourvitz et al., 2006). Fertilisation rate has an

impact on the outcome of the treatment cycle. Fertilisation rates of 60% or over are expected (Braude and Rowell, 2003). When oocytes collected during IVF cycle are few, which occur with diminished ovarian response group, it is far more critical to have a maximum number of oocytes fertilised. Researchers have attempted various interventions to improve fertilisation rates (Mokanszki et al., 2014, La Sala et al., 2015, Chansel-Debordeaux et al., 2015, Nakagawa et al., 2001, Beck-Fruchter et al., 2014).

Publication 5 critically appraises the existing evidence on multiple causes of failed fertilization and strategies to achieve a higher fertilisation rate (Jeve et al., 2017). Miscarriage is one of the most devastating pregnancy outcomes. The incidence of early embryonic demise is high as compared with other early pregnancy complications (Wilcox et al., 1988). The chromosomal anomaly is the most prevalent cause for spontaneous miscarriage (Cunningham et al., Lathi et al., 2011). When it comes to diagnosis of any miscarriage, high-quality accuracy is required to prevent the wrong diagnosis for apparent reason. In Publication 6, a critical appraisal of the available evidence was undertaken to find out the accuracy of ultrasound diagnosis of miscarriage based on existing national guideline (Jeve et al., 2011). It was the first review to investigate the evidence on ultrasound criteria. Recurrent miscarriage is one of the most debated and researched areas in early pregnancy. Recurrent miscarriages have substantial psychological, financial and clinical impact (Stirrat, 1990). Different investigations and treatments are a constant source of debate among professionals. Finally, Publication 7 is on evidence-based management of recurrent miscarriage (Jeve and Davies, 2014b).

Figure 2 provides a diagrammatic summary of the structure and content of this thesis. It illustrates how each of the seven publications relate to one another.

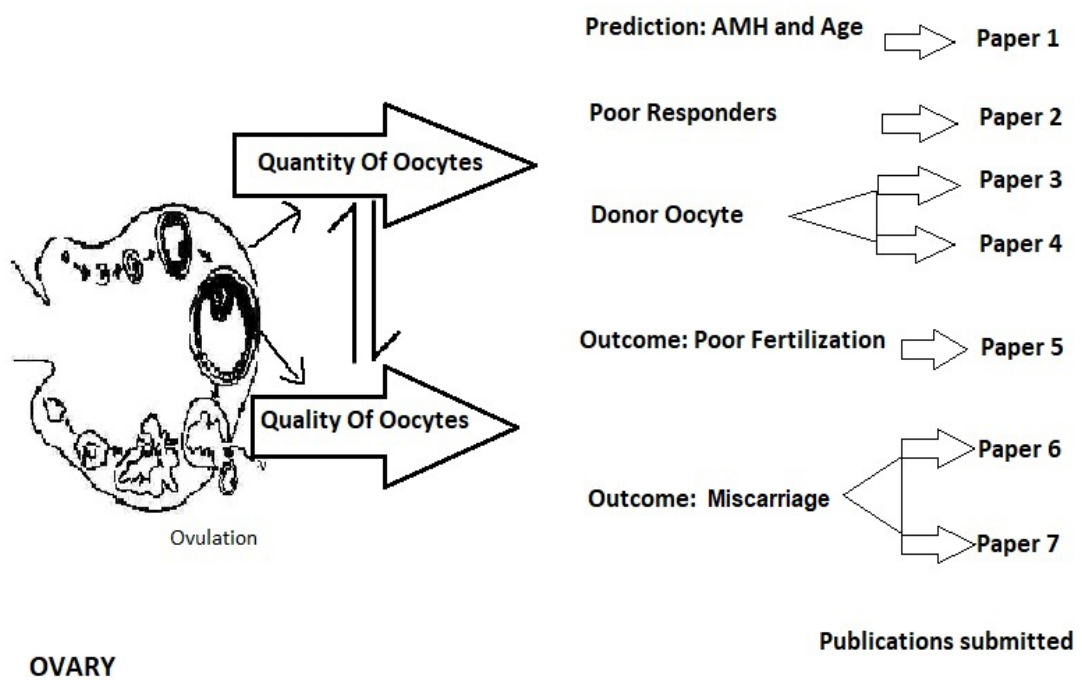


Figure 2: Diagrammatic summary of structure and contents of present thesis to explain role of ovum in reproductive outcomes

Chapter 2: Aims and objectives

Oocyte quality and quantity are closely related with each other. The reproductive outcome cannot be attributed to only one factor. To simplify, we have divided the aims and objectives as below.

2.1: To investigate the reproductive outcomes related to the oocyte quantity

Objectives:

- a) To predict the ovarian response using serum AMH and age in the poor responder group (publication 1)
- b) To evaluate all the existing protocols and strategies applied to poor responders by including evidence generated from only good quality randomised controlled trials (publication 2)
- c) To investigate the obstetric outcome of pregnancies conceived using donor oocyte IVF compared with pregnancies conceived using autologous-oocyte IVF or natural conceptions (publication 3)
- d) To analyse whether donor oocyte IVF acts as an independent risk factor for pregnancy complications (publication 4)

2.2: To investigate the reproductive outcomes related to the oocyte quality

Objectives:

- a) To classify and analyse the factors affecting fertilisation and strategies to improve the fertilisation rates (publication 5)
- b) To evaluate, by a systematic review of the literature, the accuracy of first-trimester ultrasound in diagnosing early embryonic demise (publication 6)
- c) To produce evidence-based guidance on the clinical management of recurrent miscarriage (publication 7)

Chapter 3: Methods

3.1: A mixed methods approach was used to investigate the reproductive outcomes related to the oocyte quantity.

Methods included:

- a) A cohort study of women undergoing controlled ovarian stimulation for IVF or ICSI cycle. These women were treated for either primary or secondary infertility at a teaching hospital. All women included were predicted reduced responders with serum AMH value between one and five pmol/L (publication 1).

- b) Evaluation of the existing literature with systematic review and meta-analysis. Poor responders to ovarian stimulation formed the study population. All types of intervention subjected to randomised controlled trials were included in the systematic review. Two or more trials with identical design and interventions were analysed by meta-analysis. Our outcome measures were the number of oocytes retrieved per cycle, live birth rates, and clinical pregnancy rates (publication 2).

- c) A cohort study to compare outcome in women conceived with donor oocyte and delivered a live neonate after 24 weeks of pregnancy with two age-matched control groups, including women who conceived after autologous IVF and the other group formed by women conceived spontaneously. The primary study outcome was hypertensive disorders of pregnancy (pregnancy-induced hypertension and pre-eclampsia). Multivariate analysis was performed by logistic regression (publication 3).

- d) Systematic review and meta-analysis of published literature. All observational studies comparing pregnancy outcomes in donor oocyte pregnancies with a predefined control group were included. The control group included women who had assisted conception using IVF or ICSI with autologous oocytes. The primary outcome was any hypertensive disorder in pregnancy; secondary outcome measures included the risk of caesarean section, development of gestational diabetes, and small for gestational age, preterm delivery, and intrauterine death (publication 4).

3.2: To investigate the reproductive outcomes related to the oocyte quality, qualitative and quantitative synthesis of existing evidence was performed.

- a) A systematic review of all studies analysing factors affecting fertilisation and strategies to improve the fertilisation rates. Various strategies used for improving fertilisation rates formed the intervention. The comparison group had no such intervention under research. The primary outcome measure was fertilisation rates. No secondary outcome measures. All types of study designs including case-control, cohort, and randomised studies were included (publication 5).
- b) Systematic review used to investigate the accuracy of existing diagnostic criteria to diagnose early miscarriage. Studies which evaluated the accuracy of first-trimester ultrasonography in pregnant women for the diagnosis of early embryonic demise were included. Accuracy measures including sensitivity, specificity and likelihood ratios for abnormal and normal test results were calculated for each study and each test threshold (publication 6).
- c) The evidence was sought for all current recommendations as well as all unanswered questions on investigating and managing recurrent miscarriages. The evidence was searched using the individual subclass of the aetiology of recurrent pregnancy loss. The recommendations are based on evidence which was graded based on quality (publication 7).

3.3: Recent research updates published following our research

For all submitted systematic reviews (2, 4, 5 and 6th publications), the update on current evidence was searched systematically with defined search strategy. The literature search strategy used for updates is described in detail in Appendix B. The limitations applied were human, English language and publication date. The titles and abstracts were screened to include relevant evidence. Full text of the article was reviewed and critically appraised to produce the updates for submitted publications. Update review was performed by the author only. Meta-analysis was not updated. Scoping literature search for relevant updates was performed for the first, third and seventh publications.

Chapter 4: Results and discussion

4.1: Publication 1: The combined use of antimullerian hormone and age to predict the ovarian response to controlled ovarian hyperstimulation in poor responders: A novel approach (Jeve, 2013).

Results

Our cohort study showed clinical pregnancy rate per embryo transfer was 20.33%, in poor responder group. AMH and age were analysed using linear regression model which produced an equation to give predicted oocyte count if AMH and age are known.

Discussion

The number of oocytes retrieved during an IVF cycle is a crucial step. After fertilisation of oocytes, embryos are formed. The number of embryos allows statically higher chances of good quality embryos and resultant pregnancy. It also gives an opportunity to have frozen embryos for future use. The number of eggs in IVF is a robust surrogate outcome for clinical success. Live birth rate found to rise with an increasing number of eggs up to fifteen oocytes (Sunkara et al., 2011b). An inadequate response could be associated with multiple factors. The diminished ovarian reserve is the most common cause of poor response. The use of follicle stimulating hormone as an indicator of follicular health has not been very successful in predicting the outcomes. Anti-Mullerian hormone (AMH) was first discovered to be essential for the development of Mullerian tubules in infants for a male reproductive system. This hormone was later known to be important in understanding the follicular growth. The hormones are expressed in the preantral and antral stages and play an essential role in follicular development until a single dominant follicle is selected. In 2002, van Rooij suggested AMH can be used as a marker for ovarian ageing (van Rooij et al., 2002). Subsequently, Tremellen showed AMH assessments are superior to FSH in identifying women with reduced ovarian reserve. Anti-mullerian hormone assessment should be considered as a useful adjunct to FSH levels and antral follicle count when estimating ovarian reserve (Tremellen et al., 2005). The AMH levels are direct indicators of the size of the preantral and antral follicular pool. With further research work, AMH emerged as a better predictor of ovarian reserve (Kwee et al., 2008). The sensitivity

and specificity of detecting the poor responders with AMH were between 72-93%. At the same time, few researchers questioned its clinical usefulness (La Marca et al., 2009). Our study used a novel approach of using both AMH levels and age as two factors to predict for the success of pregnancy in poor responders. All the earlier studies undertaken have used AMH as a parameter and predicted age to have a significant contribution. However, no one used both predictors together and developed a simple formula for predicting the outcome. Our results show increasing AMH value is associated with increasing oocyte yield in this reduced responders group which is consistent with previous data (Nelson et al., 2007). Our results demonstrated that combining two strong predictors allow clinicians to develop a model for predicting ovarian response. Such an approach will help clinicians to counsel patient, design protocol for optimal outcome. Clinicians could offer other options like oocyte donation and adoption, without subjecting ovarian stimulation. Individualisation of treatment is possible with the use of accurate prediction (Nelson et al., 2007).

This study has a small population size and conducted at the single centre; these are limitations of this study. None the less, this study demonstrated an approach to model two good predictors together accessing quantity and quality while predicting ovarian response. It could be used very effectively for personalised treatment approaches.

4.2: Publication 2: Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis (Jeve and Bhandari, 2016).

Results

We found 61 randomised controlled trials including 4997 cycles. We divided these trials into 10 different management strategies based on interventions. Limited evidence on growth hormone supplementation and transdermal testosterone supplementation showed improved clinical pregnancy rates and live birth rates in poor responders. Various other strategies did not show significant improvement in outcome.

Discussion

Our study supplies the most comprehensive review for investigating effective treatment for poor responders. It included only randomised trials. Kyrou and team

reviewed twenty-two studies and found insufficient evidence to recommend any treatment approach proposed to improve pregnancy rates in poor responders (Kyrou et al., 2009). Bosdou, in 2012, investigated use of androgens and androgen modulating agents in poor responders undergoing IVF. The study was not conclusive with a probable indication that use of testosterone could enhance the live birth rate. No other androgen or androgen-related agents had any effect on outcome (Bosdou et al., 2012). A systematic review and meta-analysis on the role of dehydroepiandrosterone in improving the outcome in poor responders clearly showed that dehydroepiandrosterone did not affect the outcome (Narkwichean et al., 2013). Our meta-analysis shared the same view of the insufficient evidence to recommend any treatment choice to improve reproductive outcome in poor responders. These meta-analyses published before us had a methodological limitation of using data from observational studies and nonrandomized studies in their meta-analysis (Bosdou et al., 2012, Kyrou et al., 2009, Narkwichean et al., 2013). Our limitations include heterogeneity in the definition of poor responders in these trials conducted before Bologna consensus criteria (Ferraretti et al., 2011). Although some adjuvant supplementations like growth hormone and androgens may appear to improve ovarian response and reproductive outcome, we recognise that the numbers are small to recommend their routine use in poor responders.

4.3: Publication 3: Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies (Jeve et al., 2016b)

Results

Hypertensive disorders in pregnancy affected 33% of women in the study group as compared with 7% women who conceived after autologous IVF or conceived spontaneously. The risk of hypertensive disorders in pregnancy was significantly higher in the donor oocyte group.

Discussion

Although IVF using oocyte donation has enabled women at an advanced age or with ovarian failure to achieve pregnancy successfully, pregnancy following oocyte donation can subject them to a higher risk of maternal morbidity and mortality (Karnis et al., 2003). Danish national cohort study showed an increased risk of preeclampsia

and preterm labour in donor oocyte pregnancies as compared with pregnancies after autologous IVF (Malchau et al., 2013). The immunologic theory of pre-eclampsia might explain the risk of obstetric complications for women with conceiving after oocyte donation. Advanced maternal age is associated with a significantly increased risk of perinatal complications (Laskov et al., 2012). Therefore, it is necessary to eliminate bias caused by maternal age and other risk factors. Our study has a limited population size, as a significant limitation, to address other variables.

4.4: Publication 4: Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis (Jeve et al., 2016a).

Results

We included total, 11 studies (n = 81 752). The risk of developing hypertensive disorders during pregnancy was significantly higher for donor oocyte pregnancy (odds ratio 3.92; 95% confidence interval, 3.21–4.78). Further subgroup analysis showed for a singleton, and twin pregnancies showed that the risk was significantly higher for donor oocyte pregnancy in each group. Secondary outcomes including small for gestational age, caesarean section and preterm delivery were significantly higher with oocyte donation pregnancy. Meta-regression for the covariate of age suggested that risk was independent of age.

Discussion

Our findings are consistent with some previous observational studies (Serhal and Craft, 1989, Abdalla et al., 1998, Wiggins and Main, 2005). A previous systematic review discussed the immunological pathogenesis of complications but did not include meta-analysis (van der Hoorn et al., 2010). Immunologic intolerance between the mother and the fetus may play an essential role in the pathogenesis of preeclampsia due to donor oocyte conception (Levron et al., 2014). Ours is the first meta-analysis and meta-regression to quantify the risk of pregnancy complications in women with donor oocyte pregnancy. Small values for I^2 and narrow confidence intervals, low statistical heterogeneity are significant strengths of our meta-analysis. Our significant limitation is difficult to conclude that the obstetric risks are entirely independent of variables like age from any meta-analysis and systematic review. We made every attempt to neutralise the effects of confounding factors such as age by performing

subgroup analysis and meta-regression. Ideally, individual participant data (IPD) meta-analysis would be the best design. Secondary outcomes are not entirely independent of hypertensive disorders. We did not have the information on preventative measures such as the use of low-dose aspirin or ultrasound assessment for fetal growth.

4.5: Publication 5: Strategies to improve fertilisation rates with assisted conception: a systematic review (Jeve et al., 2017).

Results

Factors affecting fertilisation were allocated as sperm-related and oocyte-related factors. We divided the methods to improve fertilisation rates based on the intervention used. Various interventions are used to improve fertilisation rates including assisted oocyte activation, physiological intracytoplasmic sperm injection (PICSI) and intracytoplasmic morphologically selected sperm injection (IMSI). Optimal laboratory condition and improved procedural effects in techniques result in higher fertilisation rates.

Discussion

The most frequent cause of failed fertilisation is no availability of appropriate sperm or failed oocyte activation. Quality of oocytes is a critical factor. Delayed cytoplasmic maturation with unsynchronised nuclear maturity can lead to morphological changes in the oocyte. Oocyte maturity can influence fertilisation rates. Based on current evidence, we divided the causes influencing fertilisation rates such as gamete related, procedure related, and laboratory related. Due to the nature of the research question and vast differences between study designs, it was not possible to do a quantitative synthesis of the evidence. Various promising interventions are being studied; further evidence is awaited before recommending their use in clinical practice.

4.6 Publication 6: Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review (Jeve et al., 2011).

Results

We screened 720 citations, 23 were reviewed in detail. Eight primary papers with four different categories of tests (18, 2 × 2 tables) involving 872 women were included in this systematic review. We found a paucity of high-quality, prospective data to recommend guideline criteria for the accurate diagnosis of early miscarriage. We demonstrated an urgent need for an appropriately powered, prospective study using current ultrasound technology and an agreed reference standard for pregnancy before any guidelines are formulated.

Discussion

Ultrasonography is the most routinely used method to study the development of the foetus. It was used as early as in 1972 to predict abnormal foetus. Later many studies were done in the early 1980's and 1990's to study for any abnormalities in the foetus. Confirmation of non-viable pregnancy is very critical. Any error in judgment could be emotionally and physically disastrous to the mother.

The criteria for diagnosing a non-viable pregnancy should have a predictability rate of 100%. The national guideline at the time of publication of this paper recommended ultrasound criteria to diagnose miscarriage as CRL 5 or more and no fetal heartbeats or MSD 20 or more with no fetal poles. Our findings showed that the above recommendations are based on inadequate evidence. This review has identified many studies and various criteria for the identification of inevitable early pregnancy demise. An empty gestational sac of ≥ 25 mm diameter and a missing yolk sac with a gestational sac diameter of ≥ 20 mm appear to be the most accurate thresholds for the diagnosis of miscarriage, with an estimated specificity of 1.00. However, clinicians should note that both thresholds had a 95% CI of 0.96–1.00, indicative of up to four in every 100 diagnoses may be a false positive. The significant limitation of these studies is the use of various reference standards. Most of the studies did not use rigorous standards for the diagnosis of miscarriage. None of the studies evaluated the reproducibility and repeatability of early pregnancy measurements on ultrasound. The reviewed studies did not stipulate if a policy of two independent examiners confirming

the diagnosis was undertaken routinely to avoid operator errors. If in doubt, repeating scans at an interval is emphasised.

A cohort study published at the same time of our publication suggested that criteria to diagnose miscarriage based on growth in MSD and CRL are potentially unsafe. However, finding an empty gestational sac on two scans more than seven days apart is highly likely to indicate miscarriage, irrespective of growth (Abdallah et al., 2011a). Other group agreed with our view that current national guidelines should be reviewed to avoid inadvertent termination of wanted pregnancies. An MSD cut-off of > 25 mm and a CRL cut-off of > 7 mm could be introduced to minimise the risk of a false-positive diagnosis of miscarriage (Abdallah et al., 2011b). A study showed MSD measurement of 20 mm by the first observer, the prediction interval for the second observer was 16.8-24.5 mm. For a CRL measurement of 6 mm, the prediction interval for the second observer was 5.4-6.7 mm (Pexsters et al., 2011).

Inter-observer variation is a well-accepted concept in medicine. Pexsters's study signifies why we should account for inter observer's variations while establishing cut-offs for ultrasound diagnosis.

4.7 Publication 7: Evidence-based management of recurrent miscarriages (Jeve and Davies, 2014a).

Results

We critically appraised the evidence to produce a concise answer for clinical practice. The pathophysiology was discussed in detail along with the mechanism of proposed treatment options. We endeavoured to make clinical recommendations based on graded evidence to guide clinical practice. We graded the evidence to support management strategy so that clinicians and patients can make an informed choice for the treatment. The most common cause for single sporadic miscarriage was a chromosomal anomaly which indicates qualitative errors in gametes or during the process of fertilisation and cell division. Recurrent miscarriage may be the first presentation of some of the haematological or endocrine disorders. The evidence does not support investigations for inherited thrombophilia. No evidence to recommend low dose aspirin and low molecular weight heparin treatment for recurrent

miscarriages seen in inherited thrombophilia patients. Idiopathic recurrent miscarriage remains a challenge.

Discussion

The literature supports only a few therapeutic interventions. The progesterone act as immunomodulator which is favourable and pregnancy protective (Raghupathy et al., 2009). The results from multicentre trials (PROMISE) were awaited. Treatment of antiphospholipid syndrome with heparin and aspirin confers a significant benefit in live births (Branch et al., 2010, Ziakas et al., 2010).

Further evidence is needed for debated treatment options such as use of progesterone, use of thyroxine for sub-clinical hypothyroidism, and treatment of inherited thrombophilia. There is no conclusive evidence to support prenatal genetic screening for unexplained recurrent miscarriages as well as for structural chromosome abnormality (Simpson, 2012).

Chapter 5: Recent research updates that occurred after our research period

In this chapter we will discuss whether: a) any added evidence has been identified following an update of the searches used in the publications, and b) the impact any added evidence has on the conclusions reported in these publications. To ease the comparison of new evidence across the publications, we will tabulate the findings.

5.1 Publication 1 and 2 : The combined use of antimullerian hormone and age to predict the ovarian response to controlled ovarian hyperstimulation in poor responders: A novel approach (Jeve, 2013) and Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis (Jeve and Bhandari, 2016).

The recent update on literature as discussed in method and appendix B showed following randomised control trials using various approaches to treat poor responders are published after our study.

Table 1: Update on strategies used to treat poor responders following our publication

Intervention Strategy	Study	Conclusion
Growth hormone adjuvant therapy	Dakhly (Dakhly et al., 2018), Choe 2018 (Choe et al., 2018), Dakhly 2016 (Dakhly et al., 2016) Norman 2016 (Norman et al., 2016)	No significant difference in the outcome
Transdermal testosterone	Bosdou 2016 (Bosdou et al., 2016), Escriva 2015 (Escriva et al., 2015)	No significant difference in the outcome
Transdermal testosterone	Singh 2016 (Singh et al., 2016)	The clinical pregnancy rates were also significantly higher

Dehydroepiandrosterone (DHEA) supplementation	Narkwichean (Narkwichean et al., 2017), Viardot-Foucault 2015 (Viardot-Foucault et al., 2015)	No significant difference in the outcome
DHEA 25 mg three times daily for 12 weeks as an adjuvant in short antagonist cycle	Kotb 2016 (Kotb et al., 2016)	DHEA increases the number of oocytes, fertilisation rate, fertilised oocytes, and clinical pregnancy rate and ongoing pregnancy rate
A delayed start protocol with gonadotropin-releasing hormone (GnRH) antagonist and microdose flare-up GnRH agonist protocol	Davar 2018 (Davar et al., 2018)	No significant difference in clinical pregnancy rate, and ongoing pregnancy rates
Effect of antioxidant treatment with coenzyme Q10	Xu 2018 (Xu et al., 2018b)	No significant difference in the outcome
mid-follicular phase recombinant LH supplementation in (GnRH) antagonist protocol	Mak 2017 (Mak et al., 2017)	No significant difference in the outcome
Corifollitropin alfa followed versus recombinant FSH	Drakopoulos 2017 (Drakopoulos et al., 2017), Blasco 2017 (Blasco et al., 2017), Kolibianakis 2015 (Kolibianakis et al., 2015)	No significant difference in the outcome
Adjuvant sildenafil therapy	Ataalla 2016 (Ataalla et al., 2016)	No significant difference in the outcome

Individualised dosing in predicted poor responders	Van Tilborg 2016 (Van Tilborg et al., 2016)	AFC-based individualised gonadotropin dosing in does not improve live birth rates
Mild ovarian stimulation	Siristatidis 2016 (Siristatidis et al., 2016)	Mild ovarian stimulation is inferior to conventional regimes
Short gonadotropin-releasing hormone agonist versus flexible antagonist versus clomiphene citrate regimens	Schimberni 2016 (Schimberni et al., 2016)	The GnRH agonist protocol showed a significantly higher pregnancy rate than the clomiphene and the GnRH antagonist protocol
450 IU versus 600 IU gonadotropin	Lefebvre 2015(Lefebvre et al., 2015)	No difference was found between the outcomes

Table 2: Further evidence published on AMH following our publication

Study	Conclusion
Fleming et al., 2015 Review (Fleming et al., 2015).	AMH established as the gold-standard biomarker to evaluate ovarian reserve and predict ovarian response to stimulation
Systematic review by Iwase et al., 2014 (Iwase et al., 2014)	AMH proved to be a useful biomarker outside infertility treatment, for other conditions, such as endometriosis and ovarian tumour, as well as surgical interventions, such as cystectomy and uterine artery embolisation
Meta-analysis by Iliodromiti et al., 2014 (Iliodromiti et al., 2014)	The diagnostic odds ratio for women with unknown ovarian reserve 2.39 (95% confidence interval (CI): 1.85-3.08). AMH, independently of age, has some association with predicting live birth

Cohort study by Zheng et al., 2017 (Zheng et al., 2017)	Patients with low levels of AMH may achieve successful treatment outcomes. Low AMH levels in isolation do not represent an appropriate marker for withholding fertility treatment.
Steiner 2017, (Steiner et al., 2017)	Among women aged 30 to 44 years without a history of infertility who had been trying to conceive biomarkers indicating diminished ovarian reserve compared with normal ovarian reserve were not associated with reduced fertility. 65% of women with low AMH were predicted to get pregnant within six cycles, compared with 62% with normal AMH, the chances of getting pregnant in any given cycle was no different for women with low and normal levels of AMH (hazard ratio 1.19, 95% confidence interval CI 0.88 to 1.61). This suggest the limitation of AMH as a marker of reproductive potential in natural conception

Clinical and research implications

In recent years, further research demonstrated AMH, independently of age, has some association with predicting the outcome. The predictive accuracy is improved when it is combined with other biomarkers such as age, antral follicle count and FSH. Ageing of the women plays a vital role. IVF stimulations protocols are based on these biomarkers to achieve optimal response.

Further research is based on these biomarkers are their predictive potential. It raises further research questions such as a value of AMH in other endocrine conditions such as premature ovarian insufficiency, prediction of menopause, benign gynaecological conditions including endometriosis. AMH could provide further insight into the rate of ovarian follicular depletion. Findings from Steiner study showed the limitations of ovarian reserve markers including AMH in natural conception. Biomarkers of diminished ovarian reserve (low AMH or high FSH) were not associated with reduced fecundability or a lower cumulative probability of conceiving. The most important limitation of this study was, the primary outcome as conception not live birth. Diminished ovarian reserve could affect fecundity by increasing the risk of

miscarriage, perhaps through an effect on egg quality. Although various AMH cut-off values were explored, the study was not powered to look at very low (≤ 0.1 ng/mL) AMH values. This study shows the importance of our approach discussed in the first paper where we have suggested to use biochemical marker, serum AMH along with age to predict the reserve. AMH alone may not be enough marker for reproductive outcome.

Our study on the treatment of poor responders and literature published following our publication did not answer the best treatment strategy for poor ovarian response. The treatment stays a challenge. The clinicians can provide evidence-based information to all women who request for any adjuvant therapy. Further research could be focused on specific approaches such as DHEA, testosterone or GH supplementation.

Limitations

Small population size is one of the significant limitations of our study to develop a predictor model using AMH and age. IVF outcome is a result of many variables along with ovarian reserve. Due to limited numbers, we could not perform further analysis to address variables.

Before the Bologna criteria, heterogeneity of the definition on poor responders was a significant limitation. Our study, although tried to address this issue with careful inclusion Included studies have a small population size. Therefore, the outcome difference does not reach statistical significance. Two researchers did not perform the updated literature search, inclusion of studies and analysis. The meta-analysis was not performed using new data.

5.2 Publication 3 and 4: Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies (Jeve et al., 2016b).

Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis (Jeve et al., 2016a)

The recent update on literature as discussed in method and appendix B, showed the following studies compared donor oocyte (DO) pregnancy outcomes

Table 3: Studies comparing donor oocyte pregnancy outcome following our publication

Study	Result	Conclusion
Kamath 2017 (Kamath et al., 2017) 5929 DO and 127,856 autologous IVF	adjusted odds ratio (aOR) 1.56, 99.5% confidence interval (CI) 1.34 to 1.80 for preterm and aOR 1.43, 99.5% CI 1.24 to 1.66 for low birth weight	Significantly higher risk of preterm and low birth weight after DO conception
Elenis 2016 (Elenis et al., 2016) Donor oocyte n= 76 Spontaneous n= 149 Autologous oocyte IVF n= 63	DO higher odds for premature delivery (OR 2.36, 95 % CI 1.02-5.45), for being small for gestational age (OR 4.23, 95 % CI 1.03-17.42)	Significantly higher risk of preterm and low birth weight after DO conception
Simeone 2016(Simeone et al., 2012)	136 consecutive patients divided into DO IVF and autologous IVF. The risk of preeclampsia was 27% in the DO group and 5.6% in the autologous IVF group, (p=0.0024, OR 6.17)	Significantly higher risk of Pregnancy-induced hypertension, caesarean section, with donor oocyte IVF
Banker 2016(Banker et al., 2016) Autologous oocytes n=691, donor oocytes n=810	Pre-term delivery highest in Donor oocyte (42.58%), autologous oocyte (29.52%)	Significantly higher risk of preterm
Yu et al., 2018(Yu et al., 2018) SART CORS data, Donor oocyte n= 2703 Autologous oocyte n=4402	Preterm delivery adjusted odds ratio (aOR) 1.33 (95% CI 1.02-1.75), small for gestational age baby aOR 1.75 (95% CI 0.85-3.7)	Significantly higher risk of preterm and no significant difference for small for gestational age baby after DO conception

Clinical and research implications

Based on our publications and these studies, data further strengthen our conclusion that donor oocyte conception can cause significant pregnancy complications. There is an addition of a few more pregnancy complications such as low birth weight. Simeone et al showed significantly increased risk of pre-eclampsia which was consistent with our findings. Kamath and Elenis showed increased risk of preterm and low birth weight babies after DO conception. Banker and Yu showed high risk of preterm births. Preventative strategies such as low dose aspirin and growth monitoring with ultrasound could reduce the risks during pregnancy. Further research could be focussed on the understanding pathophysiology of these complications and preventative measures.

Limitations

The definitions used for preterm delivery, small baby, intra-uterine growth restriction was not uniform in original publications. These definitions evolved over a period. Some of the measured the outcomes such as mode of delivery, preterm or small for gestational age baby may be the result of hypertensive disorders during pregnancy. The information was not available on any preventative measures used to reduce the risk of complications. 5.3 Publication 5: Strategies to improve fertilisation rates with assisted conception: a systematic review (Jeve et al., 2017).

The recent update on literature as discussed in method and appendix B, showed following studies to analyse strategies to improve fertilisation rates

Table 4: Update on strategies to improve fertilisation rates following our publication

Study	Results	Conclusion
Yovich et al., 2018 (Yovich et al., 2018)	The use of ICSI to improve the fertilisation rate for non-male factor subfertility significantly more ICSI-generated embryos were utilised (2.5 vs 1.8;	Reduction in failed fertilisation rates was seen. The first treatment for unexplained infertility

	p < 0.003) with productivity rates of 67.8% for pregnancy and 43.4% for livebirths.	could be undertaken within the IVF-ICSI Split model
Xu 2018 (Xu et al., 2018a)	The predictive value of acrosomal enzymes was studied, spontaneous acrosome reaction rate is significant predictor of fertilization outcome (OR= 0.68, 95% CI 0.53-0.88, P = 0.68)	Lower acrosomal enzymes levels (<25muIU/10 ⁶) were predictive of total fertilisation failure
Kirman-Brown 2019 (Kirkman-Brown et al., 2019)	Hyaluronic Acid Binding Sperm Selection (HABSelect Trial), Live birth rate (OR 1.12, 95% CI 0.94 to 1.34; p = 0.18)	PICSI offered no clear advantage in relation to the primary outcome.
Cui 2018 (Cui et al., 2018)	Spermatozoa PIWI-interacting RNAs (piRNAs) levels , There were significant increases in the levels of all 3 piRNAs in spermatozoa from the group with higher 2PN rates (for piR-31704, P = .002; for piR-39888, P < .001; for piR-40349, P < .001; respectively)	Spermatozoa levels of piR-31704 and piR-39888 were decreased in male factor infertility group piRNAs plays a role in fertilisation process
Ahmed 2018(Ahmed et al., 2018)	The study analyses the influence of extended incubation time on sperm chromatin condensation and DNA strand breaks and their effect on the fertilisation rate, Fertilisation rate in the (Group 1- no extended time)	No influence on fertilisation rates.

	was 62.45% and 69.17% (Group 2-extended incubation).	
de Moura 2017(de Moura et al., 2017)	Randomized trial analysed using diluted hyaluronidase 8IU/mL (n=104) versus normal concentration 80IU/mL(n=88) used for denudation of sibling-oocytes	Diluted group showed better results in fertilisation rates (92.3% vs. 80.6%),
Bartoli 2017(Bartoli et al., 2017)	Study to examine the impact of advanced paternal age (mean 51.0+/-5.7 years), 14.9±7.7 normally fertilized zygotes (60%) observed on day 1 of embryonic development which are significantly lower than non-advanced paternal age male (Control = 82%; p<0.0001)	Fertilisation rates were significantly reduced utilising advanced paternal age sperm
Morin 2017	Retrospective cohort study of a total of 8345 cycles, on linear regression analysis, Fertilization Rate was correlated with Total motile sperm count ($r^2 = 0.13$, regression coefficient: 3.63, p=0.02)	Total motile sperm count is correlated with fertilisation rates in IVF/ICSI cycles
Stanhiser 2017 (Stanhiser et al., 2017) (n= 3,281)	Study compared the fertilisation, implantation, and pregnancy rates following ICSI versus conventional insemination for unexplained infertility, ICSI reduced the probability of pregnancy by	ICSI significantly reduces fertilisation, pregnancy, and implantation rates compared to conventional fertilisation for

	10% (relative risk [RR] 0.90 95% CI 0.82 – 0.99)	couples with unexplained subfertility.
Zheng 2016 (Zheng et al., 2016)	966 cycles with severe oligozoospermia (count $\leq 5 \times 10^6/\text{mL}$ and motile spermatozoa $< 2 \times 10^6/\text{ml}$) analysed	The rates of fertilisation are reduced ($p < 0.001$) with reduced motile spermatozoa in severe oligozoospermia
Lesoine 2016 (Lesoine and Regidor, 2016)	Myoinositol group 136/ 233 oocytes collected were fertilised and 128/300 oocytes in the placebo group were fertilised.	Myoinositol therapy in women with PCOS resulted in better fertilisation rates
Modarres 2016 (Modarres et al., 2016)	The fertilisation rate was 44% in the group requiring surgical sperm extraction and 49% in the Sildenafil only group	No significant improvement in the semen analysis, fertilisation rates or pregnancy outcomes with Sildenafil
Rodriguez-Purata 2016 (Rodriguez-Purata et al., 2016)	Use of ICSI versus conventional IVF in donor sperm, low fertilization rate was higher with ICSI when compared to conventional 9.1% (25/389) vs. 7.9% (35/681) and failed fertilization was also higher with ICSI than in conventional insemination 3.3% (13/389) vs. 2.5% (17/681).	This study shows that fertilisation rates are correlated with patient's age and with the numbers of the oocytes inseminated, but not with the method of insemination.

