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ENDOMETRIAL RECEPTIVITY

By

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ABSTRACT

Establishment of successful pregnancy depends upon implantation, involving complex interactions between the endometrium and the blastocyst. It is well accepted that the implantation window is a narrow time frame with maximal endometrial receptivity, surrounded by a refractory endometrial status. Suboptimal endometrial receptivity and altered embryo–endometrial dialogue are responsible for two-thirds of implantation failures manifesting as miscarriage or failed in vitro fertilisation (IVF) treatment following embryo transfer.

The overarching aim of the present thesis was to understand endometrial receptivity and explore the development of potential endometrial receptivity tests that are cost-effective and may be implemented in clinical practice. The thesis is structured into seven chapters with individualised objectives.

Chapter one introduces the topic and summarises the current body of knowledge in relation to endometrial receptivity. Endometrial receptivity is complementary to endometrial selectivity and explains the pathophysiological antithesis between recurrent implantation failure and recurrent miscarriage of endometrial cause.

Chapter two is a Cochrane review of the evidence supporting the use of intrauterine human chorionic gonadotropin (hCG) administration before embryo transfer through its effect on endometrial receptivity. Seventeen randomised controlled trials (RCTs) including 4751 women were meta-analysed to identify an increase in live birth rate (RR 1.57, 95% CI 1.32 to 1.87; three RCTs; 914 participants; $I^2 = 0\%$; moderate-quality evidence) and clinical pregnancy rate (RR 1.49, 95% CI 1.32 to 1.68; 12 RCTs; 2186 participants; $I^2 = 18\%$; moderate-quality evidence) for women undergoing cleavage-stage embryo transfer with a

hCG dose ≥ 500 IU. There were no substantive differences in live birth (RR 0.92, 95% CI 0.80 to 1.04; two RCTs; 1666 participants; $I^2 = 0\%$; moderate-quality evidence) and clinical pregnancy (RR 0.99, 95% CI 0.85 to 1.15; four RCTs; 2091 participants; $I^2 = 42\%$; moderate-quality evidence) among women having blastocyst-stage embryo transfer with a hCG dose ≥ 500 IU.

Chapter three is a comprehensive review of the literature on conventional and modern markers of endometrial receptivity. A total of 163 studies including 88 834 women were reviewed to assess over 40 markers of endometrial receptivity. Associations were identified between clinical pregnancy and various endometrial receptivity markers (endometrial thickness, endometrial pattern, Doppler indices, endometrial wave-like activity and various molecules); however, their poor ability to predict clinical pregnancy prevents them from being used as diagnostic tests of endometrial receptivity.

Chapter four is an assessment of women's views as part of a target product profile for developing a test of endometrial receptivity. The results from a questionnaire answered by 131 women who suffered recurrent miscarriages support the use of an endometrial receptivity test after two miscarriages and its timing in a window of three to four days within the menstrual cycle with results available within one to two days. The invasiveness of testing should not extend beyond a vaginal examination and repeating the test should not be required more than twice with the results remaining useful for at least six menstrual cycles.

Chapters five and six explore the use of transcriptomics and metabolomics for the base of an endometrial receptivity test. A total of 24 women who suffered unexplained recurrent miscarriages underwent endometrial biopsies during the window of implantation to identify differently expressed genes and metabolites between 1) women who suffered low order

miscarriages and those who suffered high order miscarriages; and 2) women who achieved a live birth and those who suffered another miscarriage in the subsequent pregnancy. Women who suffered higher order miscarriages had 19 differently expressed genes and perturbations in the fatty acid metabolism and poorer mitochondrial health. Women who achieved a subsequent live birth had 421 differently expressed genes and perturbed cholesterol - cholesterol sulphate metabolism, fatty acid metabolism, and improved mitochondrial health.

Chapter seven integrates the findings from previous chapters and concludes the thesis. All five original studies have been published in peer reviewed journals.

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CHAPTER ONE:

THESIS INTRODUCTION

1. Background

1.1 Prepubertal endometrial development

The paramesonephric ducts (Mullerian ducts) have intermediate mesodermal origin and give rise to the oviducts, uterus, and upper third of the vagina during the embryonic development of a girl [1]. In the absence of a Y chromosome containing the SRY gene, the gonads develop into ovaries, thus allowing the paramesonephric ducts to persist in the absence of Anti-Mullerian Hormone.

The single-layered paramesonephric duct epithelium differentiates into varying morphologies ranging from ciliated columnar epithelium in the fallopian tubes to stratified squamous epithelium in the vagina under the influence of Hox genes [2]. The muscular layers of the female genital tract originate from the mesenchyme surrounding the paramesonephric ducts [3].

Histologically, the endometrium is a single layer of columnar epithelium supported by a thick layer of fibroblastic stroma until the 20th gestational week when the surface epithelium invaginates to form glandular structures. At birth, the endometrial surface and glands are lined by a low columnar to cuboidal epithelium, devoid of proliferative or secretory changes, and remains inactive until puberty [4].

1.2 Endometrial cycle

The endometrium is highly sensitive to changes in the ovarian-derived steroid hormones levels. Following the menarche the endometrium undergoes a cyclical preparation for receiving a fertilized oocyte. This involves the proliferation and differentiation of the endometrial tissue. If the implantation does not occur, the functional layer of the endometrium is shed and released in the form of menstruation [5].

In normally ovulating women, the endometrium undergoes dramatic changes within a 28-day menstrual cycle. The high mitotic indices evidenced in the follicular phase promote the proliferation of the cellular constituents of the endometrium leading to an increase in endometrial thickness from 2 mm in the post-menstrual repair phase to 14 mm before ovulation [6]. The increased DNA synthesis and the numerous mitoses in the epithelium, stroma and vascular endothelium result in the development of a glandular network and an elaborate system of blood vessels under the influence of estradiol. Inappropriate proliferation of the endometrium in the follicular phase may be associated with subfertility and miscarriage [7].

After ovulation, the progesterone released by the corpus luteum promotes glandular secretion and decidualisation. The basalis region of the glandular epithelium accumulates glycogen [8] and excretes it in the glandular lumen at a peak that coincides with the time of blastocyst implantation. The DNA synthesis and cellular division in glandular cells decrease at the same time [9]. The implantation window is defined as a short interval during the mid-luteal phase, when the endometrium is most receptive for implantation. Changes in the stroma are prominent in mid-luteal phase when the capillary permeability is increased and leads to stromal oedema, endothelial cell proliferation with coiling of the spiral arterioles on a background of frequent stromal mitoses [10].

The corpus luteum stops secreting progesterone in the absence of implantation which leads to endometrial cellular apoptosis, vascular basement membrane breakdown, tissue desquamation and menstruation.

1.3 Recurrent miscarriage and recurrent implantation failure

Miscarriage is the most frequent complication of pregnancy and represents the spontaneous loss of the embryo or foetus before it is able to survive independently (24 weeks of gestation). It affects up to 50% of pregnancies with the vast majority (80%) occurring at pre-clinical stage before the woman recognises the pregnancy [11, 12]. Up to 5% of couples suffer recurrent miscarriage (RM) defined as 2-3 or more (depending on the defining organisation) miscarriages leading to physical, emotional and financial consequences for couples, doctors and medical systems [13, 14].

In the context of assisted reproduction, recurrent implantation failure (RIF) is generally defined as a failure to achieve pregnancy after three or more unsuccessful transfers of high-quality embryos or transfers of more than ten embryos in multiple transfers [15], but evidence from published literature identified a broad range of definitions worldwide [16] (Table 1).

Table 1: Various definitions of recurrent miscarriage (RM) and recurrent implantation failure (RIF) as reported by Royal College of Obstetricians and Gynaecologists (RCOG), European Society of Human Reproduction and Embryology (ESHRE), American Society for Reproductive Medicine (ASRM), International Glossary on Infertility and Fertility Care (IGIFC), British Fertility Society (BFS) and the international Survey [16].

Diagnostic	**Defining organisation**	**Definition**
RM	RCOG	the loss of three or more consecutive pregnancies
	ESHRE	the loss of two or more pregnancies
	ASRM	two or more failed clinical pregnancies
	IGIFC	the spontaneous loss of two or more clinical pregnancies
	BFS	absence of a positive pregnancy test after three consecutive transfers of good quality embryos

RIF	ESHRE	variable definitions based on the estimated chance of implantation in each individual couple (no absolute numbers)
	Survey	broad range of definitions involving at least 2 to at least 4 fresh or frozen embryos (cleavage or blastocyst stage) of good quality (measured in various systems) transferred in at least 2 to at least 4 separate embryo transfer procedures

Recurrent miscarriage and recurrent implantation failure lay at opposite margins of the endometrial receptivity and selectivity balance. Receptivity enables the endometrium to provide an optimal environment for embryo development while selectivity allows the endometrium to recognise and reject embryos with reduced development potential.

Endometrium characterised by increased receptivity and reduced selectivity leads to recurrent miscarriage due to a failure in recognising embryos with poor developmental potential which may initiate implantation, progress to biochemical or clinical pregnancy, but ultimately fail to reach ongoing pregnancy and live birth.

On the contrary, endometrium characterised by increased selectivity and reduced receptivity leads to recurrent implantation failure due to the rejection of all embryos, including those with good developmental potential.

Figure 1 displays pregnancy outcomes based on the normal and altered endometrial receptivity and selectivity balance. Normal receptivity coupled with normal selectivity leads to the successful implantation of euploid embryos while aneuploid embryos are denied implantation. Reduced receptivity coupled with increased selectivity leads to the implantation failure of euploid embryos, while increased receptivity coupled with reduced selectivity leads to the early miscarriage of aneuploid embryos.

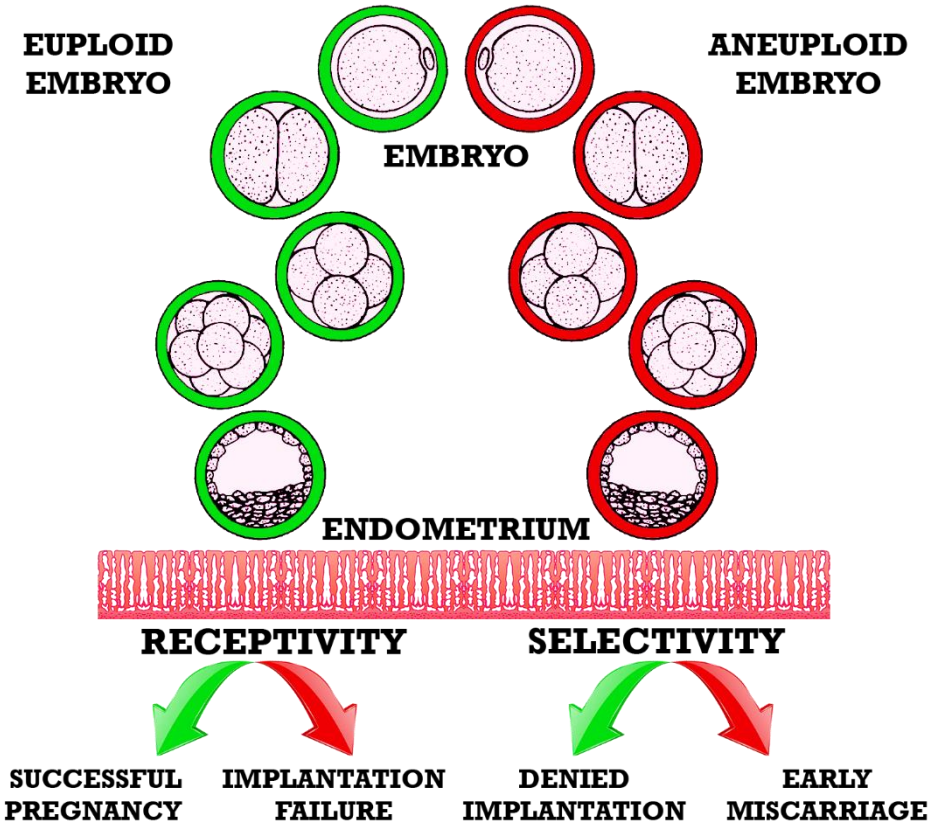

Figure 1: Endometrium as a biosensor of embryo developmental potential based on its intrinsic receptivity and selectivity functions.

There is very little evidence to guide practice when faced with these difficult clinical problems. A survey of 79 assisted reproduction units based in the UK [17] identified a diversity of approaches in both investigations and management for recurrent in vitro fertilisation (IVF) failure. The paucity of data coupled with the intense desire for achieving a successful pregnancy led to the widespread adoption of IVF add-ons consisting in tests and interventions of uncertain benefit, usually at the expense of couples.

Table 2: Investigations undertaken by assisted reproduction units based in the UK in the context of recurrent in vitro fertilisation treatment failure.

Investigation	**Proportion of units (%), n= 44**
Lupus anticoagulant and anticardiolipin antibodies	75
Karyotype (both partners)	70.4
Hysteroscopy	70.4
Thrombophilia screen	59
Thyroid stimulating hormone (TSH)	27
Glycated haemoglobin (HBA1c)	20.4
Timed endometrial biopsy	20.4
Untimed endometrial biopsy	2.2
Saline sonography	6.8

Table 3: Management strategies considered by assisted reproduction units based in the UK in the context of recurrent in vitro fertilisation treatment failure.

Management strategy	**Proportion of units (%), n= 65**
Blastocyst culture	46.2
Assisted hatching	33.8
Modify stimulation protocol	30.7
Day three transfer	27.6
Aneuploidy screen	23
No change in management	23
Donor oocytes	20
Donor sperm	12.3
Steroids	6.1
Immunotherapy	3.1

2. Endometrial receptivity investigations

Establishment of successful pregnancy depends upon implantation, involving complex interactions between the endometrium and the blastocyst. It is well accepted that the implantation window is a narrow time frame with maximal endometrial receptivity,

surrounded by a refractory endometrial status [18, 19]. Several investigations have been proposed in order to assess the endometrial receptivity and explain reproductive failure. They could be generally classified in three main groups: tests involving imaging, tests involving endometrial sampling and tests involving endometrial inspection by hysteroscopy.

2.1 Tests involving imaging

High resolution ultrasound is a well-tolerated, non-invasive investigation which may provide useful information regarding events involved in endometrial preparation. In addition to the morphologic abnormalities (e.g., septum, polyps, fibroids) identifiable during an ultrasound scan, important data is available in relation to endometrial thickness, echogenicity and pattern, uterine vascular network and uterine contractility.

Dynamic changes in endometrial thickness are routinely assessed by transvaginal ultrasound in assisted reproduction. Endometrial thickness is measured in the sagittal plane of the uterus, with the entirety of the endometrial tissue in view. The thickest echogenic area from one basal endometrial interface across the endometrial canal to the other basal surface is identified and measured to one decimal of a millimetre without including hypoechoic myometrium or intrauterine fluid.

As a receptivity marker, endometrial thickness has the main advantage of a high negative predictive value which means 87-100% of cases with thin endometrium will not achieve a pregnancy [20]. A cut-off value of 7 mm is accepted as a reliable sign of sub-optimal endometrial receptivity based on the infrequent occurrence (less than 3% of cases) and association of negative pregnancy outcomes (implantation failure or early miscarriage) [21]. Endometrial volume calculated using 3D ultrasound was introduced as a marker of

endometrial receptivity in order to counteract the lack of specificity from the endometrial thickness.

Endometrial pattern represents the variation in echogenicity between the endometrium and the adjacent myometrium observed on a longitudinal ultrasound scan plane. The "triple line" pattern has a high negative predictive value which means its absence has a very high correlation with failure to achieve a pregnancy. It accounts for stromal oedema and secretory activity which characterise the luteal endometrium [22].

The development of power-Doppler technology facilitated investigations of the relation between endometrial vascularization and implantation; however, the findings are not consistent and require further research [23, 24].

The uterine quiescence of the mid-luteal phase facilitates the adequate positioning of the embryo in the middle section of the endometrial cavity [25]. The persistence of constant contractile activity which normally characterises the follicular phase may be identified by ultrasound as a marker of sub-optimal endometrial receptivity.

2.2 Tests involving endometrial sampling

The histologic endometrial dating criteria described by Noyes in 1950 [26] became the gold standard method for assessing the luteal function and endometrial receptivity. Advances in biochemistry, molecular biology, immunology, genomics and assisted reproduction facilitated the development of a broad range of investigations aiming to characterise the exact frames of the window of implantation. Endometrial samples obtained by Pipelle biopsy, dilatation and curettage, biopsy under hysteroscopic view or endometrial flushing have been used to find clinically useful biomarkers in relation to endometrial receptivity.

The selective expression of progesterone receptor [27], matrix metalloproteinases (MMP2, Cathepsin H [28]), connexins (Cx43 [29]), and developmental factors (FrpHE [30]) has been found to be downregulated in association with the optimal endometrial receptivity.

On the other hand, the expression of stromal oestrogen receptor ER-α [31], growth factors, cytokines and chemokines (VEGF [32], IL-1β and IL-6 receptor [33]), anti-adhesion molecules (Muc-1 [34]), free radicals (CuZnSOD [35]), and prostaglandins (PGF-2α [36]) is increased in order to facilitate the embryo-endometrial interaction.

Omics- refer to the application of high-throughput techniques which simultaneously examine changes in different molecular compartments: genomics, transcriptomics, proteomics, metabolomics etc. The understanding of human endometrial physiology and pathophysiology is being revolutionised by the use of omics-; however, our understanding of different complex phenotypes related to fertility remains incomplete, inconsistent and without strong clinical application [37].

Two new molecular diagnostic tools have recently been introduced in clinical practice in order to facilitate personalised embryo transfers in women undergoing assisted reproduction. Endometrial Receptivity Analysis (ERA©), developed and patented by IGENOMIX, uses 134 selected genes relevant to endometrial receptivity [38]. Endometrial receptivity map (ER Map©), developed and patented by iGLS, is based on qRT-PCR and uses a panel of 16 genes involved in endometrial proliferation and immune response [39].

2.3 Tests involving endometrial inspection by hysteroscopy

In addition to morphologic abnormalities (e.g., septum, polyps, adhesions), hysteroscopy may identify direct visual appearances relevant to endometrial receptivity. Previous studies have characterised the optimal receptive endometrium as containing ring-

type glandular openings as opposed to sub-optimal endometrium characterised by the presence of varicose-like vessel networks on the endometrial surface [40].

Chromohysteroscopy involves flushing the endometrial surface with a dye (methylene blue) in order to increase the sensitivity of hysteroscopy for the diagnosis of subtle endometrial pathologies which have not produced macroscopic changes. It was first described by Küçük in 2008 for a study aiming to diagnose endometritis in otherwise normal looking endometrium [41]. Chromoendoscopy is used routinely in gastroenterology based on the ability of normal gastrointestinal epithelium to absorb methylene blue. However, the normal endometrium lining does not absorb methylene blue. Methylene blue is only absorbed and visualised as dark patches if cellular necrosis is present, allowing dye passage through the cellular membrane [42].

3. Objective

The overarching aim of the present research package is to understand endometrial receptivity and explore the development of potential endometrial receptivity tests that are cost-effective and may be implemented in clinical practice. In order to achieve my aim we have designed work packages with individual objectives as follows:

1. To review the literature on the effect of intrauterine human chorionic gonadotropin (hCG) administration before embryo transfer.

2. To review the literature on conventional and modern tests of endometrial receptivity.

3. To assess women's views as part of a target product profile for developing a test of endometrial receptivity.

4. To explore the use of transcriptomics for the base of an endometrial receptivity test.

5. To explore the use of metabolomics for the base of an endometrial receptivity test.

The succession of hypotheses and objectives followed a dynamic approach. First, I focused on identifying an intervention that could potentially improve reproductive outcomes by modulating endometrial receptivity. Based on previous work, intrauterine hCG administration met the criteria for such an intervention and was selected as a 'proof of concept' (e.g., endometrial receptivity can be targeted and modulated). Second, based on my clinical experience, I hypothesised the lack of robust evidence to support a particular test for evaluating endometrial receptivity. This led to the extensive review of the literature to justify subsequent financial investments into developing a new test for endometrial receptivity.

Obstetrics and Gynaecology as a specialty in general, and Reproductive Medicine as a subspecialty in particular, have a relatively low reliance on patients' input when deciding the introduction of new diagnostic tests and treatments in clinical practice. The hypothesis that women have specific views on the characteristics of a potential test for endometrial receptivity led to the initiation of a Target Product Profile by surveying women who suffered recurrent miscarriages.

Once the need for a new test for endometrial receptivity was established, evidence from the review of current tests coupled with characteristics suggested by women who would benefit from such a test, led to the development of an -OMICS based project to set the foundations for a modern test for endometrial receptivity. This hypothesised the existence of various molecular differences between women who suffered high versus low order miscarriages, and between those who will suffer another miscarriage or achieve a live birth in a subsequent pregnancy.

4. Work package 1: Intrauterine hCG administration before embryo transfer

Intrauterine administration of hCG prior to embryo transfer is a novel approach that has been suggested to improve the outcomes of assisted reproduction treatment based on the fundamental role of hCG in embryo implantation through modulation of endometrial receptivity and embryo-endometrial cross-talk. The intervention involves the use of an embryo transfer catheter to administer a small dose of hCG before the actual embryo transfer.

The aim of this work package was to quantify the effect of intrauterine hCG administration by performing a high-quality systematic review and meta-analysis of randomised controlled trials following Cochrane methodology.

5. Work package 2: Conventional and modern markers of endometrial receptivity

Various interventions such as endometrial injury, hormonal endometrial preparation, periimplantation administration of heparin and aspirin, and the use of fibrin sealant have been attempted in order to increase endometrial receptivity. Studies assessing the efficiency of these interventions report on clinical outcomes such as biochemical pregnancy, miscarriage, clinical pregnancy as surrogate markers for endometrial receptivity due to the lack of a robust test to quantify endometrial receptivity directly.

The aim of this work package was to assess the evidence from observational studies supporting the use of endometrial receptivity markers as prognostic factors for pregnancy outcome in women wishing to conceive in order to aid clinicians in choosing the most useful markers for clinical practice and for informing further research.

6. Work package 3: Target product profile for an endometrial receptivity test

A target product profile (TPP) highlights the ideal characteristics of a product aimed at treating, diagnosing or preventing a medical condition in order to meet the expectations of clinicians, patients and stake-holders. TPPs set the framework for the proposed use, target populations and other desired attributes of products, including safety and efficacy-related features. TPPs are not commonly used in the field of reproductive medicine, which led to the development, marketing and commercialisation of multiple add-ons with questionable cost efficiencies, controversial evidence base and unethical use.

The aim of this work package was to evaluate women's perspective on endometrial receptivity diagnostics in order to inform future research by incorporating the findings into a TPP for an endometrial receptivity test. This was a descriptive cross-sectional study involving 131 women who suffered recurrent miscarriages.

7. Work package 4: Transcriptomics of endometrial receptivity

Transcriptomics refer to the comprehensive analysis of the complete set of RNA transcripts that are produced by the genome in a biological specimen and holds promise to inform the practice of precision medicine. Endometrial transcriptomics rely on obtaining an endometrial sample in a precise moment of the menstrual cycle given the dynamic nature of the endometrial transformations.

The aim of this work-package was to characterise the endometrial transcriptomic profiles of women who suffered recurrent miscarriages and to set the foundation for the development of an endometrial receptivity test that could predict the fate of subsequent pregnancies. This was a prospective multicentre cohort study involving endometrial biopsies obtained in the mid-luteal phase from 24 women diagnosed with unexplained recurrent miscarriages.