Clinical and Research implications

Based on very few further publications, the results and conclusion from this publication remain unchanged. The most important addition on this topic was HAB select trial. The trial did not show clear advantage with PICSi in relation to the primary outcome. The trial has some limitations. Embryologists were not blinded, and limited data were available from poorer samples and non-random sample selection in the mechanistic cohort. Prepared rather than raw semen was used for tests of DNA integrity. The interesting finding was PICSi showed a reduced miscarriage risk. Therefore, it opened a new question to re-evaluate PICSi focusing on miscarriage rates and consider aspects of sperm quality that PICSi favours. Assisted oocyte activation and sperm selection using IMSi and PICSi remain widely studied interventions.

5.4 Publication 6: Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review (Jevic et al., 2011).

An updated literature search (as described in methods section and detailed in appendix B) showed following studies that have published after our study

Table 5: Update on studies to analyse ultrasound diagnosis of early miscarriage following our publication

Study	Results	Conclusion
Abuelghar 2013 (Abuelghar et al.)	A CRL of 2 SD or less from that expected for gestational age as a cut-off point had a sensitivity of 56.6, specificity of 81.9, the positive predictive value of 36.6, the negative predictive value of 91.1	Small for gestational age CRL was associated with a higher probability of a miscarriage
Tan 2014 (Tan et al.)	N= 305 with a gestational age of 6 to 9 weeks	The pregnancies with a yolk sac diameter \geq 5 mm had a significantly

		higher risk of miscarriage (p = 0.005).
Wie 2015 (Wie et al.) N= 188 Miscarriage=30 (16.0%)	Fetal heart rate below the 5th percentile (OR, 6.43), MSD below the 5th percentile (OR, 4.87), gestational sac volume below the 5th percentile (OR, 5.25), and yolk sac diameter below the 2.5th or above the 97.5th percentile (OR, 15.86) were significant predictors of miscarriage	A small-for-gestational-age gestational sac volume, fetal bradycardia, a small gestational sac diameter, and a small or large yolk sac diameter are significant sonographic predictor of miscarriage.
Plevakova 2015 (Plevakova et al., 2015)	A retrospective cohort study	No correlation between the volume of gestational sac and the development of the pregnancy
Preisler et al., 2015 (Preisler et al., 2015)	They found mean gestational sac diameter ≥ 25 mm with an empty sac (specificity: 100%), embryo with crown-rump length ≥ 7 mm without visible embryo heart activity (specificity: 100%), mean gestational sac diameter ≥ 18 mm for gestational sacs without an embryo presenting after 70 days' gestation (specificity: 100%), embryo with crown-rump length ≥ 3 mm without visible heart activity presenting after 70 days' gestation (specificity: 100%)	This study concluded, changed the cut-off values of gestational sac and embryo size defining miscarriage are appropriate. They noted that the timing between scans and expected findings on repeat scans are still too liberal.
Gabbay-Benziv et al.,	IVF pregnancies with a live embryo, a	Small CRL $< 10^{\text{th}}$ centile can predict miscarriage

2017(Gabbay-Benziv et al., 2017)	small CRL (CRL \leq tenth percentile for gestational age) at 40-80 days' gestation may predict miscarriage	
Datta 2017 (Datta and Raut) N=800 Miscarriage =140 (17.5%)	CRL, MSD, and fetal heartbeat values below the 5th percentile (odds ratio 26.48, 26.94, and 100.63, respectively), and YSD above the 95th percentile (OR 1.04)	First-trimester ultrasound measurements are predictors of early miscarriage.

Clinical and research implications

Before our publication, various international societies used clear cut off values and parameters to diagnose miscarriage in their guidelines (Bickhaus et al., 2013). Our publication demonstrated that the cut off used were based on poor quality evidence. The academics and professionals widely acclaimed our paper. The royal college of Obstetricians and Gynaecologists (RCOG) accepted the findings and released a statement to welcome the research and to declare amendment in their guideline (RCOG, 2011). Our paper, along with other publications made headlines in national media which attracted more extensive public attention (BBC, 2011, Telegraph, 2011, Mail, 2011, Time, 2011, Medscape, ScienceDaily, 2011). To explain the results to the member of public following media stories NHS released its statement (NHS, 2011).

It is axiomatic that decisions about embryonic viability must not be open to doubt. So it is surprising how little evidence exists to support previous guidance (Bourne and Bottomley, 2012). Review published in the New England Journal of Medicine, Recent research has shown the need to adopt more stringent criteria for the diagnosis of nonviability to minimise or avoid false positive test results.

Regarding the diagnostic criteria for nonviable pregnancy during the 1st trimester, a previous study used the following guideline for EPL: an MSD \geq 25 mm and either no embryo or a CRL \geq 7 mm and no heartbeat (Doubilet et al., 2013). This view is strengthened by the BMJ (the British Medical Journal) editorial on the diagnosis of miscarriage (McCarthy and Tong, 2015). Preisler study added strong evidence to support our suggested cut-off points for CRL (\geq 7 mm) and MSD (\geq 25MM)

measurements. These values are widely used at present. Further research is being focused upon development of predictor models following accuracy of cut off values.

Limitations

Literature search on updates was carried out and analysed by only one researcher, the author. Small population size, single centre studies and unaddressed inter-observer variations are limitations of included studies. Technological advances in ultrasound machines over the period was not addressed.

5.5 Publication 7: Evidence-based management of recurrent miscarriages (Jeve and Davies, 2014a).

A recent scoping literature review showed following studies that have published after our study.

Table 6: Update on the management of recurrent miscarriage following our publication

Study	Results	Conclusion
Iews et al., 2018 (Iews et al., 2018)	A systematic review to study preimplantation genetic diagnosis, the primary outcome was live birth rate (LBR), and a pooled total of 847 couples who conceived naturally had an LBR ranging from 25-71% compared with 26.7-87% among 562 couples who underwent IVF and PGD.	Preimplantation genetic diagnosis does not improve reproductive outcome in couples with recurrent pregnancy loss
Kangatharan et al., 2017 (Kangatharan et al., 2017).	A systematic review and meta-analysis to analyse inter-pregnancy interval (IPI). Sixteen studies (n=1043840) were included. With an IPI of less than 6	Inter-pregnancy interval of fewer than six months following miscarriage is not

	months, the overall risk of further miscarriage (Risk ratio (RR) 0.82 95% CI 0.78-0.86) and preterm delivery (RR 0.79 95% CI 0.75-0.83) were significantly reduced.	associated with adverse outcomes
Saccone et al., 2017 (Saccone et al., 2017)	Pooled data from the ten trials (1,586 women) on use of progesterone. Studies span more than 60 years. Use of progestogens lowers risk of recurrent miscarriage (RR 0.72, 95% CI 0.53-0.97) and higher live birth rate (RR 1.07, 95% CI 1.02-1.15)	Supplementation with progestogens might reduce the incidence of recurrent miscarriages
Santos et al., 2017 (Santos et al., 2017).	A systematic review and meta-analysis anti-phospholipid syndrome the meta-analysis of 9 studies showed association between antiphospholipid antibodies (aPLs) and/or APS compared to the patients with RM (OR: 0.279; 95% CI: 0.212-0.366)	Positive association between antiphospholipid antibodies and antiphospholipid syndrome in patients with recurrent miscarriage
Pils et al., 2016 (Pils et al., 2016)	A study to evaluate AMH and estradiol , Anti-Mullerian hormone and estradiol were significantly lower in women with idiopathic recurrent miscarriage (median 1.2 ng/ml, IQR 0.6-2.1, and median 36.5 pg/ml, IQR 25.8-47.3, respectively) than in women with explained recurrent miscarriage (median 2.0 ng/ml, IQR 1.1-2.7, and median 42.5 pg/ml, IQR 32.8-59.8, respectively; p<0.05).	Idiopathic recurrent miscarriage is associated with lower basal estradiol and anti-Mullerian hormone levels compared to explained recurrent miscarriage

Maraka et al., 2016 (Maraka et al., 2016).	A meta-analysis to investigate use of thyroxine, eighteen cohort studies at low-to-moderate risk of bias were included. Compared with euthyroid pregnant women, pregnant women with Subclinical hypothyroidism were at higher risk for pregnancy loss (relative risk [RR] 2.01 [confidence interval (CI) 1.66-2.44]),	Subclinical hypothyroidism during pregnancy is associated with multiple adverse outcomes, however the value of levothyroxine therapy in preventing these adverse outcomes remains uncertain
Skeith et al., 2016 (Skeith et al., 2016)	A meta-analysis to study inherited thrombophilia, Analysis of Eight trials and 483 patients (relative risk, 0.81; 95% confidence interval, 0.55-1.19;P= .28),	No benefit of LMWH in preventing recurrent pregnancy loss in women with inherited thrombophilia
Grimstad and Krieg 2016 (Grimstad and Krieg, 2016)	A review article , Larger database studies are needed with stricter control criteria before reasonable conclusions can be drawn.	No further evidence to support any treatment in inherited thrombophilia treatment
Zhang et al., 2015 (Zhang et al., 2015).	A meta-analysis to examine the role of LMWH and aspirin. A total of 2391 patients were included in this analysis (OR 1.45 CI 0.84-2.79)	Patients without the antiphospholipid syndrome, the use of combined low molecular weight heparin (LMWH) and aspirin cannot be supported
Egerup et al., 2015 (Egerup et al., 2015).	A meta-analysis on the use of intravenous immunoglobulins in women with recurrent miscarriages. 11 trials analysed, (RR 0.92, 95% CI 0.75-1.12, p = 0.42).	No significant benefit but increase the risk of adverse events
Coomarasamy et al., 2015	A multicentre randomised PROMISE trial on Progesterone treatment,	There is no evidence to support first-trimester

(Coomarasamy et al., 2015)	progesterone used in 404 women and placebo 432 women, (RR 1.04; 95% CI- 0.94 to 1.15)	progesterone therapy to improve outcomes in women with a history of unexplained recurrent miscarriage
Tang et al., 2013 (Tang et al., 2013).	A feasibility trial of screening women with idiopathic recurrent miscarriage for high uterine natural killer cell density and randomising to prednisolone or placebo. The live birth rate was 12/20 (60%) with prednisolone and 8/20 (40%) with placebo (Risk Ratio 1.5, 95% CI 0.79-2.86)	No significant difference
Dhillon-Smith et al. 2019 (Dhillon-Smith et al., 2019)	A double-blind, placebo-controlled (TABLET) trial to investigate whether levothyroxine treatment would increase live-birth rates among euthyroid women who had thyroid peroxidase antibodies and a history of miscarriage or infertility (RR- 0.97; C 0.83 to 1.14, P=0.74)	Levothyroxine in euthyroid women with thyroid peroxidase antibodies did not result in a higher rate of live births

Clinical and research implications

Our publication provides an evidence-based approach managing recurrent miscarriage clinically. The guidance is graded based on the hierarchy of the evidence. Further updates do not change the original recommendations made. Much awaited PROMISE trial did not show any benefit of using progesterone for unexplained recurrent miscarriage (RR 1.04; 95% CI- 0.94 to 1.15). However, this trial has some limitations. The progesterone treatment was started after confirmed pregnancy, and thus this study cannot address whether progesterone supplementation could be more effective in reducing the risk of miscarriage if administered during the luteal phase of the cycle, before the confirmation of pregnancy. The preparation used was vaginal, it is

suggested that intramuscular preparations of progesterone may provide greater therapeutic benefit than vaginal preparations. Treatment of subclinical hypothyroidism, inherited thrombophilia associated with recurrent miscarriage did not show any benefit. Recent TABLET trial showed that the use of levothyroxine in euthyroid women with thyroid peroxidase antibodies did not result in a higher rate of live births than placebo ,(RR-0.97; CI- 0.83 to 1.14, P=0.74). The significant limitation of this trial was inclusion of women with history of miscarriage or infertility, which is a heterogeneous population. Zhang reported no role of low molecular weight heparin (LMWH) and aspirin to reduce the risk of miscarriage in women without antiphospholipid syndrome. Further research is focussed upon idiopathic recurrent miscarriage as it is still a big challenge.

Limitations

This publication was structured to be clinically relevant and, every attempt was made to perform the comprehensive literature search. However, this is not a systematic review and meta-analysis of original studies. We have accepted the evidence-based on the hierarchy of evidence in evidence-based medicine pyramid. This is the major limitation of this publication. The literature search for updates was performed as a scoping search for the individual debated topic and idiopathic recurrent miscarriage. Therefore, we cannot be sure that all relevant literature is being included and evaluated. None the less, this publication and updates provide a clear guidance on the most controversial issues in the management of the recurrent miscarriage and evaluates all major publications.

Chapter 6: Conclusion

The ovary is the critical organ in human reproduction which controls reproductive lifespan, pregnancies, and their outcomes. Prediction of ovarian qualitative and quantitative reserve, number of quality of oocytes remaining in the ovaries, is one of the critical aspects in assisted conception. Age is the significant predictor for the quality of oocytes and reproductive outcome. Serum AMH is widely accepted as the most accurate biochemical marker of ovarian reserve. Age and serum AMH together offer the better prediction. Such prediction is vital for choosing appropriate treatment strategies to optimise the reproductive outcome in assisted conception. Quantity and quality of the oocytes and resultant ovum determines the successful reproductive outcome, a healthy live baby. Poor ovarian reserve markers including low serum AMH may not predict poor outcome in natural fertility. The researchers have trialled various strategies to treat poor responders, but unfortunately, no single strategy has shown a promising success. The ongoing research is focussed on testosterone supplementation to improve the quantity and quality of follicles recruited during ovarian stimulation. Other option to treat the women with low or no ovarian reserve is with the use of donor oocyte assisted conception. Donor oocyte pregnancy is the high risk of many obstetric complications. Therefore, the ovum, whether it is autologous or from the donor, it significantly impacts the reproductive outcome. Poor quality oocytes result in lower fertilisation rates, lower implantation rates and higher miscarriage rates. Accurate diagnosis and management of miscarriage are crucial to optimising the reproductive outcome.

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Appendix A

Statements of contribution signed by all co-authors and copyright permissions as required

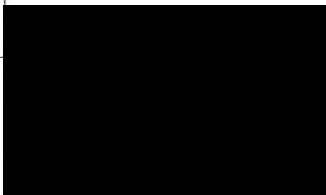
Publication	Authorship statement	Copyright permission
1: The combined use of antimullerian hormone and age to predict the ovarian response to controlled ovarian hyperstimulation in poor responders: A novel approach	Not required as no co-author	Not required as per journal policy
2: Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis	Attached below	Not required as per journal policy
3: Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies	Attached below	
4: Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis	Attached below	
5: Strategies to improve fertilisation rates with assisted conception: a systematic review	Attached below	
6: Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review	Attached below	
7: Evidence-based management of recurrent miscarriages	The co-author is deceased	Not required as per journal policy

Paper to be considered as part of the PhD by Published work:

JEVE, Y. B. & BHANDARI, H. M. 2016. Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis. J Hum Reprod Sci, 9, 70-81.

Contribution of Dr Y B Jeve:

I confirm that Dr Y B Jeve contributed significantly to this manuscript. He suggested the meta-analysis on this topic, designed study protocol, performed literature search, quality analysis and data extraction as the first author. He analysed the data, interpreted results and prepared the manuscript incorporating my comments and suggestions.

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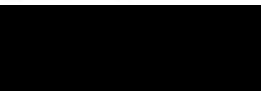
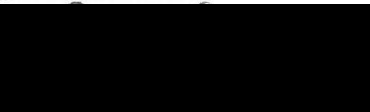
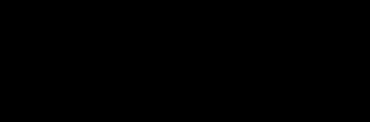
Paper to be considered as part of the PhD by Published work:

Jeve YB, Potdar N, Opoku A, Khare M. Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2016;133(2):156-8.

Contribution of the candidate:

Yadava Jeve as a first author, contributed to this manuscript significantly. He developed study design, data extraction sheet and he collected data from all co-authors. He analysed data and interpreted the results. He drafted the manuscript and incorporating co-authors' comments and feedbacks. He subsequently revised the manuscript following the reviewer's and editorial comments

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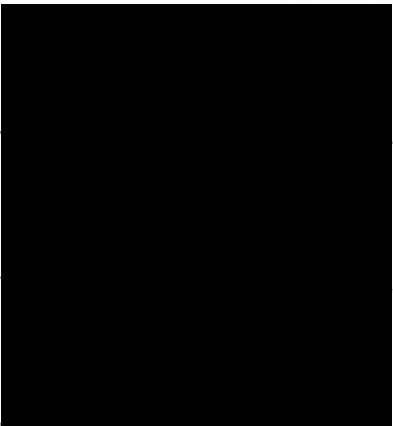
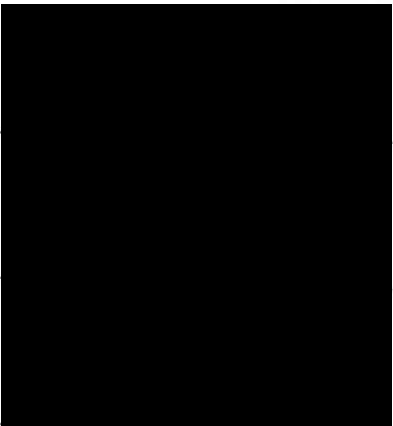
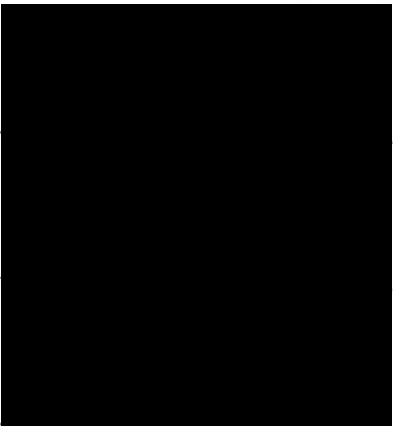
Paper to be considered as part of the PhD by Published work:

Jeve YB, Potdar N, Opoku A, Khare M. Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis. BJOG : an international journal of obstetrics and gynaecology. 2016;123(9):1471-80.

Contribution of the candidate:

Yadava Jeve as a first author, contributed to this manuscript substantially. He performed the literature search, reviews, data extraction and meta-analysis. He prepared the manuscript and addressed all comments and feedbacks by co-authors. He revised the manuscript incorporating co-authors' reviewer's and editorial comments.

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Paper to be considered as part of the PhD by Published work:

JEVE, Y. B., POTDAR, N., BLOWER, J. A. & GELBAYA, T. 2017. Strategies to improve fertilisation rates with assisted conception: a systematic review. Hum Fertil (Camb), 1-19

Contribution of the candidate:

Yadava Jeve as a first author, contributed to this manuscript significantly. He developed study design and searched for literature. He, along with other authors, included studies and analysed quality of evidence. He drafted the manuscript and incorporating co-authors' comments and suggestions. He subsequently revised the manuscript following the reviewer's and editorial comments.

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Paper to be considered as part of the PhD by Published work:

JEVE, Y., RANA, R., BHIDE, A. & THANGARATINAM, S. 2011. Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review. *Ultrasound Obstet Gynecol*, 38, 489-96..

Contribution of the candidate:

Yadava Jeve as a first author, contributed to this manuscript significantly. He developed study design, data extraction sheet and he collected data with help of co-authors. He analysed data and interpreted the results with other authors. He drafted the manuscript and incorporating co-authors' comments and feedbacks. He subsequently revised the manuscript following the reviewer's and editorial comments.

I agree that Yadava Jeve made the aforementioned contribution to the paper.

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Prof. Shakila Thangaratinam		14/06/18
Mr Amar Bhide		04/08/2018
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Title: Strategies to improve fertilisation rates with assisted conception: a systematic review

Author: Yadava Bapurao Jeve, Neelam Potdar, Jane A. Blower, et al

Publication: HUMAN FERTILITY

Publisher: Taylor & Francis

Date: May 26, 2017

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Licensed Content Author	Y. Jeve, R. Rana, A. Bhide, et al
Licensed Content Date	Oct 13, 2011
Licensed Content Volume	38
Licensed Content Issue	5
Licensed Content Pages	8
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Appendix B:

Literature search strategy and inclusion of studies in updated reviews

1. **Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis**

Database: AMED (Allied and Complementary Medicine) <1985 to September 2018>, Books@Ovid <September 10, 2018>, Full text journals from OVID, PsycARTICLES Full Text, Embase Classic+Embase <1947 to 2018 September 14>, International Political Science Abstract <1989 to June 2018>, Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to September 14, 2018>, PsycINFO <1806 to September Week 2 2018>, PsycTESTS <1910 to September 2018>

Search Strategy:

- 1 (poor responders* or Diminished ovarian response* or low Responders* or inadequate ovarian response* or low ovarian reserve*).mp. [mp=ab, hw, ti, tx, bt, ct, tn, ot, dm, mf, dv, kw, fx, dq, an, jn, tt, nm, kf, px, rx, ui, sy, tc, id, tm, td] (8987)
- 2 limit 1 to human [Limit not valid in AMED,Books@Ovid,Your Journals@Ovid,Journals@Ovid,International Political Science Abstract; records were retained] (6965)
- 3 limit 2 to yr="2015 -Current" (1590)
- 4 (Low AMH* or low anti-mullerian hormone* or the Bologna Criteria*).mp. [mp=ab, hw, ti, tx, bt, ct, tn, ot, dm, mf, dv, kw, fx, dq, an, jn, tt, nm, kf, px, rx, ui, sy, tc, id, tm, td] (705)
- 5 limit 4 to human [Limit not valid in AMED,Books@Ovid,Your Journals@Ovid,Journals@Ovid,International Political Science Abstract; records were retained] (604)
- 6 limit 5 to yr="2015 -Current" (336)
- 7 3 or 6 (1843)

- 8 (IVF* or Assisted conception* or ICSI* or In-vitro fertilization*).mp. [mp=ab, hw, ti, tx, bt, ct, tn, ot, dm, mf, dv, kw, fx, dq, an, jn, tt, nm, kf, px, rx, ui, sy, tc, id, tm, td] (96142)
- 9 limit 8 to human [Limit not valid in AMED,Books@Ovid,Your Journals@Ovid,Journals@Ovid,International Political Science Abstract; records were retained] (77484)
- 10 limit 9 to yr="2015 -Current" (16578)
- 11 7 and 10 (527)
- 12 remove duplicates from 11 (384)
- 13 limit 12 to (clinical trial or randomized controlled trial or controlled clinical trial or multicenter study) [Limit not valid in AMED,Books@Ovid,Your Journals@Ovid,Journals@Ovid,International Political Science Abstract,PsycINFO,PsycTESTS; records were retained] (99)
- 14 limit 13 to humans [Limit not valid in AMED,Books@Ovid,Your Journals@Ovid,Journals@Ovid,International Political Science Abstract,PsycINFO,PsycTESTS; records were retained] (99)
- 15 limit 14 to yr="2015 -Current" (99)

Included 21 citations after screening full text.

Reasons for exclusions include: non randomized trials, absence of appropriate outcome measure, poor description of study design.

2. Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis

Database: AMED (Allied and Complementary Medicine) <1985 to September 2018>, Books@Ovid <September 10, 2018>, Full text journals from OVID, PsycARTICLES Full Text, Embase Classic+Embase <1947 to 2018 September 14>, International Political Science Abstract <1989 to June 2018>, Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to September 14, 2018>, PsycINFO <1806 to September Week 2 2018>, PsycTESTS <1910 to September 2018>

Search Strategy:

- 1 (oocyte* or ovum* or ova* or egg*).mp. [mp=ab, hw, ti, tx, bt, ct, tn, ot, dm, mf, dv, kw, fx, dq, an, jn, tt, nm, kf, px, rx, ui, sy, tc, id, tm, td] (1264648)
- 2 limit 1 to human [Limit not valid in AMED,Books@Ovid,Your Journals@Ovid,Journals@Ovid,International Political Science Abstract; records were retained] (630493)
- 3 limit 2 to yr="2016 - 2018" (76311)
- 4 (Assisted Conception* or IVF* or ICSI).mp. [mp=ab, hw, ti, tx, bt, ct, tn, ot, dm, mf, dv, kw, fx, dq, an, jn, tt, nm, kf, px, rx, ui, sy, tc, id, tm, td] (73844)
- 5 limit 4 to yr="2016 - 2018" (12062)
- 6 Donor*.mp. [mp=ab, hw, ti, tx, bt, ct, tn, ot, dm, mf, dv, kw, fx, dq, an, jn, tt, nm, kf, px, rx, ui, sy, tc, id, tm, td] (774325)
- 7 3 and 6 (1767)
- 8 4 and 7 (588)
- 9 remove duplicates from 8 (479)
- 10 ((Obstetric* or Pregnancy*) and Complications*).mp. [mp=ab, hw, ti, tx, bt, ct, tn, ot, dm, mf, dv, kw, fx, dq, an, jn, tt, nm, kf, px, rx, ui, sy, tc, id, tm, td] (328613)
- 11 limit 10 to yr="2016 - 2018" (30841)
- 12 9 and 11 (33)

Full text screened 13

Studies included 5

Reasons for exclusion- absence of comparison group, absence of well-defined obstetric complications and review articles.

3. **Strategies to improve fertilisation rates with assisted conception: a systematic review** Database: Embase <1974 to 2018 September 14>, Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily <1946 to September 14, 2018>

Search Strategy:

- 1 (IVF* or Assisted conception* or ICSI* or In-vitro fertilization*).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, an, ui, sy] (93000)
- 2 limit 1 to human (74502)
- 3 limit 2 to yr="2016 -Current" (11521)
- 4 ((Fertilisation* or Fertilization*) and rate*).m_titl. (2148)
- 5 limit 4 to human (1602)
- 6 limit 5 to yr="2016 -Current" (156)
- 7 3 and 6 (148)
- 8 remove duplicates from 7 (113)

Title screened 113

Abstracts screened 20

Studies included 13.

4. Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review

Database: Embase <1980 to 2018 Week 38>, Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily <1946 to September 14, 2018>

Search Strategy:

- 1 (ultrasound* or ultrasonography* or ultrasound or Scan* or Sonography*).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, an, ui, sy] (2205961)
- 2 (miscarriage* or abortion* or pregnancy loss*).mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, fx, dq, nm, kf, px, rx, an, ui, sy] (195503)
- 3 limit 2 to human (160319)
- 4 1 and 3 (13894)
- 5 limit 4 to "diagnosis (best balance of sensitivity and specificity)" (2049)
- 6 limit 5 to human (2049)
- 7 limit 6 to embryo <first trimester> [Limit not valid in Ovid MEDLINE(R),Ovid MEDLINE(R) Daily Update,Ovid MEDLINE(R) In-Process,Ovid MEDLINE(R) Publisher; records were retained] (750)
- 8 limit 7 to English (676)

9 limit 8 to (clinical study or clinical trial, all or comparative study or observational study) [Limit not valid in Embase; records were retained] (186)

10 limit 9 to yr="2012 -Current" (54)

11 limit 4 to "diagnosis (best balance of sensitivity and specificity)" (2049)

12 limit 11 to human (2049)

13 limit 12 to embryo <first trimester> [Limit not valid in Ovid MEDLINE(R),Ovid MEDLINE(R) Daily Update,Ovid MEDLINE(R) In-Process,Ovid MEDLINE(R) Publisher; records were retained] (750)

14 limit 13 to English (676)

15 limit 14 to (clinical study or clinical trial, all or comparative study or observational study) [Limit not valid in Embase; records were retained] (186)

16 limit 15 to yr="2012 -Current" (54)

17 remove duplicates from 16 (53)

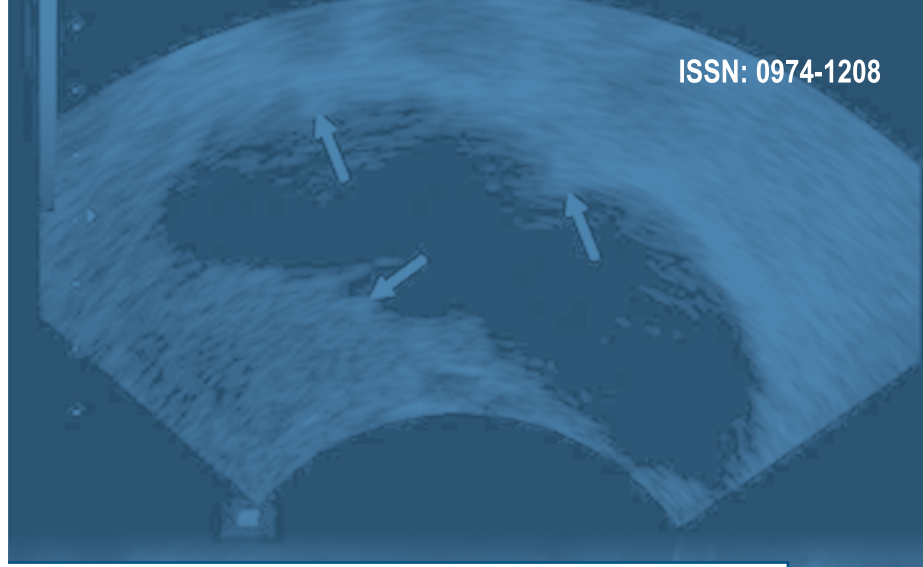
Title screened 53, Abstracts screened 9

Included: 7, Reason for exclusion: reviews 2

Appendix C:

Submitted Publications:

1. The combined use of antimullerian hormone and age to predict the ovarian response to controlled ovarian hyperstimulation in poor responders: A novel approach J Hum Reprod Sci. 2013 Oct;6(4):259-62.
2. Effective treatment protocol for poor ovarian response: A systematic review and meta analysis J Hum Reprod Sci. 2016 Apr-Jun;9(2):70-81
3. Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies Int J Gynaecol Obstet. 2016 May; 133(2):156-8.
4. Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis BJOG. 2016 Aug;123(9):1471-80
5. Strategies to improve fertilisation rates with assisted conception: a systematic review Hum Fertil (Camb). 2017 May 26:1-19
6. Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review Ultrasound Obstet Gynecol. 2011 Nov;38(5):489-96
7. Evidence based management of recurrent miscarriages J Hum Reprod Sci. 2014 Jul;7(3):159-69.

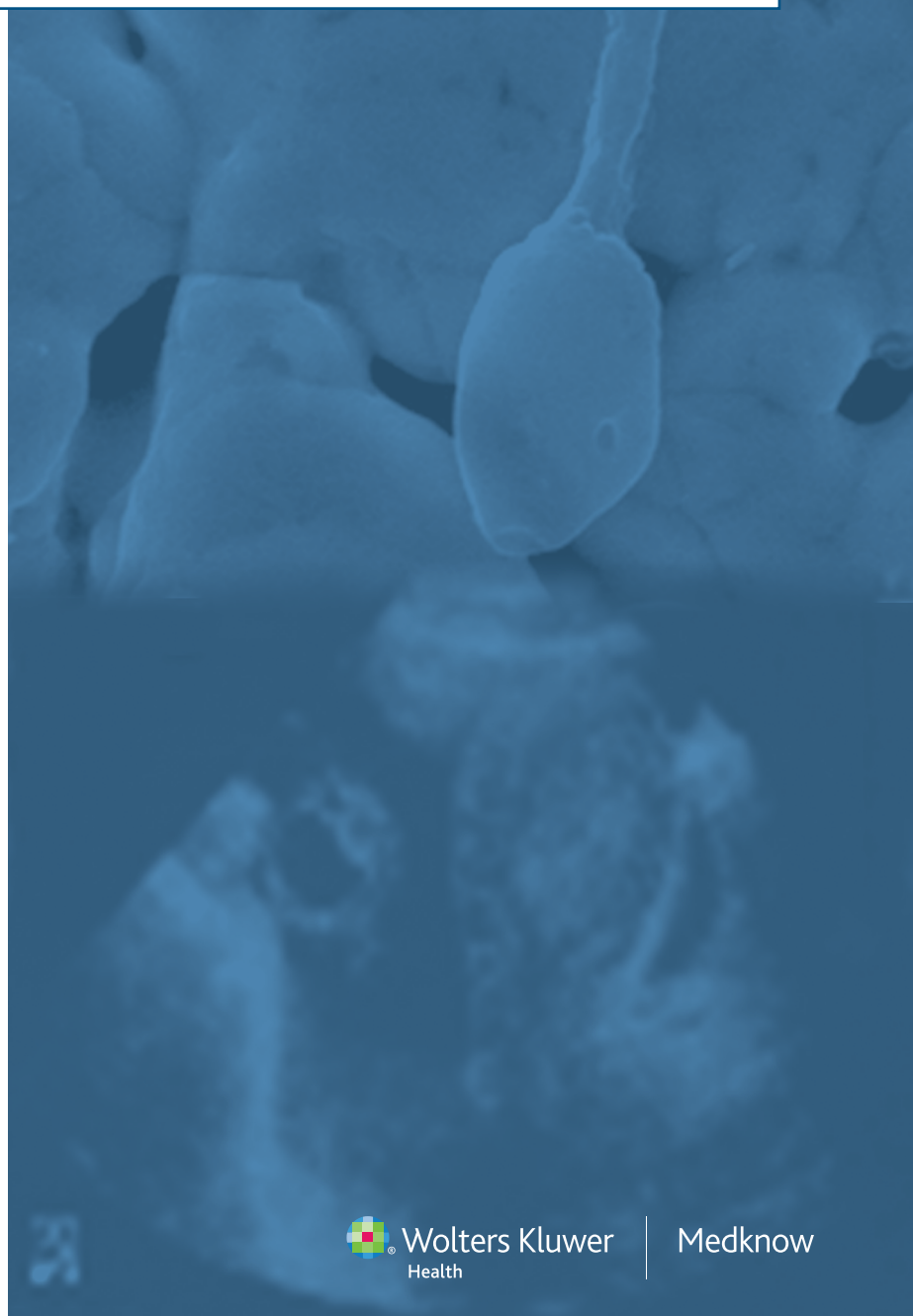


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The combined use of antimullerian hormone and age to predict the ovarian response to controlled ovarian hyperstimulation in poor responders: A novel approach

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ABSTRACT

CONTEXT: Reduced ovarian response to stimulation represents one of the most intractable problems in infertility treatment. As failed cycle can cause considerable amount of emotional and economical loss, there are various attempts made to predict ovarian response. **AIMS:** To evaluate different factors influencing outcome of assisted reproduction in women with predicted reduced response (antimullerian hormone between 1 and 5 pmol/L) and to develop a model using of AMH and age to predict the number of oocytes in poor responders. **SETTINGS AND DESIGN:** Retrospective study in a teaching hospital. **MATERIALS AND METHODS:** We analyzed 85 cycles (57 women) with predicted reduced response with serum AMH value between 1 and 5 pmol/L. Standard ovarian stimulation protocol was used. Primary outcome measures were clinical pregnancy rates and oocytes retrieved. **STATISTICAL ANALYSIS USED:** Data were analyzed using Microsoft excel and MatlabR software. **RESULTS:** Clinical pregnancy rate/ET was 20.33%, in this group. AMH and age was analyzed using linear regression model which produced an equation to give predicted oocyte count if AMH and age are known. (Oocytes = age \times (- β) + Serum AMH \times α) (Constant $\beta=0.0102$ and $\alpha = 1.0407$). **CONCLUSIONS:** Combined use of serum AMH and age to predict ovarian response within reduced responder group should be further evaluated. For first time, we suggested combining both factors to predict ovarian response using a simple equation which allow developing tailored strategy.