8. Work package 5: Metabolomics of endometrial receptivity

Metabolomics refer to the comprehensive analysis of metabolites in a biological specimen and holds promise to inform the practice of precision medicine. They are more informative than genomics, transcriptomics, or proteomics, because they denote the final products of the cell metabolism and are closer to the functional phenotype.

The aim of this work package was to characterise the endometrial metabolomic profiles of women who suffered recurrent miscarriage using discovery metabolomics and to set the foundation for the development of an endometrial receptivity test. This was a prospective multicentre cohort study involving endometrial biopsies obtained in the mid-luteal phase from 24 women diagnosed with unexplained recurrent miscarriages.

9. Own contribution

My research programme involved several work packages based on individual studies converging under the umbrella of endometrial receptivity. I took a leading role in the design, conduct, delivery of each project, and dissemination of findings by publication in peer reviewed journals [43-47] and presentation at international meetings.

Specifically, I drafted the title proposal, wrote the protocol and led the *Cochrane* review on the use of intrauterine hCG prior to embryo transfer. I selected the studies, extracted and analysed the data, interpreted the results and drafted the final manuscript. The team of co-authors performed the search in duplicate, validated data extraction and provided feedback on the final manuscript as required by Cochrane methodology.

I liaised with *Human Reproduction Update* journal and wrote the proposal for the review and meta-analyses of endometrial receptivity tests. Following its acceptance, I designed the search strategy, identified studies, extracted and analysed data, interpreted the results and drafted the final manuscript. Co-authors validated study inclusion, data extraction and provided expert feedback on the interpretation of results and recommendations for clinical practice and further research.

I was the chief investigator of the prospective multicentre cohort study involving the target product profile, transcriptomics and metabolomics of endometrial receptivity. I proposed the study design, managed the Integrated Research Application System (IRAS) application, obtained Health Technology Assessment (HTA) approval and favourable Ethics Committee opinion. I liaised with the National Institute for Health and Care Research (NIHR) Clinical Research Network (CRN) to identify participating sites, conducted site visits and managed the study documentation. I coordinated a multidisciplinary team of research nurses, doctors, statisticians and scientists to recruit participants, obtain endometrial samples, extract RNA and metabolites, analyse data and interpret the results. I saw the completion of all the projects through and I drafted the manuscripts to disseminate the new findings.

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CHAPTER TWO:

INTRAUTERINE ADMINISTRATION OF HUMAN CHORIONIC GONADOTROPIN (HCG) FOR SUBFERTILE WOMEN UNDERGOING ASSISTED REPRODUCTION

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Intrauterine administration of human chorionic gonadotropin (hCG) for subfertile women undergoing assisted reproduction (Review)

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Craciunas L, Tsampras N, Raine-Fenning N, Coomarasamy A.
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[Intervention Review]

Intrauterine administration of human chorionic gonadotropin (hCG) for subfertile women undergoing assisted reproduction

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ABSTRACT

Background

Most women undergoing assisted reproduction treatment will reach the stage of embryo transfer (ET), but the proportion of embryos that can be successfully implanted after ET has remained small since the mid-1990s. Human chorionic gonadotropin (hCG) is a hormone that is synthesised and released by the syncytiotrophoblast and has a fundamental role in embryo implantation and the early stages of pregnancy. Intrauterine administration of hCG via ET catheter during a mock procedure around the time of ET is a novel approach that has been suggested to improve the outcomes of assisted reproduction.

Objectives

To investigate whether intrauterine (intracavity) administration of hCG (IC-hCG) around the time of ET improves clinical outcomes in subfertile women undergoing assisted reproduction.

Search methods

We performed searches on 9 January 2018 using Cochrane methods.

Selection criteria

We looked for randomised controlled trials (RCTs) evaluating IC-hCG around the time of ET, irrespective of language and country of origin.

Data collection and analysis

Two review authors independently selected studies, assessed risk of bias, extracted data from studies, and attempted to contact study authors when data were missing. We performed statistical analysis using Review Manager 5. We assessed evidence quality using GRADE methods. Primary outcomes were live birth and miscarriage; secondary outcomes were clinical pregnancy rate and complications.

Main results

Seventeen RCTs investigated the effects of IC-hCG administration for 4751 subfertile women undergoing assisted reproduction. IC-hCG was administered in variable doses at different times before the ET. hCG was obtained from the urine of pregnant women or from cell cultures using recombinant DNA technology.

Most studies (12/17) were at high risk of bias in at least one of the seven domains assessed. Common problems were unclear reporting of study methods and lack of blinding. The main limitations for evidence quality were high risk of bias and serious imprecision.

For analyses of live birth and clinical pregnancy, there was considerable heterogeneity (I^2 > 75%) and therefore we present subgroups for dosage and stage of ET. Exploration for sources of heterogeneity revealed two key prespecified variables as important determinants: stage of ET (cleavage vs blastocyst stage) and dose of IC-hCG (< 500 international units (IU) vs ≥ 500 IU). We performed meta-analyses within subgroups defined by stage of embryo and dose of IC-hCG.

Live birth rates among women having cleavage-stage ET with an IC-hCG dose < 500 IU compared to women having cleavage-stage ET without IC-hCG showed no benefit of the intervention and would be consistent with no substantive difference or disadvantage of indeterminate magnitude (risk ratio (RR) 0.76, 95% confidence interval (CI) 0.58 to 1.01; one RCT; 280 participants; I^2 = 0%; very low-quality evidence). In a clinic with a live birth rate of 49% per cycle, use of IC-hCG < 500 IU would be associated with a live birth rate ranging from 28% to 50%.

Results show an increase in live birth rate in the subgroup of women undergoing cleavage-stage ET with an IC-hCG dose ≥ 500 IU compared to women having cleavage-stage ET without IC-hCG (RR 1.57, 95% CI 1.32 to 1.87; three RCTs; 914 participants; I^2 = 0%; moderate-quality evidence). At a clinic with a live birth rate of 27% per cycle, use of IC-hCG ≥ 500 IU would be associated with a live birth rate ranging from 36% to 51%.

Results show no substantive differences in live birth among women having blastocyst-stage ET with an IC-hCG dose ≥ 500 IU compared to women having blastocyst-stage ET without IC-hCG (RR 0.92, 95% CI 0.80 to 1.04; two RCTs; 1666 participants; I^2 = 0%; moderate-quality evidence). At a clinic with a live birth rate of 36% per cycle, use of IC-hCG ≥ 500 IU would be associated with a live birth rate ranging from 29% to 38%.

Evidence for clinical pregnancy among women having cleavage-stage ET with an IC-hCG dose < 500 IU showed no benefit of the intervention and would be consistent with no substantive difference or disadvantage of indeterminate magnitude (RR 0.88, 95% CI 0.70 to 1.10; one RCT; 280 participants; I^2 = 0%; very low-quality evidence).

Results show an increase in clinical pregnancy rate in the subgroup of women having cleavage-stage ET with an IC-hCG dose ≥ 500 IU compared to women having cleavage-stage ET without IC-hCG (RR 1.49, 95% CI 1.32 to 1.68; 12 RCTs; 2186 participants; I^2 = 18%; moderate-quality evidence).

Results show no substantive differences in clinical pregnancy among women having blastocyst-stage ET with an IC-hCG dose ≥ 500 IU (RR 0.99, 95% CI 0.85 to 1.15; four RCTs; 2091 participants; I^2 = 42%; moderate-quality evidence) compared to women having blastocyst-stage ET with no IC-hCG.

No RCTs investigated blastocyst-stage ET with an IC-hCG dose < 500 IU.

We are uncertain whether miscarriage was influenced by intrauterine hCG administration (RR 1.04, 95% CI 0.81 to 1.35; 11 RCTs; 3927 participants; I^2 = 0%; very low-quality evidence).

Reported complications were ectopic pregnancy (four RCTs; 1073 participants; four events overall), heterotopic pregnancy (one RCT; 495 participants; one event), intrauterine death (three RCTs; 1078 participants; 22 events), and triplets (one RCT; 48 participants; three events). Events were few, and very low-quality evidence was insufficient to permit conclusions to be drawn.

Authors' conclusions

There is moderate quality evidence that women undergoing cleavage-stage transfer using an IC-hCG dose ≥ 500 IU have an improved live birth rate. There is insufficient evidence for IC-hCG treatment for blastocyst transfer. There should be further trials with live birth as the primary outcome to identify the groups of women who would benefit the most from this intervention. There was no evidence that miscarriage was reduced following IC-hCG administration, irrespective of embryo stage at transfer or dose of IC-hCG. Events were too few to allow conclusions to be drawn with regard to other complications.

PLAIN LANGUAGE SUMMARY

The effect of administering pregnancy hormone into the womb of subfertile women undergoing assisted reproduction

Review question

Does administering pregnancy hormone into the womb of subfertile women undergoing assisted reproduction provide any benefit?

Background

Subfertility affects 15% of couples and is defined as the inability to become pregnant naturally following 12 months of regular unprotected sexual intercourse. Assisted reproduction refers to procedures involving handling of both sperm and eggs in the laboratory to create

embryos to be transferred into the womb (embryo transfer (ET)). Administering natural or synthetic pregnancy hormone into the womb of subfertile women undergoing assisted reproduction treatment is a novel approach that might increase the chance of having a baby.

Study characteristics

We evaluated 17 studies (4751 women) comparing administration of pregnancy hormone versus no hormone. The natural or synthetic hormone was administered at variable doses at different times before ET.

Key results

Live birth rates in women having day three ET with human chorionic gonadotropin administered into the uterus (IC-hCG) at a dose < 500 IU compared to women having day three ET without pregnancy hormone showed no benefit of the intervention and would be consistent with no substantive difference or disadvantage of indeterminate magnitude (very low-quality evidence: one study; 280 women). In a clinic with a live birth rate of 49% per cycle following day three ET, use of a pregnancy hormone dose < 500 IU would be associated with a live birth rate varying from 28% to 50%.

Live birth rate was increased in a subgroup of women having day three ET with a pregnancy hormone dose of 500 IU or greater compared to women having day three ET without pregnancy hormone (moderate-quality evidence: three studies; 914 women). At a clinic with a live birth rate of 27% per cycle, use of a pregnancy hormone dose of 500 IU or greater would be associated with a live birth rate varying from 36% to 51%.

Trial results show no substantive differences in live birth among women having day five ET with a pregnancy hormone dose of 500 IU or greater compared to women having day five ET without pregnancy hormone (moderate-quality evidence: two studies; 1666 women). At a clinic with a live birth rate of 36% per cycle, use of a pregnancy hormone dose of 500 IU or greater would be associated with a live birth rate varying from 29% to 38%.

We are uncertain whether administration of pregnancy hormone into the womb at any dose or time affected miscarriage (very low-quality evidence: 11 studies; 3927 women).

Evidence for clinical pregnancy among women having day three ET with a pregnancy hormone dose < 500 IU showed no benefit of the intervention and would be consistent with no substantive difference or disadvantage of indeterminate magnitude (very low-quality evidence: one study; 280 women).

The clinical pregnancy rate was increased in the subgroup of women having day three ET with a pregnancy hormone dose of 500 IU or greater compared to women having day three ET without pregnancy hormone (moderate-quality evidence: 12 studies; 2186 women).

Trial results show no substantive difference in clinical pregnancy among women having day five ET with a pregnancy hormone dose of 500 IU or greater compared to women having day five ET with no pregnancy hormone (moderate-quality evidence: four studies; 2091 women).

No randomised controlled trials (RCTs) investigated day five ET with a pregnancy hormone dose < 500 IU.

Other complications reported in the included studies were ectopic pregnancy (where the embryo develops outside the womb), heterotopic pregnancy (where embryos develop inside and outside the womb), foetal death, and triplets. Events were few, and insufficient evidence of very low quality does not permit us to determine whether there were differences between groups.

There should be further trials with live birth as the primary outcome to identify the groups of women who would benefit the most from this intervention.

Quality of the evidence

Evidence quality varied from very low to moderate depending on the outcome. The main limitations for the overall quality of the evidence were high risk of bias and serious imprecision.

Summary of findings for the main comparison. Intrauterine administration of hCG for women undergoing assisted reproduction

Intrauterine administration of hCG for women undergoing assisted reproduction

Patient or population: subfertile women undergoing assisted reproduction
Setting: assisted reproduction units
Intervention: intrauterine human chorionic gonadotropin (hCG)
Comparison: no intrauterine hCG

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Certainty of the evidence (GRADE)
	Risk with no hCG	Risk with intrauterine human chorionic gonadotropin (hCG)			
Live birth Cleavage stage: hCG < 500 IU Follow-up: mean 40 weeks	495 per 1000	376 per 1000 (287 to 500)	RR 0.76 (0.58 to 1.01)	280 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b]
Cleavage stage: hCG ≥ 500 IU Follow-up: mean 40 weeks	273 per 1000	428 per 1000 (360 to 510)	RR 1.57 (1.32 to 1.87)	914 (3 RCTs)	⊕⊕⊕⊖ MODERATE[c]
Blastocyst stage: hCG ≥ 500 IU Follow-up: mean 40 weeks	369 per 1000	340 per 1000 (296 to 384)	RR 0.92 (0.80 to 1.04)	1666 (2 RCTs)	⊕⊕⊕⊖ MODERATE[c]
Miscarriage Follow-up: mean 40 weeks	58 per 1000	60 per 1000 (47 to 78)	RR 1.04 (0.81 to 1.35)	3927 (11 RCTs)	⊕⊖⊖⊖ VERY LOW[c,d]
Clinical pregnancy Cleavage stage: hCG < 500 IU Follow-up: mean 12 weeks	579 per 1000	509 per 1000 (405 to 637)	RR 0.88 (0.70 to 1.10)	280 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,d]
Cleavage stage: hCG ≥ 500 IU Follow-up: mean 12 weeks	307 per 1000	458 per 1000 (406 to 517)	RR 1.49 (1.32 to 1.68)	2186 (12 RCTs)	⊕⊕⊕⊖ MODERATE[c]
Blastocyst stage: hCG ≥ 500 IU Follow-up: mean 12 weeks	422 per 1000	418 per 1000 (359 to 485)	RR 0.99 (0.85 to 1.15)	2091 (4 RCTs)	⊕⊕⊕⊖ MODERATE[c]
Complications Follow-up: mean 40 weeks	Other complications reported in the included studies were ectopic pregnancy (4 RCTs; N = 1073; 4 events overall), heterotopic pregnancy (1 RCT; N = 495; 1 event), intrauterine death (3 RCTs; N = 1078; 22 events), and triplets (1 RCT; N = 48;		-	1764 (7 RCTs)	⊕⊖⊖⊖ VERY LOW[c,d]

				3 events). No evidence shows a difference between groups, but events were too few for any conclusions to be drawn.	

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).
CI: confidence interval; hCG: human chorionic gonadotropin; RCT: randomised controlled trial; RR: risk ratio.

GRADE Working Group grades of evidence.
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded two levels for very serious risk of bias: lack of blinding of participants and personnel, no clear description of allocation concealment, and premature termination of the study following interim analysis.
[b]Downgraded one level for serious imprecision: total events were fewer than 300.
[c]Downgraded one level for serious risk of bias: lack of blinding of participants and personnel, no allocation concealment.
[d]Downgraded two levels for very serious imprecision: total number of events was less than 300, and 95% confidence interval around the pooled effect includes both no effect and appreciable benefit or appreciable harm.

BACKGROUND

Description of the condition

Subfertility is defined as the inability of a couple to conceive spontaneously following 12 months of regular unprotected sexual intercourse. It is estimated that 15% of couples are affected by subfertility of different causes (female factor, male factor, unexplained). Assisted reproduction refers to procedures involving the in vitro (in a laboratory dish) handling of both human gametes (sperm and eggs) with the objective of establishing a pregnancy (Zegers-Hochschild 2009). The most vulnerable step of assisted reproduction is the embryo transfer (ET), as it involves a radical change in the embryo's environment, which makes it prone to demise (Schoolcraft 2001). Most women undergoing assisted reproduction treatment will reach the stage of ET owing to important improvements in ovarian stimulation protocols and laboratory technology, but the proportion of embryos that successfully implant following ET has remained small (less than one-third) since the mid-1990s (Kupka 2014).

The process of implantation involves a reciprocal interaction between the embryo and the endometrium, culminating in a small reception-ready phase of the endometrium, during which implantation can occur. This interaction is dependent on the temporal differentiation of endometrial cells to attain uterine receptivity. Implantation failure is thought to occur as a consequence of impairment of the embryo developmental potential or impairment of uterine receptivity, or both, and the embryo-uterine dialogue (Diedrich 2007).

Many interventions have been attempted with varying degrees of success before ET (endometrial injury (Nastri 2012), dummy ET (Mansour 1990), endometrial preparation (Derks 2009), peri-implantation (heparin (Akhtar 2013), aspirin (Siristatidis 2016)), during ET (ultrasound guidance (Brown 2010), removal of cervical mucus (Craciunas 2014)), and after ET (fibrin sealant, bed rest (Abou-Setta 2014)) to optimise the embryo-endometrial interaction and improve outcomes.

Description of the intervention

Human chorionic gonadotropin (hCG) is a hormone that is synthesised and released by the syncytiotrophoblast. It stimulates ovarian production of progesterone during the first trimester of pregnancy. Intrauterine administration of synthetic or natural hCG around the time of ET is a novel approach that has been suggested to improve the outcomes of assisted reproduction treatment based on the fundamental role of hCG in embryo implantation and the early stages of pregnancy (Cole 2010). The intervention involves intrauterine administration of hCG via an ET catheter during a mock procedure (a trial of the actual ET without using an embryo, performed to assess the difficulty of the ET) using the lowest volume of medium before the conventional ET. The hCG can be released at different points inside the uterine cavity (close to the internal cervical os, mid-cavity, or near the fundus) within minutes, hours, or days before the actual ET. hCG sources for medical treatments include extraction from the urine of pregnant women (natural) or from cell cultures using recombinant DNA technology (rhCG).

How the intervention might work

The hCG may promote peritrophoblastic immune tolerance, which facilitates trophoblast invasion by inducing an increase in endometrial T-cell apoptosis (Kayisli 2003). It also supports trophoblast apposition (the first stage of implantation - loose alignment of the trophoblast to the decidua) and adhesion (second stage of implantation - closer attachment of the trophoblast to the decidua) to the endometrium by regulating proteins involved in implantation (Racicot 2014). Intrauterine injection of urinary hCG alters endometrial secretory parameters (Licht 1998), and cell proliferation and migration are increased in the presence of hCG (Bourdiec 2013).

Why it is important to do this review

Subfertility affects a relatively large proportion of couples, and assisted reproduction treatments remain costly and stressful. All effort should be directed towards increasing the success rate of infertility treatments, and primary research should be translated into clinical practice in an efficient and timely manner. Intrauterine administration of hCG around the time of ET has the potential to improve the outcomes of assisted reproduction treatments; randomised and non-randomised trials have reported varying results (Mansour 2011; Rebolloso 2013).

Previous meta-analyses assessed the efficacy of intrauterine injection of hCG before ET in assisted reproductive cycles, but improvements could be made to the methods of analysis (Dieamant 2016; Osman 2016; Ye 2015). Different studies have evaluated variable circumstances of intrauterine hCG administration in terms of stage of the embryo at transfer (cleavage vs blastocyst), source of hCG (urine vs recombinant), dose of hCG, embryo processing (fresh vs frozen/thawed), and number of embryos transferred, leading to real uncertainties about the role of the intervention. The previous version of this review reported promising outcomes for cleavage-stage ET following intrauterine injection of hCG at a dose of 500 IU or more (Craciunas 2016), but the evidence was weak and newly published randomised controlled trials may have altered our confidence in the results.

OBJECTIVES

To investigate whether intrauterine (intracavity) administration of hCG (IC-hCG) around the time of ET improves clinical outcomes in subfertile women undergoing assisted reproduction.

METHODS

Criteria for considering studies for this review

Types of studies

We included in this review all randomised controlled trials (RCTs) evaluating intrauterine (intracavity) administration of hCG (IC-hCG) around the time of ET, irrespective of language and country of origin. We planned to include only data from the first phase of cross-over RCTs in meta-analyses.

Types of participants

We included subfertile women undergoing in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) followed by ET.

Types of interventions

RCTs comparing intrauterine administration of hCG around the time of ET versus any other active intervention, no intervention, or placebo were eligible for inclusion.

Types of outcome measures

Primary outcomes

- Live birth (delivery of a live foetus after 24 completed weeks of gestation) rate per woman or couple randomised
- Miscarriage (loss of pregnancy before 24 completed weeks of gestation) rate per woman or couple randomised

Secondary outcomes

- Clinical pregnancy (presence of a gestational sac on ultrasound scan) rate per woman or couple randomised
- Complication rate per woman or couple randomised, including ectopic pregnancy, intrauterine growth restriction, foetal or congenital defects, pelvic infection, or other adverse events, reported as an overall complication rate or as individual outcomes, or both (as reported by individual studies)

Search methods for identification of studies

We sought all published and unpublished RCTs of intrauterine hCG administration around the time of ET in consultation with the Cochrane Gynaecology and Fertility Group Information Specialist. Search dates ranged from inception of the databases to 9 January 2018, and we applied no language restrictions.

Electronic searches

We searched the following.

- Cochrane Gynaecology and Fertility Group Specialised Register (searched 9 January 2018) (PROCITE platform) (Appendix 1).
- Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library (via the CENTRAL Register of Studies Online (CRSO)) (searched 9 January 2018) (Web platform) (Appendix 2).
- MEDLINE (searched from 1946 to 9 January 2018) (OVID platform) (Appendix 3).
- Embase (searched from 1980 to 9 January 2018) (OVID platform) (Appendix 4).
- PsycINFO (searched from 1806 to 9 January 2018) (OVID platform) (Appendix 5).
- Cumulative Index to Nursing and Allied Health Literature (CINAHL) (searched from 1961 to 9 January 2018) (EBSCO platform) (Appendix 6).

We combined the MEDLINE search with the Cochrane highly sensitive search strategy for identifying RCTs, which appears in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011; Chapter 6, Section 6.4.11). We combined the Embase and CINAHL searches with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) (www.sign.ac.uk/methodology/filters.html#random).

We also searched the World Health Organization International Clinical Trials Registry Platform (apps.who.int/trialsearch/Default.aspx) and ClinicalTrials.gov for ongoing and registered trials. We searched OpenGrey (www.opengrey.eu/) and Google Scholar (scholar.google.co.uk/) for grey literature. We handsearched abstracts published following major conferences (e.g. the American Society for Reproductive Medicine (ASRM), European Society of Human Reproduction and Embryology (ESHRE)) held in the last five years to find additional studies not yet published in full.

Searching other resources

We screened the reference lists of all included studies and relevant reviews to identify further articles for possible inclusion.

Data collection and analysis

We used Review Manager 5 for input of data and statistical analysis (RevMan 2014), in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Selection of studies

Two review authors (LC and NT) independently screened the title, abstract, and keywords for each publication to exclude studies that were irrelevant for the objective of this review. We retrieved the remaining publications in full text, and the same two review authors appraised them independently to identify RCTs that were suitable for inclusion. We encountered no disagreements related to study eligibility and documented the selection process with a PRISMA flow chart.

Data extraction and management

Two review authors (LC and NT) independently extracted data using a pre-designed and pilot-tested data extraction form. For studies with multiple publications, we used the main RCT report as the reference, and we supplemented it with additional data from secondary publications. We attempted to contact study authors when published data were insufficient. We encountered no disagreements. One review author (LC) entered data into Review Manager 5 (RevMan 2014), and a second review author (NT) checked entered data against the data extraction form.

Assessment of risk of bias in included studies

We used the Cochrane 'Risk of bias' assessment tool to assess the included studies for selection, performance, detection, attrition, reporting, and other biases. We encountered no disagreements. We included the 'Risk of bias' table in the Characteristics of included studies table, describing the judgements in detail.

Measures of treatment effect

All outcomes were dichotomous. We calculated Mantel-Haenszel risk ratios (RRs) with 95% confidence intervals (CIs) using the numbers of events in the intervention and control groups of each study. For outcomes with event rates below 1%, we used the Peto one-step odds ratio (OR) method to calculate the combined outcome with 95% CI.

Unit of analysis issues

We performed analysis per randomised woman or couple for live birth, clinical pregnancy, miscarriage, and complication rates. We counted multiple live births (twins, triplets) as a single live birth event. We performed a secondary analysis for miscarriage per clinical pregnancy to broaden our understanding of the treatment effect.

If a study included multiple treatment arms based on hCG dose, we planned to split the control group proportionately with the experimental groups to avoid analysing control participants in duplicate.

Dealing with missing data

We attempted to contact authors of the RCTs to obtain missing data so we could perform analyses on an intention-to-treat basis. In the case of unobtainable data, we planned to undertake imputation of individual values for the live birth rate only. We assumed that live births had not occurred in participants without a reported outcome. For other outcomes, we analysed only available data.

Assessment of heterogeneity

We identified heterogeneity by visually inspecting forest plots and by using a standard Chi^2 test with significance set at $P < 0.1$. We used the I^2 statistic to estimate total variation across RCTs that was due to heterogeneity, when I^2 greater than 50% indicated substantial heterogeneity.

Assessment of reporting biases

We conducted a comprehensive search to minimise the potential impact of publication bias and other reporting biases. We planned to use a funnel plot to explore the possibility of small-study effects when the number of included RCTs exceeded 10.

Data synthesis

We combined the data from similar RCTs comparing similar treatments using a random-effects model. We displayed an increase in the odds of an outcome to the right of the centre line and a decrease in the odds of an outcome to the left of the centre line. For comparisons that showed considerable clinical, methodological, or statistical heterogeneity ($I^2 > 75\%$), we did not combine results of RCTs in a meta-analysis. When data were incomplete and could not be presented in the analyses, we reported available data in narrative form.

Subgroup analysis and investigation of heterogeneity

When data were available, we conducted subgroup analyses to investigate the efficacy of intrauterine hCG administration around the time of ET depending on:

- stage of the embryo at transfer (cleavage vs blastocyst);
- source of intracavity hCG (IC-hCG) (urine vs recombinant);
- embryo processing (fresh vs frozen/thawed); and
- number of embryos transferred.

If we detected substantial heterogeneity, we explored possible explanations in sensitivity analyses. Factors considered included treatment indication, age of the women, ovarian stimulation protocol, response to ovarian stimulation, timing of IC-hCG administration, IC-hCG dose and volume of infused medium, method of IC-hCG administration (i.e. type of catheter), embryo quality, endometrial thickness, source of oocytes (i.e. donated, own), and ET difficulty. We took any statistical heterogeneity into account when interpreting the results, especially if we noted variation in the direction of effect.

Sensitivity analysis

We performed sensitivity analysis to examine the stability and robustness of results for the primary outcomes in relation to the following eligibility and analysis factors.

- Inclusion of RCTs without high risk of bias in one or more domains.
- Inclusion of RCTs published as full text.
- Use of a fixed-effect model.
- Calculation of OR.

Overall quality of the body of evidence - 'Summary of findings' table

Two review authors working independently (LC and NT) prepared a 'Summary of findings' table using GRADEpro software and comparing hCG versus no hCG (GRADEpro 2015). We resolved disagreements by discussion. In this table, we evaluated the overall quality of the body of evidence for the main review outcomes (live birth rate, miscarriage, clinical pregnancy rate, and complications) using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness, and publication bias) (GRADE 2013). We justified, documented, and incorporated judgements about evidence quality (high, moderate, low, or very low) into reporting of results for each outcome.

RESULTS

Description of studies

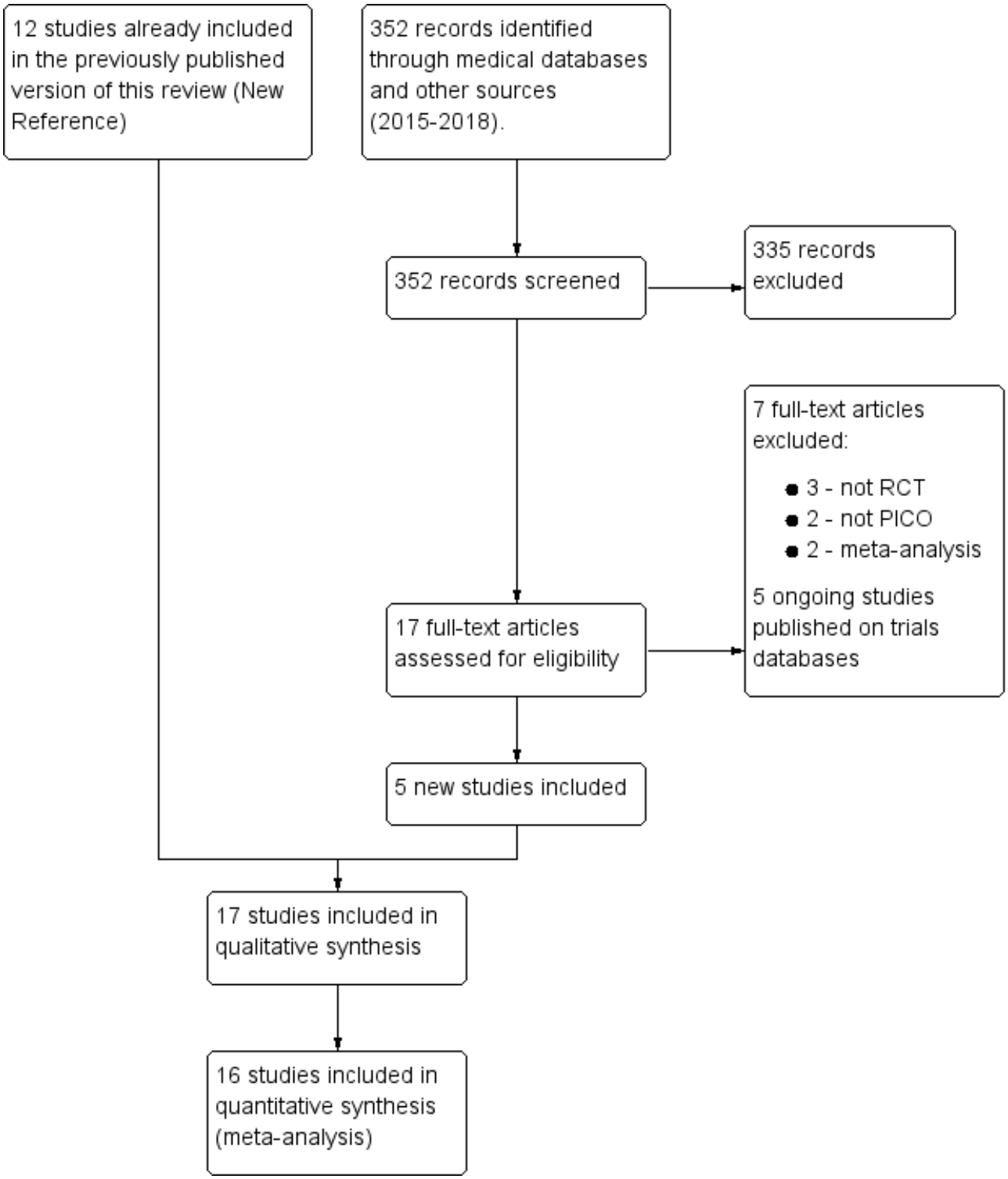
See Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification; and Characteristics of ongoing studies tables.

Results of the search

We performed the latest systematic search on 9 January 2018, and we identified 352 publications (14 from CINAHL, 91 from CENTRAL, 133 from EMBASE, 41 from CGFG, 58 from MEDLINE, 2 from PsychINFO, and 13 from other sources).

In this updated review, we have included 17 studies (12 in the previous version), excluded 13 studies (six in the previous version), and identified two studies awaiting classification and five ongoing studies. See Figure 1 for detailed search results.

Figure 1. Study flow diagram.



Included studies

Types of studies

All 17 included studies were parallel-arm RCTs. One study had two experimental arms (IC-hCG 500 IU vs IC-hCG 1000 IU vs control) (Dehghani Firouzabadi 2016), one study had two phases with three experimental arms (phase one: IC-hCG 100 IU vs IC-hCG 200 IU vs control; and phase two: IC-hCG 500 IU vs control) (Mansour 2011), and one study had two experimental arms using two different timings (IC-hCG 500 IU vs control two days before ET; IC-hCG 500 IU vs control on the day of ET) (Wirleitner 2015a).

Researchers performed randomisation at different times during treatment. Five studies randomised participants before the start

of their treatment cycle (Dehghani Firouzabadi 2016; Hong 2014; Mansour 2011; Santibañez 2014; Singh 2014), two studies randomised participants on the day of oocyte retrieval (Navali 2016; Wirleitner 2015a), four studies randomised participants on the day of embryo transfer (Aaleyasin 2015; Cambiaghi 2013; Hosseini 2016; Huang 2016), and the remaining six studies provided insufficient details about the timing of randomisation (Eskandar 2016; Kokkali 2014; Leao 2013; Mostajeran 2017; Wirleitner 2015b; Zarei 2014).

Eleven studies were published as full-text articles (Aaleyasin 2015; Dehghani Firouzabadi 2016; Hong 2014; Hosseini 2016; Huang 2016; Mansour 2011; Mostajeran 2017; Navali 2016; Santibañez 2014; Wirleitner 2015a; Zarei 2014), and six studies were published as abstracts (Cambiaghi 2013; Eskandar 2016; Kokkali 2014; Leao 2013; Singh 2014; Wirleitner 2015b).

Ten studies did not report funding (Aaleyasin 2015; Cambiaghi 2013; Dehghani Firouzabadi 2016; Eskandar 2016; Hong 2014; Hosseini 2016; Huang 2016; Leao 2013; Mostajeran 2017; Wirleitner 2015a), and seven studies reported internal funding (Kokkali 2014; Mansour 2011; Navali 2016; Santibañez 2014; Singh 2014; Wirleitner 2015b; Zarei 2014). No studies reported external funding.

Participants

Participants were couples/women recruited before undergoing assisted reproductive treatment for different subfertility causes. The number of participants varied between 36 in Leao 2013 and 1186 in Wirleitner 2015a. The studies were conducted in the USA, Austria, Greece, Iran, China, Saudi Arabia, Brazil, Egypt, Mexico, and India.

Interventions

Most studies compared intrauterine administration of urine hCG 500 IU versus controls. One study had two additional arms with lower doses (IC-hCG 100 and 200 IU) (Mansour 2011). One study had an additional arm with a higher dose (IC-hCG 1000 IU) (Dehghani Firouzabadi 2016). One study used 1000 IU (Huang 2016), and another study used 700 IU (Mostajeran 2017). One study used rhCG 250 µg (equivalent of 6500 IU) (Zarei 2014), and another study used intracavity rhCG (IC-rhCG) 40 µL (equivalent to 500 IU) (Singh 2014).

Twelve studies administered IC-hCG within minutes before ET (Aaleyasin 2015; Dehghani Firouzabadi 2016; Eskandar 2016; Hong 2014; Hosseini 2016; Kokkali 2014; Mansour 2011; Mostajeran 2017; Santibañez 2014; Singh 2014; Wirleitner 2015b; Zarei 2014), ranging from less than three minutes in Hong 2014 up to 12 minutes in Zarei 2014. Two studies administered IC-hCG six hours before ET (Cambiaghi 2013; Leao 2013). One study had four groups (two experimental and two controls) at two different timings (two days before ET and three minutes before ET) (Wirleitner 2015a). One study administered IC-hCG three days before ET (Huang 2016). Another study administered IC-hCG at the time of oocyte retrieval (Navali 2016).

For control groups, seven studies administered the same volume of transfer media (Hong 2014), culture media (Aaleyasin 2015; Singh 2014; Wirleitner 2015a; Wirleitner 2015b), or normal saline (Navali 2016; Zarei 2014), all without hCG, and 10 studies did not administer anything before ET (Cambiaghi 2013; Dehghani Firouzabadi 2016; Eskandar 2016; Hosseini 2016; Huang 2016; Kokkali 2014; Leao 2013; Mansour 2011; Mostajeran 2017; Santibañez 2014).

Outcomes

Eleven studies reported on one of our predefined primary outcomes: Aaleyasin 2015, Mansour 2011, Singh 2014, Wirleitner 2015a, and Wirleitner 2015b reported on live birth; and Aaleyasin 2015, Dehghani Firouzabadi 2016, Hong 2014, Hosseini 2016, Huang 2016, Mansour 2011, Navali 2016, Singh 2014, Wirleitner 2015a, Wirleitner 2015b, and Zarei 2014 reported on miscarriage.

Seventeen studies reported on one of our predefined secondary outcomes: Aaleyasin 2015, Cambiaghi 2013, Dehghani Firouzabadi 2016, Eskandar 2016, Hong 2014, Hosseini 2016, Huang 2016, Kokkali 2014, Leao 2013, Mansour 2011, Mostajeran 2017, Navali 2016, Santibañez 2014, Singh 2014, Wirleitner 2015a, Wirleitner 2015b, and Zarei 2014 reported on clinical pregnancy; and Aaleyasin 2015, Dehghani Firouzabadi 2016, Hosseini 2016, Mansour 2011, Navali 2016, Santibañez 2014, and Zarei 2014 reported on complications.

Studies awaiting classification

Two studies await classification (Badehnoosh 2014; Bhat 2014). These studies reported interim outcomes (implantation rate and fertilisation rate), and it is unclear whether they also collected data on clinical outcomes that might be relevant to our review. We emailed the authors of these studies in February 2016 and January 2018 to ask for more information on the methods and outcome measures of their studies.

Excluded studies

We excluded 13 studies owing to retrospective design (Huang 2017; Jeong 2013; Kanter 2017), non-randomisation (Li 2013; Rebolloso 2013; Riboldi 2013, Volovsky 2016), not meeting the PICO (Giuliani 2015; Strug 2016), and performing a meta-analysis (Dieamant 2016; Osman 2016; Ye 2015). One study was previously published as an abstract (Janati 2013); this has now been replaced by its full manuscript publication (Dehghani Firouzabadi 2016).

Risk of bias in included studies

Figure 2 shows the 'Risk of bias' graph, and Figure 3 shows the 'Risk of bias' summary. See the Characteristics of included studies table for rationales behind each judgement.

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

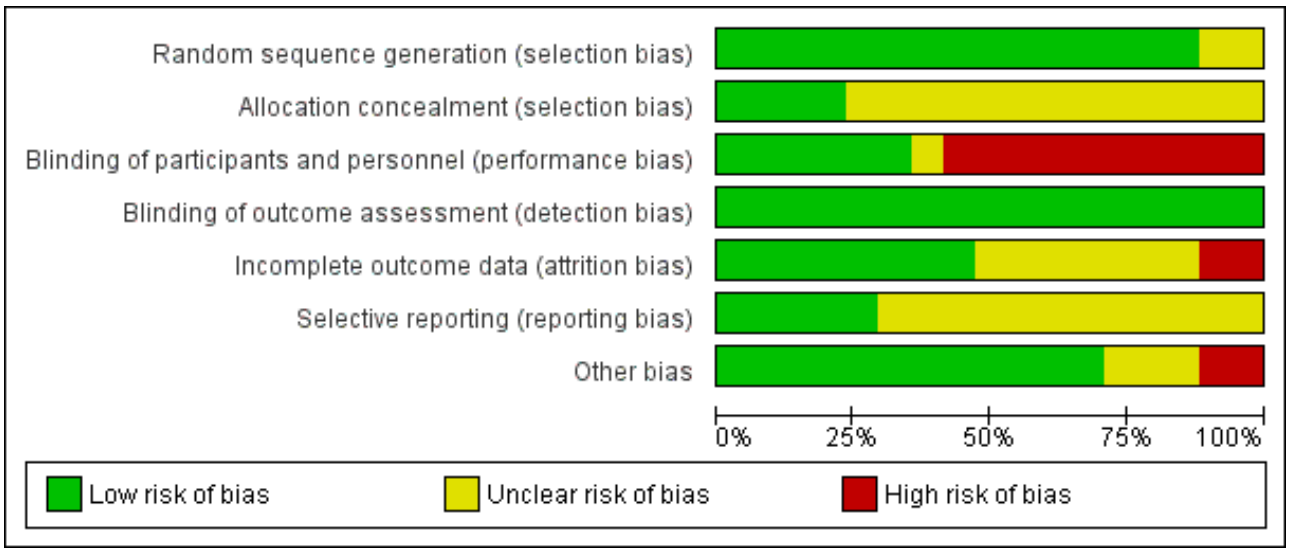
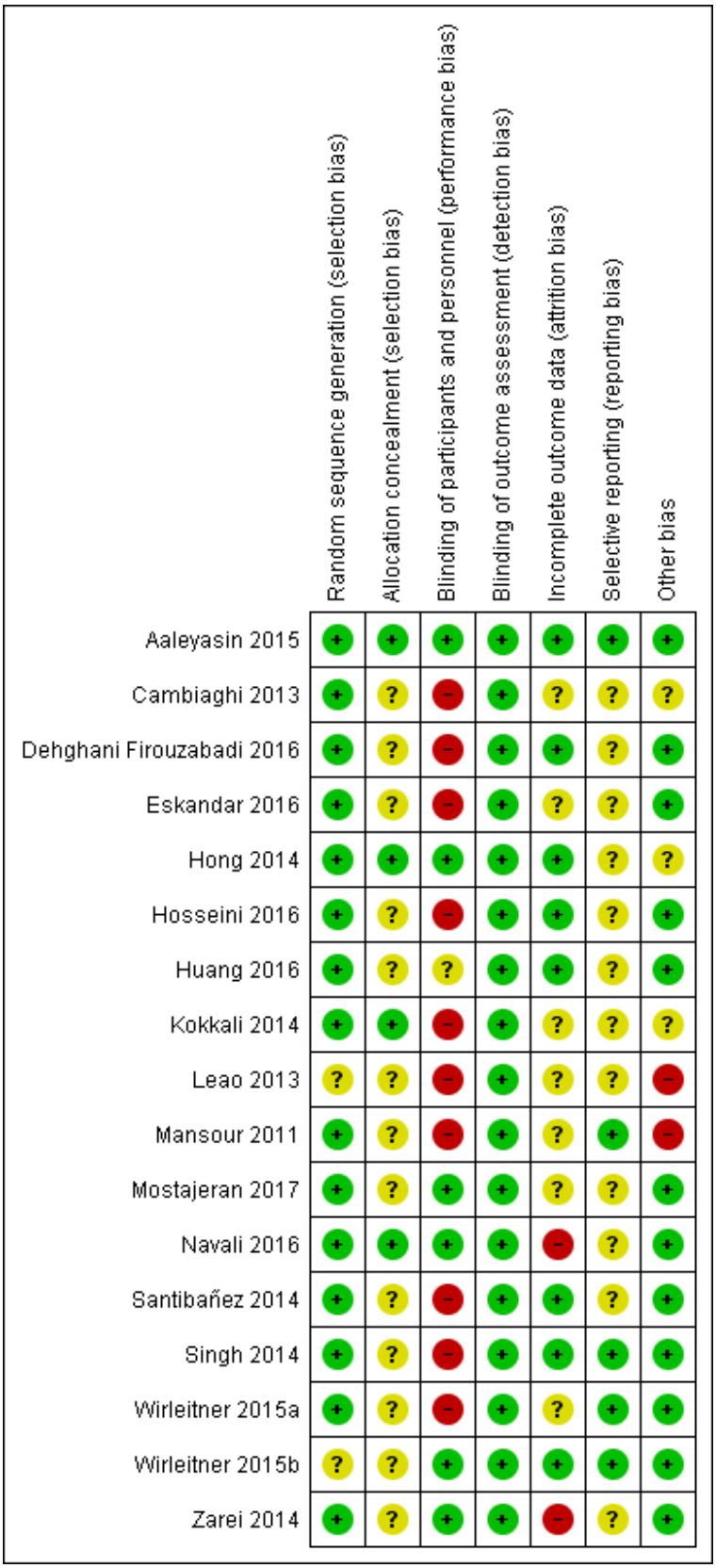


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Sequence generation

All included studies were RCTs. The randomisation technique was adequate in 15 studies (Aaleyasin 2015; Cambiaghi 2013; Dehghani Firouzabadi 2016; Eskandar 2016; Hong 2014; Hosseini 2016; Huang 2016; Kokkali 2014; Mansour 2011; Mostajeran 2017; Navali 2016; Santibañez 2014; Singh 2014; Wirleitner 2015a; Zarei 2014), which we classified at low risk of bias. Two studies lacked an adequate description of randomisation, and we classified them at unclear risk of bias (Leao 2013; Wirleitner 2015b).

Allocation concealment

Four studies mentioned adequate allocation concealment, and we classified them at low risk of bias (Aaleyasin 2015; Hong 2014; Kokkali 2014; Navali 2016). Thirteen studies lacked a description of methods of allocation concealment, and we classified them at unclear risk of bias (Cambiaghi 2013; Dehghani Firouzabadi 2016; Eskandar 2016; Hosseini 2016; Huang 2016; Leao 2013; Mansour 2011; Mostajeran 2017; Santibañez 2014; Singh 2014; Wirleitner 2015a; Wirleitner 2015b; Zarei 2014).

Blinding

Six studies documented blinding of participants or personnel (or both), and we classified them at low risk of bias (Aaleyasin 2015; Hong 2014; Mostajeran 2017; Navali 2016; Wirleitner 2015b; Zarei 2014). One study was mentioned to be single-blinded, but it was not clear who was blinded; hence, we classified it as having unclear risk of bias (Huang 2016). We classified the remaining studies at high risk of bias (Cambiaghi 2013; Dehghani Firouzabadi 2016; Eskandar 2016; Hosseini 2016; Kokkali 2014; Leao 2013; Mansour 2011; Santibañez 2014; Singh 2014; Wirleitner 2015a).

The outcome measurement was not likely to be influenced by lack of blinding; hence, we classified all studies at low risk of bias.

Incomplete outcome data

Eight studies followed up all participants and reported the results adequately (Aaleyasin 2015; Dehghani Firouzabadi 2016; Hong 2014; Hosseini 2016; Huang 2016; Santibañez 2014; Singh 2014; Wirleitner 2015b). We classified these studies at low risk of bias. We classified seven studies at unclear risk of bias (Cambiaghi 2013; Eskandar 2016; Kokkali 2014; Leao 2013; Mansour 2011; Mostajeran 2017; Wirleitner 2015a). Two studies reported large numbers of participants lost to follow-up, and we classified them at high risk of attrition bias (Navali 2016; Zarei 2014).