KEY WORDS: Ovarian hyperstimulation, poor responders, serum antimullerian hormone

INTRODUCTION

Failed-assisted conception cycle causes considerable amount of emotional and economical loss. There are various attempts made to predict reduced response. Evidence has shown convincingly that poor ovarian response is a first sign of ovarian ageing.^[1] The Bologna criteria^[2] define the poor response as presence of two or more features (i) advanced maternal age or any other risk factor for poor ovarian response; (ii) a previous poor ovarian response; and (iii) an abnormal ovarian reserve test. Two episodes of poor ovarian response after maximal stimulation deemed sufficient to define a patient as poor responder in the absence of other criteria. Ovarian ageing can occur independently of chronological age.^[3] AMH suggested being

a better marker of ovarian responsiveness, as it reflects the size of the larger resting pool of prefollicle-stimulating hormone (FSH)-dependent follicles. AMH is a more direct and independent measure of the growing preantral and antral follicular pool.^[4-6] Thus age and serum AMH are the most successful predictors of reduced ovarian response. Our aim was to evaluate the role of age and AMH influencing outcome of assisted reproduction in women with predicted reduced response (AMH between 1 and 5 pmol/L) and to develop a model using of AMH and age together to predict the number of oocytes in poor responders. Such model will help clinicians to predict the response and design appropriate protocol. It will also help in patient counselling and advice on alternative management options.

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MATERIALS AND METHODS

We have analyzed 85 cycles undergoing controlled ovarian stimulation for IVF or ICSI cycle. These women were treated for either primary or secondary infertility at teaching hospital. All women included were predicted reduced responders with serum AMH value between 1 and 5 pmol/L. We defined reduced responders based on AMH level between 1 and 5 pmol/L based on previous study by.^[7] There was no age cut off. Population was from different ethnic background. We have excluded all cases with AMH 5 pmol/L and above as they proved to have normal response and different strategies were used in these cases. We have excluded women with AMH of less than 1 pmol/L, as they demonstrated to have maximum cycle cancellation and no pregnancy occurred in this group. Ovarian stimulation was performed with exogenous gonadotropins initiated on the third or fourth cycle day in the form of either Menogon (Ferring Pharmaceuticals, Langley, UK) or Gonal-F (Serono, Feltham, UK). The starting daily dose of FSH was either 225 or 300 IU each day. Ovarian follicular responses were monitored with serum E2 concentrations and transvaginal ultrasound assessment of follicular growth. The GnRH antagonist Cetrotide 0.25 mg/day s.c. (Merck Serono Feltham, U.K.) or Ganirelix was commenced on days 4-7 if serum E2 exceeded 200 pg/mL. Follicular responses were monitored with serum E2 and transvaginal ultrasound assessment of follicular growth. Ovulation was induced with 6500 IU HCG (Ovitrelle, Serono, Feltham, UK), provided that three follicles were 17 mm in diameter and serum E2 was 200 pg/ml. Trans-vaginal oocyte retrieval was performed under ultrasound guidance 38 h after HCG administration and the number of oocytes retrieved were recorded. Women were either offered IVF-ET or ICSI-ET. The study analysis includes only fresh cycles.

Primary outcome studied was clinical pregnancy rates and secondary outcomes were positive pregnancy rate, oocytes retrieved, and cancellation rate, duration of stimulation, and FSH drug consumption.

The AMH assay was performed in batches one month before treatment cycle using the enzyme-linked immunosorbent assay provided by DSL in study period 2011 (Webster, Texas, USA), with values presented as pmol/L (conversion factor to pmol/L $\frac{1}{4}$ ng/mL \times 7.143). The Reduced responders are defined as predicted with low serum AMH (serum AMH between 1 and 5 pmol/L) and cycle cancellation is defined as less than two matured sized follicles after 2 weeks of stimulation with maximum dose of gonadotropins. Embryo cryopreservation was done with more than one high grade embryo remained after fresh embryo transfer. Data were analyzed using Microsoft excel and MetlabR software.^[8,9] Data were presented in descriptive statistic. The factors influencing outcome of assisted conception were analysed using linear

regression model. A simple matrix formula was derived to calculate predictive response when these factors are known.

RESULTS

The baseline demographic characteristics and outcome are shown in Table 1. The AMH level was ranging from 1.1 to 4.9 as per inclusion criteria. Among 44 eggs retrieved, two failed to fertilize in IVF cycle. A total of 29 (34.1%) cycles were treated by ICSI. Thus, fertilization rate was 91.6% in ICSI cycles. Duration of stimulation was from 4-14 days and from 675 IU to 5100 IU FSH was used. Out of 85 cycles started, eight (9.41%) were cancelled due to poor response. A total of 59 embryos were transferred; two embryos were frozen along with ET in those women. All cancelled cycles are shown in Table 2. Clinical pregnancy was achieved in 12 cases. Characteristics are discussed in Table 3.

Thus, there was no significant difference between these groups. Lower AMH value showed trend toward cancellation and higher AMH value showed trend toward clinical pregnancy in this group. We have analyzed age and AMH, using linear regression model.

The factors Age and AMH were put in linear regression model to produce a solution.

Table 1: Demographic characters

Number of patients cycles	85
Age at stimulation (years)	39 (31-46)
BMI (kg/m ²)	24.7±4.6
AMH (pmol/L)	2.9 (1.1-4.9)
Procedure	
IVF (%)	56 (65.9)
ICSI (%)	29 (34.1)
Length of stimulation (days)	10 (4-14)
Total dose (IU)	2100 (675-5100)
Cancelled cycles **	8 (9.41%)
Number of oocytes	3 (0-15)
Number of embryos transferred	59
Normal fertilization rate %	
IVF (%)	37 (94.8)
ICSI (%)	22 (91.6)
Cohort outcomes	
No transfer ^b	26 (30.5%)
Not pregnant	42 (49.4%)
Positive pregnancy test ^d	17 (20%)
Clinical pregnancy	12 (14.1%)
Clinical pregnancy per OR	16%
Clinical pregnancy per ET	20.33%
Clinical pregnancy per cycle	14.11%

Values are presented as median (interquartile range) or mean±standard deviation

**Percentage per cycle started, (n=85). ^aIncludes women with cancelled cycle; no eggs or failed fertilisation; ^bIncludes clinical and biochemical pregnancy. AMH=Antimullerian hormone; BMI=Body mass index; ET= Embryo transfer; IU=International units; ICSI=Intra-cytoplasmic sperm injection; IVF= *In vitro* fertilization; OR=Odds ratio

Table 2: Cancelled cycle and all cycles characters

Characteristics	All	Cancelled cycles
Total	85	8
Age	39 (31-46)	39.9 (35-45)
BMI	24.7±4.6	25.9±4.6
Amh	2.9 (1.1-4.9)	2.2 (1.1-3.3)
Total dose of FSH	2100 (675-5100)	1162.5 (675-4200)
Duration of FSH	10 (4-14)	7.5 (7-10)

BMI=Body mass index; FSH=Follicle-stimulating hormone

Table 3: Clinical pregnancy and all cycle characteristics

Characteristics	All	Clinical pregnancy
Total	85	12
Age	39 (31-46)	37 (35-39)
BMI	24.7±4.6	24.5±6
AMH	2.9 (1.0-4.9)	3.15 (1.2-4.7)
Total dose of FSH	2100 (675-5100)	2287.5 (1350-3600)
Duration of FSH	10 (4-14)	10 (8-14)
Number of oocyte retrieved	3 (0-15)	4 (2-8)

AMH=Antimullerian hormone; BMI=Body mass index; FSH=Follicle-stimulating hormone

$$x^* = \begin{pmatrix} \text{age} \\ \text{AMH} \end{pmatrix}$$

$$y = \text{oocytes}$$

$$y = \beta^T X$$

Where, $\beta = \begin{pmatrix} \beta_1 \\ \beta_2 \\ \beta_3 \end{pmatrix}$ we used the dataset to estimate β .

The solution is $\hat{\beta} = YX^T (XX^T)^{-1}$ where X is a 2×85 matrix and Y is a 1×85 matrix.

$$\text{Finally we got } \hat{\beta} = \begin{pmatrix} -0.0102 \\ 1.0407 \end{pmatrix}$$

Thus with this model when age and AMH is known (x^*), oocytes yield can be predicted by formula: $y^* = \hat{\beta}^T x^*$.

For example, woman with age 33 years and AMH value 2, the predicted response (oocyte yield) can be calculated as

$$x^* = \begin{pmatrix} 33 \\ 2 \end{pmatrix} \text{ and } \hat{\beta} = \begin{pmatrix} -0.0102 \\ 1.0407 \end{pmatrix}$$

$$\text{oocytes} = y^* = \hat{\beta}^T x^*$$

$$\text{so oocytes} = 33 \times (-0.0102) + 2 \times 1.0407$$

Thus this model, with use of simple calculator, gives approximate numbers of oocytes number two or three.

DISCUSSION

The evaluation and tailored treatment strategy for poor responder group is important to improve overall pregnancy rates. Unfortunately, lack of uniform criteria to define this group, lack of evidence to predict outcome in this particular group, and debated treatment strategy are major influencing

factors on outcome. This group of women are usually with older age and more anxious to get pregnant than younger age group. Previous failed treatment cycles add in frustration of couple and clinician. Predicting response allows tailoring individual treatment strategy. This approach was suggested by.^[7] We have further extended it for poor responder group. Our results show increasing AMH value is associated with increasing oocyte yield in this reduced responders group which is consistent with previous study.^[7] Our results show that combining two strong predictors allow clinicians to develop a model for predicting ovarian response. This will help clinicians to counsel patient, design protocol for optimal outcome. Reduced responders with predicted poor response using various factors including age and AMH will allow opting for natural cycle IVF. Other options like oocyte donation, adoption, could be discussed with couple without subjecting ovarian stimulation. At same time predicted reasonable response will allow to go ahead with antagonist and stimulation protocols. Systematic review supported antagonist protocol in this group Pandian and Marci *et al.*^[10,11] Hence, prediction of response is significant.

Age is always labelled as significant factor affecting fertility^[12,13] but biological aging and ovarian aging are not always same.^[3] Various attempts to develop ideal predictive test for ovarian reserve were failed.^[14] At same time, AMH emerged as a promising option for predicting both oocyte number and quality.^[15] AMH showed definitive advantages over other bio markers.^[5,6] It has linear relationship with age.^[4] Previous studies clearly established linear relationship between AMH and oocyte yield using AMH and age to develop a linear regression model for oocyte yield is a justified approach based on past evidence. The accuracy of multivariate models for the prediction of ovarian reserve and pregnancy in women undergoing IVF compared with the antral follicle count (AFC) as single test was reviewed by Verhagen *et al.*^[16] He reviewed age + FSH, age + inhibin B, age + AFC and different combinations for predictive model. He concluded the use of more than one single test for the assessment of ovarian reserve cannot be supported. Thus, the models incorporating inhibin B, FSH, ovarian volume, clomiphene challenge test, and so on failed to deliver successful prediction. Further evidence is required to support the multivariate model of AMH and age to predict ovarian response.

Other factors like body mass index, procedure, previous pregnancy, and duration of pregnancy were analyzed but failed to establish any significant relationship. There are various factors suggested to be significant predictors of clinical pregnancy such as age, serum E (2) concentration on the day of hCG administration, embryo quality, and number of embryos transferred sperm motility and ICSI operator.^[17] It is practically impossible to establish relationship between all factors at a time. Hence, we considered most significant

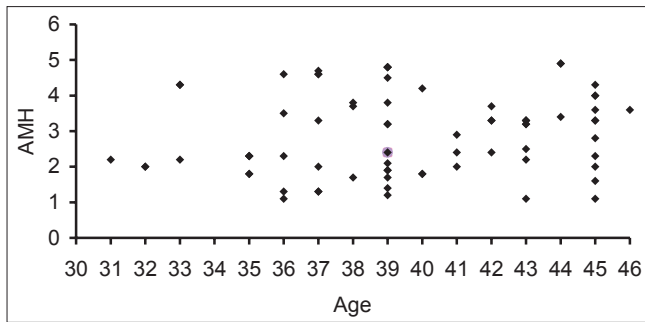


Figure 1: Age and AMH relationship

factors particularly affecting subgroup of women labelled as reduced responders and analysed to get a user friendly predictive model.

This study evaluated the relationship between two important factors, age, and AMH, influencing outcome of assisted reproduction in reduced responder group. The relationship between age and AMH is shown in Figure 1. As figure shows the value of AMH is not same within same age group. Hence it is crucial to have both predictors taken into the consideration whilst predicting ovarian response. This figure illustrates the importance of having both predictors that is age and AMH calculated together to predict more accurate response. All previous studies analyzing predictive potential were done in normal, reduced, and high responders with many variables at same time. In present study, we analyzed combined predictive potential of AMH and age affecting outcomes. This study has relatively small population size and it is a major limitation. There is a need to focus on this particular group of women to improve outcome without adding any financial burden or emotional stress. This can be achieved only with good prediction of response and individualizing therapy accordingly. Current evidence favors antagonist approach to treat reduced responder group. Current evidence supports AMH and age as good predictors of ovarian response over any other single or multiple available predictors. We have tried to introduce new approach of combining both factors using a simple equation. We accept the limitation of having small number of study subjects to draw any conclusions based on this study alone. Hence, further studies are required to establish strong evidence before recommending combination of age and AMH prediction model and consequent individualized treatment strategy in practice.

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Effective treatment protocol for poor ovarian response: A systematic review and meta-analysis

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ABSTRACT

Poor ovarian response represents an increasingly common problem. This systematic review was aimed to identify the most effective treatment protocol for poor response. We searched MEDLINE, EMBASE, and The Cochrane Library from 1980 to October 2015. Study quality assessment and meta-analyses were performed according to the Cochrane recommendations. We found 61 trials including 4997 cycles employing 10 management strategies. Most common strategy was the use of gonadotropin-releasing hormone antagonist (GnRHant), and was compared with GnRH agonist protocol (17 trials; $n = 1696$) for pituitary down-regulation which showed no significant difference in the outcome. Luteinizing hormone supplementation (eight trials, $n = 847$) showed no difference in the outcome. Growth hormone supplementation (seven trials; $n = 251$) showed significant improvement in clinical pregnancy rate (CPR) and live birth rate (LBR) with an odds ratio (OR) of 2.13 (95% CI 1.06–4.28) and 2.96 (95% CI 1.17–7.52). Testosterone supplementation (three trials; $n = 225$) significantly improved CPR (OR 2.4; 95% CI 1.16–5.04) and LBR (OR 2.18; 95% CI 1.01–4.68). Aromatase inhibitors (four trials; $n = 223$) and dehydroepiandrosterone supplementation (two trials; $n = 57$) had no effect on outcome.

KEY WORDS: Assisted conception, *in vitro* fertilization, ovarian stimulation, poor ovarian response

INTRODUCTION

Poor ovarian response (POR) is a challenging situation in assisted reproduction. There is a lack of consensus on the definition of POR and a huge variation in treating women with previous POR.^[1] However, the most common criterion to diagnose POR is retrieval of low number of oocytes despite adequate ovarian stimulation in an assisted conception cycle. The ESHRE working group on POR definition (the Bologna criteria) reached a consensus on the minimal criteria needed to define POR by the presence of two of the following three features: (i) Advanced maternal age (≥ 40 years) or any other risk factor for POR; (ii) a previous characterized POR cycle (≤ 3 oocytes with a conventional stimulation protocol); (iii) an abnormal ovarian reserve test (antral follicle count $< 5-7$ follicles or anti-Mullerian hormone (AMH) $< 0.5-1.1$ ng/ml).^[2] It was also proposed by the working group that two episodes of poor ovarian response after maximum stimulation deemed sufficient to define a patient as POR in the absence of other criteria. The

suggested incidence of POR ranges from 9% to 25%.^[3] Various controlled ovarian hyperstimulation protocols and strategies have been used in this group of women to improve reproductive outcome, but the success rate still remains low.

To date, there are various observational studies, randomized controlled trials (RCTs), and systematic reviews reported on this subject.^[4-9] However, either the studies are too specific by trying to address only one treatment strategy,^[4,7-10] or they include observational studies and nonrandomized studies in their meta-analysis.^[9] The aim of

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our systematic review is to appraise all the existing protocols applied to poor responders by including evidence generated from RCTs.

METHODS

The review was formulated using population, intervention, comparison, outcome, and design structure. Poor responders to ovarian stimulation formed the study population. All types of intervention subjected to RCTs were included in the review. The interventions were analyzed and compared with the control group used in the study. Two or more trials with identical design and interventions were analyzed by meta-analysis. Our outcome measures were number of oocytes retrieved per cycle, live birth rates (LBR), and clinical pregnancy rates (CPR).

We searched the literature on MEDLINE (1980-October 2015), EMBASE (1980-October 2015), and The Cochrane Library (2015) for relevant citations using the keywords, "poor responders, controlled ovarian hyperstimulation, reduced ovarian response, diminished ovarian response, low AMH, assisted conception, and *in vitro* fertilization (IVF)." The reference lists of all known primary and review articles were examined to identify cited articles not captured by the electronic searches. Language restrictions were not applied. A systematic search for all RCTs was carried out. Reference lists from retrieved articles and related articles were checked for relevant studies. All studies addressing the research question and satisfying our inclusion criteria were included in the review. The review protocol was registered with the PROSPERO Registry (CRD42013004190).

Data collection and analysis

The electronic searches were scrutinized, and full manuscripts of all citations that were likely to meet the predefined selection criteria were obtained. Two review authors (Yadava Bapurao Jeve and Harish Malappa Bhandari) independently assessed trial quality and extracted data. Studies which met the predefined and explicit criteria regarding population, interventions, comparison, outcomes, and study design were selected for inclusion in this review. When discrepancies occurred, they were resolved by consensus (Yadava Bapurao Jeve and Harish Malappa Bhandari). We performed meta-analysis when two or more trials were comparable in design and protocol. Data were analyzed using Review Manager (RevMan) [Computer program]. Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014. For each study, the treatment effect was measured with an odds ratio (OR) for dichotomous outcomes and mean differences for continuous outcomes and random effect models that were presented with their corresponding 95% confidence intervals (CI).

Inclusion criteria

Only RCTs that used suitable definition for POR and used different therapeutic approaches for ovarian stimulation of poor responders in assisted conception were included in the study. The trials reported after publication of the Bologna criteria for poor responders were analyzed as per this criteria.^[2]

Exclusion criteria

All observational studies or quasi-randomized studies and studies in which poor responders were not defined were excluded from the study.

Intervention groups

The interventions were grouped as below:

1. Gonadotropin-releasing hormone antagonist (GnRHant) protocols
2. Protocols using luteinizing hormone (LH) as an adjuvant
3. Protocols using growth hormone (GH) as an adjuvant
4. Protocols using transdermal testosterone as an adjuvant
5. Protocols using aromatase inhibitors as an adjuvant
6. Protocols using dehydroepiandrosterone (DHEA) as an adjuvant
7. Protocols using recombinant human chorionic gonadotropin as an adjuvant
8. Natural cycle
9. Protocols using various other adjuvants
10. Various modifications to GnRH agonist (GnRHa) protocol.

Types of outcome measures

To bring uniformity in assessment, we analyzed the most relevant primary outcomes of LBR and CPR per cycle. The secondary outcome measure was the number of oocytes retrieved per cycle.

Quality and risk of bias of included studies

We included only RCTs in this systematic review – some were blinded and/or placebo-controlled, but others were not. Quality analysis was performed using internationally accepted Cochrane tools. GRADEpro. [Computer program on www.gradepr.org]. Version [2014]. McMaster University, 2014, was used to produce a summary of findings, tables for meta-analysis; this shows significant effects with interventions. A risk of bias table was produced using Review Manager (RevMan) [Computer program]. Version 5.1. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014, and is summarized in Figure 1. Using these tools, we have classified overall quality of evidence as moderate to high grade.

RESULTS

A total of 61 RCTs (4997 assisted conception cycles) were included in this study. The treatment approaches were

categorized into 10 groups (as mentioned above), the most common being the use of GnRHant versus GnRHa for pituitary downregulation in 17 RCTs. The characteristics of the included studies are described in Table 1.

1. GnRHa versus GnRHant for pituitary downregulation: Seventeen RCTs ($n = 1696$) that met the criteria were

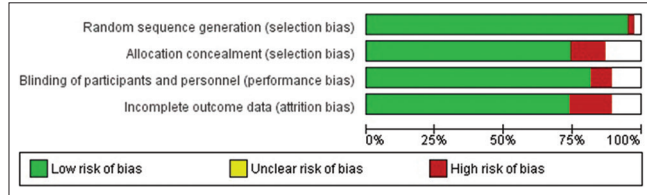


Figure 1: Methodological quality graph

subjected to meta-analysis [Figure 2]. The results suggested no significant difference in the number of oocytes retrieved (mean difference 0.09; 95% CI 0.53–0.36) and no difference in CPR with an OR of 1.24 (95% CI 0.88–1.73)

2. LH supplementation: Eight RCTs ($n = 847$) assessed the role of supplementation to ovarian hyperstimulation but found no difference in CPR (OR 1.32; 95% CI 0.93–1.87)

3. GH supplementation: None of the seven RCTs ($n = 251$) individually had shown benefit of GH supplementation in improving CPR, but the pooled data from these studies showed a significant improvement in CPR (OR 2.13; 95% CI 1.06–4.28). Of these, only four studies ($n = 27$) reported LBR and the pooled data showed

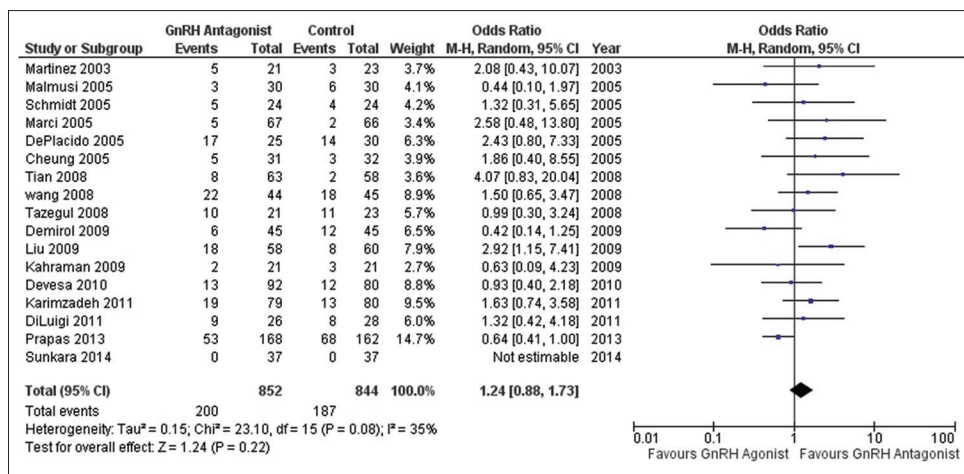


Figure 2: Gonadotropin-releasing hormone agonist (control) versus GnRH antagonist down-regulation protocols

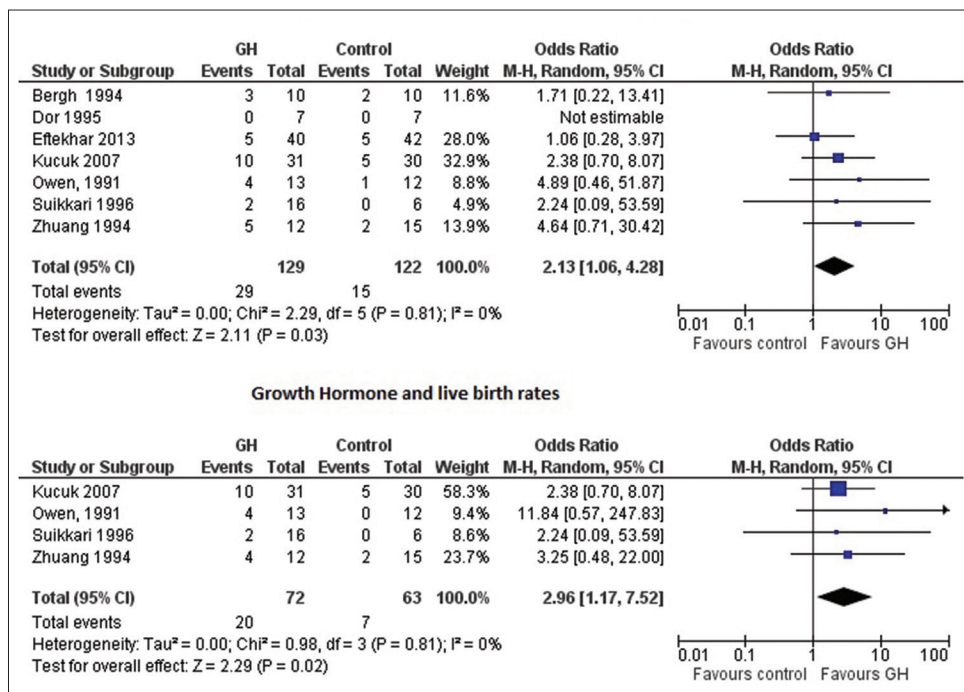


Figure 3: Use of growth hormone supplement

Table 1: Different therapeutic approaches for poor responders

Study	Population	Intervention	Comparison	Outcome	Remark
GnRHant protocols					
Martinez (2003)	n=44 (21 and 23)	Multiple dose (cetorelix)	Short, multiple dose (triptorelin)	Number of oocytes 3.7 versus 5.6 CPR 5 versus 3	GnRHant and short, multiple GnRHa
Cheung (2005)	n=66 (31 and 32)	GnRHant protocol- cetorelix 0.25 mg daily as a fixed protocol starting on day 6 of the stimulation until the day of hCG	GnRHa - Buserelin acetate nasal spray dose of 600 µg from mid-luteal phase, and co-administered with the final week of OCP pretreatment	Number of oocytes 5.89 versus 5.62 CPR 5 versus 3	Unblinded study: Fixed, multi-dose GnRHant versus - a long GnRHa protocol
Marci (2005)	n=60 (30 each)	GnRHa long protocol	GnRHant	Number of oocytes 4.3 versus 5.6 CPR 5 versus 2	Unblinded study: GnRHant versus GnRHa long protocol
Schmidt (2005)	n=48 (24 each)	Ganirelix acetate group (received 300 IU of recombinant FSH s.c.+150 IU HMG for 5 days)	21 days of OCP and the microdose leuprolide flare group	Number of oocytes 8.9 versus 9.0 CPR 5 versus 4	Unblinded study: Microdose GnRHa versus GnRHant
Malmusi (2005)	n=55 (30 and 25)	rFSH was started on the D1 ganirelix 0.25 mg daily until the hCG injection	Triptorelin 0.1 mg was initiated on D1 followed by rFSH D2 of menstruation	Number of oocytes 3.5 versus 2.5 CPR 3 versus 6	Unblinded study: GnRHa flare-up protocol versus GnRHant
De Placido (2005)	n=133 (67 and 66)	Multiple dose (cetorelix) and flare up	Multiple dose (triptorelin)	Number of oocytes 6.79 versus 6.54 CPR 17 versus 14	Multiple dose GnRHant and flare up, multiple dose
Tazegül (2008)	n=89 (44 and 45)	Multiple dose (cetorelix/ganirelix)	Long, multiple dose (leuprolide)	Number of oocytes 5.44 versus 5.44 CPR 10 versus 11	Multiple dose GnRHant versus GnRHa long protocol
Tian (2008)	n=44 (21 and 23)	Multiple dose (cetorelix)	Short, multiple dose (triptorelin)	Number of oocytes 3.25 versus 3.79 CPR 8 versus 2	Multiple dose antagonist versus GnRHa short protocol
Wang (2008)	n=121 (63 and 58)	OCP/multiple dose (cetorelix)	OCP/long, microdose GnRHa (triptorelin)	Number of oocytes 4.40 versus 5.41 CPR 22 versus 18	OCP Multiple dose GnRHant versus microdose GnRHa
Demiroglu (2009)	n=90 (45 each)	Combined OCP for 21 days. On day 3 of menstruation, 40 µg s.c./bid of leuprolide followed by 450 IU/day HMG on day 3	GnRHant multiple dose - 450 IU/day HMG starting on day 3 and 0.25 mg cetorelix daily	Number of oocytes 4.3 versus 3.1 CPR 6 versus 12	Unblinded study: GnRHa microdose flare-up versus GnRHant multiple dose
Kahraman (2009)	n=42 (21 and 21)	Multiple dose (cetorelix)	Flare-up, microdose (leuprolide)	Number of oocytes 5.60 versus 5.80 CPR 2 versus 3	Multiple dose GnRHant and GnRHa flare up
Liu (2009)	n=118 (58 and 60)	Multiple dose (cetorelix)	Short, multiple dose (triptorelin)	Number of oocytes 4.12 versus 3 CPR 18 versus 8	Multiple GnRHant and GnRHa short protocol
Devesa (2010)	n=172 (92 and 80)	OCP/multiple dose (ganirelix)	OCP/flare-up, (leuprolide)	Number of oocytes 4.89 versus 5.91 CPR 13 versus 12	OCP Multiple GnRHant and GnRHa flare-up
DiLuigi (2011)	n=54 (28 and 26)	GnRHant protocol	Microdose leuprolide acetate	Number of oocytes 5.2 versus 5.4 CPR 9 versus 8 LBR 6 versus 7	GnRHant versus GnRHa microdose protocol
Karimzadeh (2011)	n=159 (79 and 80)	Clomiphene citrate, gonadotropin and GnRHant	Microdose GnRHa flare	Number of oocytes 6.34 versus 4.1 CPR 19 versus 13	Mild protocol and microdose GnRHa flare protocols

Contd...

Table 1: Contd...

Study	Population	Intervention	Comparison	Outcome	Remark
Prapas (2013)	n=330 (168 and 162)	Fixed GnRHant protocol	Long GnRHa protocol	Number of oocytes 3.01 versus 2.64 CPR 53 versus 68	GnRHant (fixed) versus GnRHa long protocol
Sunkara (2014)	n=74 (37 and 37)	GnRHant	GnRHa long protocol	Number of oocytes 3.3 versus 4.42	GnRHant versus GnRHa long protocol
Protocols using LH as an adjuvant					
Ferraretti (2004)	n=104 (54 and 50)	Recombinant LH in addition to the increased dose of FSH	An increased dosage of FSH	Number of oocytes CPR 22 versus 11	
Demiroglu (2005)	n=106 (53 and 53)	Recombinant LH in addition to the increased dose of FSH	Increased dosage of FSH	Number of oocytes 5.89 versus 5.62 CPR 5 versus 3	
Fernández Ramírez (2006)	n=34 (16 and 18)	rLH 75 IU b.i.d. (150 IU/day) starting on the day of GnRHant initiation until hCG trigger	GnRHant without rLH	CPR 2 versus 3	
Polidoropoulos (2007)	n=136 (68 and 68)	rLH 75-150 IU/day until hCG criteria were met	GnRHa protocol	CPR 17 versus 15	
Berkkanoglu (2007)	n=97 (46 and 51)	600 IU of rFSH plus daily supplementation with 75 IU of rLH	600 IU of rFSH as the control group	Number of oocytes 4.8 versus 5.6 CPR 13 versus 14	Three-arm study with addition of r LH and hCG supplementation
Ruvolo (2007)	n=42 (24 and 18)	rLH 75-150 IU/day from 8 th day of ovarian stimulation until hCG criteria were met	GnRHa and rFSH without further LH addition	Clinical pregnancy 10 versus 4	
Barrenetxea (2008)	n=84 (42 each)	GnRHa and rFSH and rLH	GnRHa and rFSH without further LH addition	Number of oocytes 5.43 versus 5.66 CPR 10 versus 9	Unblinded study
Musters (2012)	n=116 and 128	rFSH and rLH (2:1 ratio)	rFSH alone	CPR 16 versus 15	
Protocols using GH as an adjuvant					
Owen, (1991)	n=25 (13 and 12)	GH 24 IU i.m./day on alternate days, starting simultaneously with HMG until the day of hCG administration	Use of placebo	CPR 4 versus 1 LBR 4 versus 0	
Zhuang (1994)	n=27 (12 and 15)	GH 12 IU i.m./day on alternate days	No placebo	CPR 5 versus 2 LBR 4 versus 2	
Hughes (1994)	n=33 (21 and 12)	In addition to commencing HMG, a second daily injection of GH 12 IU i.m. daily for up to 12 days or until hCG	Placebo	Number of oocytes 5 versus 5	Placebo-controlled double-blind
Bergh (1994)	n=40 (20 each)	GH 0.1 IU/kg body weight per day was given s.c. as pretreatment and during stimulation with HMG	Placebo	CPR 3 versus 2	Double-blind, placebo-controlled
Dor (1995)	n=14 (7 and 7)	GnRHa/HMG/GH (18 IU on alternate days, total dose 72 IU)	GnRHa/HMG/placebo	CPR 0 both groups	Placebo-controlled double-blind
Suikkari (1996)	n=22 (16 and 6)	GnRHa, gonadotropin in a "boost" flare-up protocol for 4, or 12 IU of human GH	Placebo	CPR 2.4 versus 2	Double-blind placebo-controlled
Howles (1999)	n=196 (96 and 100)	Long luteal buserelin 0.5 mg, 1 mg/day GH releasing factor s.c. with HMG 300 IU/day i.m. from the day of down-regulation	No supplement	CPR 8 versus 8 LBR 5 versus 4	Multicenter double-blind, placebo-controlled study

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Table 1: Contd...