Selective reporting

Five studies reported on all relevant outcomes, and we classified them at low risk of bias (Aaleyasin 2015; Mansour 2011; Singh 2014; Wirleitner 2015a; Wirleitner 2015b). All studies reported on clinical pregnancy, but if they did not report on live birth, we classified them at unclear risk of bias (Cambiaghi 2013; Dehghani Firouzabadi 2016; Eskandar 2016; Hong 2014; Hosseini 2016; Huang 2016; Kokkali 2014; Leao 2013; Mostajeran 2017; Navali 2016; Santibañez 2014; Zarei 2014).

Other potential sources of bias

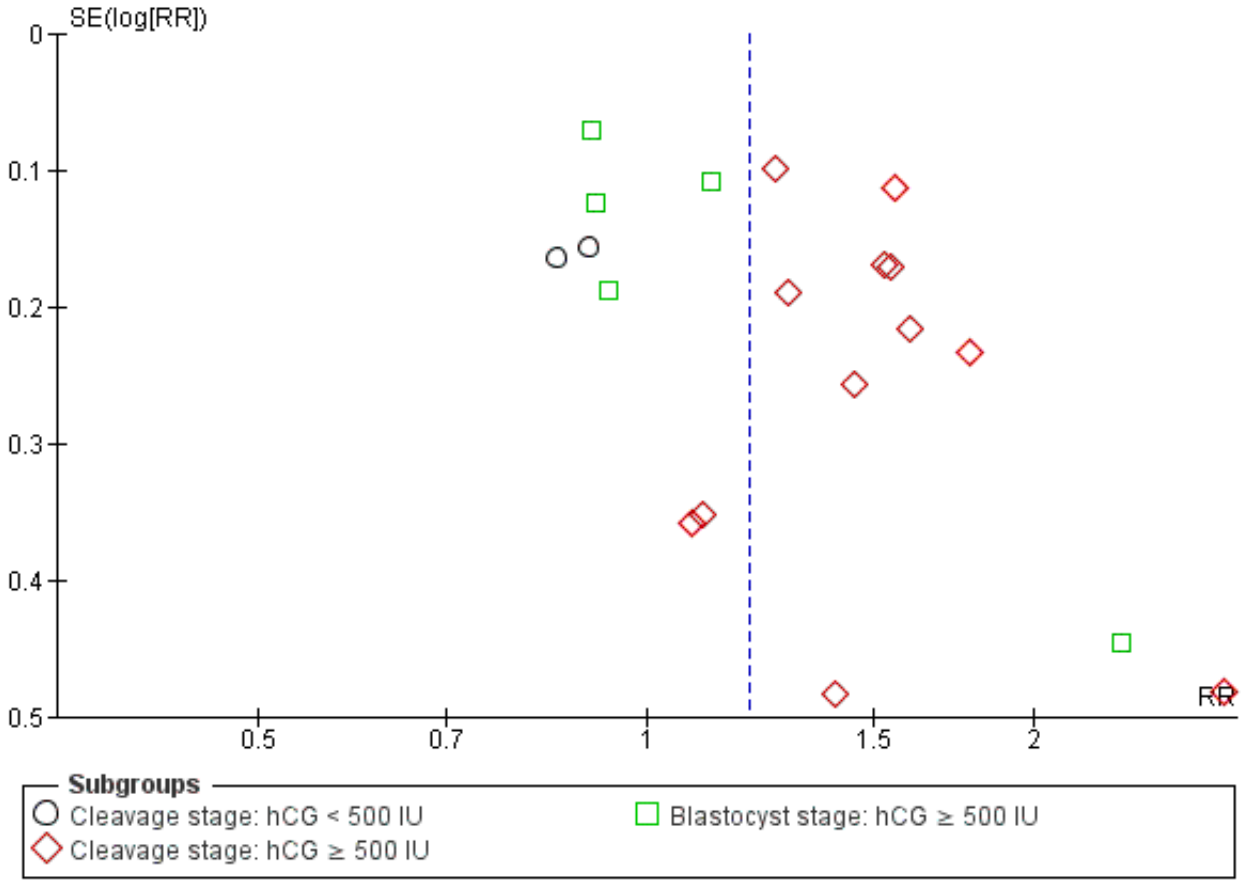
We classified 12 studies at low risk of other potential bias because groups appeared to be comparable at baseline, and we could not identify any other sources of bias (Aaleyasin 2015; Dehghani Firouzabadi 2016; Eskandar 2016; Hosseini 2016; Huang 2016; Mostajeran 2017; Navali 2016; Santibañez 2014; Singh 2014; Wirleitner 2015a; Wirleitner 2015b; Zarei 2014). We classified three studies at unclear risk of bias because they did not report on baseline characteristics between groups (probably because they were available in abstract format only) (Cambiaghi 2013; Kokkali 2014), or they reported a large number of participants who declined to participate after randomisation for various reasons (Hong 2014). We classified two studies at high risk of bias owing to lack of reporting of participant numbers in each study group in Leao 2013, and owing to performance of an interim analysis that changed the study protocol and ended the study prematurely in Mansour 2011.

The overall birth rate in the control groups in Mansour 2011 was 47%, whereas the control group live birth rate ranged from 25% to 39% in the other included studies. The reason for this was unclear. The mean age of women in Mansour 2011 was under 30 years, but this was also the case in Aaleyasin 2015, which reported a control group live birth rate of only 25%. Furthermore, Mansour 2011 randomised women at the beginning of their cycle, and Aaleyasin 2015 randomised women before embryo transfer, which should have led to higher live birth rates (by not including cancelled cycles).

Assessment of publication bias

The funnel plot for clinical pregnancy did not show any evidence of publication bias (Figure 4).

Figure 4. Funnel plot of comparison: 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, outcome: 1.4 Clinical pregnancy.



Effects of interventions

See: **Summary of findings for the main comparison** Intrauterine administration of hCG for women undergoing assisted reproduction

Note: One study included three experimental arms (Mansour 2011), and another study included two experimental arms based on intrauterine hCG dose (i.e. 100 IU, 200 IU, 500 IU, and 1000 IU, respectively) (Dehghani Firouzabadi 2016). We regarded and analysed them as separate comparisons. We split the control groups proportionately with the experimental groups to avoid analysing control participants in duplicate. One study investigated intrauterine hCG administration at two different timings (day three vs day five administration), and we regarded and analysed them as two separate comparisons (Wirleitner 2015a).

Two of the comparisons showed considerable heterogeneity (I^2 > 75%) (Analysis 1.1; Analysis 1.4), and we did not perform a global meta-analysis as prespecified in the protocol (Craciunas 2015).

Exploration for the sources of heterogeneity in these analyses revealed two key prespecified variables as important determinants: stage of ET (cleavage vs blastocyst stage) and dose of IC-hCG (< 500 IU vs ≥ 500 IU). When we subgrouped the data according to these variables, we found evidence of significant differences between subgroups. We then performed meta-analysis within the subgroups defined by stage of embryo and dose of hCG.

Primary outcomes

Live birth

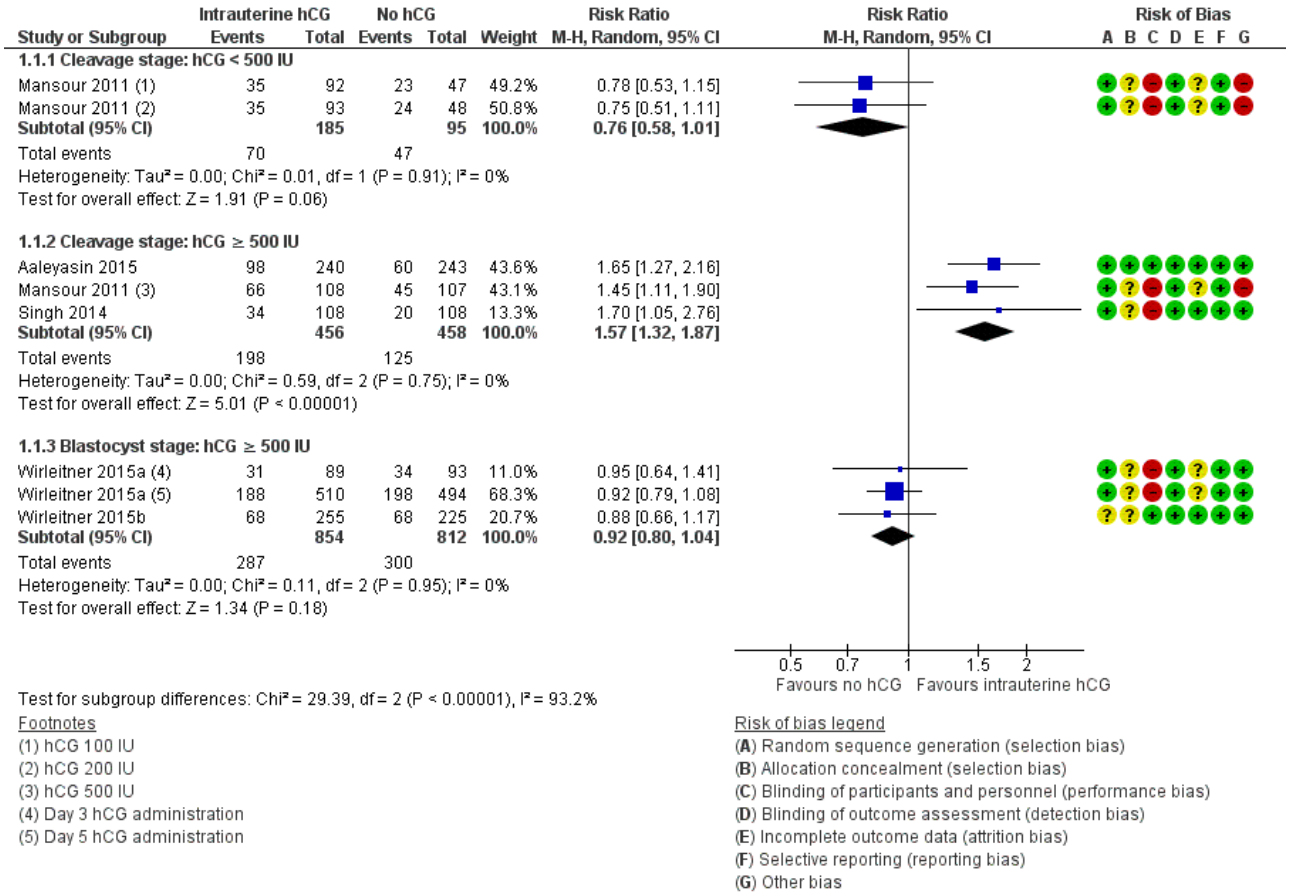
(Analysis 1.1)

Five studies with eight experimental arms reported on live birth (Aaleyasin 2015; Mansour 2011; Singh 2014; Wirleitner 2015a; Wirleitner 2015b) (Analysis 1.1).

Subgroup analysis

The forest plot displayed these studies based on the embryo stage at transfer and the hCG dose (Figure 5). The test for subgroup differences indicated a considerable difference between subgroups (Chi^2 = 29.39, degrees of freedom (df) = 2, P ≤ 0.00001, I^2 = 92.3%).

Figure 5. Forest plot of comparison: 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, outcome: 1.1 Live birth.



- Cleavage stage: IC-hCG less than 500 IU versus no IC-hCG: one RCT with two experimental arms contributed to calculation of the combined outcome (Mansour 2011). The heterogeneity was insignificant (Chi² = 0.01, df = 1, P = 0.91, I² = 0%), and findings showed no benefit of the intervention, which was consistent with no substantive difference or disadvantage of indeterminate magnitude (RR 0.76, 95% CI 0.58 to 1.01; one RCT; N = 280; I² = 0%; very low-quality evidence).
- Cleavage stage: IC-hCG 500 IU or greater versus no IC-hCG: three RCTs contributed to calculation of the combined outcome (Aaleyasin 2015; Mansour 2011; Singh 2014). The heterogeneity was insignificant (Chi² = 0.59, df = 2, P = 0.75, I² = 0%), and the live birth rate was higher in the hCG group (RR 1.57, 95% CI 1.32 to 1.87; three RCTs; N = 914; I² = 0%; moderate-quality evidence). This suggested that in women with a 27% chance of live birth without using IC-hCG, the live birth rate among those using IC-hCG 500 IU or greater will be between 36% and 51%.
- Blastocyst stage: IC-hCG 500 IU or greater versus no IC-hCG: two RCTs with three experimental arms contributed to calculation of the combined outcome (Wirleitner 2015a; Wirleitner 2015b). The heterogeneity was insignificant (Chi² = 0.11, df = 2, P = 0.95, I² = 0%), and results showed no substantive differences between groups in live birth rates (RR 0.92, 95% CI 0.80 to 1.04; two RCTs; N = 1666; I² = 0%; moderate-quality evidence).

Data were insufficient for the prespecified subgroup analyses to be performed based on embryo processing and number of embryos transferred.

Sensitivity analyses

Removing studies with high risk of bias in one or more domains did not alter the results significantly (Mansour 2011; Singh 2014; Wirleitner 2015a), but it meant that no data were available for one of the comparisons.

- Cleavage stage: IC-hCG less than 500 IU versus no IC-hCG (no data).
- Cleavage stage: IC-hCG 500 IU or greater versus no IC-hCG (RR 1.65, 95% CI 1.27 to 2.16; one RCT; N = 483).
- Blastocyst stage: IC-hCG 500 IU or greater versus no IC-hCG (RR 0.88, 95% CI 0.66 to 1.17; one RCT; N = 480).

Removing the studies available in abstract format only did not alter the results significantly (Singh 2014; Wirleitner 2015b).

- Cleavage stage: IC-hCG less than 500 IU versus no IC-hCG (RR 0.76, 95% CI 0.58 to 1.01; one RCT; N = 280; I² = 0%; very low-quality evidence).
- Cleavage stage: IC-hCG 500 IU or greater versus no IC-hCG (RR 1.55, 95% CI 1.28 to 1.87; two RCTs; N = 698; I² = 0%; moderate-quality evidence).

- Blastocyst stage: IC-hCG 500 IU or greater versus no IC-hCG (RR 0.92, 95% CI 0.80 to 1.07; one RCT; N = 1186; I^2 = 0%; moderate-quality evidence).

The calculated combined outcome based on the fixed-effect model was similar to that based on the random-effects model for the following.

- Cleavage stage: IC-hCG less than 500 IU versus no IC-hCG (RR 0.76, 95% CI 0.58 to 1.01; one RCT; N = 280; I^2 = 0%; very low-quality evidence).
- Cleavage stage: IC-hCG 500 IU or greater versus no IC-hCG (RR 1.59, 95% CI 1.33 to 1.90; three RCTs; N = 914; I^2 = 0%; moderate-quality evidence).
- Blastocyst stage: IC-hCG 500 IU or greater versus no IC-hCG (RR 0.91, 95% CI 0.80 to 1.04; two RCTs; N = 1666; I^2 = 0%; moderate-quality evidence).

Results did not differ substantially when odds ratio (OR) was used instead of risk ratio (RR).

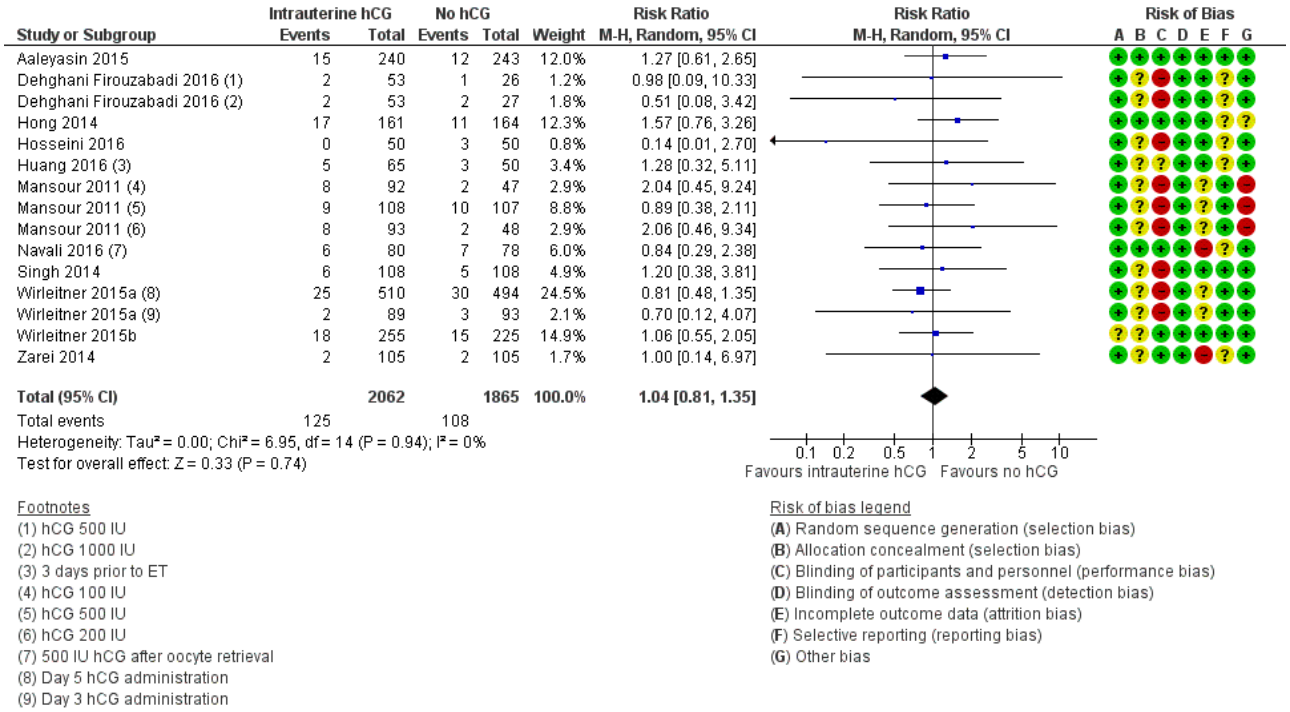
- Cleavage stage: IC-hCG less than 500 IU versus no IC-hCG (OR 0.62, 95% CI 0.38 to 1.03; one RCT; N = 280; I^2 = 0%; very low-quality evidence).
- Cleavage stage: IC-hCG 500 IU or greater versus no IC-hCG (OR 2.10, 95% CI 1.59 to 2.79; three RCTs; N = 914; I^2 = 0%; moderate-quality evidence).
- Blastocyst stage: IC-hCG 500 IU or greater versus no IC-hCG (OR 0.87, 95% CI 0.71 to 1.06; two RCTs; N = 1666; I^2 = 0%; moderate-quality evidence).

Miscarriage

(Analysis 1.2)

Eleven studies with 15 experimental arms reported on miscarriage (Aaleyasin 2015; Dehghani Firouzabadi 2016; Hong 2014; Hosseini 2016; Huang 2016; Mansour 2011; Navali 2016; Singh 2014; Wirleitner 2015a; Wirleitner 2015b; Zarei 2014; Analysis 1.2; Figure 6). Heterogeneity between studies was unsubstantial (Chi^2 = 6.95, df = 14, P = 0.74, I^2 = 0%), and studies provided no evidence of a difference between groups in miscarriage rates (RR 1.04, 95% CI 0.81 to 1.35; 11 RCTs; N = 3927; I^2 = 0%; very low-quality evidence).

Figure 6. Forest plot of comparison: 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, outcome: 1.2 Miscarriage.



Sensitivity analyses

Removing studies with high risk of bias in one or more domains - Dehghani Firouzabadi 2016, Hosseini 2016, Mansour 2011, Navali 2016, Singh 2014, and Wirleitner 2015a - did not alter the results significantly (RR 1.26, 95% CI 0.86 to 1.84; five RCTs; N = 1613; I^2 = 0%; very low-quality evidence).

Removing the two studies available in abstract format only - Singh 2014 and Wirleitner 2015b - did not alter the results significantly (RR 1.03, 95% CI 0.78 to 1.37; nine RCTs; N = 3231; I^2 = 0%; very low-quality evidence).

The calculated combined outcome based on the fixed-effect model was similar to that based on the random-effects model (RR 1.04, 95% CI 0.81 to 1.34; 11 RCTs; N = 3927; I^2 = 0%; very low-quality evidence).

Results did not differ substantially when OR was used instead of RR (OR 1.05, 95% CI 0.80 to 1.37; 11 RCTs; N = 3927; I^2 = 0%; very low-quality evidence).

Secondary analysis per clinical pregnancy

(Analysis 1.3)

Studies provided no evidence of a difference between groups in miscarriage rates calculated per clinical pregnancy (RR 0.84, 95% CI 0.62 to 1.13; 11 RCTs; N = 1620; I^2 = 24%; very low-quality evidence) (Analysis 1.3).

Secondary outcomes

Clinical pregnancy

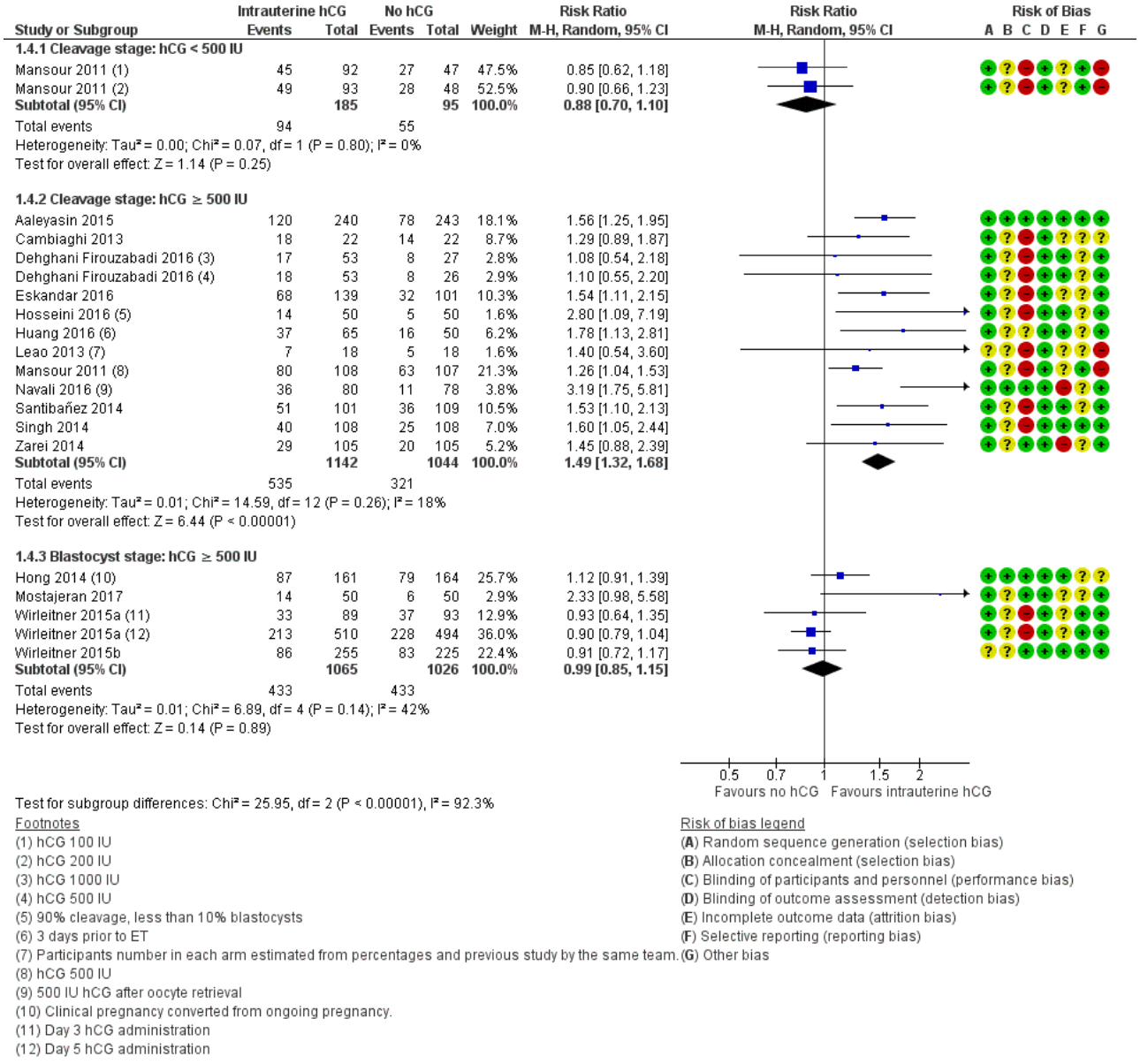
(Analysis 1.4)

All included studies reported clinical pregnancy (Analysis 1.4).