Study	Population	Intervention	Comparison	Outcome	Remark
Kucuk (2007)	n=61 (31 and 30)	GH co-treatment, daily s.c. injection of 4 mg from day 21 of preceding cycle along with GnRH α	No supplement	Number of oocytes 6.5 versus 3.2	GH co-stimulation to long luteal GnRH α regimen
Eftekhari (2013)	n=82 (40 and 42)	GH and GnRHant protocol	GnRHant protocol (no placebo)	CPR 5 versus 5 Number of oocytes 6.1 versus 4.8	
Protocols using testosterone as an adjuvant					
Massin (2006)	n=53 (27 and 26)	Transdermal application of testosterone 15 days before FSH	Placebo	CPR 4 versus 1, LBR 2 versus 1 Number of oocytes 3.17 versus 3.56	Double-blind, placebo-controlled study
Fábregues (2009)	n=62 (31 and 31)	Testosterone 20 μ g/kg/day for 5 days transdermal application preceding ovarian stimulation	High-dose gonadotropin in association with a minidose GnRH α protocol	CPR 6 versus 4, LBR 6 versus 4 Number of oocytes 4.1 versus 3.6	
Kim (2011)	n=110 (55 and 55)	12.5 mg transdermal testosterone daily for 21 days in the cycle preceding ovarian stimulation	No placebo	CPR 17 versus 8, LBR 15 versus 7 Number of oocytes 4.6 versus 3.2	
Protocols using aromatase inhibitors as an adjuvant					
Goswami (2004)	n=38 (13 and 25)	Let 2.5 mg daily from day 3 to 7, and rFSH 75 IU/day on days 3 and 8	Long GnRH α protocol and rFSH (300-450 IU/day)	CPR 3 versus 6 Number of oocytes 1.6 versus 2.1	Unblinded study, Let-FSH group versus GnRH α -FSH group
Kashyap (2005)	n=55 (29 and 26)	Let and GnRHant	No placebo, GnRHant protocol	CPR 3 versus 1	
Ozmen (2009)	n=70 (35 and 35)	Let 5 mg/day for 5 days and GnRHant 0.25 μ g/day	No placebo, GnRHant 0.25 μ g/day	CPR 8 versus 5	
Mohsen (2013)	n=60 (30 and 30)	Let supplementation	GnRH α protocol (micro dose flare)	CPR 4 versus 5	
Protocols using DHEA as an adjuvant					
Artini (2012)	n=48 (24 each)	25 mg three times a day of DHEA supplementation for 3 months previous to IVF cycle	No adjuvant	CPR 6 versus 4 Number of oocytes 3.58 versus 2.67	Prospective, randomized, controlled
Wiser (2010)	n=51 (26 and 25)	75 mg DHEA orally, once a day, at least 6 weeks before GnRH α long protocol	No adjuvant	CPR 7 versus 3 Number of oocytes 4.6 versus 3.2 3.2 and 3.5	Prospective, randomized, controlled
Protocols using rhCG as an adjuvant					
Berkkanoglu (2007)	n=99 (48 and 51)	600 IU of rFSH plus daily supplementation with 75 IU of rhCG	600 IU of rFSH	CPR 10 versus 14 Number of oocytes 3.8 versus 5.6	Three-arm study recombinant LH, hCG supplementation
Natural cycle					
Morgia (2004)	n=215 (114 and 101)	Natural cycle IVF	Microdose GnRH α flare from day 1 and 600 IU FSH	CPR 17 versus 10 Number of oocytes 0.79 versus 2.1	Unblinded study
Protocols using other adjuncts					
Jinno (1997)	n=162 (82 and 80)	Oral 1.25 mg/day bromocriptine from day 4 to 6 in preceding cycle and then 4.5 mg/day, discontinued 7 days before the beginning of HMG administration	GnRH α long protocol	CPR 25 versus 13 Number of oocytes 9.5 versus 8.4	Unblinded, controlled trial: Bromocriptine rebound method

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Table 1: Contd...

Study	Population	Intervention	Comparison	Outcome	Remark
Chung Hoon (1999)	n=70 (35 each)	Triptorelin 0.1 mg from day 21; HMG/FSH 300 IU/day i.m. from day of downregulation plus 120 mg/day pyridostigmine until hCG	No adjuvant	CPR 9 versus 4 Number of oocytes 5.9 versus 3.7	Double-blind, placebo-controlled study
Battaglia (1999)	n=34 (17 each)	GnRHa + daily supplemented (16 g) with oral L-arginine	No adjuvant	CPR 0 versus 2 Number of oocytes 1.6 versus 4.1	Oral L-arginine + GnRHa
Lok (2004)	n=60 (30 in each)	Low-dose aspirin (80 mg daily) at the time of commencement of GnRHa in the preceding cycle and continuing until hCG	Placebo	CPR 3 versus 2 Number of oocytes 4 versus 3	Double-blind, placebo-controlled study
Various modifications to GnRHa protocols					
Dirmfeld (1991)	n=54 (28 and 26)	600 µg/day of buserelin nasal spray on day 1 till hCG	1000 µg/day of buserelin nasal spray 15-30 days before ovulation induction, then 600 µg/day till hCG	CPR 3 versus 2 Number of oocytes 7.0 versus 5.6	Unblinded
Von Hoof (1993)	n=47 (25 and 22)	225 IU/day i.m. HMG from day 3 for 5 days, increasing to 450 IU/day	Unchanged dose		Unblinded study
Rombauts (1998)	n=40 (18 and 20)	GnRHa from day 2. FSH (150 IU) from day 25 of previous cycle	GnRHa from day 2. FSH 150 IU from day 3 of treatment cycle	Number of oocytes 4.5 versus 6	Controlled study
Dirmfeld (1999)	n=78 (40 and 38)	GnRHa - started in the midluteal phase and stopped before administration of gonadotropins	GnRHa from the midluteal phase and was continued throughout the follicular phase	CPR 5 versus 9 number of oocytes 6.46 versus 7.73	Unblinded study
Raga (1999)	n=30 (15 and 15)	300 IU/day rFSH + 150 IU/day HMG	Purified FSH	CPR 4 versus 1 number of oocytes 7.2 versus 5.6	Prospective randomized study
Garcia-Velasco (2000)	n=70 (34 and 36)	Leuprolide acetate 1 mg/d SC till menstruation and then stopped. 3 Amp HMG + 5 Amp FSH on days 1 and 2, 2 Amp HMG + 3 Amp FSH on days 3,4 and 5 from D6	No stop protocol, high dose protocol Long GnRH suppression and gonadotropins	CPR 5 versus 6 number of oocytes 4.1 versus 6.9	Unblinded study
Akman (2000)	n=40 (20 each)	Ovarian stimulation with no GnRHa or GnRHant administration	0.25 mg of Cetrorelix daily till hCG	CPR 1 versus 4 number of oocytes 3.46 versus 3.25	Unblinded study
Akman (2001)	n=48 (24 in each)	Low-dose oral contraceptive on cycle day 1 for 21 days. On day 2 of menstruation leuprolide acetate (40 µg s.c./day) followed by gonadotropins	Gonadotropins from day 2 and later 0.25 mg of cetrorelix daily	CPR 6 versus 5 Number of oocytes 5.5 versus 4.5	Unblinded study
Marci (2003)	n=38 (19 each)	short flare up protocol using GnRHa 0.1 mg/day triptorelin from day 2	Daily dose of 0.25 mg Cetrotide and 375 IU/day rFSH	CPR 3 and 2	Unblinded study
Mohamed (2006)	n=30 (15 and 13)	Buserelin 500 µg/day s.c. from day 1 of cycle and ovarian stimulation from day 3	Ovarian stimulation from day 3 and GnRHant 0.25 mg/day from day 8	Number of oocytes 6 each	Unblinded study
Kucuk (2007)	n=42 (21 each)	Long protocol triptorelin 0.1 mg, 150 IU rFSH, triptorelin was halved to 0.05 mg and the rFSH was increased to 450 IU	Flare-up regimen of triptorelin. Along with triptorelin, 450 IU rFSH	Number of oocytes 6.8 versus 3.8	Long protocol flare up regimen

Contd...

Table 1: Contd...

Study	Population	Intervention	Comparison	Outcome	Remark
Lainas (2008)	n=270 (180 and 90)	Ganirelix 0.25 mg and including the day of hCG administration	0.05 mg/day triptorelin from day 2 of the cycle including the day of hCG administration	CPR 29 versus 7 number of oocytes 3 and 3	

CPR=Clinical pregnancy rate, LBR=Live-birth rate, GnRH=Gonadotropin-releasing hormone, GnRHa=Gonadotropin-releasing hormone agonist, GnRHant=GnRH antagonist, FSH=Follicle-stimulating hormone, rFSH=Recombinant FSH, HCG=Human chorionic gonadotropin, rhCG=Recombinant hCG, OCP=Oral contraceptive pill, HMG=Human menopausal gonadotropin, LH=Luteinizing hormone, rLH=Recombinant LH, GH=Growth hormone, Let=Letrozole, DHEA=Dehydroepiandrosterone, s.c=subcutaneous, IVF=*in vitro* fertilization

Table 2: Summary of findings for use of growth hormone supplementation

Growth hormone supplementation to gonadotropins compared to placebo or no supplementation for poor responders

Patient or population: Patients with poor responders
 Settings: Randomized controlled trials
 Intervention: Growth hormone supplementation to gonadotropins
 Comparison: Placebo or no supplementation

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Quality of the evidence (grade)	Comments
	Assumed risk Placebo or no supplementation	Corresponding risk Growth hormone supplementation to gonadotropins				
Study population						
Clinical pregnancy rates	123 per 1000 Moderate	230 per 1000 (129-375)	OR 2.13 (1.06-4.28)	251 (7 studies)	⊕⊕⊕⊕ High	
Live birth rates	111 per 1000 Moderate	270 per 1000 (128-485)	OR 2.96 (1.17-7.52)	135 (4 studies)	⊕⊕⊕⊕ High	

*The basis for the assumed risk (e.g., the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI=Confidence interval, OR=Odds ratio. Grade working group grades of evidence - High quality=Further research is very unlikely to change our confidence in the estimate of effect, Moderate quality=Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate, Low quality=Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate, Very low quality=We are very uncertain about the estimate

Table 3: Summary of findings for the use of transdermal testosterone supplementation

Transdermal testosterone supplementation to gonadotropins compared to placebo or no supplementation for poor responders

Patient or population: Patients with poor responders
 Settings: Randomized controlled trials
 Intervention: Transdermal testosterone supplementation to gonadotropins
 Comparison: Placebo or no supplementation

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	Number of participants (studies)	Quality of the evidence (grade)	Comments
	Assumed risk Placebo or no supplementation	Corresponding risk Transdermal testosterone supplementation to gonadotropins				
Study population						
Clinical pregnancy rates	116 per 1000 Low	240 per 1000 (132-398)	OR 2.41 (1.16-5.04)	225 (3 studies)	⊕⊕⊕⊕ High	
Live birth rates	107 per 1000 Moderate	207 per 1000 (108-360)	OR 2.18 (1.01-4.68)	225 (3 studies)	⊕⊕⊕⊕ High	

*The basis for the assumed risk (e.g., the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI=Confidence interval, OR=Odds ratio. Grade working group grades of evidence - High quality=Further research is very unlikely to change our confidence in the estimate of effect, Moderate quality=Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate, Low quality=Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate, Very low quality=We are very uncertain about the estimate

significantly improved LBR (OR 2.96; 95% CI 1.17–7.52) with GH supplementation [Figure 3 and Table 2]

4. Testosterone supplementation: A relatively smaller number of trials tested transdermal testosterone

supplementation in assisted conception cycles (three RCTs; $n = 225$). The meta-analysis showed significantly improved CPR (OR 2.41; 95% CI 1.16–5.04) and LBR (OR 2.18; 95% CI 1.01–4.68), but the number of oocytes retrieved was not statistically significant (mean difference 0.94; 95% CI 0.24–1.64), [Figure 4 and Table 3]

5. DHEA supplementation: Two RCTs ($n = 99$). DHEA supplementation was found to have no significant effect on the number of oocytes (mean difference 0; 95% CI - 1.07–1.07) and CPR (OR 2.10; 95% CI 0.75–5.85)
6. Use of aromatase inhibitors: Letrozole supplementation was used in four trials ($n = 223$) and the pooled data failed to find any statistically significant CPR (OR 1.28; 95% CI 0.60–2.73)
7. Natural cycle: The natural cycle IVF was tested by only one trial ($n = 215$).^[11] The CPR and number of oocytes retrieved were statistically similar in both groups
8. Other interventions: Various authors modified the GnRH α protocols or used various supplementations such as bromocriptine, pyridostigmine, L-arginine, and low-dose aspirin which are described in Table 1. None of these interventions showed any significant improvement in outcomes.

DISCUSSION

Our systematic review updates on the evidence on various strategies to improve reproductive outcome for POR. We analyzed 61 RCTs and 4997 assisted conception cycles which were divided into 10 categories based on the interventions used.

The use of GnRHant protocol for pituitary downregulation is a commonly used approach for poor responders. GnRHant protocol offers several advantages. They cause immediate, rapid gonadotropin suppression by competitively blocking GnRH receptors in the anterior pituitary gland, thereby preventing endogenous premature release of LH and FSH. Our meta-analysis of 17 RCTs did not show any significant difference in CPR or number of oocytes retrieved with the use of GnRHant.^[12-28]

LH aids maintain adequate concentrations of intraovarian androgens and promote steroidogenesis and follicular growth. It has been proposed that addition of LH to ovarian stimulation protocol may benefit poor responders. Meta-analysis of eight trials^[13,29-33] did not show significant improvement in CPR with use of recombinant LH.

GH, insulin-like growth factor-1, and GH-releasing hormone increase the sensitivity of ovaries to gonadotropin stimulation and enhance follicular development. GH enhances oocyte quality by accelerating and coordinating cytoplasmic and nuclear maturation. There are some suggestions that GH-releasing factor supplementation may improve pregnancy rates in poor responders. The pooled data from eight RCTs in this review show significantly improved CPR and LBR with GH supplementation.^[13,29-36] There was no significant heterogeneity in the included studies ($\tau^2 = 0.00$, $\chi^2 = 0.98$, $df = 3$ [$P = 0.81$]; $I^2 = 0\%$). However, none of the studies had independently found any significant benefit with GH supplementation. The

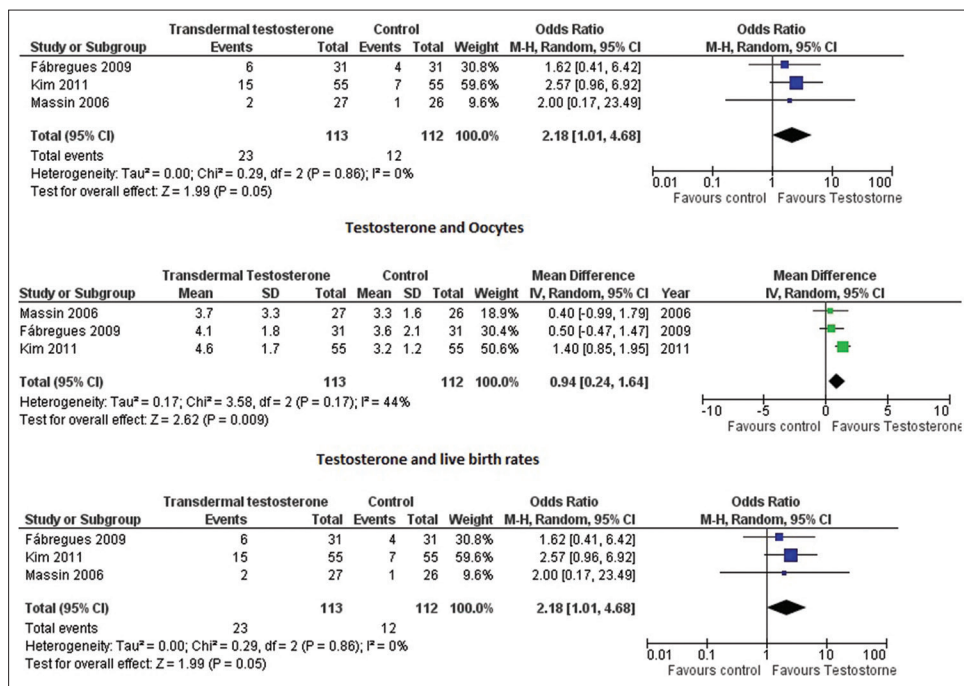


Figure 4: Use of testosterone supplement

total numbers in the meta-analysis are small to draw any definitive conclusions.

Androgen stimulates early stages of follicular growth and increases the number of preantral and antral follicles by the proliferation of granulosa and thecal cells and reduction in granulosa cell apoptosis. It is hypothesized that positive change in microenvironment in the ovaries may lead to an increase in the number and the maturity of oocytes in poor responder group.^[37] Three randomized trials^[38-40] have tested this approach and the meta-analysis shows significant improvement in LBR and CPR.

Aromatase inhibition was proposed to improve ovarian response to FSH in poor responders. Our meta-analysis included four RCTs and failed to show any improvement in outcome with the use of aromatase inhibitors.

It is proposed that DHEA changes the follicular microenvironment by reducing hypoxic inducible factor-1, thus improving the quality of oocytes. Pooled data from 2 RCTs showed no significant difference in CPR with DHEA supplementation.^[41]

Natural cycle IVF offers several advantages such as low cost and low risk of multiple pregnancies and most importantly eliminates the risk of ovarian hyperstimulation syndrome. Morgia *et al.*^[11] randomized natural cycle IVF and microdose GnRHa flare along with FSH. It was found that natural cycle IVF may be as effective as IVF using controlled ovarian hyperstimulation. No further trials with this approach were found for meta-analysis.

Strengths and limitations

Our study provides most comprehensive and up-to-date review on the topic of assessing most effective treatment for poor responders and included only RCTs. We divided different approaches into 10 categories and performed meta-analysis as appropriate. Previous reviews were very specific in addressing one treatment strategy, and they failed to provide any conclusive answer. Some reviews were methodologically limited as they included observational studies and nonrandomized studies in their meta-analysis.^[4,7,9]

The major limitation of this review is related to its small population size. Although some adjuvant supplementations may appear to improve ovarian response and reproductive outcome, we recognize that the numbers are small to recommend their routine use in poor responders. There was significant heterogeneity in the definition of poor responders in these trials conducted before Bologna consensus criteria were recommended.

Interpretation

Our meta-analysis showed no difference in the number of oocytes retrieved or the CPRs with use of GnRHant. The pooled data from seven studies show significantly improved CPR and LBR with GH supplementation in the previous review.^[4] Our meta-analysis adds a further RCT^[36] ($n = 82$) which results in a 48% increase in sample size. GH supplementation showed some promising results; however, the numbers are small to draw any convincing conclusion. Our results for testosterone supplementation are consistent with the results of previous meta-analyses as there were no new RCTs.^[5,7] Letrozole supplementation may result in improved FSH sensitivity and concentration, but this beneficial effect was not reflected in the results. A systematic review by Bosdou *et al.*^[7] previously showed no difference in outcome with the use of letrozole. Two more RCTs have been undertaken^[37,42] since the previous review, and we added a total of 68 cycles (43%) to the sample size in our review. However, the pooled data showed no significant difference in outcome with use of letrozole. The anti-aging effect of the adrenal androgen DHEA is thought to be the mechanism to improve ovarian response. Recent meta-analysis did not show significant improvement with the use of DHEA.^[9] Only two RCTs were eligible for our meta-analysis, which failed to demonstrate any benefit.

CONCLUSION

Evidence from this review suggests that GH supplementation or transdermal testosterone supplementation to assisted conception treatment cycles is associated with an improved CPR and LBR in poor responders. However, it is essential to recognize that this evidence is derived from a small number of studies; hence, we feel that the current evidence is insufficient to recommend the routine use of either of these approaches. Other treatment strategies are not found to be useful in improving clinical outcome in poor responders. We recommend that the empirical use of adjuvants should be avoided pending good quality evidence from well-designed studies.

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Conflicts of interest

There are no conflicts of interest.

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CLINICAL ARTICLE

Three-arm age-matched retrospective cohort study of obstetric outcomes of donor oocyte pregnancies

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ABSTRACT

Objective: To evaluate and compare obstetric complications between women who conceived after oocyte donation and age-matched control women who conceived spontaneously or by autologous in vitro fertilization (IVF). **Methods:** In a retrospective cohort study, data were assessed from all women who conceived after oocyte donation and delivered a live neonate after 24 weeks of pregnancy between January 2007 and December 2014 at a UK hospital. Two age-matched control groups—one containing women who conceived after autologous IVF and the other containing women who conceived spontaneously—were used for comparison. The primary study outcome was hypertensive disorders of pregnancy (pregnancy-induced hypertension and pre-eclampsia). Multivariate analysis was performed by logistic regression. **Results:** Each group included 45 women. Hypertensive disorders in pregnancy affected 15 (33%) women in the study group, 3 (7%) women who conceived after autologous IVF, and 3 (7%) who conceived spontaneously. The risk of hypertensive disorders in pregnancy was significantly higher in the donor oocyte group (odds ratio 5.85, 95% confidence interval 1.42–23.9; $P = 0.01$). **Conclusion:** Women who conceived after oocyte donation had an increased risk of hypertensive disorders. Oocyte donation should be managed as an independent risk factor, and couples should be counselled appropriately.

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1. Introduction

Oocyte donation has become widely used as a treatment option for infertile couples. In 2009, 21 604 treatment cycles using egg donation were reported in Europe alone [1]. The demand for donor oocytes has grown steadily with the trend of delayed childbearing [1]. Increasing maternal age is a social trend that is likely to continue in the years to come [2]. Other causes for the increase in oocyte donation include ovarian failure, diminished ovarian reserve, multiple failures of in vitro fertilization (IVF), availability of advanced reproductive technology, and diagnosis of genetic conditions with risk of inheritance to offspring [3].

Advanced maternal age is an independent risk factor for obstetric complications [4], particularly hypertensive disorders of pregnancy [5]. It is also associated with an increased risk of adverse neonatal outcomes [6]. Women with pregnancy after oocyte donation are at increased risk of pre-eclampsia, fetal growth restriction, preterm labor, cesarean delivery, and postpartum hemorrhage [7–9]. Therefore, for studies investigating adverse perinatal outcomes in pregnancies conceived after oocyte donation, advanced maternal age is likely to be a significant confounding factor.

The few available studies report conflicting evidence about the risk of hypertensive disorders in donor oocyte pregnancies after adjusting for maternal age [10,11], and it is unclear whether pregnancy complications and obstetric risks are due to oocyte donation or to confounding factors such as maternal age. The aim of the present study was to evaluate and compare obstetric complications between women who conceived after oocyte donation and age-matched control women with spontaneous conception or autologous IVF conception.

2. Materials and methods

The present retrospective study was conducted as a three-armed, age-matched cohort study. The study group comprised all women who conceived after oocyte donation and delivered a live neonate after 24 weeks of pregnancy at a teaching hospital in Leicester, UK, between January 1, 2007, and December 31, 2014. The two control groups comprised age-matched women who conceived after IVF via autologous oocytes, and age-matched women with spontaneous conception. The age-matched control women were identified by selecting pregnancies recorded immediately after donor oocyte pregnancies in the maternity electronic database. Pregnancies after preimplantation genetic diagnosis, or after surgical sperm retrieval or use of donor sperm were not included in the study. As per local protocol, ethical approval and individual patient consent were not required to analyze anonymized hospital data.

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All women in the study group were seen in a dedicated prenatal clinic. For the present analysis, a retrospective service review was undertaken to determine the obstetric outcomes. Data were collated from booking, prenatal, and intrapartum records, and the maternity electronic database system. For each patient, demographic data and details of assisted conception were collected at the booking visit. Data collection was completed for individual women in the study group using a prepared template.

The primary study outcome was hypertensive disorders of pregnancy, including pregnancy-induced hypertension and pre-eclampsia. Pregnancy-induced hypertension was defined as blood pressure above 140/90 mm Hg on two or more occasions at least 6 hours apart, without proteinuria, and presenting after 20 weeks of pregnancy. Pre-eclampsia was defined as blood pressure above 140/90 mm Hg on two or more occasions at least 6 hours apart, with proteinuria of 0.3 g/day or higher, after 20 weeks of pregnancy. Fetal growth restriction (FGR) was defined as a birth weight below the fifth centile for gestational age. Preterm labor was defined as delivery before 37 completed gestational weeks. Gestational age was calculated from the day of oocyte collection, which was defined as day 14 of the cycle. The gestational age for natural conception was based on booking prenatal ultrasonography performed between 8 and 13 weeks of pregnancy.

All data were analyzed via Stata version 13 (StataCorp, College Station, TX, USA). Descriptive data were compared among the three groups, including age, body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters), smoking, and parity. Histograms were produced to ascertain the distribution, and data with a normal distribution are presented as mean \pm SD. Multivariate analysis was conducted by conditional logistic regression and adjusted for age, BMI, smoking, previous medical history, parity, and multiple pregnancy. $P < 0.05$ was considered to be statistically significant.

3. Results

During the study period, 45 women who conceived after oocyte donation met the study criteria and were included in the analysis. The two control groups therefore each also contained 45 age-matched women.

There was no difference in characteristics between the three groups (Table 1). The parity of women in the study group ranged from 0 to 4, that in the autologous IVF control group ranged from 0 to 4, and that in spontaneous conception group ranged from 0 to 3.

The pregnancy complications are summarized in Table 2. In the logistic regression model, there was no difference in the incidence of FGR, preterm delivery, prepartum hemorrhage, mean birth weight, or cesarean delivery among the groups. Notably, the likelihood of hypertensive disorders in pregnancy was significantly higher in the study group than in either control group after adjusting for age, BMI, multiple gestation, and smoking status (odds ratio 5.85, 95% confidence interval 1.42–23.90; $P = 0.01$). There was a significant difference in the incidence of FGR among the three groups ($\chi^2 = 6.85$, $P = 0.03$); after including other covariates in the regression model, however, this difference was not significant ($P = 0.25$).

4. Discussion

The present results showed an increased risk of hypertensive disorders among women with donor oocyte pregnancies as compared with age-matched control women with spontaneous or assisted conception. By contrast, the results did not show a significant association between oocyte donation and FGR, preterm labor, or cesarean delivery rate. This might be explained by the small sample size, which is a significant limitation of the study.

Two studies of obstetric outcomes in donor oocyte pregnancies [7,12] have shown an increased risk of preterm labor, pre-eclampsia, prolonged labor, and cesarean delivery. However, another study [13] failed to find any association of adverse outcomes with conception after oocyte donation. A Danish national cohort study [14] showed an increased risk of pre-eclampsia and preterm labor in donor oocyte pregnancies as compared with pregnancies after autologous IVF, intracytoplasmic sperm injection, and spontaneous conception. However, these two complications overlap with cofounders such as multiple pregnancies, advanced age, underlying polycystic ovary syndrome, assisted conception, and other pre-existing medical conditions [15].

Advanced maternal age is associated with a significantly increased risk of perinatal complications [16]; therefore, it is necessary to eliminate bias caused by maternal age and other risk factors. Levron et al. [11] recently showed that oocyte donation was independently associated with a higher rate of hypertensive diseases of pregnancy after adjustment for maternal age, gravidity, parity, and chronic hypertension. The present findings are consistent with a few studies reporting high complication rates with donor oocyte pregnancies independent of recipient age, parity, plurality, and the age of the donor [17,18].

The risk of obstetric complications for women with conceiving after oocyte donation might be explained on the basis of the immunologic theory of pre-eclampsia. Although the underlying cause of pre-eclampsia is unknown, an immunologic component is clearly involved in the pathophysiology of this disorder [19], and parental human leukocyte antigen sharing is thought to have a role in the etiology of pre-eclampsia [20]. The success of pregnancy depends on appropriate implantation, and any insult during this process can cause obstetric complications such as spontaneous abortion, intrauterine growth restriction, or preterm birth, in addition to pre-eclampsia [21]. By definition, the fetus is allogeneic to the gestational carrier in donor oocyte pregnancies. As a result, the mother faces a higher degree of antigenic dissimilarity as compared with spontaneously conceived pregnancies [22]. One study [23] has reported increased immune activity and fibrinoid deposition at the maternal-fetal interface of donor oocyte pregnancies, representing a host-versus-graft rejection-like process. Thus, these pregnancies are at higher risk of perinatal complications. Further research is required to explain the pathogenesis of donor oocyte pregnancies and placental complications.

On one hand, assisted reproductive technology using oocyte donation has enabled women at advanced age or with ovarian failure to successfully achieve pregnancy. On the other hand, conception after oocyte donation can subject them to a higher risk of maternal morbidity and mortality [24]. The risk of complications resulting from oocyte donation highlights the importance of preconception counseling. Although

Table 1
Characteristics of the study women by group.^a

Characteristic	Oocyte donation (n = 45)	Autologous oocyte IVF (n = 45)	Spontaneous conception (n = 45)	P value ^b
Age, y	40.23 \pm 5.64	39.23 \pm 1.71	39.69 \pm 0.71	0.40
Body mass index ^c	25.11 \pm 4.42	25.51 \pm 4.04	26.23 \pm 4.70	0.50
Parity	0.48 \pm 0.92	0.34 \pm 0.81	0.61 \pm 0.85	0.33
Smoker	0	2 (4)	2 (4)	0.34

Abbreviation: IVF, in vitro fertilization.

^a Values are given as mean \pm SD or number (percentage), unless indicated otherwise.

^b Analysis of variance.

^c Calculated as weight in kilograms divided by the square of height in meters.

Table 2
Obstetric outcomes by group.^a

Outcome	Oocyte donation (n = 45)	Autologous oocyte IVF (n = 45)	Spontaneous conception (n = 45)	P value ^b
Maternal hypertensive disorders	15 (33)	3 (7)	3 (7)	0.01
Fetal growth restriction	5 (11)	1 (2)	2 (4)	0.25
Preterm birth	7 (16)	2 (4)	2 (4)	0.09
Prepartum hemorrhage	2 (4)	1 (1)	3 (7)	0.65
Birth weight, g	3185.20 ± 653.13	3200.30 ± 540.52	3283.23 ± 738.02	0.78
Cesarean delivery	17 (38)	16 (36)	12 (27)	0.35
Emergency	6 (13)	7 (16)	8 (18)	
Elective	11 (24)	9 (20)	4 (9)	

Abbreviations: IVF, in vitro fertilization.

^a Values are given as number (percentage) or mean ± SD, unless indicated otherwise.

^b Conditional logistic regression.

counseling is more important for women older than 40 years who choose oocyte donation, younger women should be informed of the risks [24,25]. Furthermore, obstetricians need to be aware of the increased pregnancy risks for this group of women, who should be managed appropriately during the pregnancy, delivery, and puerperium period [25].

In conclusion, oocyte donation should be treated as an independent risk factor for hypertensive disorders in pregnancy. Women should be fully informed of the risks before undergoing fertility treatment. Women who select conception using donor oocytes should be managed in high-risk obstetric clinics with appropriate surveillance and management strategies for obstetric complications. There is currently limited evidence to attribute complications to an immunologic origin; further research is needed to explain the pathogenesis and risks involved in donor oocyte pregnancies.

Conflict of interests

The authors have no conflicts of interest.

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Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis

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Background Observational studies showed that women with a donor oocyte (DO) pregnancy have an increased risk of pregnancy complications.

Objectives Systematic review and meta-analysis to compare pregnancy complications of DO pregnancy with autologous oocyte *in vitro* fertilisation (IVF), and whether DO pregnancy acts as an independent risk factor.

Search strategy Online searches of databases from 1 January 1980 to 31 January 2015 were performed using a set of relevant keywords.

Selection criteria All studies comparing pregnancy complications of women with donor oocyte IVF and autologous oocyte IVF were included.

Data collection and analysis Data collected included demographics and pregnancy complications. Methodological quality assessment was performed using the Newcastle–Ottawa scale. Statistical analysis was performed using REVIEW MANAGER 5.3 and STATA 13.0. Meta-regression was performed for age.

Main results In total, 11 studies ($n = 81\,752$) were included. Ten studies ($n = 11\,539$) examined the primary outcome. The risk of developing hypertensive disorders in pregnancy was significantly higher for DO pregnancy (odds ratio, OR 3.92; 95% confidence interval, 95% CI 3.21–4.78). Further subgroup analysis for singleton and twin pregnancies showed that the risk was significantly higher for DO pregnancy in each group. Secondary outcomes including small for gestational age (OR 1.81), caesarean section (OR 2.71), and preterm delivery (OR 1.34) were significantly higher with DO pregnancy. Meta-regression for the covariate of age suggested that risk was independent of age.

Author's conclusions Donor oocyte pregnancy acts as an independent risk factor for pregnancy complications, including hypertensive disorders, small for gestational age, and preterm delivery. Women should be counselled carefully before undergoing DO-assisted conception.

Keywords Donor oocyte pregnancy, *in vitro* fertilisation, pre-eclampsia, pregnancy-induced hypertension.

Tweetable abstract Donor oocyte conception is an independent risk factor for obstetric complications.

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Introduction

Donor oocyte (DO) conception has allowed many women of advanced age to achieve successful pregnancies. The demand has grown steadily with the trend of delayed child-bearing. This phenomenon of increased maternal age is likely to continue in the years to come.¹ The recent rise in DO pregnancies is reported by all European countries.² In addition to advanced maternal age there are various other indications for DO conception, which include primary or

secondary ovarian failure, diminished ovarian reserve, multiple failures of *in vitro* fertilisation (IVF), and diagnoses of genetic conditions.³ The treatment option of egg sharing, which involves a woman sharing her eggs with another in exchange for free or reduced-cost fertility treatment, has been regulated in the UK since 1998, and is viewed positively by the egg-sharing donors and recipients.⁴ Advanced maternal age in itself is associated with pregnancy complications, including hypertensive disorders, gestational diabetes, preterm labour, and intrauterine growth

restriction.^{5,6} A recent report from the Human Fertilization and Embryonic Authority (HFEA) showed that over half of women receiving IVF aged 45 years and older used donated oocytes, but that there has also been a substantial increase in recipients aged under 35 years.⁷ The most common complication noted in DO pregnancies is pregnancy-induced hypertension and pre-eclampsia, ranging from 16 to 40% of women.^{8–13} Pre-eclampsia affects 3–5% of all pregnancies, and is estimated to result in 60 000 maternal deaths annually worldwide.¹⁴ Although obstetric complications have been attributed to advanced maternal age in these women, the risk is reported to be independent of age.¹³ Placental pathology as a result of immunological pathogenesis is suggested to be the reason for obstetric complications in DO pregnancy. The study by Kim et al.¹⁵ showed that the incidence of hypertensive disorders is significantly higher if the oocyte donor is unrelated to the recipient, compared with a related sibling donor. It is suggested that immunologic intolerance between the mother and the fetus may play an important role in the pathogenesis of pre-eclampsia.^{16–18} Pregnancies achieved by IVF or by intracytoplasmic sperm injection (ICSI) are at a higher risk for pregnancy complications compared with spontaneous pregnancies.¹⁹ Therefore, it would be more appropriate to use autologous-oocyte IVF pregnancy as a control group to study outcome in DO pregnancies.

The aim of this systematic review and meta-analysis is to study the obstetric outcome of DO pregnancies compared with autologous-oocyte IVF pregnancy. We aim to discover whether DO pregnancy acts as an independent risk factor for pregnancy complications.