Subgroup analysis

The forest plot displayed the studies based on embryo stage at transfer and hCG dose (Figure 7). The test for subgroup differences indicated a considerable difference between subgroups (Chi^2 = 25.95, df = 2, P ≤ 0.00001, I^2= 92.3%).

Figure 7. Forest plot of comparison: 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, outcome: 1.4 Clinical pregnancy.



- Cleavage stage: IC-hCG less than 500 IU versus no IC-hCG: one RCT with two experimental arms contributed to calculation of the combined outcome (Mansour 2011). Heterogeneity was insignificant (Chi^2 = 0.07, df = 1, P = 0.80, I^2 = 0%), and studies

provided no evidence of a difference between groups in clinical pregnancy rates (RR 0.88, 95% CI 0.70 to 1.10; one RCT; N = 280; I^2 = 0%; very low-quality evidence).

- Cleavage stage: IC-hCG 500 IU or greater versus no IC-hCG: 12 RCTs contributed to calculation of the combined outcome (Aaleyasin 2015; Cambiaghi 2013; Dehghani Firouzabadi 2016; Eskandar 2016; Hosseini 2016; Huang 2016; Leao 2013; Mansour 2011; Navali 2016; Santibañez 2014; Singh 2014; Zarei 2014). Heterogeneity was insignificant (Chi^2 = 14.59, df = 12, P = 0.26, I^2 = 18%), and the clinical pregnancy rate was higher in the hCG group (RR 1.49, 95% CI 1.32 to 1.68; 12 RCTs; N = 2186; I^2 = 18%; moderate-quality evidence).

One study investigated IC-hCG 500 IU and reported no evidence of a difference between groups in clinical pregnancy rates (Kokkali 2014). Data from this study were insufficient to be included in the meta-analysis.

- Blastocyst stage: IC-hCG 500 IU or greater versus no IC-hCG: four RCTs with five experimental arms contributed to calculation of the combined outcome (Hong 2014; Mostajeran 2017; Wirleitner 2015a; Wirleitner 2015b). Heterogeneity was moderate (Chi^2 = 6.89, df = 4, P = 0.14, I^2 = 42%), and studies provided no evidence of a difference between groups in clinical pregnancy rates (RR 0.99, 95% CI 0.85 to 1.15; four RCTs; N = 2091; I^2 = 42%; moderate-quality evidence).

Data were insufficient for the predefined subgroup analyses to be performed based on embryo processing and number of embryos transferred.

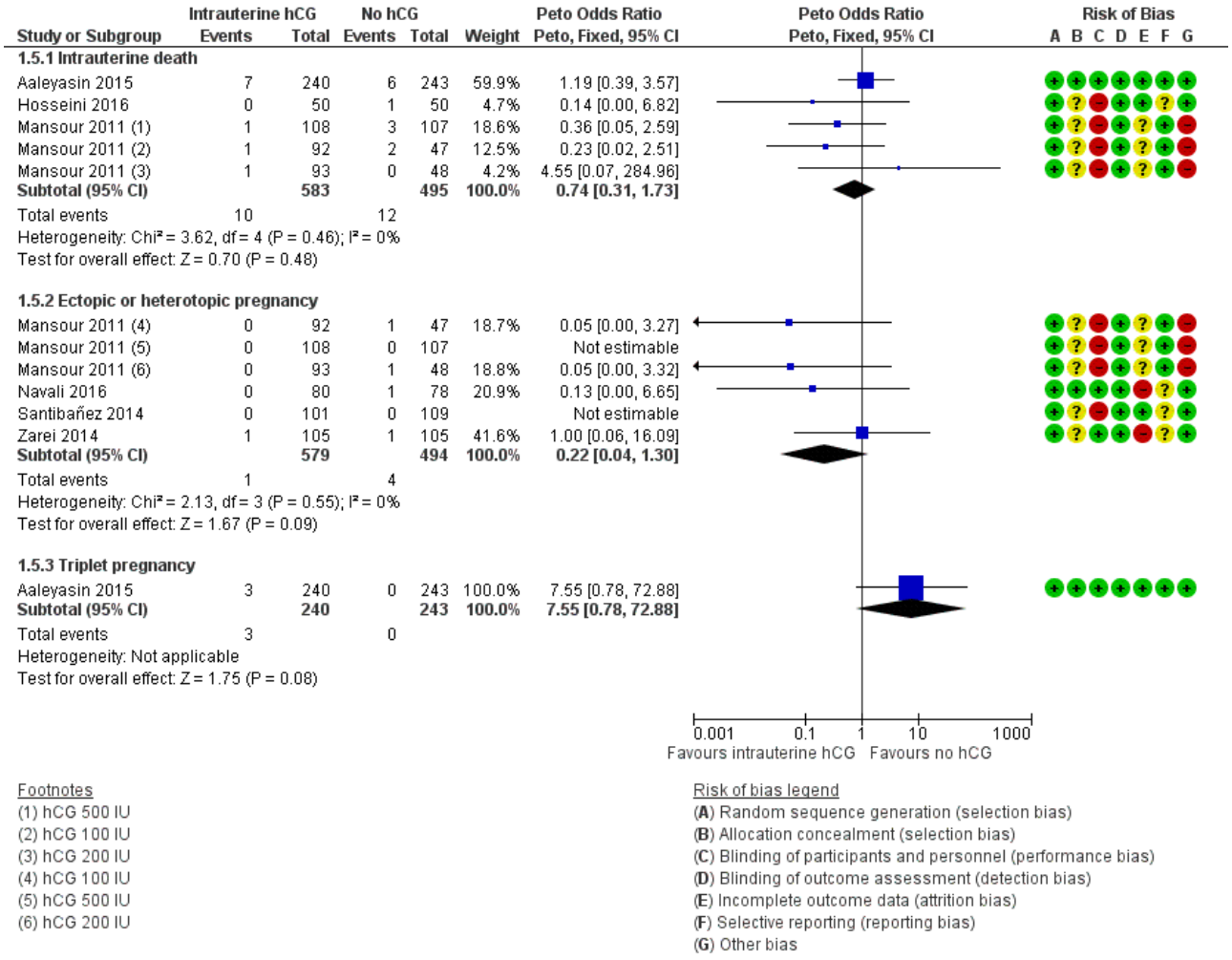
Complications

(Analysis 1.5)

Seven studies with 10 experimental arms reported complications (Aaleyasin 2015; Dehghani Firouzabadi 2016; Hosseini 2016; Mansour 2011; Navali 2016; Santibañez 2014; Zarei 2014; Analysis 1.5).

Evidence was insufficient to show whether there was a difference between groups for any of the mentioned complications: ectopic pregnancy (four studies; N = 1073; four events overall), heterotopic pregnancy (one study; N = 495; one event), intrauterine death (three studies; N = 1078; 22 events), and triplets (one study; N = 48; three events). For intrauterine death, the analysis in Figure 8 displays the Peto OR (which is the default setting for this analysis). Mantel-Haenszel random-effects RRs were almost identical (RR 0.77, 95% CI 0.33 to 1.77; three studies; N = 1078; I^2 = 0%).

Figure 8. Forest plot of comparison: 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, outcome: 1.5 Complications.



DISCUSSION

Summary of main results

This updated systematic review included 17 randomised controlled trials (RCTs) investigating the effect of intrauterine administration of human chorionic gonadotropin (hCG) to 4751 subfertile women undergoing assisted reproduction. Intracavitary hCG (IC-hCG) was administered in variable doses at different times before embryo transfer (ET). hCG was obtained from the urine of pregnant women or from cell cultures using recombinant DNA technology.

For analyses of live birth and clinical pregnancy, there was considerable heterogeneity (I^2 > 75%) and therefore we present subgroups for dosage and stage of ET. Exploration for the sources of heterogeneity revealed two key prespecified variables as important determinants: stage of ET (cleavage vs blastocyst stage) and dose of IC-hCG (< 500 IU vs ≥ 500 IU). We performed meta-analysis within the subgroups defined by stage of embryo and dose of IC-hCG.

Live birth rates among women having cleavage-stage ET with an IC-hCG dose < 500 IU compared to women having cleavage-stage ET without IC-hCG showed no benefit of the intervention and would be consistent with no substantive difference or disadvantage of indeterminate magnitude. In a clinic with a live birth rate of 49% per cycle, use of IC-hCG < 500 IU would be associated with a live birth rate ranging from 28% to 50%.

Results show an increase in live birth rate in the subgroup of women undergoing cleavage-stage ET with an IC-hCG dose ≥ 500 IU compared to women having cleavage-stage ET without IC-hCG (RR 1.57, 95% CI 1.32 to 1.87; three RCTs; 914 participants; I^2 = 0%; moderate-quality evidence). At a clinic with a live birth rate of 27% per cycle, use of IC-hCG ≥ 500 IU would be associated with a live birth rate ranging from 36% to 51%.

Results show no substantive differences in live birth among women having blastocyst-stage ET with an IC-hCG dose ≥ 500 IU compared to women having blastocyst-stage ET without IC-hCG (moderate-quality evidence). At a clinic with a live birth rate of 36% per cycle, use of IC-hCG ≥ 500 IU would be associated with a live birth rate ranging from 29% to 38%.

Evidence for clinical pregnancy among women having cleavage-stage ET with an IC-hCG dose < 500 IU showed no benefit of the intervention and would be consistent with no substantive

difference or disadvantage of indeterminate magnitude (very low-quality evidence).

Results show an increase in clinical pregnancy rate in the subgroup of women having cleavage-stage ET with an IC-hCG dose of 500 IU or greater compared to women having cleavage-stage ET with no IC-hCG (moderate-quality evidence).

Results show no substantive differences in clinical pregnancy in the subgroup of women having blastocyst-stage ET with an IC-hCG dose of 500 IU or greater (moderate-quality evidence).

No RCTs investigated blastocyst-stage ET with an IC-hCG dose < 500 IU.

We are uncertain whether miscarriage and complication rates were influenced by IC-hCG administration, irrespective of embryo stage at transfer or dose of IC-hCG (very low-quality evidence). Reported complications were few, and very low-quality evidence was insufficient to permit conclusions to be drawn.

Overall completeness and applicability of evidence

All RCTs reported on clinical pregnancy, which is an important secondary outcome, but only a few RCTs continued follow-up until live birth, which is the most important primary outcome.

Most RCTs reported miscarriage rates. RCTs rarely reported complications and adverse events, or their absence.

Data were insufficient for all planned subgroup analyses to be performed.

The inclusion criteria for participants ensured a broad range of subfertility causes and women's characteristics similar to those expected in a regular assisted reproduction unit.

Quality of the evidence

We rated most of the studies (12/17) at high risk of bias in at least one of the seven domains assessed. Common problems were unclear reporting of study methods and lack of blinding. Brief reporting of results in studies published as abstracts represents an additional potential source of bias. Ten studies did not report funding, and seven studies reported internal funding. No studies reported external funding.

The quality of the evidence as assessed via GRADE varied from very low to moderate for live birth and clinical pregnancy, which means that further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate for some subgroups. The quality of the evidence for miscarriage was very low, meaning that we are very uncertain about the estimate. The main limitations in the overall quality of the evidence were high risk of bias and serious imprecision.

Potential biases in the review process

We performed a systematic search in consultation with the Cochrane Gynaecology and Fertility Group Trials Search Co-ordinator, but we cannot be sure all relevant trials were identified for inclusion. The protocol was pre-published and was followed accordingly (Craciunas 2015). We attempted to contact study authors when data were missing, but only one study author replied, providing clarification and additional data (Mansour 2011). We performed analyses on an intention-to-treat basis. Potential bias in the review process was unlikely. Data from two studies awaiting classification and from five ongoing studies may inform future updates of this review.

Agreements and disagreements with other studies or reviews

One previously published meta-analysis concluded that women undergoing in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) may benefit from IC-hCG injection before ET (Ye 2015). One meta-analysis found no effect of IC-hCG in terms of live birth and miscarriage but reported increased clinical pregnancy following IC-hCG injection (Osman 2016). A third meta-analysis published as an abstract reported increased clinical pregnancy rates and similar implantation, miscarriage, and ongoing pregnancy rates following IC-hCG (Dieamant 2016). These previous meta-analyses included significantly fewer RCTs compared to the present review (five, eight, and six, respectively) and have not explored the sources of heterogeneity based on IC-hCG dose and embryo stage at transfer.

The reported effect of intrauterine hCG administration was consistent within the subgroups of our review, with an apparent different effect based on stage of the embryo at transfer and dose of IC-hCG.

AUTHORS' CONCLUSIONS

Implications for practice

The finding of probably improved clinical pregnancy and live birth for cleavage-stage transfers using an intracavity human chorionic gonadotropin (IC-hCG) dose of 500 IU or greater is clinically important. Given the strength of the evidence, we believe that patients will benefit from this intervention, and it could be incorporated into clinical practice. However, current evidence for IC-hCG treatment does not support its use for blastocyst transfers. Review authors found no evidence that miscarriage was influenced by intrauterine human chorionic gonadotropin (hCG) administration, irrespective of embryo stage at transfer or dose of IC-hCG. Events were too few to allow any conclusions to be drawn with regard to other complications.

Implications for research

The findings of this review should provide a strong foundation for funding and conducting further high-quality randomised controlled trials of intrauterine hCG administration for women undergoing assisted reproduction according to CONSORT (Consolidated Standards of Reporting Trials) guidelines. These trials should be powered adequately and should focus on subgroups (cleavage vs blastocyst, fresh vs frozen/thawed, single vs two or more embryo transfers, cause of subfertility, dose and timing of IC-hCG) to identify the groups of women who would benefit the most from this intervention, and they should report on potential adverse events. Live birth rate must be the primary outcome. Blinding throughout the treatment cycle and during embryo transfer may reduce potential performance bias (adjusting ovarian stimulation doses; deciding the timing of maturation triggering, oocyte retrieval, and technique during embryo transfer, respectively).

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Craciunas 2015

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Craciunas 2016

Craciunas L, Tsampras N, Coomarasamy A, Raine-Fenning N. Intrauterine administration of human chorionic gonadotropin (hCG) for subfertile women undergoing assisted reproduction. *Cochrane Database of Systematic Reviews* 2016, Issue 5. [DOI: 10.1002/14651858.CD011537.pub2; PUBMED: 27195724]

* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Aaleyasin 2015

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 483 **Women's age (mean years; experimental vs control):** 29.1 vs 28.7 **Inclusion criteria:** all infertile women who were candidates for the first IVF/ICSI **Exclusion criteria:** aged > 40 years; history of percutaneous epididymal sperm aspiration; testicular sperm extraction; myomectomy; hydrosalpinx; presence of uterine fibroma with the pressure effect on endometrium; endometriosis; azoospermia **Ovarian controlled hyperstimulation:** long GnRH agonist protocol

Aaleyasin 2015 *(Continued)*

	Fertilisation: ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** fresh **Number of embryos transferred (mean; experimental vs control):** 2.8 vs 2.9
Interventions	**Experimental (n = 240):** hCG 500 IU in a volume of 50 µL tissue culture media (Vitrolife, Göteborg, Sweden) was injected into the uterus 5 to 7 minutes before ET **Control (n = 243):** 50 µL tissue culture media (Vitrolife, Göteborg, Sweden) instead of hCG
Outcomes	Clinical pregnancy, miscarriage, live birth, intrauterine death
Notes	**Location:** Shariati Teaching Hospital, Tehran, Iran **Period:** January 2011 to July 2012 **Power calculation:** yes **Funding:** not mentioned **Trial registration:** not mentioned and not found **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated list
Allocation concealment (selection bias)	Low risk	A technician, not belonging to the study personnel, prepared and coded drugs according to the list.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	All participants and clinical care providers were blinded to the list until the end of the study.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	All participants and clinical care providers were blinded to the list until the end of the study.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Zero women were lost to follow-up.
Selective reporting (reporting bias)	Low risk	Reported on all important outcomes
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Cambiaghi 2013

Methods	**Design:** 2-arm parallel RCT

Cambiaghi 2013 *(Continued)*

Participants	**Number:** 44 **Women's age (mean years; experimental vs control):** not mentioned **Inclusion criteria:** endometrial thickness > 7 mm on the day the donor received hCG and at least 2 blastocysts on the day of ET **Exclusion criteria:** not mentioned **Ovarian controlled hyperstimulation:** donor oocytes, protocol not mentioned **Fertilisation:** not mentioned **Stage of the embryo at transfer:** blastocyst **Embryo processing:** fresh **Number of embryos transferred:** not mentioned (likely 2, from inclusion criteria)
Interventions	**Experimental (n = 22):** intrauterine injection of hCG 500 IU 6 hours before ET **Control (n = 22):** ET without any pre-intrauterine injection
Outcomes	Clinical pregnancy
Notes	**Location:** Instituto Paulista de Ginecologia, Obstetricia e Medicina Reproducao, Sao Paulo, Brazil **Period:** January to December 2012 **Power calculation:** no **Funding:** not mentioned **Trial registration:** not mentioned and not found **Publication type:** abstract

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-based randomisation
Allocation concealment (selection bias)	Unclear risk	Allocation concealment not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not mentioned
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not mentioned, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Very brief reporting on results

Cambiaghi 2013 *(Continued)*

Selective reporting (reporting bias)	Unclear risk	No reporting on adverse events, miscarriage, or live birth
Other bias	Unclear risk	No reporting on baseline characteristics between groups

Dehghani Firouzabadi 2016

Methods	**Design:** 3-arm parallel RCT
Participants	**Number:** 159 **Women's age:** 20 to 40 years **Inclusion criteria:** women aged 20 to 40 years with a male factor or unexplained infertility and basal FSH < 12 who had undergone assisted reproduction **Exclusion criteria:** azoospermia; presence of uterine myoma; endometriosis; hydrosalpinges; previous IVF/ICSI trials (successful or unsuccessful); history of endocrine disease such as diabetes and thyroid dysfunction; previous history of hysteroscopic operation due to submucosal myoma; intrauterine synechia **Ovarian controlled hyperstimulation:** antagonist protocol **Fertilisation:** ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** fresh **Number of embryos transferred:** 1 to 3
Interventions	**Experimental (n = 53):** hCG 500 IU (40 µL) intrauterine injection 7 minutes before ET **Experimental (n = 53):** hCG 1000 IU (40 µL) intrauterine injection 7 minutes before ET **Control (n = 53):** nothing before ET
Outcomes	Clinical pregnancy, miscarriage
Notes	**Location:** Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran **Period:** April 2012 to March 2013 **Power calculation:** not mentioned **Funding:** not mentioned **Trial registration:** IRCT2012091310328N3 **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Liable women were randomly assigned to 2 test groups in the ratio of 1:1 or to a control group according to computer-generated random numbers (n = 53).

Dehghani Firouzabadi 2016 *(Continued)*

Allocation concealment (selection bias)	Unclear risk	Allocation concealment not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not blinded
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not blinded, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data reported on all randomised participants
Selective reporting (reporting bias)	Unclear risk	No live birth data
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Eskandar 2016

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 240 **Women's age (mean years; experimental vs control):** 32.3 vs 31.5 **Inclusion criteria:** women undergoing embryo transfer **Exclusion criteria:** not mentioned **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** IVF and ICSI **Stage of the embryo at transfer:** not mentioned, assumed cleavage (day 3) based on other publications from the same IVF unit **Embryo processing:** not mentioned **Number of embryos transferred:** 2 to 3
Interventions	**Experimental (n = 139):** 500 IU of hCG intrauterine 10 minutes before ET **Control (n = 101):** ET without any pre-intrauterine injection
Outcomes	Clinical pregnancy
Notes	**Location:** Saudi Center for Assisted Reproduction, Abha, Saudi Arabia **Period:** not mentioned **Power calculation:** not mentioned **Funding:** not mentioned **Trial registration:** not mentioned and not found

Eskandar 2016 *(Continued)*

Publication type: abstract

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were divided randomly into 2 groups by a computer-based programme.
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not mentioned.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Blinding was not mentioned.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blinding not mentioned, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Very brief reporting on results
Selective reporting (reporting bias)	Unclear risk	No reporting on adverse events, live birth, or miscarriage
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Hong 2014

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 300 **Women's age (mean years; experimental vs control):** 35.0 vs 35.1 **Inclusion criteria:** all participants undergoing fresh or frozen ET within the ART programme when the female partner was younger than 43 years of age **Exclusion criteria:** women could not be simultaneously participating in another prospective clinical trial at the centre, but no other inclusion/exclusion criteria were applied **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** not mentioned **Stage of the embryo at transfer:** blastocyst **Embryo processing:** fresh and frozen/thawed **Number of embryos transferred:** 1 or 2
Interventions	**Experimental (n = 148):** endometrial infusion of 20 µL ET media (synthetic serum substitute and MediCult BlastAssist, Origio) laden with 500 IU of purified urinary placental hCG (Novarel, Ferring Pharmaceuticals) < 3 minutes before ET **Control (152):** endometrial infusion of 20 µL ET media only

Hong 2014 *(Continued)*

Outcomes	Miscarriage and clinical pregnancy (converted from ongoing pregnancy)
Notes	**Location:** Reproductive Medicine Associates of New Jersey, Princeton, New Jersey, USA **Period:** August 2012 to December 2013 **Power calculation:** yes, but not met (778 embryos required, 473 embryos transferred) **Funding:** not mentioned **Trial registration:** NCT01643993 **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	A random number function was used to create variable blocks of 4 to 8, with participants assigned to the 2 groups in a 1:1 allocation.
Allocation concealment (selection bias)	Low risk	Allocation concealment was achieved with sequentially numbered, opaque, sealed envelopes.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Both the physician performing the transfer and the participants were blinded to the assigned treatment group throughout the entirety of the study.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not mentioned, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	No loss to follow-up
Selective reporting (reporting bias)	Unclear risk	No reports on live births and adverse events
Other bias	Unclear risk	25 participants declined to participate for various reasons after randomisation.