Methods

A literature search was performed using Ovid MEDLINE[®] (1980–January 2015), EMBASE (1980–January 2015), Ovid OLDMEDLINE[®], Pre-MEDLINE, in-process and other non-indexed citations database consists of in-process and PubMed-not-MEDLINE records from NLM, HaPI (1985–January 2015), Google scholar, Web of Sciences (1980–2015), and the Cochrane Library. The conference proceedings citation index was also searched with no language restriction applied. A combination of medical subject headings and keywords were used to generate a subset of: citations for oocyte* ('ovum', 'ova', 'egg*'); citations including donor and oocyte; citations including assisted conception techniques ('ART* or IVF* or ICSI*'); and citations including various pregnancy complications ('gestational hypertension or pregnancy-induced hypertension or pre-eclampsia*' 'fetal growth restriction*' or IUGR* or small baby or birthweight', 'preterm labour', 'intrauterine death*', 'caesarean section*'). These subsets were combined using 'AND' or 'OR', as appropriate, to

generate a set of results addressing the research question. Duplicates were removed. The reference list of all published articles including review articles was examined to check for missed citations. No author was contacted. This review was registered in the PROSPERO database (CRD42015023739).

Data collection and analysis

Two review authors (YJ and NP) independently screened the titles and abstracts. Studies that met the predefined and explicit criteria were selected for inclusion in the review. The electronic searches were scrutinised and the full texts of all citations that were likely to meet the predefined selection criteria were obtained. The quality of included citations was independently examined and data extraction was performed. Where disagreements occurred, they were resolved by the consensus of both authors.

Criteria for including studies in the review

Types of study

Inclusion criteria: all observational studies comparing pregnancy outcomes in DO pregnancies with a predefined control group of pregnancies achieved with autologous oocyte IVF or ICSI only. Studies comparing outcome of DO singleton and twin gestations were included. All published data were used in the review. We have included both prospective and retrospective cohort and case-control studies.

Exclusion criteria: all observational studies reporting outcome in only DO pregnancies without any control group were excluded. Studies with incomplete data or with a heterogeneous control group were excluded. All studies with a spontaneous conception group used as the control group, donor sperm conception, case reports, and DO conception for Turner syndrome were also excluded, as Turner syndrome itself carries an additional risk for obstetric complications.

Types of participants

Women who conceived as a result of DO-assisted conception and delivered at 24 weeks of gestation, or later, were included in the analysis. The control group included women who had assisted conception using IVF or ICSI with autologous oocytes.

Types of outcome measures

The primary outcome was any hypertensive disorder in pregnancy, which included pregnancy-induced hypertension (PIH) and pre-eclampsia (PET). PIH was defined as a blood pressure of $\geq 140/90$ mmHg on two or more occasions, at least 6 hours apart, without proteinuria, and later than 20 weeks of gestation. PET was defined as a blood

pressure of $\geq 140/90$ mmHg on two or more occasions, at least 6 hours apart, with proteinuria of ≥ 0.3 g/day, and later than 20 weeks of gestation. This definition was consistent with the definition set by the International Society for the Study of Hypertension in Pregnancy (ISSHP).²⁰ Subgroup analysis was performed for PIH and PET separately in singleton DO pregnancies.

Secondary outcome measures included risk of caesarean section, development of gestational diabetes, small for gestational age, preterm delivery, and intrauterine death (IUD). Small for gestational age (SGA) was defined as a birthweight of less than tenth centile. For the purpose of standardisation, we have used the term SGA. The terms fetal growth restriction (FGR) or intrauterine growth restriction (IUGR) have been used by some authors, although all of these fulfil the criteria for SGA based on birthweight. Preterm labour was defined as delivery before 37 completed weeks of gestation.

Quality and risk of bias of the included studies

All studies included were cohort and case-control studies. Quality assessment was performed using the internationally accepted Newcastle-Ottawa scale.²¹ The Newcastle-Ottawa scale evaluates the quality of non-randomised studies, and focuses on the design, content, and ease of use for incorporating the quality of assessments in the interpretation of meta-analytic results. This scale is useful to evaluate potential biases at selection, comparability, exposure, and outcome stages. The selected studies were assessed for methodological quality using the domain-based risk for bias assessment tool, as recommended by the Cochrane Collaboration.²² A risk-of-bias table was produced in REVMAN 5.3,²³ using the biases described by the Newcastle-Ottawa scale.

Statistical analysis

The study characteristics and outcomes were assembled in tabular form. A formal meta-analysis was performed using REVMAN 5.3.²³ A fixed-effect model (using the Mantel-Haenszel method) was used when the I^2 statistic showed heterogeneity of $< 50\%$, whereas a random-effect model was used when the I^2 statistic showed heterogeneity $> 50\%$. The effect estimate was expressed as an odds ratio (OR) with a 95% confidence interval (95% CI), represented graphically by forest plots. Clinical heterogeneity was examined by assessing the participants, intervention used, study quality, and outcome measures. Statistical heterogeneity was examined using the χ^2 test, with $P < 0.05$ taken as suggestive of heterogeneity. Publication bias was assessed visually using a funnel plot and by formal testing. Meta-regression was performed using STATA 13.0 to determine whether age at conception was a limiting factor between studies (Figure S1).

Results

The process of literature search and study selection for the quantitative meta-analysis is shown in Figure 1. We reviewed 43 full-text articles after the screening search and excluded 32 articles, with the most common reason for exclusion being inappropriate control group. A total of 11 studies including 81 752 cycles were included in the review and meta-analysis. The characteristics of the included studies are shown in Table S1.

Meta-analysis

Primary outcome

The primary outcome measure was hypertensive disorders of pregnancy, which included PIH and PET. We found that the risk of developing hypertensive disorders in pregnancy was significantly higher in the DO pregnancy group than in autologous oocyte IVF pregnancy. The fixed-effect model for ten studies showed women with DO pregnancy ($N = 970$) had a significant risk of developing hypertensive disorders in pregnancy [$n/N = 341/970$ (35%) in the DO group versus $n/N = 1831/10569$ (17%) in the autologous IVF group; OR 3.92, 95% CI 3.21–4.78, $I^2 = 10\%$; Figure 2]. We performed subgroup analysis for four studies with twin pregnancies,^{13,24–26} and the fixed-effect model showed that hypertensive disorders of pregnancy were significantly higher in DO twin pregnancy than in the autologous twin pregnancy group [$n/N = 89/229$ (38%) versus $n/N = 948/5630$ (16%), OR 3.69, 95% CI 2.62–5.19, $I^2 = 0\%$; Figure 2]. The subgroup analysis for PET in singleton DO pregnancies included four studies,^{18,24,27,28} and the fixed-effect model showed that the risk of PET was significantly higher in singleton DO pregnancy [$n/N = 66/606$ (10%) versus $n/N = 333/10193$ (3%), OR 2.90, 95% CI 1.98–4.24, $I^2 = 0\%$; Figure 3]. The fixed-effect model meta-analysis of five studies showed that the risk of PIH in singleton DO pregnancy is significantly higher when compared with singleton IVF pregnancy [$n/N = 99/577$ (17%) versus $n/N = 522/10211$ (5%), OR 3.08, 95% CI 2.26–4.19, $I^2 = 0\%$; Figure 3].^{11,24,26,28,29} Additionally, the subgroup analysis fixed-effect model of two studies of women older than 40 years of age showed that the risk for hypertensive disorders was still significantly higher with DO pregnancy compared with IVF pregnancy [$n/N = 30/129$ (23%) versus $n/N = 18/168$ (10%), OR 2.33, 95% CI 1.21–4.49, $I^2 = 2\%$; Figure 3].^{29,30} Meta-regression for the covariate of age showed that between-study variance was minimal ($\tau^2 = 0.008$), and that the occurrence of hypertensive disorders in the studies was independent of age, with P

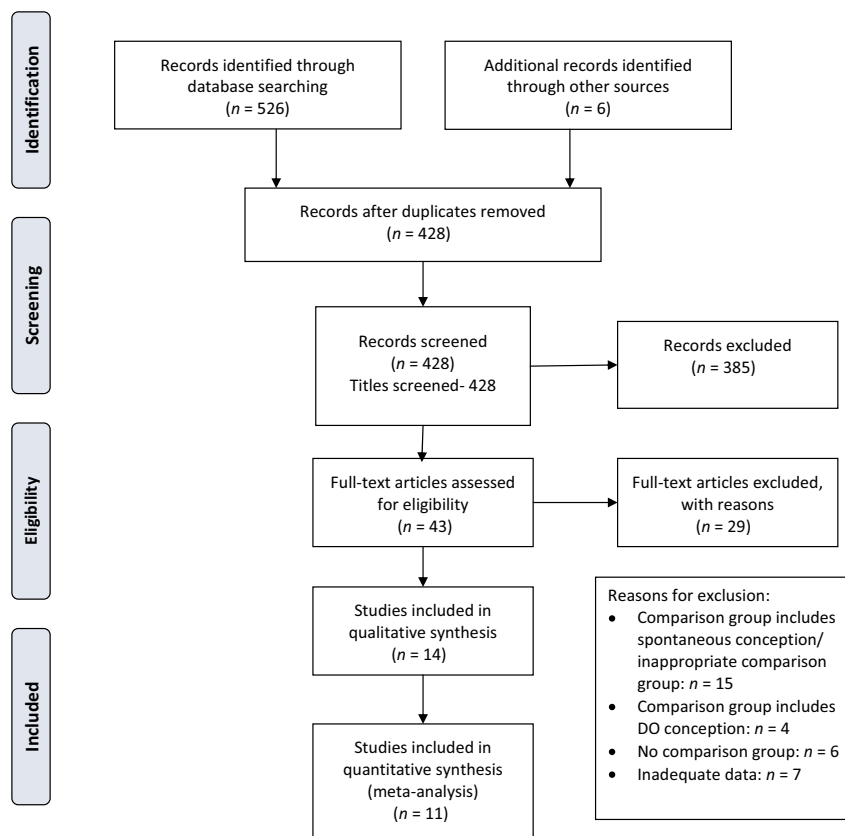


Figure 1. PRISMA 2009 flow diagram.

values above the level of significance ($P = 0.473$, 95% CI 0.90–1.05).

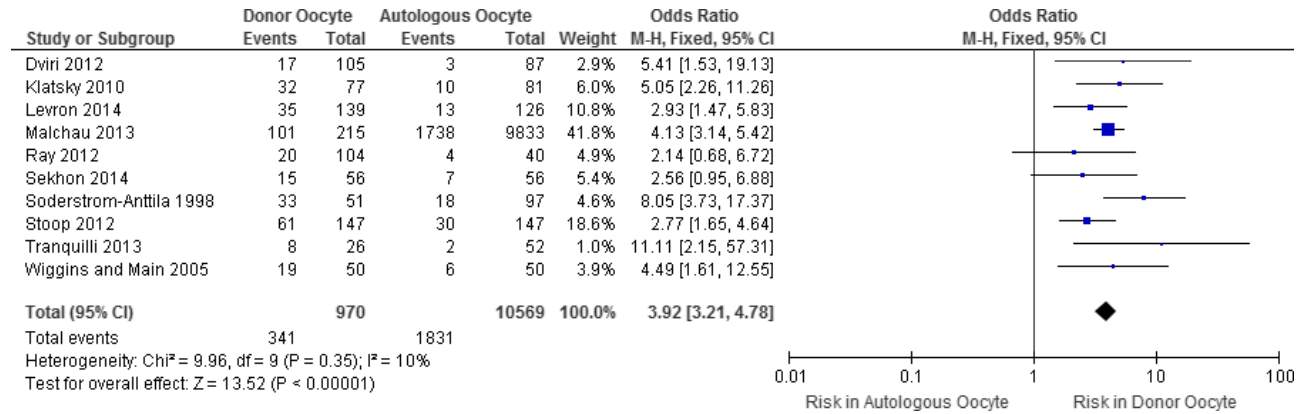
As both cohort and case–control studies were included in the meta-analysis, to assess the robustness of the results we performed sensitivity analysis after the exclusion of the case–control studies. Six cohort studies were included in the meta-analysis for the primary outcome measure of hypertensive disorders of pregnancy (Figure S2).^{18,24,25,30–32} The analysis showed that the risk of hypertensive disorders was significantly raised in all DO pregnancies (OR 3.63, 95% CI 2.92–4.51, $I^2 = 0\%$). This effect was significant for singleton (OR 3.05, 95% CI 2.19–4.24, $I^2 = 0\%$) as well as twin pregnancies (OR 3.64, 95% CI 2.57–5.16, $I^2 = 0\%$). Risk for PET in the singleton DO subgroup also showed a significant difference (OR 2.62, 95% CI 1.75–3.93 $I^2 = 0\%$).

Secondary outcome

Secondary outcome measures included SGA, preterm delivery, risk of caesarean section, gestational diabetes, and intrauterine death (IUD). Meta-analysis of six studies showed that the risk of SGA was significantly raised in DO pregnancy [$n/N = 58/630$ (9%) versus $n/N = 594/11262$ (5%), OR 1.81, 95% CI 1.26–2.60, $I^2 = 21\%$].^{11,18,24,27,31,32}

As a result of uncertainty about the definition used to define SGA in the original papers, however, subgroup analysis was performed for three studies with consistent definition, and the results gave OR 1.44 [$n/N = 34/422$ versus $n/N = 585/11\ 044$, OR 1.44, 95% CI 0.93–2.23, $I^2 = 0\%$].^{11,18,24} Because of the discrepancy in the definitions used for identifying FGR, IUGR, and SGA, these results should be interpreted with caution. Birthweight reported in six studies showed no significant difference (Figure 4).^{11,24,29,32–34} The risk of preterm delivery was analysed in nine studies, and the fixed-effect model showed that the risk of preterm birth was significantly higher in DO pregnancy compared with autologous IVF pregnancy [$n/N = 194/1011$ (19%) versus $n/N = 1078/11\ 651$ (9%), OR 1.34, 95% CI 1.08–1.66, $I^2 = 38\%$; Figure 4].^{18,24,26,29–32,35–37} The risk of caesarean section was significantly increased in DO pregnancy [$n/N = 435/690$ (88%) versus $n/N = 3452/10\ 283$ (33%), OR 2.71, 95% CI 2.23–3.30, $I^2 = 43\%$; Figure 4].^{11,18,24,26,37,38} Neither the increased risk of IUD, analysed in two studies [$n/N = 4/303$ (1.3%) versus $n/N = 3/346$ (0.8%), OR 1.39, 95% CI 0.32–6.15],^{35,37} nor the increased risk of developing gestational diabetes for the mother, analysed in five studies [$n/N = 58/524$ (11%) versus $n/N = 52/519$ (10%),

Hypertensive disorders in DO pregnancy



Hypertensive disorders in twin pregnancy

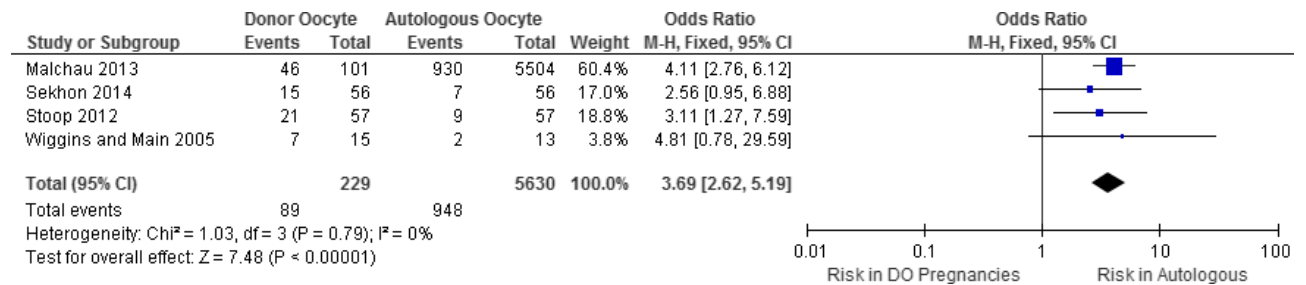


Figure 2. Primary outcome- Hypertensive disorders in DO pregnancy.

OR 1.25, 95% CI 0.68–2.30],^{11,18,26,32,37} was statistically significant.

Discussion

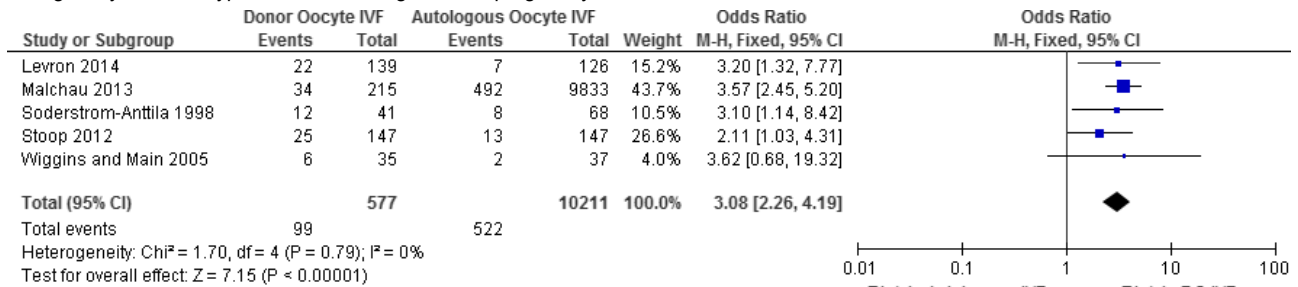
Main findings

The results of the meta-analysis described above show that the risk of developing hypertensive disorders in pregnancy is significantly higher with DO pregnancy when compared with autologous IVF pregnancy. The studies included singletons as well as twins; therefore, we studied the effect in singleton and multiple pregnancies separately. The increased risk was found in all subgroups, including women of advanced maternal age. When the risk of hypertensive disorders in pregnancy was subdivided into two groups, PIH and PET, the risk of both complications was higher in DO pregnancies. These findings are consistent with some previous observational studies.^{8–12,29,30} Multiple pregnancy is a known risk factor for developing hypertensive disorders; however, our analysis suggested that DO pregnancy is independent of multiple pregnancies for the development of hypertensive disorders. The risks of SGA and preterm delivery are significantly higher in DO pregnancy. Our meta-analysis showed that the chances of caesarean delivery for singletons are significantly higher with DO pregnancy. Similar findings are reported by other authors.^{3,9,10,12}

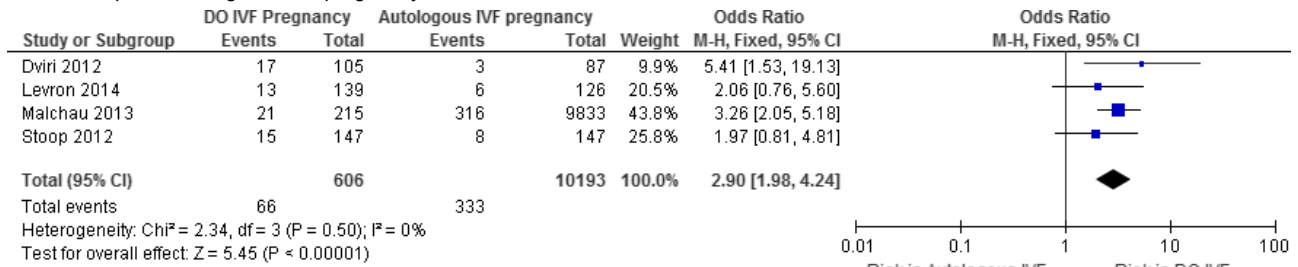
Strengths and limitations

This is the first meta-analysis and meta-regression to quantify the risk of pregnancy complications in women with DO pregnancy. A previous systematic review by van der Hoorn focused on the immunological pathogenesis of complications, but did not include meta-analysis.³⁹ We have quantified the risk taking into account potential confounding factors; therefore, this meta-analysis provides evidence-based information for the clinical care of women with DO pregnancy. The outcomes of DO IVF pregnancies have been compared with autologous oocyte IVF pregnancies in various subgroups, adjusting for maternal age, multiple gestation, and the assisted reproductive technique. The meta-analysis showed small values for I^2 and narrow confidence intervals. This suggests that the accuracy of the meta-analysis is of good quality, and that the estimated value is relatively stable. Low statistical heterogeneity is a major strength of our meta-analysis. A previous systematic review and meta-analysis performed by Pecks showed that DO is a risk factor for the development of PIH; however, the control group included all types of conventional reproductive therapy, including spontaneous conception.⁴⁰ Shanis showed that IVF itself increases the risk of PET.⁴¹ The systematic review by van-der Hoorn concluded that the higher risk of maternal morbidity in DO pregnancy is

Pregnancy-induced hypertension and singleton DO pregnancy



Pre-eclampsia and singleton DO pregnancy



Hypertensive disorders in pregnancy with maternal age > 40 years

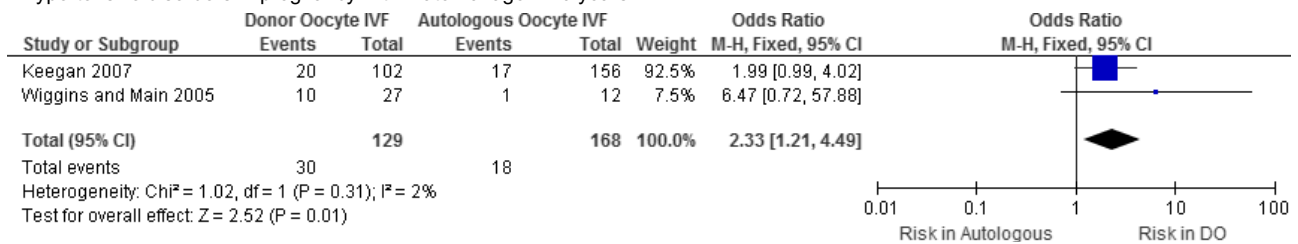


Figure 3. Primary outcome – subgroup analysis.

linked to the high degree of antigenic dissimilarity.¹⁷ In original publications the definitions of FGR, IUGR, or SGA are not standardised. These definitions have evolved over a period of time. We have performed sensitivity analysis to address this limitation. We have used the term SGA, which covers all babies with birthweights of less than the tenth centile. Although we made every attempt to neutralise the effects of confounding factors such as age by performing subgroup analysis and meta-regression, it is difficult to prove that the risk is totally independent of these variables from any meta-analysis and systematic review. It requires a multivariate analysis on an original data set. Ideally, individual participant data (IPD) meta-analysis would be the best design to assess the effect of various other confounding factors. The secondary outcomes of preterm delivery, SGA, and caesarean section may be the result of hypertensive disorders in pregnancy. It is not possible to identify the magnitude of these complications independent of hypertensive disorders. Analysis of any complication as an outcome depends upon prevention and management strategies. The information was not available on preventative

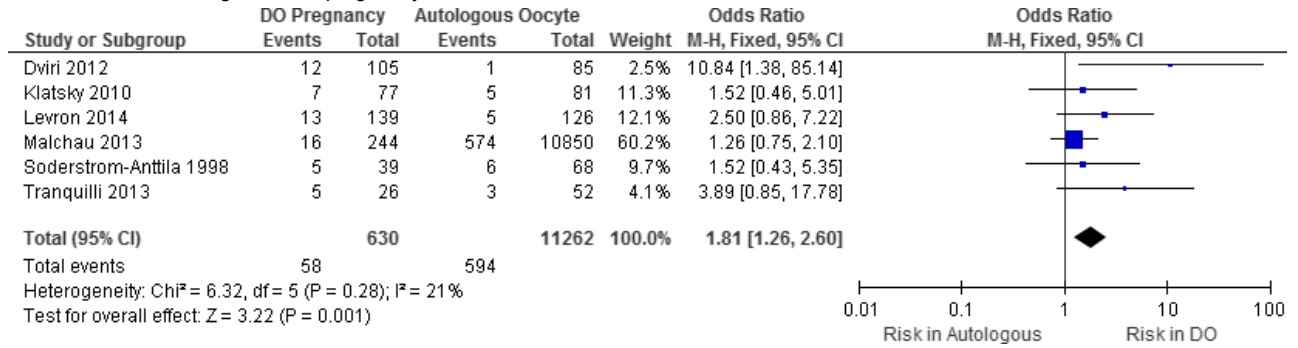
measures such as the use of low-dose aspirin or ultrasound assessment for fetal growth.

Interpretation

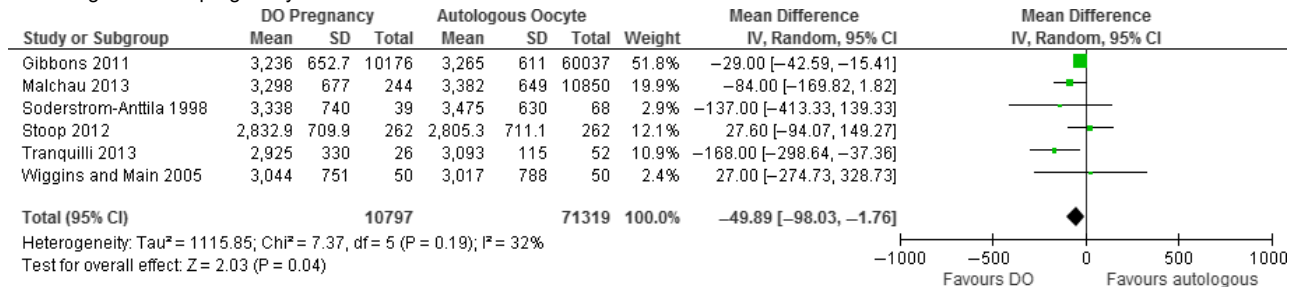
Donor oocyte (DO) IVF provides the opportunity of pregnancy for many women, but at the same time increases the risks associated with pregnancy. Multiple gestations, advanced age, and underlying polycystic ovary syndrome are constant confounding factors for all studies examining the association between assisted reproductive techniques (ARTs) and hypertensive disorders in pregnancy.⁴² Thomopoulos showed that ART pregnancies, especially IVF techniques, are accompanied by increased risks for gestational hypertension and PET, as compared with non-ART pregnancies, even after adjustment for confounding factors.⁴²

The success of pregnancy depends upon an appropriate implantation and placental function. Any insult during the process of implantation and placentation leads to obstetric complications, including spontaneous miscarriage, SGA, preterm birth, and PET.⁴³ The risk of hypertensive disor-

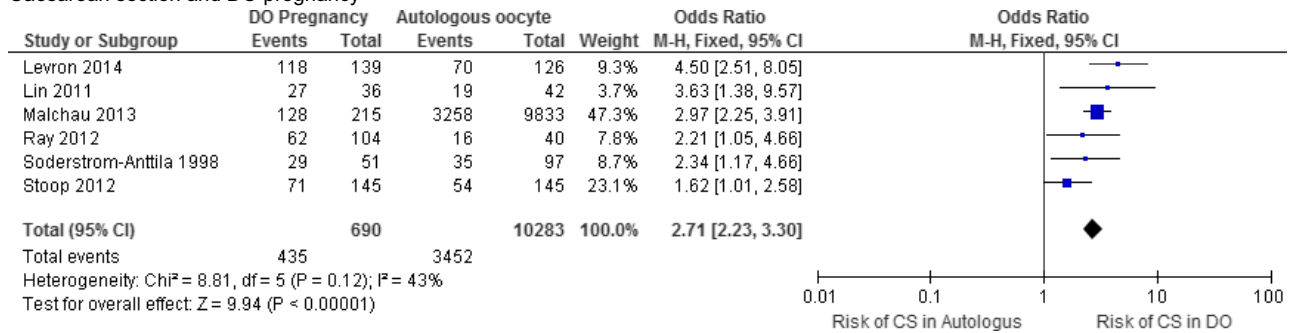
Small for Gestational Age and DO pregnancy



Birth weight and DO pregnancy



Caesarean section and DO pregnancy



Preterm delivery and DO pregnancy

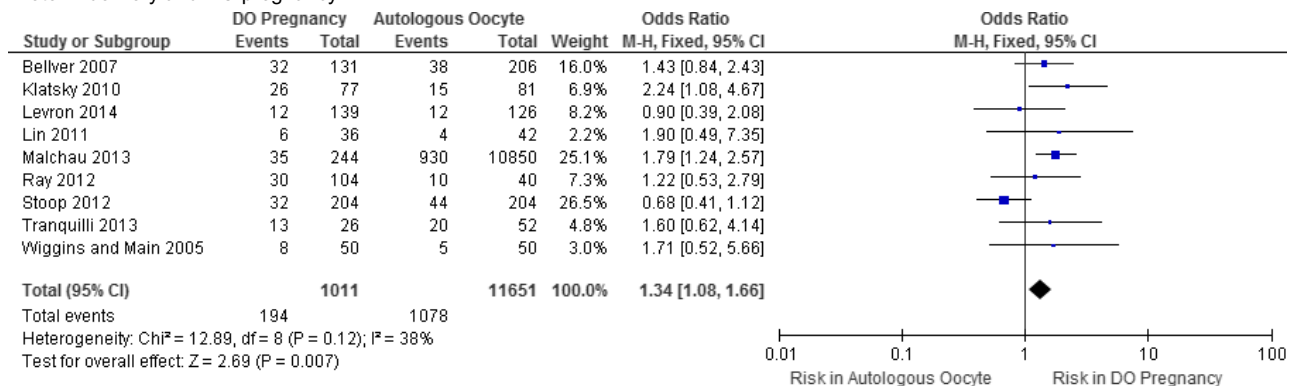


Figure 4. Secondary outcomes.

ders of pregnancy in DO pregnancies can be explained on the basis of an immunological mechanism.^{44,45} In DO pregnancies the fetus is allogeneic to the gestational carrier. Therefore, the mother has to cope with a higher degree of

antigenic dissimilarity compared with spontaneously conceived pregnancies.^{46,47} Increased immunological activity and fibrinoid deposition was noted at the maternal-fetal interface in DO pregnancies.

This represents a host versus graft rejection-like phenomenon.⁴⁸ Parental human leukocyte antigen sharing plays a role in the aetiology of PET.⁴⁹ Considering the immunologic mechanisms at work in DO, it might be worthwhile performing human leukocyte antigen (HLA) typing of the donor and the recipient in order to select haplo-identical combinations that would be more comparable with spontaneously conceived pregnancies than fully HLA mismatched combinations.³⁹ This makes DO pregnancies at higher risk of perinatal complications. Further research is required to explain the pathogenesis of DO pregnancies and placental complications.

Although the DO technique proved to be an excellent treatment option for many women to achieve pregnancy, it exposes them to higher risks of many maternal complications, including maternal death.^{50–52} Women undergoing DO conception should be counselled before conception about the increased risks during DO pregnancy, and that the risk is independent of age or multiple pregnancies.^{51,52} Obstetricians should be aware of the increased pregnancy risks in this particular group of patients, and appropriate surveillance strategies should be in place during antenatal, intrapartum, and postnatal care.⁵¹ The use of serial growth scans to diagnose SGA has resource implications. Therefore, an individualised surveillance and management strategy should be considered. The use of low-dose aspirin in DO pregnancy in the absence of any other risk factors requires further evaluation. Oocyte cryopreservation for future fertility is suggested as an alternative for avoiding DO in selected cases;³⁴ however, data on success rates, the effect on continuing pregnancy, and adverse effects are limited.⁵³

Conclusion

In the light of current evidence, DO pregnancy should be considered as an independent risk factor for pregnancy complications, including hypertensive disorders of pregnancy, SGA, preterm delivery, and caesarean section. Women should be counselled carefully about these risks before undergoing DO-assisted conception. These women should be managed in high-risk obstetric clinics with individualised monitoring and management strategies to reduce complications. The role of low-dose aspirin in DO pregnancy in the absence of any other risk factors requires further research. The use of serial growth scans in DO pregnancies needs further evaluation. Limited evidence attributed these complications to immunological origin; further research is required to explain the pathogenesis involved in donor oocyte pregnancies.

Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship

YJ performed the literature search, reviews, data extraction, meta-analysis, and prepared the manuscript. NP reviewed papers as a second reviewer, performed data extraction, data analysis, and meta-regression. NP contributed to the study design and to writing the article. AO helped with data extraction and the literature search. MK contributed to the design of the study, overall quality assessment, and preparation of the final article.

Details of ethics approval

Not required.

Funding

None.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Risk of bias.

Figure S2. Sensitivity analysis for cohort studies.

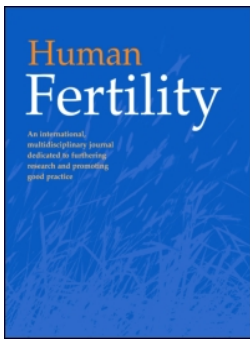
Table S1. Study characteristics. ■

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Strategies to improve fertilisation rates with assisted conception: a systematic review

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ABSTRACT

Successful fertilisation is one of the key steps determining success of assisted conception. Various factors including sperm or oocyte pathology and environmental factors have a significant impact on fertilisation rates. This systematic review is aimed to evaluate the existing evidence about factors affecting fertilisation and strategies to improve fertilisation rates. A literature search was performed using Ovid MEDLINE[®] (Jan 1950–April 2016), EMBASE (Jan 1950–April 2016), Ovid OLDMEDLINE[®], Pre-MEDLINE (Jan 1950–April 2016) and the Cochrane Library. Relevant key words were used to combine sets of results and a total 243 papers were screened. Only qualitative analysis was performed, as there was major heterogeneity in study design and methodology for quantitative synthesis. Factors affecting fertilisation were divided into sperm- and oocyte-related factors. The methods to improve fertilisation rates were grouped together based on the approach used to improve fertilisation rates. Optimising laboratory condition and procedural effects in techniques is associated with improved fertilisation rates. Various techniques are described to improve fertilisation rates including assisted oocyte activation, physiological intracytoplasmic sperm injection (PICSI) and intracytoplasmic morphologically selected sperm injection (IMSI). This review highlights the promising strategies under research to enhance fertilisation rates. Adequately powered multicentre randomised trials are required to evaluate these techniques before considering clinical application.

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KEYWORDS

Fertilisation; assisted oocyte activation; IVF; ICSI; PICSI; IMSI

Introduction

Fertilisation is defined as the process resulting from the fusion of the two parental gametes: the oocyte and the spermatozoon. It induces a cascade of critical events that result in the development of the zygote. For assisted conception, fertilisation is aided by procedures such as IVF and ICSI. Total fertilisation rate is calculated as the total number of zygotes divided by the total number of mature oocytes inseminated (Buffone, Schindler, & Schultz, 2009). Over the last 40 years, since the inception of IVF, fertilisation rates of 60% or over are expected (Braude & Rowell, 2003). Undoubtedly, the live birth rates are related to the fertilisation rates and subsequent development of the zygote to the embryo stage. Structural and functional defects in gametes and laboratory factors can result in reduced fertilisation rates. Fertilisation rates are considered surrogate indicators of the quality of services and laboratory environment (Heitmann et al., 2015).

If all mature oocytes fail to fertilise, it is called total failed fertilisation (TFF). Total failure of fertilisation may

occur due to undetected sperm or oocyte abnormalities (Braude & Rowell, 2003). Sperm-related activation deficiency is seen in globozoospermia and extreme oligoasthenoteratozoospermia (Heindryckx, Van der Elst, De Sutter, & Dhont, 2005). Intracytoplasmic sperm injection (ICSI) has overcome many gamete defects, but total fertilisation failure still occurs in 2–3% of ICSI cycles (Kang et al., 2015; Palermo, Neri, Takeuchi, & Rosenwaks, 2009). Lack of viability, inability of the sperm nucleus to decondense, and chromatin abnormalities can be reasons behind failed fertilisation after ICSI (Nasr-Esfahani, Deemeh, & Tavalaee, 2010). Various sperm function tests are proposed and endorsed by different researchers in addition to the routine semen analysis (Oehninger, Franken, & Ombelet, 2014; Talwar & Hayatnagarkar, 2015). However, sperm function tests are not part of routine fertility investigations as they are complex, expensive, not rigorously tested, do not always provide clinically useful information and typically do not affect treatment outcome (Talwar & Hayatnagarkar, 2015). The most reliable predictor of failed fertilisation is failed fertilisation in the previous

IVF cycle (Roest, Van Heusden, Zeilmaker, & Verhoeff, 1998).