Hosseini 2016

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 100 **Women's age (mean years; experimental vs control):** 30.5 vs 31.3 **Inclusion criteria:** women undergoing assisted reproduction **Exclusion criteria:** history of uterine surgery such as myomectomy; history of recurrent miscarriage; known hydrosalpinx, endometrioma, or endometriosis

Hosseini 2016 *(Continued)*

	Ovarian controlled hyperstimulation: frozen/thawed cycles; preparation of endometrium initiated with hormone replacement protocol, which involved administration of oestrogen, followed by progesterone without ovarian downregulation **Fertilisation:** not mentioned **Stage of the embryo at transfer:** 90%+ cleavage, < 10% blastocysts **Embryo processing:** frozen/thawed **Number of embryos transferred:** 2 to 3
Interventions	**Experimental (n = 50):** case group received intrauterine injection of 40 µL of a 5000-unit hCG vial (Choriomon, IBSA, Lugano) mixed with 0.4 mL of IMSI-type media (equivalent to 500 hCG units) through Labotect catheter (Labotect, Labor-Technik-Gottingen GmbH, Germany). Seven minutes later, embryo transfer was performed with a sterile Labotect catheter, guided by abdominal ultrasound at 1 to 1.5 cm from uterine fundus. **Control (n = 50):** in the control group, embryo transfer was carried out with no intervention
Outcomes	Clinical pregnancy, miscarriage, still birth
Notes	**Location:** Al-Zahra Hospital Fertility Center, Tabriz, Iran **Period:** May 2014 to April 2015 **Power calculation:** no **Funding:** not mentioned **Trial registration:** not mentioned **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	During embryo transfer, participants were randomly divided (according to table of random numbers) into control and case groups (50 patients each).
Allocation concealment (selection bias)	Unclear risk	Allocation concealment was not mentioned.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Blinding was not possible owing to the nature of the intervention (control group received no placebo).
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not blinded, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	All participants were accounted for by outcome measures.
Selective reporting (reporting bias)	Unclear risk	No live birth data
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Huang 2016

Methods	**Design:** 3-arm parallel RCT, only data from control group (not placebo) used
Participants	**Number:** 161 total, 115 used for comparison **Women's age (mean years; experimental vs control):** 33.95 vs 33.08 **Inclusion criteria:** 2 instances of failed transfer of good-quality embryos; undergoing FET cycles; aged 38 years; body mass index (BMI) of 18 to 24; normal endometrial thickness (8 to 16 mm); frozen preservation of ≥ 2 embryos, with at least 1 good-quality embryo **Exclusion criteria:** diseases such as endometrial polyps, intrauterine adhesion, or uterine submucosal myomas, which might cause endometrial abnormalities; adenomyosis; hydropic fallopian tubes, PCOS, or endometriosis ≥ stage III **Ovarian controlled hyperstimulation:** frozen/thawed cycles; preparation of endometrium was conducted with letrozole and progesterone **Fertilisation:** not mentioned **Stage of the embryo at transfer:** cleavage **Embryo processing:** frozen/thawed **Number of embryos transferred:** 2
Interventions	**Experimental (n = 65):** the perfusion group received 1000 IU of hCG (Lizhu, Zhuhai, China) mixed with 1 mL of normal saline via intrauterine injection 3 days before ET **Control (n = 50):** no intrauterine injection
Outcomes	Clinical pregnancy, miscarriage
Notes	**Location:** Center of Reproductive Medicine of Liuzhou Maternity and Child Healthcare Hospital, Guangxi Province, China **Period:** January 2015 and December 2015 **Power calculation:** no **Funding:** not mentioned **Trial registration:** not mentioned **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation via a computerised random digit generator based on patient registration number in order of referral
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Single-blinded mentioned, but not clear who was blinded

Huang 2016 *(Continued)*

Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not mentioned, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	Data reported on all randomised participants
Selective reporting (reporting bias)	Unclear risk	No live birth data
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Kokkali 2014

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 194 **Women's age (years):** > 40 **Inclusion criteria:** women aged > 40 years receiving donor eggs **Exclusion criteria:** not mentioned **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** not mentioned **Stage of the embryo at transfer:** not mentioned **Embryo processing:** not mentioned **Number of embryos transferred:** not mentioned
Interventions	**Experimental (n = 97):** intrauterine hCG 500 IU injection 7 minutes before ET **Control (n = 97):** no intrauterine injection
Outcomes	Clinical pregnancy
Notes	**Location:** Genesis Athens Hospital, Centre for Human Reproduction, Athens, Greece **Period:** July 2012 to September 2013 **Power calculation:** no **Funding:** Genesis Athens Clinic **Trial registration:** not registered **Publication type:** abstract

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was performed in a 1:1 fashion to 1 of 2 groups [...] prepared from a computer-generated list.

Kokkali 2014 *(Continued)*

Allocation concealment (selection bias)	Low risk	Adequate allocation concealment was ensured by sequentially numbered, opaque, sealed envelopes prepared from a computer-generated list.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not blinded
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not blinded, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Very brief reporting on results
Selective reporting (reporting bias)	Unclear risk	No reporting on live birth and adverse events
Other bias	Unclear risk	No reporting on baseline characteristics between groups

Leao 2013

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 36 **Women's age:** not mentioned **Inclusion criteria:** women with 2 previous failures in IVF cycles with ET **Exclusion criteria:** not mentioned **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** not mentioned **Stage of the embryo at transfer:** not mentioned **Embryo processing:** not mentioned **Number of embryos transferred:** not mentioned
Interventions	**Experimental (n = 18):** intrauterine injection of hCG 500 IU 6 hours before ET **Control (n = 18):** women were forwarded straight to ET
Outcomes	Clinical pregnancy
Notes	**Location:** IPGO, Sao Paulo, Brazil **Period:** January to December 2012 **Power calculation:** no **Funding:** not mentioned **Trial registration:** not mentioned and not found

Leao 2013 *(Continued)*

Publication type: abstract presented as poster at 5th IVI International Congress, Seville, Spain, 2013

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation mentioned with no details
Allocation concealment (selection bias)	Unclear risk	Allocation concealment not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not mentioned
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not mentioned, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Very brief reporting on results
Selective reporting (reporting bias)	Unclear risk	No reporting on adverse events, miscarriage, or live birth
Other bias	High risk	Number of participants in each arm was not reported, but was deduced based on percentages and previous study by the same team

Mansour 2011

Methods	**Design:** 2 RCTs within the same study analysed as 4-armed parallel RCT
Participants	**Number:** 280 + 215 = 495 **Women's age (mean years; experimental 100, 200 vs control; 500 vs control):** 29 vs 28.5 vs 29.1; 28.3 vs 28.4 **Inclusion criteria:** women aged < 40 years old with infertility due to male factor **Exclusion criteria:** previous IVF/ICSI trials, including a successful trial; azoospermia; uterine myoma or previous myomectomy; endometriosis; presence of hydrosalpinges **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** fresh **Number of embryos transferred (mean; experimental 100, 200 vs control; 500 vs control):** 2.9 vs 2.8 vs 2.9; 2.9 vs 2.8

Mansour 2011 *(Continued)*

Interventions	**Experimental 100 (n = 92):** 40 µL of tissue culture medium (G-2 plus ref. 10132, Vitrolife, Göteborg, Sweden) containing hCG 100 IU injected intrauterine approximately 7 minutes before ET **Experimental 200 (n = 93):** 40 µL of tissue culture medium (G-2 plus ref. 10132, Vitrolife, Göteborg, Sweden) containing hCG 200 IU injected intrauterine approximately 7 minutes before ET **Experimental 500 (n = 108):** 40 µL of tissue culture medium (G-2 plus ref. 10132, Vitrolife, Göteborg, Sweden) containing hCG 500 IU injected intrauterine approximately 7 minutes before ET **Control (n = 95 + 107):** no intrauterine hCG injection before ET
Outcomes	Live birth, miscarriage, clinical pregnancy, ectopic pregnancy
Notes	**Location:** The Egyptian IVF-ET Center, Cairo, Egypt **Period:** January 2010 to January 2011 **Power calculation:** yes, but not met **Funding:** The Egyptian IVF-ET Center **Trial registration:** NCT01030393 **Publication type:** full text Live birth rate was established by personal communication with study authors, June 2015. Study publication reported number of deliveries, which included 6 women who had stillbirths (3 in each group).

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomised to 2 groups with the use of sealed dark envelopes.
Allocation concealment (selection bias)	Unclear risk	Allocation concealment not mentioned. Could explain different withdrawal rates between groups
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not blinded
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not blinded, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Women lost to follow-up live birth (similar numbers between groups)
Selective reporting (reporting bias)	Low risk	Reported on all important outcomes
Other bias	High risk	Interim analysis with change of protocol and premature ending of study. Relatively high live birth rate in control group, reasons unclear

Mostajeran 2017

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 100 **Women's age:** mean 31.3 ± 5.2 years **Inclusion criteria:** women 20 to 40 years old with body mass index 18 to 30 kg/m² were eligible if they were infertile owing to male factor, had a regular menstrual cycle of 24 to 35 days, and were presumed to be ovulatory **Exclusion criteria:** presence of polycystic ovary syndrome, with uterine pathologies, endometriosis, or presence of hydrosalpinges and any endocrine disease or chronic systemic illness; azoospermia; history of successful IVF or ICSI **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** IVF and ICSI **Stage of the embryo at transfer:** blastocyst **Embryo processing:** frozen/thawed **Number of embryos transferred:** 1 to 3
Interventions	**Experimental (n = 50):** injection of 700 IU of intrauterine hCG (chorionic gonadotropin human, Darou Pakhsh Company, Iran) 10 minutes before embryo transfer **Control (n = 50):** did not receive hCG before embryo transfer
Outcomes	Clinical pregnancy
Notes	**Location:** Fertility and Infertility Center of Isfahan in Iran **Period:** September 2013 to April 2014 **Power calculation:** yes, but inadequate **Funding:** not mentioned **Trial registration:** not mentioned **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly divided into two `50-member` groups by random allocation software. Saghaei, 2004
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Embryo transfer in both groups was done by the attending gynaecologist, who was blinded to the study.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not blinded, but unlikely to induce bias

Mostajeran 2017 *(Continued)*

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	6 participants lost to follow-up
Selective reporting (reporting bias)	Unclear risk	No data on miscarriage or live birth
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Navali 2016

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 158 **Women's age (mean years; experimental vs control):** 30.6 vs 32 **Inclusion criteria:** normal ovarian reserve (anti-Müllerian hormone ≥ 0.7 ng/mL); age ≤ 41 years; undergoing ICSI and fresh ET; normal levels of thyroid-stimulating hormone and prolactin **Exclusion criteria:** uncontrolled chronic maternal disease such as endocrinopathy and autoimmune disease, severe endometriosis, severe hydrosalpinx, or non-obstructive azoospermia; high risk for severe ovarian hyperstimulation syndrome (development of > 20 follicles > 10 mm at ovarian stimulation or retrieval of > 16 oocytes on the day of oocyte retrieval); morphological embryo deficiencies **Ovarian controlled hyperstimulation:** flexible antagonist protocol **Fertilisation:** ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** fresh **Number of embryos transferred:** 2 to 3
Interventions	**Experimental (n = 80):** 0.1 mL (500 IU hCG) and 0.4 mL normal saline were pulled into an insulin syringe and injected into the uterus immediately after oocyte retrieval under general anaesthesia **Control (n = 78):** 0.5 mL normal saline injected into the uterus at the same time as experimental group
Outcomes	Clinical pregnancy, miscarriage, ectopic pregnancy
Notes	**Location:** Reproductive Medical Center, Al-Zahra University Hospital, Tabriz University of Medical Sciences, Tabriz, Iran **Period:** September 2015 to February 2016 **Power calculation:** yes, but not met **Funding:** Women's Health Research Center, Tabriz University of Medical Sciences, Iran. No external funds were used. **Trial registration:** IRCT201206165485N4 **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement

Navali 2016 *(Continued)*

Random sequence generation (selection bias)	Low risk	A computer-generated randomisation list with a block size of 4 with 1:1 allocation was used to randomise participants.
Allocation concealment (selection bias)	Low risk	Treatment allocation was placed in a sealed, opaque envelope that was picked up consecutively by an operating room technician during the oocyte retrieval procedure.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Only the technician was aware of the participant's allocation; she prepared and handed the intervention drug to the physician.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Only the technician was aware of the participant's allocation; she prepared and handed the intervention drug to the physician.
Incomplete outcome data (attrition bias) All outcomes	High risk	20 (12%) participants were lost to follow-up or were excluded following randomisation.
Selective reporting (reporting bias)	Unclear risk	No data on live birth
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Santibañez 2014

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 210 **Women's age (mean years; experimental vs control):** 36.4 vs 37.3 **Inclusion criteria:** infertile women aged < 40 years who had an indication for an IVF/ICSI **Exclusion criteria:** azoospermia **Ovarian controlled hyperstimulation:** indicated based on individual participant characteristics **Fertilisation:** IVF or ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** fresh and frozen/thawed **Number of embryos transferred (mean):** 2.1
Interventions	**Experimental (n = 101):** 20 µL of embryo culture medium (G-2, Vitrolife, Göteborg, Sweden) that contained hCG 500 IU was administered intrauterine before ET **Control (n = 109):** no intrauterine hCG was administered
Outcomes	Clinical pregnancy, ectopic pregnancy
Notes	Study authors mention "prospective observational study", but the design was in fact RCT. **Location:** Reproductive Medicine Centre, PROCREA, Mexico City **Period:** August 2011 to November 2012

Santibañez 2014 *(Continued)*

Power calculation: yes

Funding: PROCREA

Trial registration: not mentioned and not found

Publication type: full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	A simple randomisation sample and assignment were generated in a computer-based programme.
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not mentioned
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not mentioned, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	All women followed up until pregnancy test/ultrasound scan
Selective reporting (reporting bias)	Unclear risk	No reporting on live birth and miscarriage despite mention of follow-up
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Singh 2014

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 216 **Women's age (mean years; experimental vs control):** 35 vs 34.5 (from ESHRE 2014 oral presentation) **Inclusion criteria:** infertile women aged < 42 years; recurrent implantation failure **Exclusion criteria:** not mentioned **Ovarian controlled hyperstimulation:** based on individual participant characteristics (from ESHRE 2014 oral presentation) **Fertilisation:** ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** not mentioned **Number of embryos transferred (mean; experimental vs control):** 2.7 vs 2.5 (from ESHRE 2014 oral presentation)

Singh 2014 *(Continued)*

Interventions	**Experimental (n = 108):** intrauterine administration of rhCG 500 IU in 40 µL 5 minutes before ET **Control (n = 108):** culture medium administered only before ET (from ESHRE 2014 oral presentation)
Outcomes	Clinical pregnancy, miscarriage, live birth (from ESHRE 2014 oral presentation)
Notes	**Location:** Bhopal Test Tube Baby Centre, Infertility, Bhopal, India **Period:** 2006 to 2013 **Power calculation:** not mentioned **Funding:** Bhopal Test Tube Baby Centre **Trial registration:** BTTB/2006/19 (?) **Publication type:** abstract

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly divided into 2 groups via a computer-generated list.
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not mentioned
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not mentioned, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	Zero women lost to follow-up
Selective reporting (reporting bias)	Low risk	Reported on all important outcomes
Other bias	Low risk	Similar baseline characteristics between groups after randomisation

Wirleitner 2015a

Methods	**Design:** 4-arm parallel RCT (same intervention on day 3 or 5)
Participants	**Number:** 182 + 1004 = 1186 **Women's age (mean years; experimental vs control):** 36.1 vs 35.5; 37.1 vs 36.7 **Inclusion criteria:** fresh autologous blastocyst transfer on day 5; woman aged ≤ 43 years **Exclusion criteria:** oocyte donation cycles; women with reported recurrent implantation failure (≥ 3 negative IVF cycles)

Wirleitner 2015a *(Continued)*

	Ovarian controlled hyperstimulation: GnRH agonist long protocol **Fertilisation:** IVF or IMSI **Stage of the embryo at transfer:** blastocyst **Embryo processing:** fresh **Number of embryos transferred:** 1 or 2
Interventions	**Experimental (day 3) (n = 89):** intrauterine hCG 500 IU (Pregnyl, Organon, Netherlands) dissolved in 40 µL embryo culture medium G-2 PLUS (Vitrolife, Göteborg, Sweden) administered on day 3 (2 days before ET) **Control (day 3) (n = 93):** administration of 40 µL culture medium without hCG on day 3 (2 days before ET) **Experimental (day 5) (n = 510):** intrauterine hCG 500 IU (Pregnyl, Organon, Netherlands) dissolved in 40 µL embryo culture medium G-2 PLUS (Vitrolife, Göteborg, Sweden) administered on day 5 (3 minutes before ET) **Control (day 5) (n = 494):** administration of 40 µL culture medium without hCG on day 3 (3 minutes before ET)
Outcomes	Clinical pregnancy, miscarriage, live birth
Notes	**Location:** IVF Centers Prof. Zech, Bregenz, Austria **Period:** February 2013 to February 2014 **Power calculation:** met only for day 5 administration **Funding:** not mentioned **Trial registration:** not mentioned and not found **Publication type:** full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomisation was done electronically with a random number generator.
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Participants blinded, but not personnel
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not blinded, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	19 participants lost to follow-up

Wirleitner 2015a *(Continued)*

Selective reporting (reporting bias)	Low risk	Reports on all relevant outcomes
Other bias	Low risk	Baseline characteristics of participants were comparable between 2 study groups.

Wirleitner 2015b

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 480 **Women's age (mean years; experimental vs control):** 40.3 vs 40.4 **Inclusion criteria:** women aged 38 to 43 years **Exclusion criteria:** recurrent implantation failure **Ovarian controlled hyperstimulation:** GnRH agonist long protocol **Fertilisation:** IMSI **Stage of the embryo at transfer:** blastocyst **Embryo processing:** fresh **Number of embryos transferred:** 1 or 2
Interventions	**Experimental (n = 255):** intrauterine hCG 500 IU dissolved in 40 µL embryo culture medium administered 3 minutes before ET **Control (n = 225):** administration of 40 µL culture medium without hCG 3 minutes before ET
Outcomes	Clinical pregnancy, miscarriage, live birth
Notes	**Location:** IVF-Centers Prof. Zech, Bregenz, Austria **Period:** not mentioned **Power calculation:** yes **Funding:** funded by hospital/clinic(s) - this study was not externally funded **Trial registration:** CRT 355 **Publication type:** abstract

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation was mentioned without further details.
Allocation concealment (selection bias)	Unclear risk	Not mentioned

Wirleitner 2015b *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Low risk	Participants were blinded.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Not blinded, but unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	All participants were followed up.
Selective reporting (reporting bias)	Low risk	Reports on all relevant outcomes
Other bias	Low risk	Baseline characteristics of participants were comparable between 2 study groups.

Zarei 2014

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 210 **Women's age (mean years; experimental vs control):** 29.9 vs 31.2 **Inclusion criteria:** 18- to 40-year-old women with infertility **Exclusion criteria:** women with autoimmune disorders, endocrinopathies, who had previous successful IVF/ICSI trials; endometriosis; azoospermia; hydrosalpinges **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** not mentioned (likely fresh) **Number of embryos transferred (mean; experimental vs control):** 6.1 vs 5.7
Interventions	**Experimental (n = 105):** rhCG 250 µg (0.5 mL, 6500 IU) (Ovitrelle, Merck Serono, France) through intrauterine injection 12 minutes before ET **Control (n = 105):** intrauterine injection of normal saline (0.5 mL) 12 minutes before ET
Outcomes	Clinical pregnancy, miscarriage, ectopic pregnancy, stillbirth
Notes	**Location:** Reproductive Medicine Center of Mother and Child Hospital, Shiraz, Iran **Period:** December 2011 to November 2012 **Power calculation:** yes **Funding:** Shiraz University of Medical Sciences **Trial registration:** IRCT2012121711790N1

Zarei 2014 *(Continued)*

Publication type: full text

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Participants were randomly assigned to 2 study groups via a computerised random digit generator based on their registration number in order of referral.
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding of participants and personnel (performance bias) All outcomes	Low risk	The syringes with volume of 0.5 mL from each group were prepared by the fellowship student and injected blinded by the attending gynaecologist.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Double-blinding mentioned (? women ? outcome assessors - in addition to gynaecologists performing the transfer), unlikely to induce bias
Incomplete outcome data (attrition bias) All outcomes	High risk	23/105 participants in intrauterine rhCG group and 7/105 participants in placebo group were lost to follow-up after receiving the allocated treatment (unclear why).
Selective reporting (reporting bias)	Unclear risk	No report on live births
Other bias	Low risk	Baseline characteristics of participants were comparable between 2 study groups.

ART: assisted reproductive technology; BMI: body mass index; ET: embryo transfer; ESHRE: European Society of Human Reproduction and Embryology; FET: frozen embryo transfer; FSH: follicle-stimulating hormone; GnRH: gonadotropin-releasing hormone; hCG: human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; IMSI: intracytoplasmic morphologically selected sperm injection; IU: international unit; IVF: in vitro fertilisation; PCOS: polycystic ovary syndrome; RCT: randomised controlled trial; rhCG: recombinant human chorionic gonadotropin.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Dieamant 2016	Meta-analysis
Giuliani 2015	Participants were oocyte donors who did not undergo embryo transfer
Huang 2017	Retrospective
Janati 2013	Included in the first review; replaced now by more recent full publication (Dehghani Firouzabadi 2016)
Jeong 2013	Retrospective
Kanter 2017	Retrospective
Li 2013	Not randomised

Study	Reason for exclusion
Osman 2016	Meta-analysis
Rebolloso 2013	Not randomised
Riboldi 2013	Not randomised
Strug 2016	Participants were oocyte donors who did not undergo embryo transfer.
Volovsky 2016	Case control
Ye 2015	Meta-analysis

Characteristics of studies awaiting assessment *[ordered by study ID]*

Badehnoosh 2014

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 80 **Women's age (mean years; experimental vs control):** 29.5 vs 29.3 **Inclusion criteria:** women undergoing ICSI **Exclusion criteria:** not mentioned **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** ICSI **Stage of the embryo at transfer:** not mentioned **Embryo processing:** not mentioned **Number of embryos transferred (mean; experimental vs control):** 2.9 vs 2.8
Interventions	**Experimental:** intrauterine injection of hCG 500 IU dissolved in 40 µL of ET media 10 minutes before ET **Control:** 40 µL of ET media 10 minutes before ET
Outcomes	Implantation rate defined as positive pregnancy test at 2 weeks after ET (biochemical pregnancy)
Notes	We emailed the study authors in February 2016 and January 2018 for more information on study design and outcomes. No reply has yet been received. **Location:** Avicenna Infertility Clinic, Tehran, Iran **Period:** not mentioned **Power calculation:** not mentioned **Funding:** not mentioned **Trial registration:** not mentioned and not found **Publication type:** abstract

Bhat 2014

Methods	**Design:** 2-arm parallel RCT
Participants	**Number:** 32 **Women's age (mean years; experimental vs control):** 29.6 vs 29.6 **Inclusion criteria:** women undergoing IVF **Exclusion criteria:** not mentioned **Ovarian controlled hyperstimulation:** not mentioned **Fertilisation:** IVF or ICSI **Stage of the embryo at transfer:** cleavage **Embryo processing:** fresh and frozen/thawed **Number of embryos transferred (mean; experimental vs control):** 2.9 vs 2.9
Interventions	**Experimental:** intrauterine administration of hCG 500 IU 7 minutes before ET **Control:** ET without hCG
Outcomes	Fertilisation rate
Notes	We emailed the study authors in February 2016 and January 2018 for more information on study design and outcomes. No reply has yet been received. **Location:** Radhakrishna Multispeciality Hospital and IVF Centre in Bengaluru in Southern India **Period:** April 2013 to March 2014 **Power calculation:** not mentioned **Funding:** none **Trial registration:** not mentioned and not found **Publication type:** full text

ET: embryo transfer; hCG: human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; IU: international unit; IVF: in vitro fertilisation; RCT: randomised controlled trial.