Different strategies have been investigated to overcome gamete dysfunction to improve fertilisation rates. This review is aimed to study the factors affecting fertilisation and strategies to improve fertilisation rates.

Materials and methods

A literature search was performed using Ovid MEDLINE® (Jan 1950–April 2016), EMBASE (Jan 1950–April 2016), Ovid OLDMEDLINE®, Pre-MEDLINE, in-Process & Other Non-Indexed Citations database consists of In-Process and PubMed-not-MEDLINE records from NLM, Google scholar, Web of Sciences (Jan 1950–April 2016) and the Cochrane Library. Searches were conducted using various combinations of the keywords, 'IVF', 'ICSI', 'Fertilisation rates', 'sperm', 'Oocyte', 'laboratory', 'assisted oocyte activation', 'IMSI' and 'PICSI'. These subsets were combined using 'AND' or OR as appropriate to produce sets of results relevant to the research question. The duplicates were removed and searches were limited to human participants and restricted to English language. The reference list of all published articles including review articles was examined for missed citations. No author was contacted. Titles were screened first and then relevant abstracts were obtained. Full text of the articles addressing research question were reviewed. Two reviewers assessed the quality of the evidence (Y. J. and T. G.) and extracted data. Inclusion criteria involved all studies analyzing factors affecting fertilisation and strategies to improve the fertilisation rates. The population was couples undergoing IVF or ICSI cycles. Interventions were various strategies used for improving fertilisation rates. The comparison group was those cycles with no intervention. Primary outcome measure was fertilisation rates. No secondary outcome measure analysis was planned. All types of study designs including case control, cohort and randomized studies were included. Case reports and studies with no comparison group were excluded from qualitative analysis. Quantitative synthesis of suitable studies was planned. This review was registered with PROSPERO database (CRD42016043931).

Results

The search generated 243 articles, which were screened. Total of 51 studies used various methods to improve fertilisation rates (Table 1) and were included in the qualitative analysis. The studies were grouped

based on the different strategies used to improve fertilisation rate. Studies included in the qualitative analysis were not found to be suitable for quantitative synthesis due to extensive heterogeneity in study design and methodology. The factors affecting fertilisation were classified into two groups: (i) sperm- and (ii) oocyte-related factors. Each has been discussed in detail below.

Sperm-related factors affecting fertilisation rates

Sperm origin

Conflicting results for fertilisation are available in the literature after use of ejaculated or surgically retrieved sperm for male factor infertility (Esteves & Agarwal, 2013; Oron et al., 2014). It is important to compare type of sperm abnormality while comparing the source of sperm. Lower fertilisation rates are reported with surgically retrieved sperm from non-obstructive azoospermia men compared with those from obstructive azoospermia (Esteves & Agarwal, 2013; Oron et al., 2014). Few studies show similar fertilisation rate between surgically retrieved sperm and ejaculated spermatozoa (Amirjannati et al., 2012; Jamal et al., 2012). The combination of enzymatic digestion, density gradient centrifugation and stimulation of motility (where indicated) is suggested to improve fertilisation rates (58.4% versus 45%) in testicular sperm extraction (Wober et al., 2015). However, evidence is not adequate to recommend these methods. A recent study suggested that neither the source of spermatozoa nor the aetiology of severe male infertility has any relevant impact on the results of ICSI cycles as long as fresh motile, morphologically normal spermatozoa are used (Gnoth et al., 2015).

Sperm structural defects

The role of sperm morphology for the prediction of fertilisation rate is debated in the literature (Demir et al., 2012; Figueiredo, Tavares, Ferras, Couceiro, & Chaves, 1996). A global impairment of spermatogenesis is supposed to be the origin of the morphological alterations (Perdrix et al., 2011). There is a strong correlation between high relative vacuole area and poor sperm morphology (Perdrix et al., 2012). Sperm head vacuoles were related to impaired fertilisation rate after IVF (Fekonja et al., 2014). In 2001, Bartoov, Berkovitz, and Eltes (2001) introduced the motile sperm organelle morphology examination (MSOME), in which the fine nuclear morphology of motile spermatozoa was examined in real time with an inverted light microscope equipped with high-power differential

Table 1. Strategies to improve fertilisation rates.

Method	Study	Design and population	Intervention	Comparison	Results
Optimizing laboratory condition and procedural effects in techniques Operator dependent	Shen et al. (2003)	Retrospective cohort with first time ICSI ($n = 441$)	Well-trained operators with >5 years' experience in ICSI	Operators compared among themselves	Significant differences in the 2PN rate among the four ICSI operators
ICSI technique	Ebner et al. (2004)	Cohort study with previous ICSI failures ($n = 127$)	Modified ICSI technique	Standard ICSI	Modified ICSI led to significant fertilisation rates
Improvement in laboratory environment	Heitmann et al. (2015)	Retrospective cohort, first autologous IVF or ICSI ($n = 820$)	Improvement in laboratory environment and air quality	Previous facility	Significantly improved outcome
Change in culture media	Huang et al. (2016)	RCT ($n = 750$)	Synthetic serum substitute (SSS)	Various concentration of SSS	Supplemental protein concentration in the ET medium does not affect the treatment outcomes
Assisted oocyte activation Chemical methods	Ceschin et al. (2016)	RCT ($n = 60$)	G1-PLUS™/G2-PLUS™ sequential	GV BLAST™ sole	No difference
	Nakagawa et al. (2001)	Cohort study, oocytes that showed no evidence of normal fertilisation 18 h after ICSI ($n = 170$)	Calcium ionophore A23187 (A23187) and puromycin	Previously failed fertilisation	The sequential treatment of calcium ionophore A23187 and puromycin activates unfertilised oocytes after ICSI
	Moaz et al. (2006)	Cohort study, consecutive cycles with different abnormal sperm morphology, previous failed or low fertilisation rate ($n = 56$)	10 $\mu\text{mol/l}$ ionophore	Control group without AOA	Significantly improved fertilisation rates
	Nasr-Esfahani et al. (2008)	Randomized control study, ICSI for severe teratozoospermia ($n = 87$)	10 μM Ionomycin for 10 min	No AOA	Significant increase in fertilisation rate
	Borges et al. (2009)	RCT, ICSI for male factor ($n = 314$)	Calcium ionophore A23187	Control group without AOA	No significant difference
	Heindryckx et al. (2005)	RCT, Globozoospermia and oligoasthenoteratozoospermia ($n = 30$)	Calcium ionophore A23187 and injection of CaCl_2	Control group without AOA	Fertilisation rate improved
	Ebner et al. (2012)	Cohort study, ICSI for cryptoospermia and azoospermic ($n = 66$)	Ready-to-use Ca^{2+} ionophore solution	Preceding cycle without activation	Both azoospermic (64.4%) and cryptozoospermic (48.4%) men statistically significantly improved fertilisation
	Montag and van der Ven (2012)	Cohort study, poor fertilisation (<50%) in a previous ICSI cycle ($n = 27$)	Ready-to-use Ca^{2+} ionophore solution	Preceding cycle without activation	Higher fertilisation rates for previous poor fertilisation rates
	Vanden Meerschaut et al. (2012)	RCT, history of TFF or low fertilisation	Calcium ionophore (calcimycin) combined with CaCl_2 injection	No AOA	Improve fertilisation rates
	Eftekhar et al. (2013)	RCT, ICSI for teratoospermia ($n = 38$)	Oocyte activation with calcium ionophore	No AOA	No significant difference
	Caglar Aytac et al. (2015)	RCT, normal sperm, diminished ovarian reserve ($n = 296$)	Calcium ionophore solution	No AOA	60.7% and 55.4% no difference

(continued)

Table 1. Continued

Method	Study	Design and population	Intervention	Comparison	Results
	Ebner et al. (2015)	Cohort study, previous <50% fertilisation rate with ICSI (n = 101)	Ready-to-use Ca ²⁺ ionophore solution (A23187)	Preceding cycle without activation	The fertilisation rate of 48% in the study cycles was significantly higher compared with the 25% in the control cycles No effect
Electrical methods	Liu et al. (2014)	RCT, failed to fertilize, mature oocytes (n = 386)	AOA with 7% anhydrous alcohol	No AOA	No effect
	Zhang et al. (1999)	RCT, failed-to-fertilize oocytes (n = 104)	Electrical activation	ICSI with no activation	Improve fertilisation rates
	Manipalviratn et al. (2006)	RCT, failed-to-fertilize oocytes (n = 100)	Electrical activation	ICSI with no activation	Improve fertilisation rates
	Mansour et al. (2009)	Randomized control study, teratospermia or totally immotile spermatozoa (n = 241)	Electro activation and ICSI	ICSI with no activation	The fertilisation rate was statistically significantly higher in the electro activated group
	Baltaci et al. (2010)	Randomized control study, one previous TFF (n = 71)	Electrical activation	ICSI with no activation	Improved fertilisation
Mechanical methods	Tesarik et al. (2002)	Randomized control study (n = 6)	Mechanical manipulation that facilitated entry of calcium into the oocyte	Standard ICSI	Improved fertilisation
TESE and selection of motile sperm in complete asthenozoospermia, necrozoospermia or cryptozoospermia	EH-Nour, Al Mayman, Jaroudi, and Coskun (2001)	Randomized control study, ICSI cycles with no motile sperm (n = 30)	Sperm selection using hypo-osmotic swelling test (HOST)	No HOST	No statistical significance
Selection of sperm using hypo-osmotic swelling test	Sallam, Farrag, Agameya, El-Garem and Ezzeldin, (2005)	Cohort study, immotile testicular spermatozoa treated with ICSI (n = 79)	Spermatozoa used for injection were selected using the modified HOS test	Spermatozoa were selected on the basis of their morphology	Fertilisation rate was significantly higher in the HOS test group (43.6%) compared with the no-HOS test group (28.2%)
Selection of sperm using laser	Aktan et al. (2004)	Cohort study, human-ejaculated spermatozoa /TESE from patients with complete asthenozoospermia (n = 10)	A single laser shot applied at the end of the tail	Selection using hypo-osmotic swelling test	Laser selection gave significantly higher fertilisation rates (45.4% versus 20.4%; p < 0.0001)
Selection of sperm using mechanical Touch	de Oliveira et al. (2004)	Cohort study, ICSI cycles using frozen-thawed testicular sperm (n = 17)	Motile sperm selected using mechanical touch	Morphologically normal appearance	Significant increase in fertilisation rate
Use of pentoxifylline	Kovacic et al. (2006)	Cohort study, surgically retrieved sperms (n = 77)	Short exposure of testicular samples with only immotile sperm to pentoxifylline (PF)-sperm motility stimulator	Untreated immotile sperm	PF group had a higher fertilisation rate (66% versus 50.9%; p < 0.005)
Use of ICSI and rescue ICSI	Beck-Fruchter et al. (2014)	Systematic review, meta-analysis (n = 1863)	Early rescue ICSI, in failed fertilisation	Previously failed or poor fertilisation	Improved fertilisation rates
	Huang et al. (2015)	Retrospective cohort study, early rescue ICSI (n = 13,232)	Early rescue ICSI, in failed fertilisation	Planned ICSI	Similar fertilisation and pregnancy rates as planned ICSI. No increased malformation
	Cao et al. (2016)	Cohort study, cycles with a fertilisation rate of <50% to conventional IVF (n = 313)	Rescue ICSI	Conventional <i>in vitro</i> fertilisation cycle	The fertilisation rates not significantly improved. Improved rate of rescue ICSI for fertilisation rates of <25%

(continued)

Table 1. Continued

Method	Study	Design and population	Intervention	Comparison	Results
Use of PICS	Parmegiani et al. (2012)	Randomized control study (n = 100)	HA culture dish (PICS Sperm Selection Device)	Viscous medium containing HA (Sperm Slow)	No difference in fertilisation rates
	Worrirow et al. (2013)	Randomized control study (n = 802)	Hyaluronan-bound sperm selection	Visual selection	The fertilisation rates not significantly different
	Majumdar and Majumder (2013)	Randomized control study (n = 156)	PICS	Visual selection	The fertilisation rates not significantly different
	Mokanszki et al. (2014)	Non randomized study compared conventional ICSI (n = 140) to PICS (n = 110)	PICS	Visual selection	The fertilisation rates significantly improved
	Choe et al. (2012)	Randomized control study (n = 219)	HA-bound	Visual selection	The fertilisation rates not significantly different
	Bartoov et al. (2002)	Cohort study, motile sperm organelle morphology examination (n = 100)	MSOME	Conventional ICSI	The fertilisation rates significantly improved.
	Bartoov et al. (2003)	Cohort study with of least two previous consequent pregnancy failed ICSI cycles, subjected to IMSI (n = 112)	IMSI	ICSI	The fertilisation rates not significantly different
	Gatimel et al. (2016)	Retrospective comparative study with two previous ICSI failures (n = 216)	IMSI	ICSI	The fertilisation rates not significantly different
	Luna et al. (2015)	Cohort study (n = 31)	IMSI	ICSI	The fertilisation rates not significantly different
	La Sala et al. (2015)	Randomized control study, infertile couples scheduled for their first ICSI cycle (n = 121)	IMSI	ICSI	The fertilisation rates not significantly different
	Setti et al. (2011)	Randomized control study (n = 500)	IMSI	ICSI	The fertilisation rates not significantly different
	Knez et al. (2012)	Randomized control study (n = 122)	IMSI	ICSI	The fertilisation rates not significantly different
	De Vos et al. (2013)	Prospective sibling-oocyte study (n = 700)	IMSI	ICSI	The fertilisation rates not significantly different
Use of polarization microscopy Sperm selection	El Khattabi et al. (2013)	Prospective non-randomized observational study (n = 478)	IMSI	ICSI	The fertilisation rates not significantly different
	Malgorzata et al. (2007)	Cohort study (n = 49)	Electron microscopic spermatozoa morphology check	Conventional IVF	The fertilisation rates significantly improved with normal ultrastructure spermatozoa percentage
	Gianaroli et al. (2008)	Prospective randomized study (n = 231)	Birefringent spermatozoa selected using poloscope for ICSI	Conventional ICSI cycle	The fertilisation rates not significantly different
	Ghosh et al. (2012)	Cohort study of ICSI cycles for azoospermia and	Birefringent spermatozoa selected using poloscope	ICSI using	The fertilisation rates significantly improved in (continued)

Table 1. Continued

Method	Study	Design and population	Intervention	Comparison	Results
Identify the meiotic spindle	Picinato et al. (2014)	complete asthenozoospermia (n = 96)	for ICSI		complete asthenozoospermia but not different in azoospermic men
	Korkmaz, Cinar and Akyol (2011)	Cohort study of couples undergoing ICSI (n = 450) Cohort study (n = 190)	Polarization microscopy (PM) before ICSI Spindle imaging with a PolScope microscope	No-PM group consisted of No visualization	The fertilisation rates significantly improved The fertilisation rates significantly improved
Postcosomal WW binding protein	Aarabi et al. (2014)	Cohort study (n = 110)	Postcosomal WW binding protein levels	Control low PAWP	High levels of PAWP are associated with higher fertilisation rates
Micro electrophoresis Sperm selection	Simon et al. (2015)	Cohort study (n = 88)	Use of negatively charged sperm through micro-electrophoresis	Use of positively charged or neutral sperm	The percentage of negatively charged sperm was associated with improved fertilisation rate

interference contrast (Nomarski/DIC) optics (magnification, $\times 150$) enhanced by digital imaging (magnification, $\times 44$) which achieved a total magnification of over 6000. This is much higher magnification than routinely used for ICSI procedure. MSOME can identify a motile spermatozoon with a normal nucleus and a normal nuclear content (with $< 4\%$ of the nucleus occupied by vacuoles) (Bartoov, Berkovitz, & Eltes, 2001). Although, few studies have showed improved fertilisation and pregnancy rates with MSOME, its clinical use remains unclear (Perdrix & Rives, 2013). Again, no consensus has been established concerning normal or abnormal MSOME criteria (Perdrix et al., 2012). Large-headed spermatozoa are commonly associated with a lower chance of pregnancy and ICSI is advised in these cases (Guthauser et al., 2011). Globozoospermia is characterized by round-headed spermatozoa lacking an acrosome and is found in 0.1% of infertile men (Karaca, Akpak, Oral, Durmus, & Yilmaz, 2015). Similarly, oligoasthenozoospermic men were reported to have lower fertilisation rates, poor embryo quality and lower pregnancy rates (Zhu et al., 2015). Necrozoospermia is defined as sperm viability ≥ 5 to $\leq 45\%$. The aetiological factors include infections, epididymis dysfunction; however, in many cases, no aetiology can be found (Nduwayo, Barthelemy, Lansac, Tharanne, & Lecomte, 1995). Few authors suggest that testicular sperm should be favoured over ejaculated sperm in cases of persistent necrozoospermia (Negri et al., 2014; Tournaye et al., 1996).

Sperm motility and progression

Normal structural appearance of sperm on light microscopy cannot rule out functional deficiencies. Sperm motility disorder associated with bronchiectasia called immotile cilia syndrome (ICS), or primary ciliary dyskinesia (PCD), shows morphologically normal immotile spermatozoa on light microscopy but ultra-structural studies reported a wide spectrum of axonemal defects (Rossman, Forrest, Lee, Newhouse, & Newhouse, 1981). Severe asthenozoospermia or total immotility have also been reported in men with dysplasia of the fibrous sheath (DFS), a multigenic disease with common pattern of flagellar abnormalities (Chemes, Brugo, Zanchetti, Carrere, & Lavieri, 1987; Chemes et al., 1998). Therefore, it is vital to identify truly immotile sperm as it is possible that the sperm may be dead.

The role of ion channels in human sperm function was studied by Brown et al. (2016) found that impaired potassium channel conductance and/or resting membrane potential (V_m) regulation is both

common and complex in human spermatozoa and importantly is associated with impaired fertilisation capacity when the V_m of cells is completely depolarized. The practical implication of this knowledge would be to develop novel diagnostic tools to assess semen quality using membrane permeable voltage-sensitive dyes.

In human spermatozoa, Ca^{2+} influx induced by progesterone is mediated by CatSper, a sperm-specific Ca^{2+} channel. A suboptimal Ca^{2+} influx is significantly associated with abnormal semen parameters and failed fertilisation. Functional failure of CatSper compromises fertility of human sperm. Such defects are rare. Sperm with a near absence of CatSper current fail to respond to activation of CatSper by progesterone. It is associated with fertilisation failure at IVF (Williams et al., 2015). CatSper is a suitable and specific target for further studies.

Premature chromosomal condensation (PCC)

Sperm PCC is one of the prevalent causes of fertilisation failure in both IVF and ICSI (Schmiady, Sperling, Kentenich, & Stauber, 1986). It has been observed that ICSI is associated with higher rate of PCC and higher variations in the degree of condensation as compared with IVF (Schmiady & Kentenich, 1989). When the sperm nucleus can act with the chromosome condensing factors and the oocyte does not become activated, this will lead to the induction of PCC. When a cell, with chromosomes in MII, fuses with an inter-phase cell, the nuclear membrane of the cell in the inter-phase dissolves and its chromatin condenses. This is known as PCC (Johnson & Rao, 1970). The cytogenetic-cytological data on unfertilised human oocyte following ICSI have led to two predominant results including presence of non-activated sperm head in the cytoplasm and PCC (Schmiady, Tandler-Schneider, & Kentenich, 1996). PCC was also observed in fertilised eggs that had formed two or three pronuclei which resulted either from distinct pronuclear asynchrony or interchromosomal asynchrony within the chromosome set (Schmiady & Kentenich, 1989).

Sperm deoxyribonucleic acid (DNA) damage

Sperm DNA integrity tests have been proposed as a mean to assess sperm competence. Several methodologies have been developed to assess sperm DNA fragmentation including the SCSA (Sperm chromatin structure assay) (Evenson, Darzynkiewicz, & Melamed, 1980), sperm chromatin dispersion (SCD) assays (Fernández et al., 2005) and TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) assay

(Gorczyca, Traganos, Jesionowska, & Darzynkiewicz, 1993). There is insufficient evidence to recommend the routine use of sperm DNA integrity tests in the evaluation and treatment of the infertile couple (Barratt et al., 2010). Recent meta-analysis suggests high sperm DNA damage is related to lower pregnancy rates in IVF but not in ICSI cycles, whereas it is associated with higher miscarriage rates in both IVF and ICSI cycles (Zhao, Zhang, Wang, & Li, 2014). Oxidative stress is a major cause of defective sperm function and DNA fragmentation in cases of male infertility (Aitken, Jones, & Robertson, 2012). Potential contribution of antioxidant therapy in the clinical management of this condition is under investigation (Lewis et al., 2013). A Cochrane review found low-quality evidence synthesised from four small randomised controlled trials suggesting that antioxidant supplementation in sub fertile males may improve live birth rates (Showell et al., 2014).

Oocyte-related factors affecting fertilisation rates

Oocyte morphology

Oocyte selection based on morphology has been discussed in the literature (Van Blerkom & Henry, 1992). Oocyte grading based on the three factors of first polar body, size of perivitelline space and cytoplasmic inclusions is found to be significantly related to fertilisation rate and embryo quality after ICSI (Xia, 1997). However, the presence of one or two abnormal factors such as large perivitelline space, spots, vacuoles or refractile bodies did not correlate with fertilisation rate (Dozortsev, De Sutter, & Dhont, 1994). Delayed cytoplasmic maturation with unsynchronized nuclear maturity can lead to morphological changes in oocyte. It has been suggested that oocytes with extreme morphological abnormalities should not be discarded as ICSI may overcome the barriers to fertilisation and cleavage (Esfandiari, Burjaq, Gotlieb, & Casper, 2006; Esfandiari, Ryan, Gotlieb, & Casper, 2005).

Oocyte maturity

Oocyte maturity influences fertilisation rates (Esfandiari, Javed, Gotlieb, & Casper, 2005). Fifteen percent of the oocytes obtained remain at prophase I (~10%) and MI (~5%) stage (Cha & Chian, 1998; De Vos, Van de Velde, Joris, & Van Steirteghem, 1999; Schultz, 2002). It would be logical to hypothesise that the potential use of these oocytes may increase the number of available embryos. Rescue *in vitro* maturation (IVM) is currently not a routine procedure. Previous literature evaluated the clinical outcomes

derived from short-term *in vitro* cultured MI oocytes that progressed to MII oocytes. Majority of studies showed significantly reduced fertilisation rates for MI oocytes compared with MII oocytes (Balakier, Sojecki, Motamedi, & Librach, 2004; De Vincentiis, De Martino, Buffone, & Brugo-Olmedo, 2013; De Vos et al., 1999; Huang et al., 1999; Shin et al., 2013; Strassburger et al., 2004, 2010; Vanhoutte, De Sutter, Van der Elst, & Dhont, 2005). Few authors suggested higher fertilisation rates (up to 60%) in these oocytes (De Vincentiis, De Martino, Buffone, & Brugo-Olmedo, 2013; Huang et al., 1999; Strassburger et al., 2004). Safety is a major concern for embryos derived after *in vitro* maturation of MI oocyte. In one study, nearly 80% of embryos from MI oocytes were genetically abnormal. The rate of aneuploidy falls with the duration of incubation, with 40% after 2 h of incubation period (Strassburger et al., 2010). Current evidence does not support use of MI oocytes.

Oocyte activation

Oocyte activation is a series of events triggered by the fertilising spermatozoon and necessary for the beginning of the zygote development (Tesarik, 1994). Few oocytes fail to get activated during fertilisation. During IVF cycles, the majority of oocytes (60–90%) that fail to fertilise do not contain sperm nuclei. This suggests fertilisation failure is due to failure of sperm to penetrate or sperm ejection (Mahutte & Arici, 2003). After ICSI, the majority of unfertilised MII oocytes show swollen sperm head which suggests failed oocyte activation. Oocyte activation failure could account for 40–70% of unfertilised oocytes exposed to ICSI (Heindryckx et al., 2005; Mahutte & Arici, 2003; Yanagida, 2004). Globozoospermia results in oocyte activation failure due to abnormalities in acrosome formation and oocyte activation capacity (Dam et al., 2007). Even morphologically normal sperm can fail to activate the oocyte. Assisted oocyte activation (AOA) is discussed below.

Strategies to improve fertilisation rates

Optimising laboratory condition and procedural effects in techniques

The technique of performing ICSI procedure is a significant predictor of fertilisation (Shen, Khabani, Klein, & Battaglia, 2003). Immobilization of the spermatozoon and rupture of the oolemma are the two key steps for successful fertilisation (Svalander, Forsberg, Jakobsson, & Wikland, 1995; Van den Bergh, Bertrand, Biramane, & Englert, 1995). Aspiration of the ooplasm is always

used to make sure that the oocyte membrane is broken during injection. Injection of motile sperm without immobilization leads to poor fertilisation rates (Vanderzwalmen et al., 1996). The degeneration of oocytes after ICSI may be due to a faulty ICSI technique. It has been suggested that sperm-borne and oocyte-borne oocyte activation failures can be overcome by modifying the ICSI technique (Tesarik, Rienzi, Ubaldi, Mendoza, & Greco, 2002). Pushing the needle tip close to the membrane opposite the puncture site, aspirating the cytoplasm at this point and releasing the sperm in the centre of oocyte are the modifications suggested to improve the fertilisation rates in oocyte-dependent activation failure (Ebner, Moser, Sommergruber, Jesacher, & Tews, 2004). Laser-mediated ICSI has been suggested to overcome technical difficulty of passing the zona pellucida. A micro-hole with diameter 5–10 μm can be created in the zona pellucida by a laser beam just prior to ICSI and this will allow the injection pipette to pass without any trauma. Laser-mediated ICSI has shown to improve fertilisation rates by a few authors (Choi et al., 2011; Demiroglu, Benkhalifa, Sari, & Gurgan, 2006). It has been proposed that performing ICSI through a laser-drilled hole in the zona pellucida reduces the risk of oocyte damage related to deformation during the initial phase of the microinjection procedure. This technique appears to be suitable for patients whose oocyte show inherent fragility and high degeneration rates after the standard ICSI procedure (Rienzi et al., 2001). Improvements in IVF laboratory conditions and air quality have profound positive effects on outcomes (Heitmann et al., 2015). A recent review suggested that successful and consistent outcomes are associated with optimization of each procedure associated with the collection and processing of gametes (Balaban, Sakkas, & Gardner, 2014). Change in culture media did not show any difference in fertilisation rate (Ceschin et al., 2016). A recent randomised trial suggested supplemental protein concentration in the embryo transfer medium does not affect the treatment outcomes (Huang et al., 2016). In contrast, a review of the literature suggested that out of 11 studies five studies showed that the culture medium not only influences immediate outcome but also has influence on phenotypic characteristics such as birthweight (Zandstra, Van Montfoort, & Dumoulin, 2015). However, recently published large retrospective cohort study of 7295 singletons showed no difference in birthweight despite of changes in clinical care and laboratory practice (Maas, Galkina, Thornton, Penzias, & Sakkas, 2016). There are various limitations of these studies including retrospective design with consecutive use of different culture media,

different composition, limited human studies and limited sample sizes in those studies. There is insufficient evidence on the impact of culture media on birth-weight. Culture medium or oxygen concentration used in *in vitro* culture does not have significant effect on the global gene expression profile of preimplantation embryos (Mantikou et al., 2016). External pH of culture media influences sperm binding, motility (Dale, Menezes, Cohen, DiMatteo, & Wilding, 1998; Emmens, 1947) oocyte maturation (Bagger, Byskov, & Christiansen, 1987; Downs & Mastropolo, 1997) and embryo development. Improper intracellular pH impedes sperm function (Hamamah & Gatti, 1998; Marquez & Suarez, 2007) frequent fluctuations in environmental conditions, including pH of media, could be harmful. They can be transduced into deleterious intracellular perturbations (Phillips, Leveille, Claman, & Baltz, 2000). Embryos have a limited ability to regulate their internal pH (pHi), oocytes lack robust mechanisms (Swain, 2010). Therefore, careful attention to external pH (pHe) of culture media is imperative in IVF. Optimal pHe conditions needed for oocyte maturation, fertilisation and various stages of developing embryos may vary. Increasing buffering capacity of media, using combination buffers or some other novel approach may be beneficial during fertilisation (Swain, 2010).

Spindles are formed from microtubules. They are exquisitely sensitive to changes in temperature. The maintenance of temperature at 37 °C during *in vitro* manipulation is important for spindle integrity, normal fertilisation and subsequent embryo development (Wang, Meng, Hackett, Odenbourg, & Keefe, 2001). They get rapidly depolymerised even after a slight reduction in temperature to 33 °C. Damage to the meiotic spindle may be the cause of aneuploid embryos (Mandelbaum et al., 2004; Zenzes, Bielecki, Casper, & Leibo, 2001). Optimization of laboratory condition and techniques is the most important factor and the only practically controllable method of improving fertilisation rates. Unfortunately, these factors are not studied widely with robust study designs. Therefore, what are optimal laboratory conditions required to maximise the fertilisation rates is an unanswered question.

Assisted oocyte activation (AOA)

Several methods of AOA have been described, which employ mechanical, electrical or chemical stimuli. The use of chemical oocyte activation is the most popular method. Calcium plays a pivotal role in triggering all downstream nuclear and cytoplasmic changes in fertilised oocytes, leading to successful oocyte activation

and the onset of embryogenesis (Ramadan, Kashir, Jones, & Coward, 2012; Tesarik, 1994). Chemical AOA could be performed for predominantly sperm-related or oocyte-related activation failures. Commonly used agents are calcium ionophores. These agents are lipid soluble and they transport calcium ions across the oocyte cell membrane inducing a single transient surge in intracellular calcium concentration (Swann & Ozil, 1994). Calcium ionophores such as ionomycin and calcimycin (A23187, GM508) are used successfully for oocyte activation (Borges, de Almeida Ferreira Braga, de Sousa Bonetti, Iaconelli, & Franco, 2009; Heindryckx et al., 2005; Moaz, Khattab, Foutouh, & Mohsen, 2006; Montag & van der Ven, 2005, Reply: Auer & Schwaerzler, 2005; Nasr-Esfahani et al., 2010; Vanden Meerschaut et al., 2012).

Various randomised and non-randomised studies have shown significantly improved fertilisation rates following AOA using calcium ionophore A23187 (Moaz et al., 2006; Nakagawa et al., 2001; Vanden Meerschaut et al., 2012) whereas some studies failed to find significant improvement (Borges et al., 2009; Caglar Aytac et al., 2015; Eftekhari, Aflatoonian, Mohammadian, & Eftekhari, 2013). AOA with Ca²⁺ ionophore (GM508 Cult-Active) showed significantly better fertilisation rates for both azoospermic and cryptozoospermic men (Ebner et al., 2012). Systematic review showed that oocyte activation (with calcium ionophore) improved pregnancy rate significantly with globozoospermia (Chansel-Debordeaux, Dandieu, Bechoua, & Jimenez, 2015).

The electrical activation with direct current voltage leads to rearrangement of the proteins of the cell membrane, which allow the influx of extracellular calcium (Mansour & Buckett, 2008; Yanagida et al., 1999). Electrical activation significantly increases the fertilisation rates (Manipalviratn et al., 2006; Mansour et al., 2009; Zhang et al., 1999). Mechanical oocyte activation includes advancing the microinjection pipette and aspirating peripheral cytoplasm, followed by deposition of the aspirated cytoplasm and the spermatozoon in the centre of the oocyte. The peripheral cytoplasm, which is rich in mitochondria, increases the energy source at the central site of subsequent pronuclear formation (Ebner et al., 2004). Failed oocyte activation can be overcome by modifying the ICSI technique with mechanical stimulation (Tesarik et al., 2002). The meta-analysis by Sfontouris et al., (2015) concluded that the evidence available from the current RCTs is not sufficient to judge the efficacy or safety of ICSI-AOA on key reproductive outcomes, therefore, these interventions should be further examined by

well-designed RCTs before the introduction of ICSI-AOA as a standard treatment.

AOA has been studied for fertilisation of aged oocyte. It has been suggested that 3-d-old unfertilised human oocytes after IVF or ICSI could be activated by the calcium ionophore A23187 or strontium chloride (Liu et al., 2014). In another study using combination of calcium ionophore A23187 with puromycin, the best results of AOA were achieved in unfertilised oocytes 20 h after ICSI, which exhibited an activation rate of 91.2% (31/34), a cleavage rate of 64.7% (22/34) and 44.1% (15/34) high-quality embryos (Lu et al., 2006). A live birth is reported after calcium ionophore A23187 activation on 1-d-old unfertilised oocytes in a patient with repeated near-total fertilisation failure after ICSI (Lu et al., 2012). A recent study demonstrated that activating post-ICSI aged human unfertilised oocytes with a combination of a calcium ionophore and a cytokine can produce good-morphology euploid blastocysts (Economou et al., 2016). Current evidence on oocyte activation shows early promise but no sufficient clinical evidence to make recommendation for practice.

TESE and selection of motile sperm in complete asthenozoospermia, necrozoospermia or cryptozoospermia

Absolute immotile spermatozoa is one of the most important causes of reduced fertilisation and pregnancy rates after ICSI. In total necrozoospermia, TESE is the only possible option, but retrieving motile or viable spermatozoa for ICSI may not be possible (Tournaye et al., 1996). Although TESE yields more viable sperm, testicular sperm are immotile immediately after biopsy. There are various techniques described to differentiate between immotile and non-viable sperm. Live spermatozoa with normal membrane function show swelling of the cytoplasm and curling of the tail due to water influx when exposed to hypo-osmotic conditions. Mechanical touch technique or the sperm tail flexibility test (STFT) is an easy and cost-effective way for selecting viable immotile spermatozoa. The principle of this technique is to evaluate the tail flexibility by touching it with the ICSI pipette (Soares et al., 2003). Pentoxifylline (PTX) is a 3'5'-nucleotidase phosphodiesterase inhibitor of the methylxantine group that enhances sperm motility by increasing intracellular c-AMP. Its short exposure does not affect early embryo development (Ortega et al., 2011). Short exposure of testicular samples with immotile sperm to pentoxifylline (PTX)-sperm motility stimulator showed higher fertilisation rate (Kovacic, Vlaisavljevic, & Reljic, 2006). A laser is used to

discriminate between viable and dead immotile spermatozoa, curling of the sperm tail seen in viable sperm. Laser-assisted immotile sperm selection showed higher fertilisation rate as compared with hypo-osmotic swelling test (HOS) (Aktan et al., 2004). However, it remains unclear which is the best technique to improve the pregnancy outcomes in cases with absolute immotile spermatozoa (Ortega et al., 2011).

Use of ICSI and rescue ICSI

A retrospective cohort study of 1,395,634 fresh IVF cycles showed that the use of ICSI increased from 36.4% in 1996 to 76.2% in 2012 in the United States, with the largest relative increase among cycles without male factor infertility. However, ICSI use was not associated with improved post fertilisation reproductive outcomes, irrespective of male factor infertility diagnosis when compared with conventional IVF. On the contrary, among cycles without male factor infertility ($n = 317,996$), ICSI use was associated with lower rates of implantation, live births, as compared with conventional IVF (Boulet et al., 2015). Fertilisation rates were not reported in this study. The systematic review and meta-analysis of 11 studies with well-defined unexplained infertility showed marginally higher true fertilisation rate with ICSI as compared with conventional IVF (RR 1.49, 95% confidence interval [CI] 1.35–1.65). The number of subjects needed to be treated with ICSI to prevent one case of TFF was five (Johnson, Sasson, Sammel, & Dokras, 2013). The use of ICSI in the absence of a male factor can adversely affect reproductive outcome when used for poor responders. Probably because of aging-related defects overcoming the advantage of sperm selection, the choice of IVF technique is not relevant to reproductive success when oocyte quality is compromised by reproductive aging (Artini et al., 2013). Early rescue ICSI is proposed as a safe alternative method for couples with total fertilisation failure or near total fertilisation failure when compared with conventional IVF treatment. The fertilisation rates were improved with rescue ICSI but the rate of polyploidy was high; therefore, rescue ICSI is not recommended for patients with a fertilisation rate >25% as the procedure is associated with a greater risk and low returns (Cao et al., 2016). Systematic review on rescue ICSI analysed 38 studies including 1863 patients with failed fertilisation showed pooled pregnancy rate of 14.4% per cycle and rate of malformations was not elevated (Beck-Fruchter, Lavee, Weiss, Geslevich, & Shalev, 2014). However, rescue ICSI is not permitted in the United Kingdom due

to safety concerns. There is a risk of injecting sperm into unfertilised oocyte with prior insemination, which may already contain sperm head. Further evidence on safety and efficacy is required before recommending rescue ICSI.

Use of physiological intracytoplasmic sperm injection (PICSI)

PICSI is based on the uses of hyaluronic acid to select mature sperm for injection. The formation of hyaluronic-acid (HA)-binding sites on the sperm plasma membrane is one of the signs of sperm maturity. PICSI-selected sperm show less DNA damage, and fewer chromosomal aneuploidies (Huszar et al., 2007). A small non-randomised study reported significantly improved fertilisation rates with PICSI (Mokanszki et al., 2014). Other studies did not show any difference in fertilisation rate following the selection of hyaluronan-bound (HB) sperm for ICSI (Choe et al., 2012; Majumdar & Majumdar, 2013; WorriLOW et al., 2013). A Cochrane review concluded that the evidence is insufficient to support sperm selection by hyaluronic acid binding (McDowell et al., 2014). Results from multicentre trial in the United Kingdom, HABSelect, are awaited (Trial registration ISRCTN99214271). This study will provide further evidence on the use of PICSI.

Use of intracytoplasmic morphologically selected sperm injection (IMSI)

IMSI is a technique of selecting a spermatozoon after use of 6000× or higher magnification. The MSOME was significantly and positively associated with both fertilisation rate and pregnancy outcome (Bartoov et al., 2002). The use of MSOME method for ICSI procedure led to the development of IMSI. It is suggested that IMSI improves clinical outcome (Shalom-Paz et al., 2015) but other authors failed to find any difference in fertilisation rates (Bartoov et al., 2003). Observational studies did not find any improvement in clinical outcome after the use of IMSI for two previous ICSI failures (Gatimel, Parinaud, & Leandri, 2016; Luna et al., 2015). Results from many randomised and non-randomised studies suggest that the fertilisation rates are not significantly different with IMSI as compared with ICSI (De Vos et al., 2013; El Khattabi et al., 2013; Knez, Tomazevic, Zorn, Vrtacnik-Bokal, & Virant-Klun, 2012; La Sala et al., 2015; Setti, Figueira, Braga, Iaconelli, & Borges, 2011; Teixeira et al., 2013). Further trials are required to before recommending IMSI in clinical practice.

Use of polarization microscopy

Transmission electron microscope studies have shown that the presence of birefringence in the sperm head indicates a normal sperm structure with the highest potential of development. The use of birefringent spermatozoa is associated with higher fertilisation rate (Malgorzata et al., 2007) and higher clinical pregnancy rates (Gianaroli et al., 2008). Selection of birefringent spermatozoa with the aid of polarised light microscope, called polscope (Polscope, CRi Oosight imaging system, model: MA20511 Cambridge Research Instrumentation, Woburn, MA), shows promising results in asthenozoospermic men and men undergoing TESA or TESE before ICSI (Ghosh et al., 2012). Polarization microscopy to identify the meiotic spindle in oocytes is suggested to improve fertilisation rates (Cohen et al., 2004; Wang, Meng, Hackett, & Keefe, 2001). This may result in potential deleterious impact on embryo development due to additional oocyte handling (Picinato et al., 2014; Rienzi et al., 2003). Therefore, no significant difference in clinical pregnancy, or live-birth rates was found (Picinato et al., 2014). Polarization microscopy is not a widely practiced method and there is insufficient evidence of its usefulness to improve the fertilisation rates.

Postacrosomal WW binding protein (PAWP)

Increased levels of PAWP in sperm are correlated with higher fertilisation rates independently of age, DNA fragmentation index and other sperm parameters. Considering its proposed role in the initiation of oocyte activation, PAWP could have potential applications in the diagnosis and treatment of infertility (Aarabi et al., 2014).

Micro electrophoresis

Selection of negatively charged sperm through micro-electrophoresis has the potential to isolate sperm relatively free of DNA damage to be used in ICSI. The percentage of negatively charged sperm is positively associated with fertilisation rate and blastocyst development and inversely associated with embryo arrest (Simon et al., 2015). Further evidence is awaited on this sperm selection method.

Magnetic activated cell sorting (MACS)

MACS is a novel sperm preparation technique that separates apoptotic and non-apoptotic spermatozoa based on the expression of phosphatidylserine (Makker, Agarwal, & Sharma, 2008). Externalization of

phosphatidylserine (PS) in the inner plasma membrane is one of the earliest signs of apoptosis (Lee, Meng, Flatten, Loegering, & Kaufmann, 2013). Annexin-V magnetic-MACS is based on the binding of superparamagnetic Annexin-microbeads to externalized phosphatidylserine at the outer leaflet of the plasma membrane of sperm with activated apoptosis signaling or membrane damage (Grunewald & Paasch, 2013). The spermatozoa prepared by density gradient centrifugation (DGC) and further processed by MACS showed a reduced level of apoptotic markers and a high fertilisation potential (Lee et al., 2010). In recent study, when the MACS ART annexin V reagent kit (Miltenyi Biotec, Auburn, CA) was used to filter apoptotic sperm using a binding protein with high affinity for PS, annexin V conjugated to microbeads, combined with DCG, was shown to be an effective method to isolate high-quality sperm with progressive motility, non-apoptosis, high DNA integrity, and low protamine deficiency for clinical use (Chi et al., 2016). It has been suggested that this technique could be a potential tool to improve sperm quality on cryopreserved spermatozoa of cancer patient and improve ICSI outcome (Herrero et al., 2013). A meta-analysis of five studies (total $n = 499$) showed sperm selection using MACS resulted in higher clinical pregnancy rates. However, this analysis was based on poor-quality studies (Gil, Sar-Shalom, Melendez Sivira, Carreras, & Checa, 2013). A randomised triple blinded controlled trial involving 237 infertile couples undergoing ICSI as part of an oocyte donation programme showed its use for unselected males does not improve the reproductive outcome (Romany et al., 2014). MACS did not show any clinically relevant adverse effects on obstetric and perinatal outcomes (Romany et al., 2017). The use of this technology for males with suspected cell damage and poor semen quality would be an area for future research.

Conclusion

The commonest cause of failed fertilisation is non-availability of appropriate sperm or failed oocyte activation. Repeated ICSI attempts may result in 85% fertilisation rates. Optimization of laboratory environment and techniques is associated with improved fertilisation rates. Assisted oocyte activation and sperm selection using IMSI and PCSI are widely studied methods to improve assisted conception outcome. Various other novel approaches such as polarised microscopy and micro-electrophoresis are suggested to improve the fertilisation rates. However, multicentre randomized controlled trial are needed to generate

conclusive evidence to support any of these methods before routine clinical application.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Accuracy of first-trimester ultrasound in the diagnosis of early embryonic demise: a systematic review

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KEYWORDS: crown–rump length; fetal cardiac activity; gestational sac; miscarriage; pregnancy loss; study quality; yolk sac

ABSTRACT

Objectives To evaluate, by systematic review of the literature, the accuracy of first-trimester ultrasound in diagnosing early embryonic demise.

Methods We searched MEDLINE (1951–2011), Embase (1980–2011) and the Cochrane Library (2010) for relevant citations. The reference lists of all known primary and review articles were examined. Language restrictions were not applied. Studies which evaluated the accuracy of first-trimester ultrasonography in pregnant women for the diagnosis of early embryonic demise were selected in a two-stage process and their data extracted by two reviewers. Accuracy measures including sensitivity, specificity and likelihood ratios (LRs) for abnormal and normal test results were calculated for each study and for each test threshold.

Results Eight primary articles with four test categories (18 2 × 2 tables), involving 872 women, evaluated the accuracy of ultrasound in diagnosing early embryonic demise. The lower limit of the 95% CI for specificity was > 0.95 in only two tests. These were an empty gestational sac with mean diameter of ≥ 25 mm and absent yolk sac with a mean gestational sac diameter of ≥ 20 mm (specificity, 1.00; 95% CI, 0.96–1.00 for both).

Conclusions There is a paucity of high-quality, prospective data on which to base guidelines for the accurate diagnosis of early pregnancy demise. The findings are limited by the small number of studies and patients, the age of the studies, inclusion of symptomatic and asymptomatic women and variable reference standards for diagnosis of early pregnancy demise. Before guidelines for the safe management of threatened miscarriage can be formulated, there is an urgent need for an appropriately

powered, prospective study using current ultrasound technology and an agreed reference standard for pregnancy success or loss. Copyright © 2011 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Ultrasound examination is the method of choice in the diagnosis of early embryonic demise¹. One in three women miscarries at some time during reproductive life and the incidence of early embryonic demise is high compared with other early pregnancy complications². The diagnosis of failed pregnancy has implications for further management, with associated emotional impact on the mother. Most of the current recommendations regarding the diagnosis of early embryonic demise arose as a result of the public enquiry report investigating the misdiagnosis of the death of embryos³.

Recommendations regarding the ultrasound criteria for the diagnosis of pregnancy failure in the first trimester vary. The American College of Radiologists (ACR) recommends a diagnosis of early embryonic demise when the embryo has a crown–rump length (CRL) > 5 mm without cardiac activity⁴. In the UK, the joint report of the Royal College of Obstetricians and Gynaecologists (RCOG) and the Royal College of Radiologists (RCR) recommends using the following criteria to diagnose pregnancy of ‘uncertain viability’: an intrauterine gestational sac of < 20 mm in mean diameter with no obvious yolk sac, or presence of a fetus or fetal echo of < 6 mm CRL with no obvious fetal heart activity^{5,6}. The Society of Gynaecologists of Canada (SOGC) recommends diagnosis of early embryonic demise with certainty when the mean gestational sac diameter exceeds 8 mm without a yolk sac or when the mean

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gestational sac diameter exceeds 16 mm without an embryo on transvaginal scan⁷. The evidence for all of the above recommendations came from very small studies.

We undertook a systematic review of the literature to assess the accuracy of the various ultrasound criteria in diagnosing early embryonic demise.

METHODS

This review was carried out with a prospective protocol using well accepted methodology⁸.

Search strategy

We searched MEDLINE (1951–2011), Embase (1980–2011) and the Cochrane Library (2010) for relevant citations. We used a combination of MeSH and text words to generate two subsets of citations, one indexing ultrasound ('ultrasound', 'ultrasonography', 'exp ultrasound') and the other indexing outcomes ('miscarriage', 'abortion', 'pregnancy loss', 'early' AND 'pregnancy' AND 'failure', 'fetal OR foetal OR fetus OR foetus' AND 'death OR demise'). These two subsets were then combined with 'AND' to generate a subset of citations relevant to our research question. The reference lists of all known primary and review articles were examined to identify cited articles not captured by the electronic searches. Language restrictions were not applied. A comprehensive database of relevant articles was constructed.

Study selection

Primary studies which evaluated the accuracy of first-trimester ultrasonography in pregnant women for the diagnosis of early embryonic demise were selected in a two-stage process. We included studies that assessed patients symptomatic or asymptomatic of threatened miscarriage in the first trimester. First, the electronic searches were scrutinized and full manuscripts of all citations that were likely to meet the predefined selection criteria were obtained. Second, final inclusion or exclusion decisions were made by the reviewers (Y.J. and S.T.) after examination of these manuscripts. Studies which met the predefined and explicit criteria regarding population, tests, outcomes and study design were selected for inclusion in the review. When disagreements occurred, they were resolved by consensus (Y.J. and S.T.). In cases of duplicate publication, the most recent and complete version was selected. Subjective ultrasound criteria for diagnosis of early embryonic demise were not included.

From each selected article we extracted information on study characteristics, quality and accuracy results. Accuracy data were used to construct 2 × 2 tables of ultrasound findings and pregnancy outcomes.

Methodological quality assessment

All manuscripts meeting the selection criteria were assessed for their methodological quality. Quality was defined as the confidence that the study design, conduct and analysis minimized bias in the estimation of test accuracy. Based on existing checklists, quality assessment involved scrutinizing the study design and relevant features of the population, test and outcomes of the study. A study was considered to be of good quality if it used a prospective design, consecutive enrolment, full verification of the test result with reference standard, and had adequate description of the test.

Data synthesis

Accuracy measures, including sensitivity, specificity and likelihood ratios (LRs) for abnormal and normal test results, were calculated for each study, separately for each test threshold. Heterogeneity of diagnostic odds ratio was assessed graphically using forest plot and statistically using chi-square test to aid in decisions regarding how to proceed with quantitative synthesis. Because, for some tests and outcomes, there was either graphical or statistically significant heterogeneity, we planned to use random effects model meta-analysis. When a quantitative approach was not appropriate due to significant clinical heterogeneity, we refrained from pooling and described the results narratively and reported the accuracy measures estimated in each study. All statistical analyses were performed using the Meta Disc statistical package⁹.

RESULTS

From 720 citations, 23 were reviewed in detail. Eight primary articles with four different categories of tests (18 2 × 2 tables) involving 872 women were included in this systematic review^{10–17} (Figure 1).

Clinical characteristics of the included studies

Four primary articles included women symptomatic of threatened miscarriage^{10–13}, three included both symptomatic and asymptomatic women^{14–16} and one included only asymptomatic women¹⁷ in the first trimester. It was not possible to separate the data of asymptomatic women from those who were symptomatic of threatened miscarriage. The sonographic criteria for diagnosis of early embryonic demise included varied CRL measurements with absent cardiac activity, size of empty gestational sac, absent yolk sac with varied gestational sac sizes and combined criteria (Table 1). The reference standard for pregnancy loss included diagnosis of miscarriage on further scan, clinically diagnosed miscarriage, histopathology, failure of embryo development, falling levels of beta-human chorionic gonadotropin (β -hCG) and evaluation of fetal status on second-trimester ultrasound.

Table 1 Clinical characteristics of studies included in this review of accuracy of early pregnancy ultrasound examination in diagnosing early embryonic demise

Study	Study type & quality	Pop. (n)	Inclusion criteria	Diagnostic test		Reference standard for pregnancy success or loss	Outcome measures
				Machine & operator	Sonographic criteria		
Nyberg <i>et al.</i> ¹⁰ (1986)	Cohort study, consecutive recruitment, no blinding of test, blinding of reference standard, follow-up adequate, test insufficiently described, reference standard described	168	Symptomatic for threatened miscarriage	Machine: commercially available real-time sector and linear array systems Operator: not described	No embryo and MSD ≥ 25 mm; no YS and MSD ≥ 20 mm	Clinical outcome considered normal if living fetus visualized on subsequent sonogram or if clinical record confirmed normal pregnancy progression; outcome considered abnormal if spontaneous miscarriage or absent growth in subsequent sonograms; pathologic examination of uterine curettings and fall in β -hCG considered to indicate abnormal gestation	Clinical miscarriage, US showing absent growth or confirmed normal pregnancy progression
Nyberg <i>et al.</i> ¹⁴ (1987)	Cohort study, arbitrary recruitment, prospective, no blinding of test, no blinding of reference standard, follow-up adequate, test insufficiently described, reference standard described	83	In first trimester with pelvic pain/bleeding/confirmation of pregnancy (symptomatic and asymptomatic)	Machine: real-time sector and linear array systems using 3.5- or 5-MHz transducer Operator: not described	No embryo and MSD ≥ 25 mm; no YS and MSD ≥ 20 mm	Clinical outcome determined by review of medical records and subsequent sonograms; clinical outcome considered abnormal if spontaneous miscarriage or follow-up sonogram demonstrated absent GS growth or absent embryonic development despite adequate (> 14 days) follow-up	Spontaneous miscarriage, US showing absent GS/no embryo development after 14 days' follow-up
Scott <i>et al.</i> ¹¹ (1987)	Cohort study, consecutive recruitment, prospective, no blinding of test, no blinding of reference standard, follow-up adequate, test insufficiently described, reference standard described	102	Presenting as threatened miscarriage	Machine: high-resolution real-time sector scanner using 3.5- or 5-MHz transducer Operator: not described	Empty GS diameter > 26 mm	Viability of GS determined by follow-up US and review of clinical records showing successful outcome of pregnancy	Clinical miscarriage, US showing absent growth or normal growth

Continued over.

Table 1 (Continued)

Study	Study type & quality	Pop. (n)	Inclusion criteria	Diagnostic test		Reference standard for pregnancy success or loss	Outcome measures
				Machine & operator	Sonographic criteria		
Levi et al. ¹⁵ (1988)	Cohort study, consecutive recruitment, prospective, no blinding of test, no blinding of reference standard, follow-up adequate, test insufficiently described, reference standard described	55	Symptomatic and asymptomatic with < 10-week pregnancy	Machine: ESI 1000 or ESI 2000 (Elscent) with 6.5-MHz mechanical sector endovaginal probe Operator: not described	No YS and MSD ≥ 8 mm; no embryo and MSD ≥ 16 mm; no cardiac activity; no embryo or cardiac activity and MSD ≥ 16 mm	All patients except those who opted for termination followed up at least until middle of second trimester; pregnancy considered normal if cardiac pulsation identified on subsequent US	Clinical miscarriage, US showing absent growth or normal growth at least late in second trimester
Levi et al. ¹⁶ (1990)	Cohort study, consecutive recruitment, retrospective, no blinding of test, no blinding of reference standard, follow-up adequate, test insufficiently described, reference standard described	71	Symptomatic and asymptomatic	Machine: ESI 1000 or ESI 2000 (Elscent) with 6.5-MHz mechanical sector endovaginal probe Operator: not described	No cardiac activity and CRL < 5 mm; no cardiac activity and CRL < 4 mm	All patients followed up until termination of pregnancy or at least until late second trimester	Clinical miscarriage or follow-up US showing absent growth or normal growth at least late in second trimester
Ismail and Kishk ¹² (1991)	Cohort study, consecutive recruitment prospective, no blinding of test, no blinding of reference standard, follow-up adequate, test sufficiently described, reference standard described	86	Symptomatic for threatened miscarriage	Machine: SDV3000 abdominal real-time (Philips) with 3.5- and 5-MHz sector transducer Operator: both gynecologist and radiologist	Empty GS largest diameter > 20 mm	Viability of GS determined by follow-up US after 1–2 weeks; GS considered viable if subsequent US demonstrated live fetus	Miscarriage or live fetus on subsequent US
Goldstein ¹⁷ (1992)	Cohort study, arbitrary recruitment, no blinding of test, no blinding of reference standard, follow-up adequate, test sufficiently described, reference standard described	96	Positive pregnancy test at first visit (asymptomatic)	Machine: Aloka 633 (Corometrics) with 5-MHz vaginal probe or Siemens SL1 with 5- or 7.5-MHz vaginal probe Operator: simultaneous observations by physician and nurse	No cardiac activity and CRL < 4 mm; no cardiac activity and CRL < 5 mm; no cardiac activity and CRL > 5 mm; no cardiac activity and CRL > 6 mm	All women followed up until delivery or completion of failed pregnancy	Subsequent miscarriage or delivery of healthy newborn

Continued over.

Table 1 (Continued)

Study	Study type & quality	Pop. (n)	Inclusion criteria	Diagnostic test		Reference standard for pregnancy success or loss	Outcome measures
				Machine & operator	Sonographic criteria		
Tongsong <i>et al.</i> ¹³ (1994)	Cohort study, consecutive, prospective, no blinding of test, no blinding of reference standard, follow-up adequate, test sufficiently described, reference standard described	211	Symptomatic for threatened miscarriage	Machine: real-time Aloka 650 with 5-MHz transvaginal probe Operator: single sonographer	Empty GS MSD ≥ 17 mm; no YS and MSD ≥ 13 mm	Clinical outcome considered normal if living fetus visualized on subsequent US or if clinical records confirmed normal pregnancy progression; GS considered non-viable if subsequent US demonstrated absent growth or spontaneous miscarriage	Miscarriage or live fetus in subsequent US or in medical records

CRL, crown-rump length; GS, gestational sac; MSD, mean gestational sac diameter; Pop., population; US, ultrasound examination; YS, yolk sac.

Study quality

Six of the eight (75%) primary articles were prospective cohort studies which recruited consecutive women (Figure 2). In no study was the operator blinded to the test and in only one study was the operator blinded to ascertainment of reference standard. The test was described in sufficient detail in three of the eight (37%) primary studies. The follow-up was adequate in all studies.

Accuracy of ultrasound

The sensitivity of the included studies for early pregnancy demise ranged from 14% to 100% (Table 2). The highest sensitivity (1.00; 95% CI, 0.54–1.00) was observed in studies using the sonographic criteria of absence of embryo or cardiac activity in gestational sac with a mean diameter of or above 16 mm¹⁵.

Fourteen of the 18 evaluations had specificity > 90% in diagnosing early pregnancy demise (Figure 3, Table 2). The lower limit of the 95% CI for specificity was > 0.95 for only two sonographic criteria: an empty gestational sac with mean diameter ≥ 25 mm (specificity, 1.00; 95% CI, 0.96–1.00) and absent yolk sac with mean gestational sac diameter ≥ 20 mm (specificity, 1.00; 95% CI, 0.96–1.00).

The positive LR for the sonographic diagnosis of early embryonic demise was > 10 in 13/18 (72.2%) of the studies and the negative LR was < 0.1 in 3/18 (16.7%). Table 2 provides the accuracy estimates sensitivity, specificity and positive and negative LRs and false-positive rates for various cut-off levels of ultrasound features.

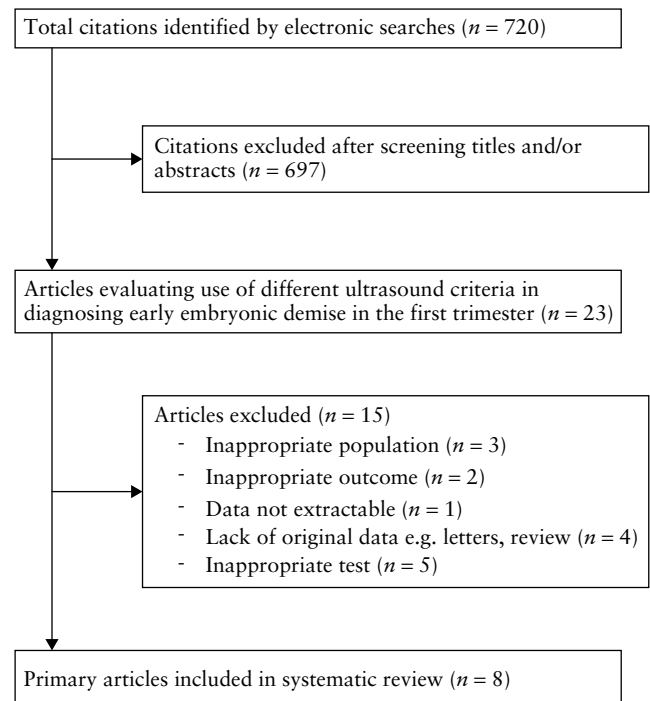


Figure 1 Flow chart of study selection in this systematic review of accuracy of ultrasound examination in diagnosing early embryonic demise.

Table 2 Accuracy of early pregnancy sonographic features in diagnosing early embryonic demise

Sonographic criteria	Study (year)	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR- (95% CI)	FPR
No cardiac activity						
With CRL < 4 mm	Goldstein ¹⁷ (1992)	0.50 (0.01–0.99)	0.59 (0.41–0.75)	1.21 (0.28–5.14)	0.85 (0.21–3.50)	0.41
	Levi <i>et al.</i> ¹⁶ (1990)	0.64 (0.41–0.83)	0.82 (0.63–0.94)	3.56 (1.52–8.38)	0.44 (0.25–0.79)	0.18
With CRL < 5 mm	Goldstein ¹⁷ (1992)	0.50 (0.12–0.88)	0.65 (0.48–0.79)	1.43 (0.58–3.53)	0.77 (0.34–1.77)	0.35
	Levi <i>et al.</i> ¹⁶ (1990)	0.65 (0.45–0.81)	0.88 (0.73–0.96)	5.16 (2.19–12.20)	0.41 (0.25–0.66)	0.12
With CRL > 5 mm	Goldstein ¹⁷ (1992)	0.50 (0.12–0.88)	1.00 (0.90–1.00)	36.00 (2.08–622.64)	0.51 (0.24–1.07)	0
With CRL > 6 mm	Goldstein ¹⁷ (1992)	0.50 (0.07–0.93)	1.00 (0.87–1.00)	27.00 (1.51–482.2)	0.51 (0.21–1.23)	0
Empty gestational sac (GS)						
Diameter > 26 mm	Scott <i>et al.</i> ¹¹ (1987)	0.46 (0.35–0.56)	1.00 (0.69–1.00)	10.05 (0.66–152.18)	0.57 (0.45–0.71)	0
MSD ≥ 25 mm	Nyberg <i>et al.</i> ¹⁴ (1987)	0.20 (0.08–0.39)	1.00 (0.93–1.00)	22.65 (1.32–388.50)	0.80 (0.66–0.96)	0
	Nyberg <i>et al.</i> ¹⁰ (1986)	0.29 (0.19–0.40)	1.00 (0.96–1.00)	50.17 (3.10–811.70)	0.71 (0.62–0.82)	0
Largest diameter > 20 mm	Ismail and Kishk ¹² (1991)	0.81 (0.70–0.89)	0.57 (0.29–0.82)	1.88 (1.02–3.48)	0.34 (0.18–0.65)	0.43
MSD ≥ 17 mm	Tongsong <i>et al.</i> ¹³ (1994)	0.50 (0.43–0.58)	1.00 (0.88–1.00)	31.17 (1.99–489.13)	0.51 (0.43–0.59)	0
MSD ≥ 16 mm	Levi <i>et al.</i> ¹⁵ (1988)	0.50 (0.12–0.88)	1.00 (0.88–1.00)	30.00 (1.74–516.92)	0.51 (0.24–1.07)	0
Absent yolk sac						
With MSD ≥ 20 mm	Nyberg <i>et al.</i> ¹⁴ (1987)	0.14 (0.04–0.33)	1.00 (0.93–1.00)	16.76 (0.93–300.55)	0.85 (0.73–1.00)	0
	Nyberg <i>et al.</i> ¹⁰ (1986)	0.41 (0.30–0.52)	1.00 (0.96–1.00)	70.64 (4.40–1133.7)	0.59 (0.50–0.71)	0
With MSD ≥ 13 mm	Tongsong <i>et al.</i> ¹³ (1994)	0.96 (0.92–0.99)	1.00 (0.69–1.00)	21.12 (1.41–316.93)	0.04 (0.02–0.10)	0
With MSD ≥ 8 mm	Levi <i>et al.</i> ¹⁵ (1988)	0.67 (0.38–0.88)	1.00 (0.92–1.00)	59.06 (3.67–951.17)	0.35 (0.18–0.69)	0
Combined criteria						
No cardiac activity in GS MSD ≥ 16 mm	Levi <i>et al.</i> ¹⁵ (1988)	1.00 (0.54–1.00)	1.00 (0.88–1.00)	55.71 (3.54–877.02)	0.07 (0.01–1.05)	0
No embryo or cardiac activity in GS MSD ≥ 16 mm	Levi <i>et al.</i> ¹⁵ (1988)	1.00 (0.54–1.00)	1.00 (0.88–1.00)	55.71 (3.54–877.02)	0.07 (0.01–1.05)	0

CRL, crown–rump length; FPR, false-positive rate; LR+, positive likelihood ratio; LR-, negative likelihood ratio; MSD, mean gestational sac diameter.

DISCUSSION

For the diagnosis of early embryonic demise there are various ultrasound criteria used which have relatively high specificity and poor sensitivity. The ultrasound features of an empty gestational sac with mean diameter ≥ 25 mm and absent yolk sac with mean gestational sac diameter ≥ 20 mm were the thresholds with the highest and most precise estimates of specificity for diagnosing early embryonic demise.

Specificity versus sensitivity

Most pregnancy screening tests, such as Down syndrome or gestational diabetes screening, strive for optimal

sensitivity whilst tolerating a low false-positive rate. With threatened early pregnancy loss, it is imperative to have a highly specific test with a zero false-positive rate, as the diagnosis of early embryonic demise leads to evacuation of the uterus. While it would be ideal to have both a highly sensitive and highly specific test for early pregnancy loss, it is critical to realize that a false-positive diagnosis of early embryonic demise is likely to result in inadvertent termination of pregnancy. Positive results from highly specific tests would rule in a diagnosis of early pregnancy demise (Specific, Positive, In = SpPIIn)¹⁸. The application of this rule and test performance in the diagnosis of early pregnancy demise will be affected by the following: spectrum bias of different stages of

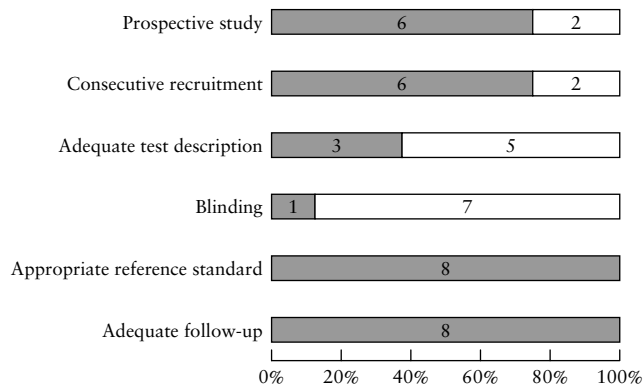


Figure 2 Quality of primary studies ($n = 8$) included in this review evaluating accuracy of ultrasound examination in diagnosing early embryonic demise. ■, Yes; □, No.

pregnancy demise, differential verification bias due to varied application of different reference standards for confirmation of the diagnosis and the small number of included studies¹⁸. The power to rule in a diagnosis of early pregnancy demise will depend on both specificity and sensitivity.

Ultrasound criteria for diagnosis of early pregnancy demise

This review has identified a number of studies and varied criteria for the identification of inevitable early pregnancy demise. An empty gestational sac of ≥ 25 mm diameter and a missing yolk sac with a gestational sac diameter of ≥ 20 mm appear to be the most accurate thresholds for the diagnosis of early embryonic demise, with an estimated specificity of 1.00. However, it should be noted that both thresholds had a 95% CI of 0.96–1.00, indicating that up to four in every 100 diagnoses may be a false-positive one. Although other criteria may have equally high specificities reported, all of these studies involved very few patients.

There was a fair amount of inconsistency among the reported specificities. For instance, empty gestational sacs ≥ 16 mm and ≥ 17 mm were reported to have a high specificity in two studies^{13,15}, but the use of a more conservative threshold (> 20 mm) gave very poor specificity for the diagnosis of early embryonic demise¹². None of the studies evaluated the reproducibility and repeatability of these early pregnancy measurements. Furthermore, only half had access to an endovaginal probe. The latter two criteria are relevant when considering the very small measurements that are taken to make the diagnosis of early pregnancy demise. In clinical practice, operator error is

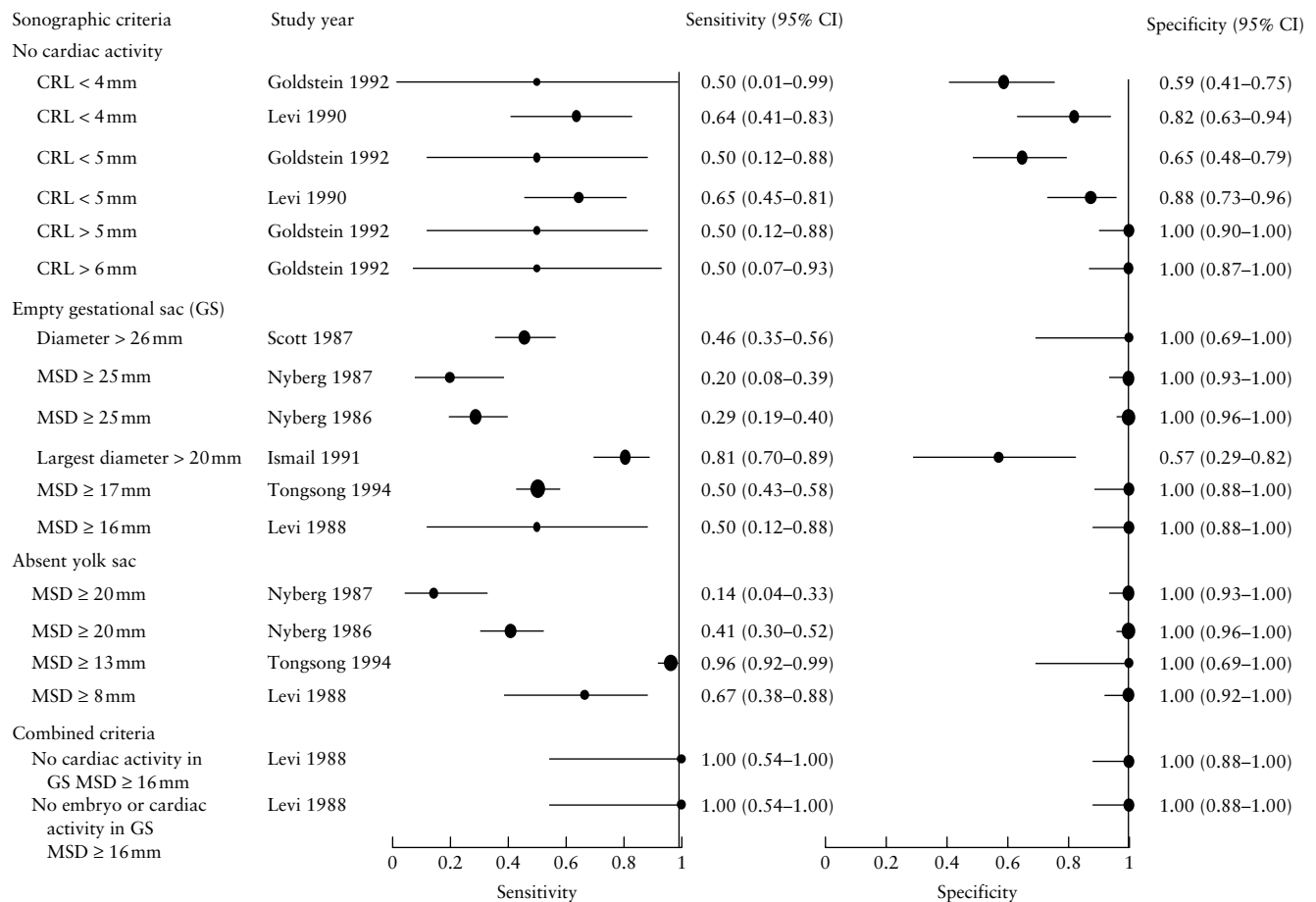


Figure 3 Sensitivity and specificity of first-trimester ultrasound criteria in diagnosing early embryonic demise. CRL, crown–rump length; MSD, mean sac diameter.

common and it is not unusual for small embryos to be missed by inexperienced examiners. The reviewed studies did not stipulate if a policy of two independent examiners confirming the diagnosis was undertaken routinely.