Characteristics of ongoing studies *[ordered by study ID]*

IRCT2017041733486N1

Trial name or title	Evaluation effect of intrauterine injection of human chorionic gonadotropin before embryo transfer on implantation rate and pregnancy rate in frozen cycles on IVF/ICSI
Methods	Randomised controlled trial
Participants	Women undergoing embryo transfer
Interventions	**Experimental:** interventional group (n 130) was injected with 500 IU of intrauterine hCG before embryo transfer **Control:** the second group (n = 130) did not receive administration of 500 IU hCG

IRCT2017041733486N1 *(Continued)*

Outcomes	Chemical and clinical pregnancy, implantation, miscarriage, ectopic pregnancy
Starting date	October 2017
Contact information	Ziaee Zohreh; 00982188896692; ziaee-z@razi.tums.ac.ir
Notes	

NCT02668965

Trial name or title	The effects of intrauterine infusion of hCG at the time of embryo transfer
Methods	Randomised controlled trial
Participants	Women undergoing embryo transfer
Interventions	**Experimental:** intrauterine infusion with hCG (500 IU) 10 microliters before embryo transfer **Control:** intrauterine infusion with standard embryo culture media 10 microliters before embryo transfer
Outcomes	Implantation, chemical and clinical pregnancy
Starting date	December 2015
Contact information	Savinee Boonsuk, MD; +66818706643; noomnim_mu@hotmail.com
Notes	

NCT02825108

Trial name or title	Intrauterine injection of human chorionic gonadotropin injection (hCG) before embryo transfer on pregnancy outcomes in frozen embryo transfer cycles
Methods	Randomised double-blind clinical trial to evaluate the effect of intrauterine injection of human chorionic gonadotropin (hCG) before frozen embryo transfer (ET)
Participants	All patients with primary infertility who have only 1 fresh implantation failure and are undergoing frozen embryo transfer cycles were enrolled.
Interventions	**Experimental:** participants receive 40 µL of tissue culture medium (G.2plus ref. 10132, Vitrolife, Göteborg, Sweden) containing 500 IU of hCG (Choriomon, IBSA SA, Switzerland), which is injected intrauterine, approximately 7 minutes before embryo transfer **Control:** patients receive only 40 µL of tissue culture medium (G.2plus ref. 10132, Vitrolife, Göteborg, Sweden), which is injected intrauterine, approximately 7 minutes before embryo transfer
Outcomes	Implantation, pregnancy loss/miscarriage
Starting date	January 2015
Contact information	Nasser Aghdami, MD, PhD; (+98)23562000 ext 516; nasser.aghdami@royaninstitute.org Leila Arab, MD; (+98)23562000 ext 414; leara91@gmail.com

NCT02825108 *(Continued)*

Notes	**Contact:** Leila Arab, MD

NCT02870855

Trial name or title	Effect of intrauterine injection of hCG on pregnancy outcome in repeated implantation failure patients
Methods	Randomised controlled trial
Participants	Women who undergo frozen ET
Interventions	**Experimental:** intrauterine injection of hCG before blastocyst transfer **Control:** no hCG injection
Outcomes	Clinical pregnancy, miscarriage, ectopic pregnancy
Starting date	July 2017
Contact information	Yuan Li, doctor; +86-731-82355100; 1002251255@qq.com
Notes	

NCT03238807

Trial name or title	Effect of intrauterine injection of hCG before ET on clinical outcomes in IVF/ICSI cycles
Methods	Randomised controlled trial (RCT) to detect whether intrauterine injection of hCG before ET improves clinical outcomes in IVF/ICSI cycles
Participants	Women undergoing IVF/ICSI
Interventions	**Experimental:** 0.1 mL of the tissue culture medium with 500 IU hCG will be injected inside the uterus before ET **Control:** 0.1 mL of the tissue culture medium without hCG will be injected inside the uterus before ET
Outcomes	Implantation, clinical pregnancy, miscarriage, live birth
Starting date	October 2017
Contact information	KArim S Abdallah, MSc; +201000024188; karimsayed88@hotmail.com
Notes	

ET: embryo transfer; hCG: human chorionic gonadotropin; ICSI: intracytoplasmic sperm injection; IVF: in vitro fertilisation; RCT: randomised controlled trial.

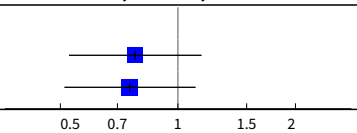
DATA AND ANALYSES

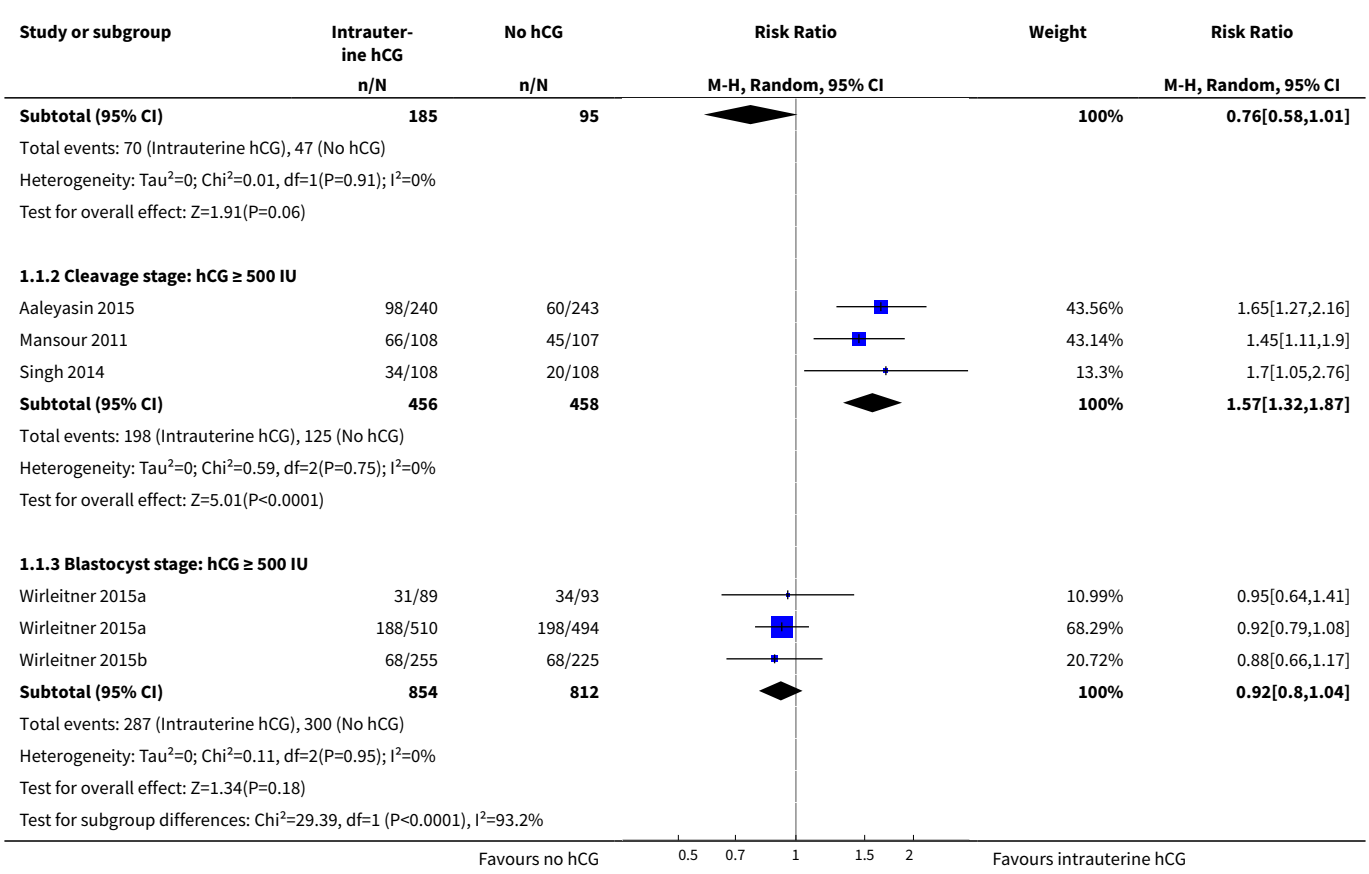
Comparison 1. Intrauterine human chorionic gonadotropin (hCG) versus no hCG

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth	5		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
1.1 Cleavage stage: hCG < 500 IU	1	280	Risk Ratio (M-H, Random, 95% CI)	0.76 [0.58, 1.01]
1.2 Cleavage stage: hCG ≥ 500 IU	3	914	Risk Ratio (M-H, Random, 95% CI)	1.57 [1.32, 1.87]
1.3 Blastocyst stage: hCG ≥ 500 IU	2	1666	Risk Ratio (M-H, Random, 95% CI)	0.92 [0.80, 1.04]
2 Miscarriage	11	3927	Risk Ratio (M-H, Random, 95% CI)	1.04 [0.81, 1.35]
3 Miscarriage per clinical pregnancy	11	1620	Risk Ratio (M-H, Random, 95% CI)	0.84 [0.62, 1.13]
4 Clinical pregnancy	16		Risk Ratio (M-H, Random, 95% CI)	Subtotals only
4.1 Cleavage stage: hCG < 500 IU	1	280	Risk Ratio (M-H, Random, 95% CI)	0.88 [0.70, 1.10]
4.2 Cleavage stage: hCG ≥ 500 IU	12	2186	Risk Ratio (M-H, Random, 95% CI)	1.49 [1.32, 1.68]
4.3 Blastocyst stage: hCG ≥ 500 IU	4	2091	Risk Ratio (M-H, Random, 95% CI)	0.99 [0.85, 1.15]
5 Complications	6		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
5.1 Intrauterine death	3	1078	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.74 [0.31, 1.73]
5.2 Ectopic or heterotopic pregnancy	4	1073	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.22 [0.04, 1.30]
5.3 Triplet pregnancy	1	483	Peto Odds Ratio (Peto, Fixed, 95% CI)	7.55 [0.78, 72.88]

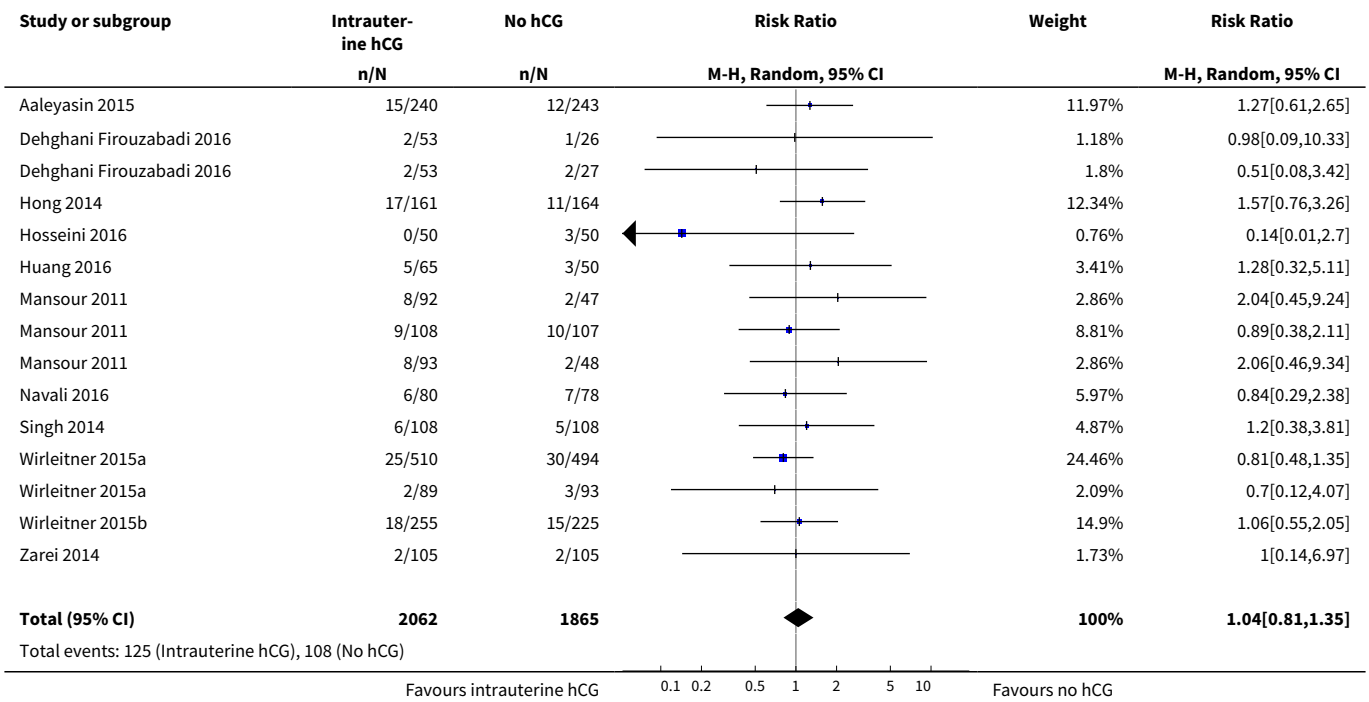
Analysis 1.1. Comparison 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, Outcome 1 Live birth.

Study or subgroup	Intrauterine hCG n/N	No hCG n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 Cleavage stage: hCG < 500 IU					
Mansour 2011	35/92	23/47		49.2%	0.78[0.53,1.15]
Mansour 2011	35/93	24/48		50.8%	0.75[0.51,1.11]

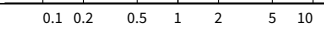




Analysis 1.2. Comparison 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, Outcome 2 Miscarriage.



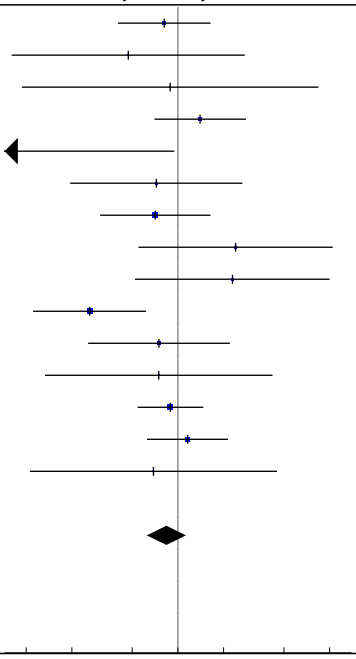
Study or subgroup	Intrauterine hCG n/N	No hCG n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Heterogeneity: Tau²=0; Chi²=6.95, df=14(P=0.94); I²=0%					
Test for overall effect: Z=0.33(P=0.74)					



Favours intrauterine hCG — Favours no hCG

Analysis 1.3. Comparison 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, Outcome 3 Miscarriage per clinical pregnancy.

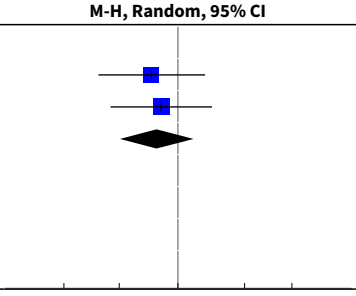
Study or subgroup	Intrauterine hCG n/N	No hCG n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
Aaleyasin 2015	15/120	12/78		11.5%	0.81[0.4,1.64]
Dehghani Firouzabadi 2016	2/17	2/8		2.63%	0.47[0.08,2.76]
Dehghani Firouzabadi 2016	2/18	1/8		1.68%	0.89[0.09,8.44]
Hong 2014	17/87	11/79		11.69%	1.4[0.7,2.81]
Hosseini 2016	0/14	3/5		1.1%	0.06[0,0.95]
Huang 2016	5/37	3/16		4.52%	0.72[0.2,2.66]
Mansour 2011	9/80	10/63		9.09%	0.71[0.31,1.64]
Mansour 2011	8/45	2/27		3.66%	2.4[0.55,10.48]
Mansour 2011	8/49	2/28		3.64%	2.29[0.52,10.02]
Navali 2016	6/36	7/11		8.82%	0.26[0.11,0.62]
Singh 2014	6/40	5/25		6.22%	0.75[0.26,2.2]
Wirleitner 2015a	2/33	3/37		2.75%	0.75[0.13,4.2]
Wirleitner 2015a	25/213	30/228		16.84%	0.89[0.54,1.47]
Wirleitner 2015b	18/86	15/83		13.51%	1.16[0.63,2.14]
Zarei 2014	2/29	2/20		2.36%	0.69[0.11,4.5]
Total (95% CI)	**904**	**716**		**100%**	**0.84[0.62,1.13]**
Total events: 125 (Intrauterine hCG), 108 (No hCG)					
Heterogeneity: Tau²=0.07; Chi²=18.32, df=14(P=0.19); I²=23.56%					
Test for overall effect: Z=1.14(P=0.26)					



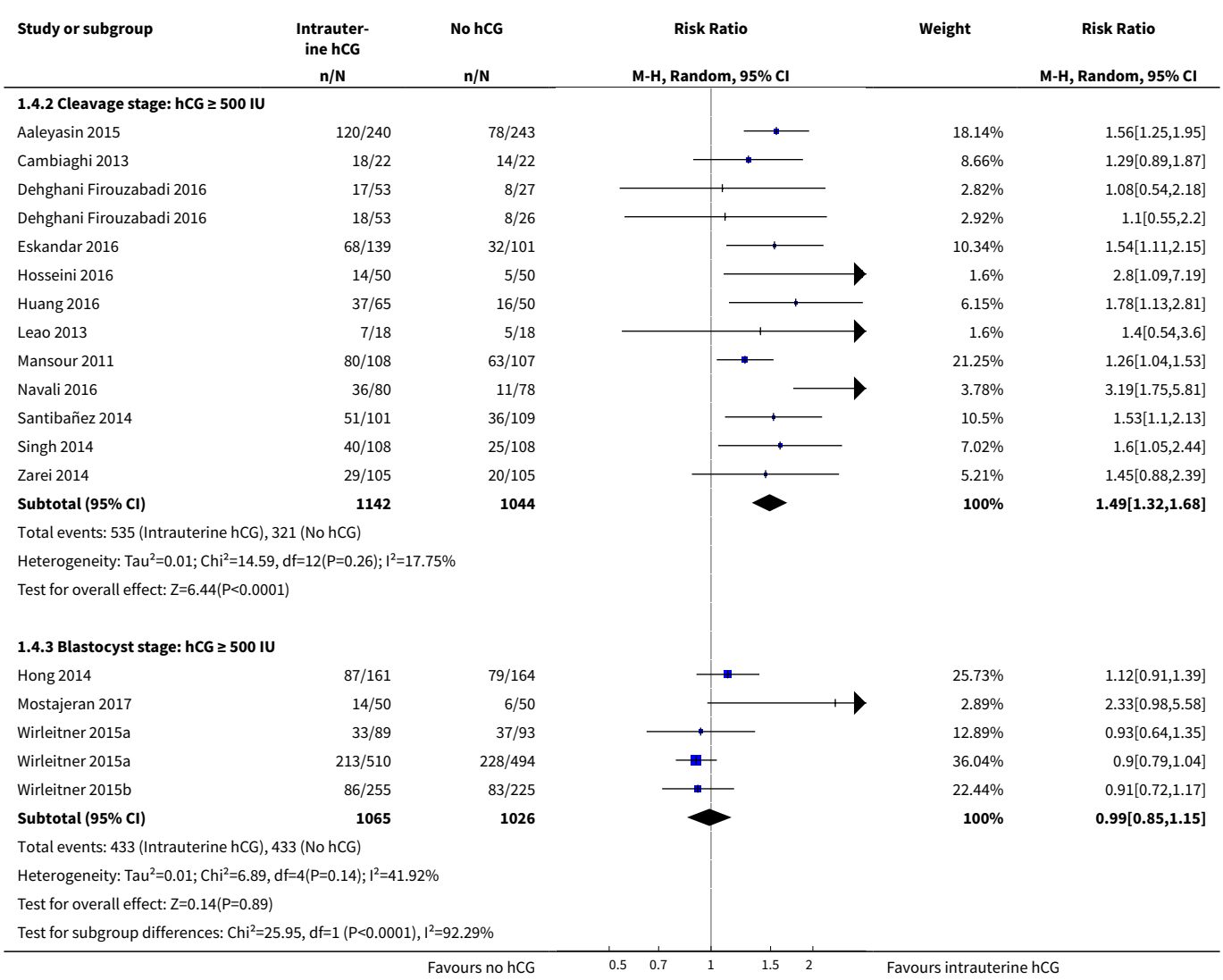
Favours intrauterine hCG — Favours no hCG

Analysis 1.4. Comparison 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, Outcome 4 Clinical pregnancy.