Reference standards

The main problem with these studies is the use of varied reference standards. The only conclusive criterion to diagnose miscarriage is documentation of spontaneous expulsion of histologically confirmed pregnancy tissue or the finding of retained products in the uterus in a woman with previous evidence of intrauterine gestational sac on ultrasound examination. The patient follow-up in such studies needs to be of sufficient length to allow a conclusive diagnosis of early pregnancy demise to be made. This will depend on many factors, such as presumed gestational age, growth of gestational sac, presence of intragestational sac structures and presence of an embryo. It is possible that some abnormal pregnancies will develop much more slowly than do normal pregnancies and that the heartbeat will eventually appear at a much later gestation than normal. Finally, a biochemical threshold, such as a decline in serum hCG on follow-up measurements, may be a powerful predictor of miscarriage, but does not preclude laboratory error or a physiological drop in hCG late in the first trimester. Most studies did not use rigorous standards for the diagnosis of early pregnancy demise, relying rather on medical chart reviews or subsequent ultrasound thresholds to 'confirm' early pregnancy loss, thereby potentially biasing the results.

Conclusion

This review is the first to comprehensively collate evidence of the role of ultrasound in diagnosing early embryonic demise. The review is strengthened by its broad search strategy without language restrictions and assessment of quality of the included studies. The findings were limited by the small number and poor quality of the studies, small number of patients evaluated and heterogeneity in the tests and outcome assessment. Most studies were also conducted some two decades ago and changes in ultrasound technology and the introduction of transvaginal probes would have affected the accuracy and generalizability of the findings. An appropriately powered study using current ultrasound technology, a transvaginal approach and the appropriate reference standard for pregnancy success or loss is urgently required before setting future standards for the accurate diagnosis of early embryonic demise. In order for these studies to be successful, however, a consensus about an appropriate methodological approach should be reached before embarking on further projects.

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A slide presentation, prepared by Dr Tommaso Bignardi, one of UOG's Editors for Trainees, is available online.

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Evidence-based management of recurrent miscarriages

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ABSTRACT

Recurrent miscarriages are postimplantation failures in natural conception; they are also termed as habitual abortions or recurrent pregnancy losses. Recurrent pregnancy loss is disheartening to the couple and to the treating clinician. There has been a wide range of research from aetiology to management of recurrent pregnancy loss. It is one of the most debated topic among clinicians and academics. The ideal management is unanswered. This review is aimed to produce an evidence-based guidance on clinical management of recurrent miscarriage. The review is structured to be clinically relevant. We have searched electronic databases (PubMed and Embase) using different key words. We have combined the searches and arranged them with the hierarchy of evidences. We have critically appraised the evidence to produce a concise answer for clinical practice. We have graded the evidence from level I to V on which these recommendations are based.

KEY WORDS: Aspirin, antiphospholipids syndrome, immunotherapy, low molecular weight heparin, recurrent pregnancy loss, recurrent miscarriage, unexplained

INTRODUCTION

Spontaneous miscarriage is a major loss for all pregnant women. It affects 1% of all women.^[1] The incidence of spontaneous miscarriage may be much greater than is clinically recognized. Spontaneous miscarriage occurs in 12% to 15% of all pregnancies. Thirty percent pregnancies are lost between implantation and sixth week. Maternal age and previous miscarriages increases risk of subsequent miscarriages.^[2] The management of recurrent miscarriages is an unsolved problem; up to 50% of cases of recurrent losses will not have a clearly defined etiology. The investigations and management of recurrent miscarriages is one of the most debated topics. This review is aimed to provide evidence-based approach to manage recurrent pregnancy loss. This review is structured to be clinically relevant.

MATERIALS AND METHOD

Literature search was performed using electronic databases, Embase, and PubMed (1950 to Jan 2014). We have used different keywords and MeSH terms to generate set of results with were combined

to generate most relevant results. The evidence was searched using individual subclass of etiology of recurrent pregnancy loss. Different key words were used such as recurrent miscarriage, recurrent pregnancy loss, habitual abortions, pregnancy failures, unexplained, and idiopathic miscarriage; and these words were combined with various factors known to cause or treat miscarriages. The search results were combined and most relevant results were grouped together for critical appraisal. The evidence was sought for all current recommendations as well as all unanswered questions on investigating and managing recurrent miscarriages. The good-quality meta-analysis was critically appraised and accepted. The recommendations are based on evidence. The evidence is graded as (I-IV).

- I. High-quality meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with a very low risk of bias
- II. Meta-analyses, systematic reviews of randomized controlled trials or randomized controlled trials with a high risk of bias or high-quality case-control or cohort studies

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- III. Well-conducted case-control or cohort studies with risk of confounding, bias
- IV. Nonanalytical studies, e.g. case reports, case series
- V. Expert opinion

Clinical Guideline

Definition

Recurrent miscarriages are defined as three or more consecutive miscarriages.^[1] The Practice Committee of the American Society for Reproductive Medicine^[3] defines recurrent pregnancy loss as by two or more failed clinical pregnancies. The risk of recurrent spontaneous miscarriage is much higher in patients with previous losses. The risk of miscarriage after two consecutive losses is 17% to 25% and the risk of miscarrying fourth pregnancy after three consecutive losses is between 25% and 46%. The risk gets worse with increasing maternal age.^[4] The evidence suggests higher frequency of spontaneous miscarriages amongst subfertile couples and a higher prevalence of subfertility in women with recurrent spontaneous miscarriages when compared with the general population.^[5] Self-reported losses by patients may not be accurate. In one study, only 71% of self-reported clinical pregnancy losses could be verified in hospital records.^[6] It is important to define pregnancy as a clinical pregnancy documented by ultrasonography or histo-pathological examination (Evidence level IV).

Investigations

Genetic

The prevalence of chromosome abnormalities in women facing a single sporadic miscarriage is to be 45%.^[7] Approximately 50% to 60% of early spontaneous miscarriages are associated with a chromosomal anomaly of conceptus. Most common abnormality is aneuploidy, with autosomal trisomy accounting for more than 50% of chromosomally abnormal abortuses.^[8] A strong family history of recurrent miscarriage or genetic anomaly suggests a parental karyotypic abnormality, and a chromosomal analysis of the affected partner is appropriate in the primary evaluation. Chromosomal analysis of the miscarriage offers an explanation in at least 50% of cases.^[9] Parental karyotyping is not predictive of a subsequent miscarriage.^[10] Routine karyotyping of couples with recurrent miscarriage is not recommended. Cytogenetic analysis may be performed on products of conception to avoid unnecessary evaluation and treatment and because an aneuploid conceptus indicates a somewhat greater likelihood of success with a subsequent pregnancy^[10] (Evidence Level III).

Anatomical defects

Women with recurrent pregnancy loss have a 3.2% to 6.9% likelihood of having a major uterine anomaly and 1.0% to 16.9% chance of having an arcuate uterus.^[11] Ultrasound is quick, readily available, economical, and lacks radiation.

2D US can only identify about half of the congenital uterine anomalies present, but it has very low false positive rate.^[12] Some authors consider that this combination of hysteroscopy and laparoscopy can be the gold standard in evaluating congenital uterine anomalies.^[13-16] However, these are invasive tests. Three-dimensional ultrasound by experienced hands is a more accurate than two-dimensional ultrasound and equal to MRI at assessing uterine anomaly.^[17] Sonohysterography is a noninvasive, cost-effective method with 95% accuracy in identifying uterine anomalies.^[18] MRI is a highly sensitive and specific method available because of its superior ability to reliably visualize complex uterovaginal anatomy.^[19] Two-dimensional ultrasound can be used as an initial screening tool. Combined hysteroscopy and laparoscopy, sonohysterography, MRI, and 3D US can be used for a definitive diagnosis (Evidence level II).

Infections

Bacterial vaginosis is a risk factor for preterm delivery and a strong risk factor for late miscarriages.^[20] Vaginal swabs should be considered as screening tests during pregnancy in high risk women with previous history of late miscarriages.^[21] TORCH test is not recommended (Evidence level II).

Haematological disorders

Acquired thrombophilia

Antiphospholipid syndrome (APS) is the only proven thrombophilia that is associated with adverse pregnancy outcomes.^[22] Five to fifteen percent of women with recurrent miscarriage have clinically significant antiphospholipid antibody titres, as compared with 2% to 5% of unselected obstetrical patients.^[23] Antiphospholipid syndrome (APS) is an autoimmune disease with the presence of antiphospholipid autoantibodies (aPL) formed against the person's own tissues. These autoantibodies interfere with coagulation. Recurrent pregnancy loss will test positive for antiphospholipid antibodies (aPLs), the actual reported range varies between 8% and 42%.^[24,25] Laboratory testing for aPL Abs should generally be limited to patients who present with the thrombotic and/or the pregnancy manifestations of the disorder. Weak positive test results for aPL immunoassays are unlikely to have any clinical significance. The two assays that were preferred were the dilute Russell viper venom time (dRVVT) panel, which is widely used in clinical laboratories and is believed to be specific for detecting LA in those patients at high risk of thrombosis,^[26] and an LA-insensitive aPTT.^[27] Testing positive for aCL Abs does not necessarily mean that a patient has APS. The positive test may be triggered by a preceding infection, such as syphilis, Lyme disease, EBV, CMV, HIV, and hepatitis C virus. These patients do not have LA or elevated Abs against β 2GPI.^[28] Anti- β 2GPI immunoassays are more specific but less sensitive for APS

than aCL Ab assays.^[29] The antiphospholipid syndrome should be diagnosed only when two tests performed 12 or more weeks apart are positive (Evidence level I). International Consensus classification criteria for diagnosis of the antiphospholipid syndrome is based on the presence of at least one of the clinical criteria and one of the laboratory criteria.^[25,30] Laboratory criteria includes the presence of Lupus anticoagulant (LA) or Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer Anti- β 2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA. Clinical criteria include vascular thrombosis or pregnancy morbidity. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, severe pre-eclampsia or eclampsia, or recognized features of placental insufficiency before 34 weeks gestation and three or more unexplained consecutive spontaneous abortions before the 10th week of gestation are features of pregnancy morbidity. The presence of any one of these clinical features plus abnormal laboratory test diagnose antiphospholipid syndrome (Evidence level I). When a patient has the clinical appearance of APS but negative standard aPL assay results, there is possibility of seronegative APS. Noncriteria tests such as aCL and anti- β 2GPI IgA Abs and antiphosphatidylserine Abs may help to clarify the picture^[28] (Evidence level II) Ruffatti showed that pregnant women with APS reported that patients with triple aPL Ab-positivity (ie, positivity for LA, aCL, and anti- β 2GPI Abs) and/or previous thromboembolism had an increased likelihood of poor neonatal outcomes than patients with double or single aPL Ab positivity and no thrombosis history.^[31] However, other study showed that lupus anticoagulant is the primary predictor of adverse pregnancy outcome in aPL-associated pregnancies^[32] (Evidence level III).

Inherited Thrombophilia: Antithrombin activity, Protein C activity, Protein S levels, Factor V Leiden (F5), and/or prothrombin G20210A (F2)

Inherited thrombophilias such as factor V Leiden mutation, prothrombin gene mutation (PT 20210A), and deficiencies of natural anticoagulants protein C, protein S, and antithrombin are associated with recurrent miscarriage.^[33] The existence of a causal role for heritable thrombophilia and pregnancy failure is controversial.^[22] A combination of risk factors, including multiple inherited thrombophilic defects are associated with secondary hypercoagulable states.^[34] Case-control studies have shown a modest association (odds ratios of 2 to 3) between recurrent miscarriage and thrombophilias such as the factor V Leiden mutation and the prothrombin G20210A mutation.^[35] This association is stronger for fetal deaths, such as stillbirths after 20 weeks' gestation, than for recurrent early losses.

Many other large prospective cohort studies have not shown significant associations between thrombophilias and sporadic pregnancy loss.^[36-38] However, the strength of the association between inherited thrombophilia and recurrent miscarriage is not very strong, and more importantly, no evidence indicates that the use of anticoagulants improves the chance of live birth in these women.^[39] A disadvantage of testing patients with a VTE for thrombophilia is the high costs of testing. Thrombophilia testing should not be performed routinely in women with recurrent miscarriage except in the context of scientific studies^[39] (Evidence level I).

MTHFR mutation

MTHFR (EC 1.5.1.20) is a key enzyme in one-carbon metabolism. The enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the predominating circulating form of folate.^[40] MTHFR gene polymorphisms are commonly associated with hyperhomocysteinemia^[41,42] Thus, hyperhomocysteinemia is considered as a risk factor for neural tube defects (NTD)^[43,44] and recurrent embryo loss^[44,45] Homocysteine levels vary, depending on an individual's state when measured (intake of folic acid, vitamin B12); therefore, it is difficult to achieve representative results. Mild hyperhomocysteinemia has been identified as a risk factor for arterial disease and venous thrombosis. The evidence is conflicting on hyperhomocysteinemia as a risk factor for recurrent miscarriage.^[46,47] Therefore, testing for MTHFR mutation is not a part of routine evaluation for recurrent miscarriage. (Evidence level II).

Thromboelastography

The test is not recommended as routine investigation for recurrent miscarriage currently as little evidence to support it (Evidence level III). It is argued that thromboelastography identifies a proportion of women with recurrent early miscarriages who are in a pro-thrombotic state outside of pregnancy.^[48] Thromboelastography, a "near patient" test of whole blood hemostasis by dynamically assessing the kinetics, strength and stability of the fibrin clot.^[49]

Endocrine:

PCO, elevated LH, and insulin resistance

Polycystic ovaries (PCO) is the most commonly identified ultrasound abnormality amongst women with recurrent miscarriages.^[50] The prevalence of PCO is 40% among women with recurrent miscarriage; however, polycystic ovarian morphology is not predictive of pregnancy loss amongst ovulatory women with recurrent miscarriage conceiving spontaneously. The ultrasound diagnosis of PCO in women with a history of recurrent miscarriage does not necessarily predict a poor outcome in subsequent pregnancy and therefore it is not recommended^[51] (Evidence level III).

It has been reported that hypersecretion of basal LH with or without polycystic ovaries is a risk factor for miscarriage. Women with elevated LH, a frequent feature of the polycystic ovary syndrome, are at increased risk for miscarriage after either spontaneous or assisted conception. However, suppression of endogenous LH release before conception, in women with elevated circulating LH concentrations and a history of recurrent miscarriage, did not improve the live birth rate. Neither an elevated serum luteinizing hormone concentration (>10 IU/l) nor an elevated serum testosterone concentration (>3 nmol/l) was associated with an increased miscarriage rate.^[52]

Insulin resistance plays a significant role in recurrent pregnancy loss.^[53] Insulin resistance can be independent of polycystic ovarian status. Women with a history of recurrent miscarriage are at an increased risk for insulin resistance during the first trimester of a new pregnancy.^[54,55] Recent meta-analysis concluded that insulin resistance is associated with the susceptibility to recurrent miscarriages, and it may contribute to the occurrence of recurrent miscarriages.^[56] Therefore, insulin resistance might be one of the direct causes that lead to recurrent miscarriage.^[57]

Luteal Phase defect

The shortened luteal phase has been associated with pregnancy loss but the assessment and interpretation of a putative luteal phase defect is problematic. The use of histological and biochemical endpoints as diagnostic criteria for endometrial dating are unreliable (Evidence level III).

Diabetes Mellitus

Evaluation for diabetes is advised with clinical suspicion. Glycated hemoglobin test is advised to screen diabetes. The best test is the oral glucose tolerance test (OGTT), but it is the most expensive, is inconvenient. Fasting plasma glucose would miss people with impaired glucose tolerance. Glycated hemoglobin does not require fasting, and may be the best compromise^[58] (Evidence level III).

Thyroid Disorders

Thyroid Function Tests and Thyroid antibody (Thyroid Peroxidase Antibody -TPO) Tests: Recurrent miscarriages are associated with clinical and sub clinical thyroid disorders. Thyroid function tests are recommended based on clinical history while evaluating miscarriages. Evidence is controversial about role of TPO antibodies.^[59] Pregnant women with subclinical hypothyroidism or thyroid antibodies have an increased risk of recurrent miscarriage.^[60,61] TPO antibody screening is not recommended^[62] (Evidence level II).

Immunology

A significant proportion of recurrent pregnancy loss is associated with immune aetiologies.^[63] Various mechanisms

are suggested. Peripheral natural killer (pNK) and uterine NK (uNK) cells have been associated with reproductive failure. Abnormally functioning immunocompetent cells, including natural killer (NK) cells, in the endometrium, are thought to be responsible and treatment trials including oral prednisolone and intravenous immunoglobulins are now underway.^[64] Peripheral immunological dysfunction is observed with recurrent miscarriage.^[65] Chronic histiocytic intervillitis is a rare type of placental pathology that is associated with reproductive loss. It is considered to be an immunologic origin.^[66] Many studies have suggested that women with recurrent miscarriages have signs of generally exaggerated inflammatory immune responses both before and during pregnancy and signs of breakage of tolerance to autoantigens and fetal antigens.^[67] There is neither an adequate standardization of counting uterine NK cells nor consensus as to what constitutes an abnormal level.^[64] The prognostic value of measuring pNK or uNK cell parameters is uncertain. Further evidence is required to confirm or refute the role of NK cell assessments as a predictive test for screening women who may benefit from immunotherapy.^[68] No immunological test is currently recommended in the recurrent miscarriage work up (Evidence level I).

Male factors

Sperm samples from recurrent pregnancy loss couples have an increase in their sperm DNA fragmentation.^[69-71] Meta-analysis showed a significant increase in miscarriage in patients with high DNA damage compared with those with low DNA damage.^[72] The associating sperm quality with recurrent pregnancy loss emphasizes the importance of evaluating male factor by tests. Several different tests are available, but no consensus has yet been reached as to which tests are most predictive. Among terminal uridine nick-end labeling assay (TUNEL), sperm chromatin structure assay (SCSA), sperm chromatin dispersion (SCD), and alkaline Comet assays, the alkaline COMET assay showed better prediction for male infertility.^[73] A chromosomal abnormality was found in 15.2% men with azoospermia and in 2.3% nonazoospermic men. Male factors abnormality is a significant cause for recurrent pregnancy loss after assisted conception. The number of azoospermic men who needs to be screened to prevent one miscarriage is 80–88 and the number need to screen is 315–347 in the nonazoospermic group.^[74] Although there is some evidence of association between DNA defragmentation and recurrent miscarriage, well-designed prospective studies are needed before using these tests in clinical practice.^[75] Routine testing for sperm ploidy (e.g. fluorescence *in situ* hybridization [FISH]) or DNA fragmentation is not recommended (Evidence level II).

Management

Referral to recurrent miscarriage clinic and expert advice help us improve the reproductive outcome

Tender loving care and lifestyle advice

A cause for recurrent miscarriage can be identified approximately 50–60% of the time. There is tremendous psychological impact of recurrent miscarriage.^[76] Psychological support in the form of frequent discussions and sympathetic counseling are crucial to the successful evaluation and treatment of the anxious couple. When no etiologic factor is identified, no treatment started at 60% to 80% fetal salvage rate still may be expected. Therefore, couples with unexplained recurrent miscarriage should be offered appropriate emotional support and reassurance^[77] (Evidence level III). Obesity,^[78] cigarette smoking,^[79] alcohol use,^[80] and moderate-to-heavy caffeine use^[81] may be associated with sporadic miscarriage, but its association with recurrent miscarriage is uncertain. Cigarette smoking has been suggested to have an adverse effect on trophoblastic function and is linked to an increased risk of sporadic pregnancy loss.^[82] The Cochrane review concludes that any vitamin supplements prior to pregnancy or in early pregnancy do not prevent women experiencing miscarriage or stillbirth.^[83] Lifestyle modification and stress reduction should be emphasized by pointing out that a healthier lifestyle, free from tobacco, alcohol, illicit drugs, and undue stress cannot hurt and may significantly improve the couple's chances for a successful pregnancy (Evidence level III).

Genetic anomalies

In vitro fertilization (IVF) plus prenatal genetic testing is a suggested strategy in the management of couples with chromosomal abnormalities and recurrent miscarriages.^[84] It is proposed as a faster method of conceiving a live child than natural conception, at least for translocation carriers with recurrent miscarriages.^[85] However, this evidence is being questioned. Systematic reviews showed that there is no conclusive evidence to support prenatal genetic screening for unexplained recurrent miscarriages as well as structural chromosome abnormality.^[84,86,87] The new technologies such as trophoblast-laser-assisted blastocyst biopsy and molecular karyotyping via whole genome amplification and either comparative genomic hybridization (CGH) or single nucleotide polymorphism (SNP) arrays helped to revitalize the concept of preimplantation genetic screening. The evidence from these newer technologies is awaited. Because of the lack of evidence, assisted conception with preimplantation genetic screening as a treatment of recurrent miscarriage is not recommended (Evidence level II).

Anatomical defects

Almost 65% to 85% of patients with bicornuate or septate uteri have a successful pregnancy outcome after metroplasty. However, 59.5% of the patients with such anomalies have a successful subsequent pregnancy

without surgery, with a cumulative live birth rate of 78.0%. Further evidence is needed to recommend metroplasty surgery in these women^[11] (Evidence level II). The clinical management of pregnancy-loss patients with Asherman syndrome/intrauterine synechiae, uterine fibroids, and uterine polyps is also controversial, and there is no conclusive evidence that surgical treatment reduces the risk of pregnancy loss. Minimally invasive surgeries are the better option for the treatment of structural defects. Cervical incompetence is treated with cervical encirclage; however, the CERVO trial demonstrated no added benefit of cerclage.^[88] Trans-vaginal Ultrasound examination in subsequent pregnancy is indicated with history of midterm loss due to cervical incompetence. The current data suggest that emergency cerclage is associated with a longer latency and period better pregnancy outcomes when compared with bed rest.^[89] (Evidence level III). The accuracy of cervical length in predicting preterm delivery is relatively poor.^[90] Compared to the McDonald technique, the Shirodkar technique was more effective in prolonging pregnancy in patients with singleton pregnancies undergoing ultrasound-indicated cerclage^[91] (Evidence level III). Cerclage, vaginal progesterone, or pessary are equally efficacious in the prevention of preterm birth in women with a short cervix detected on sonography at the midtrimester in singleton gestation^[92,93] (Evidence level II).

Infection

Treatment of asymptomatic abnormal vaginal flora and bacterial vaginosis with oral clindamycin early in the second trimester significantly reduces the rate of late miscarriage and spontaneous preterm birth in a general obstetric population^[20,94] (Evidence II).

Endocrine disorders

It is generally agreed that maternal endocrine disorders (e.g. diabetes, thyroid dysfunction) should be evaluated and treated.^[95,96] Though elevated LH is associated with increased risk of miscarriage suppression of LH secretion with GnRH agonist prior to ovulation induction yielded no difference in outcome. Hyperprolactinemia may be associated with recurrent pregnancy loss through alterations in the hypothalamic-pituitary-ovarian axis, resulting in impaired folliculogenesis and oocyte maturation, and/or a short luteal phase. Normalization of prolactin levels with a dopamine agonist improved subsequent pregnancy outcomes in patients with recurrent pregnancy loss. Hyper-prolactinemia is treated with dopamine agonist.^[97] Thyroid disorders can be treated medically to achieve euthyroid status and medications should be modified with pregnancy appropriately. There is lack of consensus regarding the definition of a normal upper limit of TSH. A consensus is emerging that TSH values more than 2.5 mIU/L are outside the normal range.^[3]

Thyroid hormone requirement in early pregnancy is higher. The aim is to maintain baseline TSH < 2.5 mU/L. Some evidence suggests association of raised thyroid (TPO) antibodies with recurrent miscarriage.^[62,98] Levothyroxine 50 µg daily for the women with raised TPO antibodies with normal TSH is suggested intervention. Observational study suggest TPO Ab-positive status does not have a prognostic value regarding the outcome of a subsequent pregnancy, and empirical thyroxine therapy in those who tested positive did not seem to improve outcome.^[59] The thyroid antibodies and levothyroxine study (TABLET) study is a randomized controlled trial of the efficacy and mechanism of levothyroxine treatment on pregnancy and neonatal outcomes in women with thyroid antibodies. This study will help to find the role of thyroxin treatment for women with normal thyroid function tests but raised thyroid peroxidase antibody (TPO) (<http://www.controlled-trials.com/ISRCTN15948785/>). Until robust evidence is available, thyroxine treatment is not recommended in raised thyroid antibody status with normal thyroid function tests (Evidence level III).

Progesterone supplementation

The progesterone act as immunomodulator and it shift from proinflammatory Th-1 cytokine responses to anti-inflammatory Th-2 cytokine response which is more favorable and pregnancy protective.^[99,100] Dihydroprogesterone is a potential immunomodulator, it produces progesterone-induced blocking factors (PIBF) which is protein produced by pregnancy lymphocyte following exposure to progesterone. PIBF inhibits cell-mediated cytotoxicity and natural killer cell activity. Thus, it is immunoprotective for pregnancy. Administration of progesterone to women with sporadic miscarriages is ineffective.^[97,101] However, in patients with three or more consecutive miscarriages immediately preceding their current pregnancy, empiric progestogen administration may be of some potential benefit.^[97,101-103] A large multicenter study called promise study (<http://www.medscinet.net/promise>) is currently underway to assess the benefit of progesterone supplementation in women with unexplained recurrent miscarriages. Most commonly used regime is micronized progesterone tablets 400 mg daily. The route of administration may be either vaginal or oral. The argument for use of progesterone is that there is no evidence of harm and some evidence of benefit, although not coming from huge multicentric trials. The decision should be based on clinician's discretion until strong evidence is available to recommend routine use (Evidence level III).

Metformin

Data on the use of metformin to decrease the chance of miscarriage are contradictory as no adequately powered

trials have been published. Insulin resistance is an independent risk factor for spontaneous miscarriage in spontaneous pregnancy. Patients with insulin resistance should be advised to improve their insulin sensitivity through lifestyle change or medical intervention before infertility treatment to reduce their risk of spontaneous miscarriage. Nonrandomized studies have shown that the reduction in insulin levels with metformin in insulin-resistant individuals may reduce miscarriage risk by restoring normal hemostasis and improving the endometrial milieu^[104,105] Metformin is not recommended as a treatment of recurrent miscarriage (Evidence level III).

Hematological disorders:

Antiphospholipid syndrome

Low doses of acetylsalicylic acid and low molecular weight heparin (LMWH) are the best solution in women suffering from recurrent spontaneous miscarriage. This treatment combination of low dose aspirin and low molecular weight heparin reduces the miscarriage rate by 54%.^[106] The role of low molecular weight heparin and aspirin treatment specifically for the prevention of recurrent miscarriage remains controversial. The meta-analysis showed the combination of unfractionated heparin and aspirin confers a significant benefit in live births. However, the efficacy of low molecular weight heparin plus aspirin remains unproven as LMWH data were based on only two trials. These trials were criticized as studies were not blinded and the randomization procedure had been criticized in one of the trials and inclusion criteria were very different. Third trial showed no significant difference in live birth rate with LMWH treatment versus aspirin or a combination of both versus aspirin in women with recurrent miscarriage.^[107] A small trial showed comparable results with LMWH plus aspirin as an alternative to unfractionated heparin and aspirin in the management of recurrent miscarriage secondary to APS.^[108] The consensus is combination of low molecular weight heparin and aspirin is superior to aspirin alone in achieving more live births. Therefore, it is recommended treatment for recurrent miscarriages with antiphospholipid syndrome^[109,110] (Evidence level I). Glucocorticoids should not be given in antiphospholipid antibodies syndrome without connective tissue disorder. Low-dose prednisone is given when lupus is present and with the advice of rheumatologist. Prednisone does not prevent recurrent fetal death in women with antiphospholipid antibody.^[111] Women with a history of thrombosis in whom the antiphospholipid syndrome or a heritable thrombophilia is diagnosed should receive an appropriate dose of unfractionated heparin or low-molecular-weight heparin^[23] (Evidence level I). A third trial could not find that heparin/aspirin was better than aspirin alone.

Inherited Thrombophilia

Role of anticoagulation therapy in the treatment of recurrent miscarriages with hereditary thrombophilia is debatable. Few studies suggested low molecular weight heparin therapy during pregnancy may improve the live birth rate of women with second-trimester miscarriage associated with inherited thrombophilia.^[112-114] However, there is currently no evidence supporting treatment, because observational research is hampered by poor methodology or inconsistent results.^[115] Recent meta-analysis showed that the use of LMWH in women with inherited thrombophilia with recurrent pregnancy loss is not indicated.^[116] Women with thrombophilia should be followed closely without routine prophylactic low molecular weight heparin other than for prevention of venous thromboembolism in limited circumstances (Evidence level I).

Hyperhomocysteinemia and MTHFR mutation

High-dose folic acid (5 mg) and vitamin B12 (0.5 mg) once daily has been reported to reduce levels of homocysteine; however, a randomized-controlled trial on the effect of variable doses of both vitamins on pregnancy has yet to be conducted. High-dose folic acid is considered with women with high BMI and diabetes. There is no evidence to support usage of 5 mg folic acid from pre-pregnancy stage purely to reduce the risk of recurrent miscarriage (Evidence level III).

Fibrinolytic anomalies

Activators and inhibitors of the fibrinolytic system are frequently abnormal in recurrent miscarriage.^[117] Plasma levels of tissue factor activity, thrombomodulin activity, and procoagulant phospholipids were significantly higher in recurrent miscarriage group.^[118] Clinical evaluation of recurrent miscarriage does not include investigating fibrinolytic anomalies. It is limited to the research interest.

Immunotherapy

Both organ-specific and systemic autoimmunity are associated with an increased prevalence of recurrent miscarriage. Immune modulating therapies have been mooted as potential therapeutic strategies. There is no specific immunological test or clinical method, which will predict the need for treatment. Mechanisms of possible efficacy of high dose of intravenous immunoglobulin therapy for recurrent miscarriage may include enhancement of CD94 expression and subsequent suppression of NK cell cytotoxicity.^[119] Evidence does not support routine use of intralipid therapy.^[120] The Cochrane review analyzed various strategies including paternal cell immunization, third-party donor leukocytes, trophoblast membranes, and intravenous immune globulin. None of these interventions proved beneficial over placebo in improving the live birth rate.^[121] This Cochrane review has been widely criticized for not making the necessary sub-analyses between primary and

secondary recurrent miscarriage. Meta-analysis showed that IVIG increased the rates of live birth in secondary recurrent miscarriage, but there was insufficient evidence for its use in primary recurrent miscarriage.^[122] There is risk of possible complications such as undesirable immune responses and the possibility of transmitting infectious diseases like cytomegalovirus. The risk of transmitting infectious disease with intravenous immunoglobulin is extremely small. Most recent systematic review and meta-analysis concludes that NK cell analysis and immune therapy should be offered only in the context of clinical research.^[123] The current recommendation is immunotherapy should not be advised.^[121] (Evidence level II)

Unexplained recurrent miscarriage

- Psychological support: Stress itself is a risk factor for miscarriage^[124] and recurrent miscarriage is a stressful condition so that the vicious cycle can be broken by strong psychological support. Women should be reassured for a successful future pregnancy with supportive care.^[125,126] (Evidence level III)
- Aspirin 75 mg OD: Evidence is debatable. There is paucity of evidence to make any recommendation on aspirin for treating recurrent miscarriage in women without antiphospholipid syndrome.^[115] Few RCT suggested clear benefit of using aspirin for such women.^[127] Recent trial failed to support any role of Aspirin in unexplained recurrent miscarriage.^[128] Aspirin helps in improving uterine perfusion.^[129] Aspirin is useful in many undiagnosed implantation failure patients. However, in the absence of strong evidence, routine use of Aspirin is not recommended (Evidence level II)
- Progesterone: Meta-analysis of 4 randomized trials and only 132 women in total showed a statistically significant reduction in miscarriages.^[130] Further, the evidence is awaited before making recommendation on use of progesterone in explained miscarriage. (Evidence level III)
- LMWH: Use of LMWH to prevent miscarriage is not recommended in the absence of antiphospholipid syndrome (Evidence level II)
- Human chorionic gonadotrophin (hCG): Recent Cochrane review failed to find quality evidence to support use of hCG for preventing miscarriage.^[131] A well-designed randomized controlled trial of adequate power and methodological quality is required. Therefore, the use of hCG is not recommended (Evidence level II)
- Steroids: The effect of prednisolone therapy for some women with recurrent miscarriage may be due to altered endometrial angiogenic growth factor expression and reduced blood vessel maturation.^[132] The role is mostly limited to recurrent miscarriage with known connective tissue disorders. Rheumatologic advice should be taken

with patients diagnosed having recurrent pregnancy loss and connective tissue disorder. The results from the Prednisolone Trial are awaited; it is a randomized controlled trial of prednisolone for women with idiopathic recurrent miscarriage and raised uNK cells in the endometrium.^[133] There is no robust evidence to recommend steroid use for unexplained recurrent miscarriage (Evidence level III)

- Immunoglobulins: IVIG administration for treatment of recurrent miscarriage is not justified outside the context of research as discussed earlier (Evidence level II)
- Intravenous intralipid solution: No evidence of benefit with use of intralipid. Well controlled, large-scale, and confirmatory studies required before it can be recommended for routine use^[118,120] (Evidence level III)

CONCLUSION

Recurrent miscarriage is one of most the widely researched areas in medicine. Recurrent miscarriage may be the first presentation of some of the hematological or endocrine disorders. Many investigations such as genetic thrombophilia screening are not based on strong evidence. The management of unexplained miscarriage is a challenge. Role of aspirin and low molecular weight heparin is controversial in genetic thrombophilias. Any form of immunotherapy is not recommended until further evidence is available. We look forward for results of various ongoing multicentre trials to produce an answer.

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