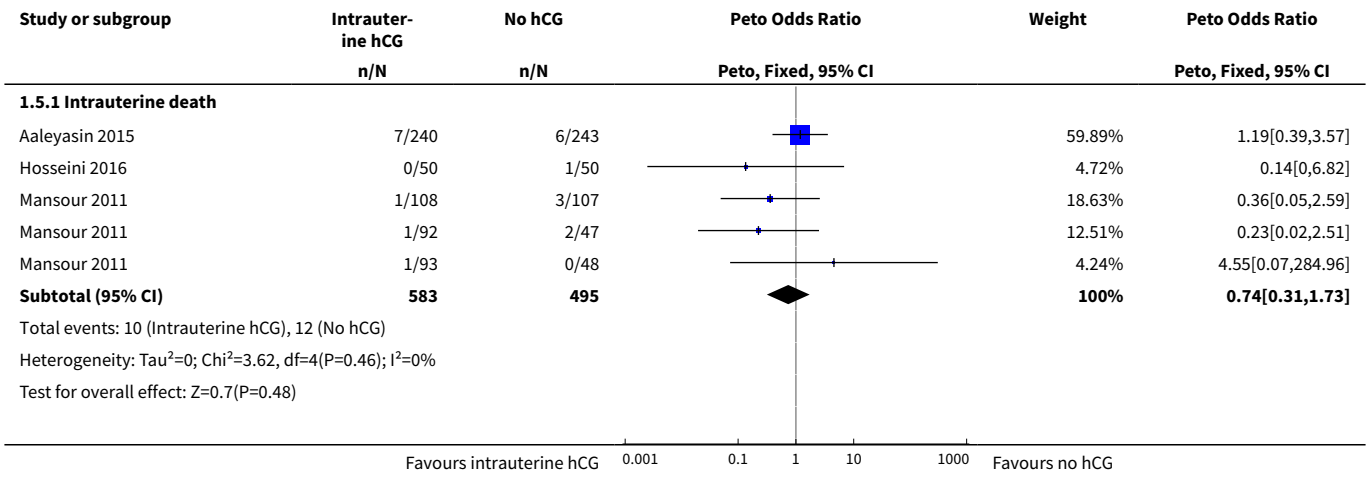
Study or subgroup	Intrauterine hCG n/N	No hCG n/N	Risk Ratio M-H, Random, 95% CI	Weight	Risk Ratio M-H, Random, 95% CI
1.4.1 Cleavage stage: hCG < 500 IU					
Mansour 2011	45/92	27/47		47.51%	0.85[0.62,1.18]
Mansour 2011	49/93	28/48		52.49%	0.9[0.66,1.23]
Subtotal (95% CI)	**185**	**95**		**100%**	**0.88[0.7,1.1]**
Total events: 94 (Intrauterine hCG), 55 (No hCG)					
Heterogeneity: Tau²=0; Chi²=0.07, df=1(P=0.8); I²=0%					
Test for overall effect: Z=1.14(P=0.25)					

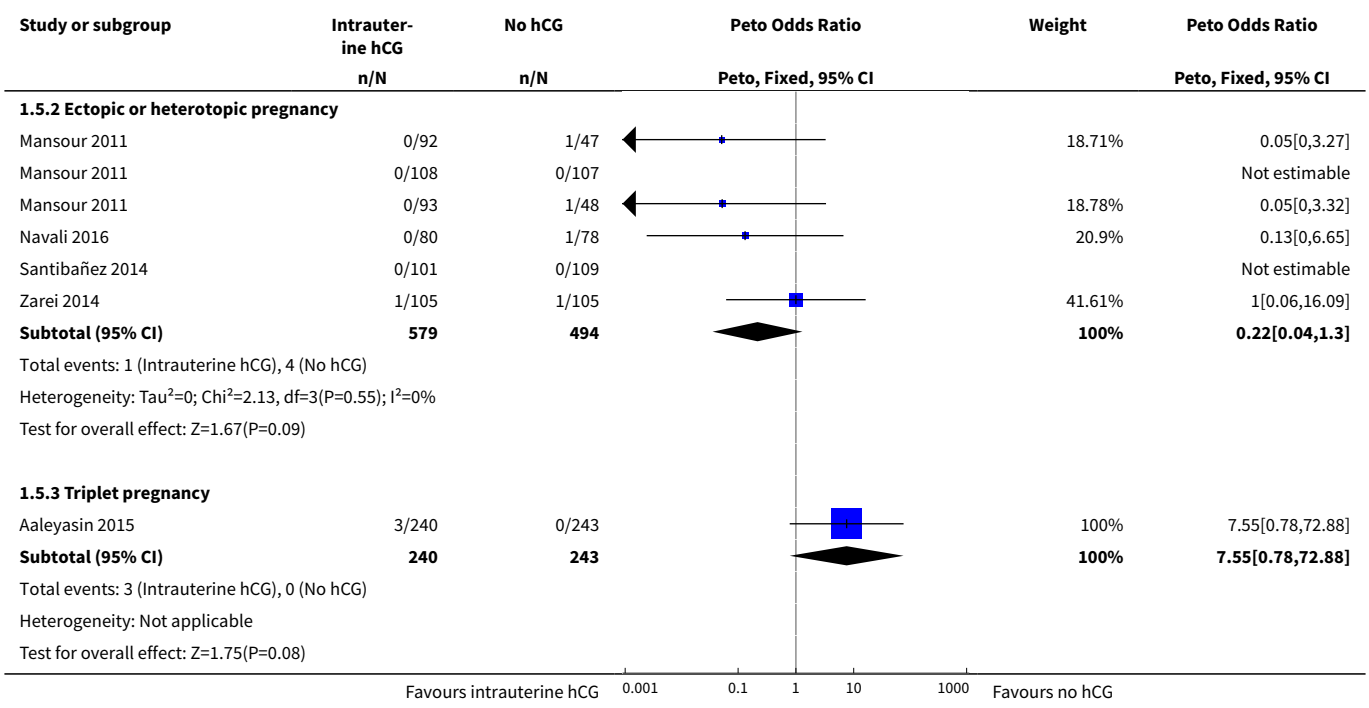


Favours no hCG — Favours intrauterine hCG



Analysis 1.5. Comparison 1 Intrauterine human chorionic gonadotropin (hCG) versus no hCG, Outcome 5 Complications.





APPENDICES

Appendix 1. Cochrane Gynaecology and Fertility Group (CGF) Specialised Register search strategy

PROCITE Platform

Searched 9 January 2018

Keywords CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "ET" or "Embryo" or "in-vitro fertilization" or "Embryo Transfer" or "Embryo Transfer-uterine" or "blastocyst transfer" or Title CONTAINS "IVF" or "in vitro fertilization" or "in-vitro fertilisation" or "ICSI" or "intracytoplasmic sperm injection" or "Embryo" or "in-vitro fertilization" or "ET" or "Embryo" or "in-vitro fertilization" or "Embryo Transfer" or "Embryo Transfer-uterine" or "blastocyst transfer"

AND

Keywords CONTAINS "HCG " or "human chorionic gonadotrophin" or "human chorionic gonadotropin" or "recombinant HCG" or "rhCG" or Title CONTAINS "HCG " or "human chorionic gonadotrophin" or "human chorionic gonadotropin" or "recombinant HCG" or "rhCG"

AND

Keywords CONTAINS "intrauterine human chorionic gonadotrophin" or "intrauterine" or "Intrauterine injection" or "intrauterine instillation "or "uterine cavity injection" or "endometrial" or "Endometrium" or "uterine" or Title CONTAINS "intrauterine human chorionic gonadotrophin" or "intrauterine" or "Intrauterine injection" or "intrauterine instillation "or "uterine cavity injection" or "Endometrium" or "uterine" (113)

Appendix 2. CENTRAL search strategy

Web Platform via CENTRAL Register of Studies online (CRSO)

Searched 9 January 2018

#1 MESH DESCRIPTOR Reproductive Techniques, Assisted EXPLODE ALL TREES 2881

#2 (embryo* adj2 transfer*):TI,AB,KY 2522

#3 (blastocyst* adj2 transfer*):TI,AB,KY 264

#4 (assisted reproduct*):TI,AB,KY 851

#5 (ivf or icsi):TI,AB,KY 4126

#6 (in vitro fertili?ation):TI,AB,KY 2200

#7 (intracytoplasmic sperm injection*):TI,AB,KY 1350

#8 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 6556

#9 MESH DESCRIPTOR Chorionic Gonadotropin EXPLODE ALL TREES 697

#10 (Human Chorionic Gonadotrop?in adj7 intrauter*):TI,AB,KY 21

#11 (Human Chorionic Gonadotrop?in adj7 uter*):TI,AB,KY 6

#12 ((endometri* adj2 infusion*) and chorionic):TI,AB,KY 3

#13 ((intra?uter* adj2 infusion*) and chorionic):TI,AB,KY 6

#14 ((intra?uter* adj2 instillation) and chorionic):TI,AB,KY 3

#15 ((endometri* adj2 injection*) and chorionic):TI,AB,KY 3

#16 ((intra?uter* adj2 injection*) and chorionic):TI,AB,KY 36

#17 ((intra?uter* adj2 administration) and chorionic):TI,AB,KY 28

#18 (intrauter* adj7 ?hcg):TI,AB,KY 50

#19 #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 767

#20 #8 AND #19 493

Appendix 3. MEDLINE search strategy

OVID Platform

Searched from 1946 to 9 January 2018

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ (40939)
2 embryo transfer$.tw. (11209)
3 in vitro fertili?ation.tw. (22663)
4 assisted reproduct*.tw. (13334)
5 (ivf or et).tw. (260179)
6 icsi.tw. (7528)
7 intracytoplasmic sperm injection$.tw. (6590)
8 (blastocyst adj2 transfer$).tw. (843)
9 or/1-8 (297500)
10 exp Chorionic Gonadotropin/ad, tu, th [Administration & Dosage, Therapeutic Use, Therapy] (5410)
11 (Human Chorionic Gonadotrop?in adj7 intrauter$).tw. (87)
12 (Human Chorionic Gonadotrop?in adj7 uter$).tw. (159)
13 (Human Chorionic Gonadotrop?in adj7 intra-uter$).tw. (0)
14 ((endometri$ adj2 infusion$) and chorionic).tw. (1)
15 ((endometri$ adj2 ?instillation) and chorionic).tw. (0)
16 ((intra?uter$ adj2 infusion$) and chorionic).tw. (6)
17 ((intra?uter$ adj2 ?instillation) and chorionic).tw. (6)
18 ((endometri$ adj2 injection$) and chorionic).tw. (5)
19 ((intra?uter$ adj2 injection$) and chorionic).tw. (16)
20 ((intra?uter$ adj2 administration) and chorionic).tw. (14)
21 ((endometri$ adj2 administration) and chorionic).tw. (7)
22 (intrauter$ adj7 ?hcg).tw. (198)
23 (intra-uter$ adj7 ?hcg).tw. (15)
24 (uter$ adj7 ?hcg).tw. (342)
25 or/10-24 (6018)
26 9 and 25 (1776)

27 randomized controlled trial.pt. (515870)
28 controlled clinical trial.pt. (101741)
29 randomized.ab. (452787)
30 randomised.ab. (91845)
31 placebo.tw. (215895)
32 clinical trials as topic.sh. (202549)
33 randomly.ab. (311971)
34 trial.ti. (203432)
35 (crossover or cross-over or cross over).tw. (83358)
36 or/27-35 (1322190)
37 exp animals/ not humans.sh. (4813914)
38 36 not 37 (1219196)
39 26 and 38 (369)

Appendix 4. Embase search strategy

OVID Platform

Searched from 1980 to 9 January 2018

1 exp embryo transfer/ or exp fertilization in vitro/ or exp intracytoplasmic sperm injection/ (58311)
2 embryo$ transfer$.tw. (17900)
3 in vitro fertili?ation.tw. (26272)
4 assisted reproduct*.tw. (18775)
5 icsi.tw. (13770)
6 intracytoplasmic sperm injection$.tw. (8240)
7 (blastocyst adj2 transfer$).tw. (1906)
8 (ivf or et).tw. (606759)
9 or/1-8 (659662)
10 (Human Chorionic Gonadotrop?in adj7 intrauter$).tw. (122)
11 (Human Chorionic Gonadotrop?in adj7 uter$).tw. (149)
12 (intrauter$ adj7 ?hcg).tw. (286)
13 chorionic gonadotropin/dt, ut [Drug Therapy, Intrauterine Drug Administration] (4766)
14 (uter$ adj3 ?hcg).tw. (127)
15 ((endometri$ adj2 infusion$) and chorionic).tw. (2)
16 ((endometri$ adj2 ?instillation) and chorionic).tw. (0)
17 ((intra?uter$ adj2 infusion$) and chorionic).tw. (8)
18 ((intra?uter$ adj2 ?instillation) and chorionic).tw. (7)
19 ((endometri$ adj2 injection$) and chorionic).tw. (5)
20 ((intra?uter$ adj2 injection$) and chorionic).tw. (44)
21 ((intra?uter$ adj2 administration) and chorionic).tw. (30)
22 ((endometri$ adj2 administration) and chorionic).tw. (14)
23 or/10-22 (5333)
24 9 and 23 (2692)
25 Clinical Trial/ (962568)
26 Randomized Controlled Trial/ (479015)
27 exp randomization/ (76661)
28 Single Blind Procedure/ (30048)
29 Double Blind Procedure/ (142111)
30 Crossover Procedure/ (53667)
31 Placebo/ (302487)
32 Randomi?ed controlled trial$.tw. (169852)
33 Rct.tw. (26427)
34 random allocation.tw. (1709)
35 randomly allocated.tw. (28558)
36 allocated randomly.tw. (2271)
37 (allocated adj2 random).tw. (788)
38 Single blind$.tw. (20051)
39 Double blind$.tw. (177385)
40 ((treble or triple) adj blind$).tw. (725)
41 placebo$.tw. (258956)
42 prospective study/ (414841)
43 or/25-42 (1837099)

44 case study/ (51204)
45 case report.tw. (342456)
46 abstract report/ or letter/ (1012507)
47 or/44-46 (1397981)
48 43 not 47 (1790343)
49 24 and 48 (861)

Appendix 5. PsycINFO search strategy

OVID Platform

Searched from 1806 to 9 January 2018

1 exp reproductive technology/ (1682)
2 in vitro fertili?ation.tw. (684)
3 icsi.tw. (68)
4 intracytoplasmic sperm injection$.tw. (52)
5 (blastocyst adj2 transfer$).tw. (4)
6 (embryo$ adj2 transfer$).tw. (140)
7 or/1-6 (1957)
8 exp Gonadotropic Hormones/ (4096)
9 (Human Chorionic Gonadotrop?in adj7 intrauter$).tw. (0)
10 (Human Chorionic Gonadotrop?in adj7 uter$).tw. (0)
11 (intrauter$ adj7 ?hcg).tw. (0)
12 (uter$ adj7 ?hcg).tw. (0)
13 or/8-12 (4096)
14 7 and 13 (8)

Appendix 6. CINAHL search strategy

EBSCO Platform

Searched from 1961 to 9 January 2018

#	Query	Results
S15	S8 AND S14	59
S14	S9 OR S10 OR S11 OR S12 OR S13	697
S13	TX(Chorionic Gonadotrop?in N7 intrauter*)	1
S12	TX(Chorionic Gonadotrop?in N7 uter*)	3
S11	TX(Human Chorionic Gonadotrop?in N7 intrauter*)	0
S10	TX(Human Chorionic Gonadotrop?in N7 intrauter*)	1
S9	(MM "Gonadotropins, Chorionic")	588
S8	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7	5290
S7	TX embryo* N3 transfer*	1159
S6	TX ovar* N3 hyperstimulat*	456
S5	TX ovari* N3 stimulat*	419
S4	TX IVF or TX ICSI	2204

(Continued)

S3	(MM "Fertilization in Vitro")	1803
S2	TX vitro fertilization	3895
S1	TX vitro fertilisation	3895

WHAT'S NEW

Date	Event	Description
23 October 2018	Amended	Correction of text in Declarations of interest section

HISTORY

Protocol first published: Issue 2, 2015
Review first published: Issue 5, 2016

Date	Event	Description
15 June 2018	New search has been performed	New study data were added, leading to a change to the conclusions of this review.
15 June 2018	New citation required and conclusions have changed	New searches were performed for this major update, and additional RCTs have contributed data (Dehghani Firouzabadi 2016; Eskandar 2016; Hosseini 2016; Huang 2016; Mostajeran 2017; Navali 2016).
22 June 2016	Amended	Links to an analysis and to a figure were inserted.

CONTRIBUTIONS OF AUTHORS

LC and NT performed the literature search, assessed studies for eligibility, and extracted the data.

LC performed the analyses and drafted the review.

NT, NRF, and AC provided feedback and edited the review.

All review authors agree with the final version of the review.

DECLARATIONS OF INTEREST

LC, NT and AC do not have any conflicts of interest to disclose. NRF has received travel costs or advisory board honoraria from GE Healthcare, Merck Serono and Ferring and provides medico-legal reports for court proceedings. He has shares in two fertility clinics.

SOURCES OF SUPPORT

Internal sources

- None, Other.

External sources

- None, Other.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We slightly narrowed the Cochrane Gynaecology and Fertility Group Specialised Register search strategy.

We performed a subgroup analysis based on IC-hCG dose to address the heterogeneity.

For outcomes with event rates below 1%, we used the Peto one-step odds ratio (OR) method to calculate the combined outcome with 95% confidence interval.

If a study included multiple treatment arms receiving different doses of hCG, we split the control group proportionately with the experimental groups to avoid analysing control participants in duplicate.

INDEX TERMS

Medical Subject Headings (MeSH)

*Embryo Transfer [adverse effects] [statistics & numerical data]; Abortion, Spontaneous [epidemiology] [etiology]; Chorionic Gonadotropin [*administration & dosage]; Embryo Implantation [drug effects]; Infertility, Female [*drug therapy]; Live Birth [epidemiology]; Pregnancy Rate; Reproductive Control Agents [*administration & dosage]; Uterus

MeSH check words

Adult; Female; Humans; Pregnancy

CHAPTER THREE:

CONVENTIONAL AND MODERN MARKERS OF ENDOMETRIAL RECEPTIVITY

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human reproduction update

Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis

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BACKGROUND: Early reproductive failure is the most common complication of pregnancy with only 30% of conceptions reaching live birth. Establishing a successful pregnancy depends upon implantation, a complex process involving interactions between the endometrium and the blastocyst. It is estimated that embryos account for one-third of implantation failures, while suboptimal endometrial receptivity and altered embryo–endometrial dialogue are responsible for the remaining two-thirds. Endometrial receptivity has been the focus of extensive research for over 80 years, leading to an indepth understanding of the processes associated with embryo–endometrial cross-talk and implantation. However, little progress has been achieved to translate this understanding into clinically meaningful prognostic tests and treatments for suboptimal endometrial receptivity.

OBJECTIVE AND RATIONALE: The objective of this systematic review was to examine the evidence from observational studies supporting the use of endometrial receptivity markers as prognostic factors for pregnancy outcome in women wishing to conceive, in order to aid clinicians in choosing the most useful marker in clinical practice and for informing further research.

SEARCH METHODS: The review protocol was registered with PROSPERO (CRD42017077891). MEDLINE and Embase were searched for observational studies published from inception until 26 February 2018. We included studies that measured potential markers of endometrial receptivity prior to pregnancy attempts and reported the subsequent pregnancy outcomes. We performed association and accuracy analyses using clinical pregnancy as an outcome to reflect the presence of receptive endometrium. The Newcastle–Ottawa scale for observational studies was employed to assess the quality of the included studies.

OUTCOMES: We included 163 studies (88 834 women) of moderate overall quality in the narrative synthesis, out of which 96 were included in the meta-analyses. Studies reported on various endometrial receptivity markers evaluated by ultrasound, endometrial biopsy, endometrial fluid aspirate and hysteroscopy in the context of natural conception, IUI and IVF. Associations were identified between clinical pregnancy and various endometrial receptivity markers (endometrial thickness, endometrial pattern, Doppler indices, endometrial wave-like activity and various molecules); however, their poor ability to predict clinical pregnancy prevents them from being used in clinical practice. Results from several modern molecular tests are promising and further data are awaited.

WIDER IMPLICATIONS: The post-test probabilities from our analyses may be used in clinical practice to manage couples' expectations during fertility treatments (IUI and IVF). Conventionally, endometrial receptivity is seen as a dichotomous outcome (present or absent), but we propose that various levels of endometrial receptivity exist within the window of implantation. For instance, different transcriptomic signatures could represent varying levels of endometrial receptivity, which can be linked to different pregnancy outcomes. Many studies reported the means of a particular biomarker in those who achieved a pregnancy compared with those who did not. However, extreme values of a biomarker (as opposite to the means) may have significant prognostic and diagnostic implications that are not captured in the means. Therefore, we suggest reporting the outcomes by categories of biomarker levels rather than reporting means of biomarker levels within clinical outcome groups.

Key words: endometrial receptivity / window of implantation / ultrasound / endometrial biopsy / endometrial fluid aspirate / hysteroscopy / clinical pregnancy / IUI / IVF

Introduction

Early reproductive failure is the most common complication of pregnancy since 70% of conceptions cease development prior to reaching viability (Roberts and Lowe, 1975). More than 50% of pregnancies are lost at pre-clinical stages through implantation failure or biochemical miscarriage (Wilcox *et al.*, 1988; Chard, 1991). Miscarriage then affects 25% of clinical pregnancies (Macklon *et al.*, 2002), with more than 90% of these occurring in the first trimester of pregnancy (Regan and Rai, 2000).

Establishing a successful pregnancy depends upon implantation, a complex process involving interactions between the endometrium and the blastocyst. The window of implantation is described as a narrow time frame with maximal endometrial receptivity, surrounded by a refractory endometrial status (Navot *et al.*, 1986; Tabibzadeh and Babaknia, 1995).

Endometrial receptivity and selectivity are two complementary concepts introduced to describe the endometrium as a biosensor of embryo quality (Macklon and Brosens, 2014). Selectivity is an intrinsic programmed function of the endometrium to recognize and reject embryos with reduced development potential. In contrast, receptivity enables the endometrium to provide an optimal environment for embryo development and placenta formation.

Implantation failure is a consequence of impaired embryo development potential or impaired endometrial selectivity/receptivity, both having a negative effect on the embryo–endometrial cross-talk (Diedrich *et al.*, 2007). It is estimated that embryos account for one-third of implantation failures, while suboptimal endometrial receptivity and altered embryo–endometrial dialogue are responsible for the remaining two-thirds (Edwards, 1994; Simon *et al.*, 1998; Franasiak *et al.*, 2014).

Endometrial receptivity and the characteristics of the window of implantation have been the focus of extensive research for over 80 years, since Rock and Bartlett (1937) described the histological changes of the endometrium around the time of implantation. More recently, microscopy, flow cytometry and molecular advancements have allowed further investigations into the cross-talk between the embryo and the endometrium (Strowitzki *et al.*, 2006). Omics- refer to the application of high-throughput techniques which simultaneously examine changes in different molecular compartments: genomics, transcriptomics, proteomics, metabolomics, etc. The understanding of human endometrial physiology and pathophysiology is being revolutionized by the use of omics (Altmäe *et al.*, 2014).

Despite the indepth understanding of the processes associated with embryo–endometrial cross-talk and implantation, little progress has been achieved for its clinical integration in terms of prognostic tests and treatments for suboptimal endometrial receptivity. The objective of this systematic review was to examine the evidence from observational studies supporting the use of endometrial receptivity markers as prognostic factors for pregnancy outcome in women wishing to conceive in order to aid clinicians in choosing the most useful markers for clinical practice and for informing further research.

Methods

PROSPERO registration and systematic search

The review protocol was registered with PROSPERO (CRD42017077891) on the 1 November 2017 prior to starting the preliminary searches (Craciunas et al., 2017). A comprehensive literature search was then performed in two steps (L.C. and I.G.). An initial search of MEDLINE and Embase was conducted using a very broad search strategy covering keywords and Medical Subject Headings (MeSH) relevant to the review question. This was followed by a more targeted search aiming at identifying additional studies similar to the ones included from the initial search.

Search terms included keywords such as endometrium, uterus, implantation, luteal phase, biopsy, hysteroscopy, ultrasonography, Doppler, thickness, pattern, -omics, natural killer, marker, pregnancy and miscarriage. The search strategy for MEDLINE is published in Supplementary Table SI. Both MEDLINE and Embase were searched for studies published from inception until the date of the final search (26 February 2018) with no restrictions. The 'Similar articles' function in PubMed and 'Related articles' function in Google Scholar were used to identify further relevant publications. The reference lists of all relevant publications were screened to complete the literature search.

Study selection, data extraction and quality assessment

Primary observational studies that reported original data regarding potential markers of endometrial receptivity were included in the present systematic review if they provided clinical outcomes from either natural conceptions or fertility treatments (IUI or IVF). Interventional studies, commentaries, narrative reviews and letters were excluded. Case reports, case series, cohort studies with fewer than 15 participants and studies published as abstracts only were also excluded.

We only included studies that measured the markers of endometrial receptivity prior to pregnancy events (implantation failure, miscarriage, clinical pregnancy, live birth) to avoid the potential bias secondary to changes caused by the pregnancy event itself. Studies were deemed eligible irrespective of the country of origin, authors or affiliations, language or year of publication.

One author (L.C.) screened the titles and abstracts to compile a list of potentially eligible studies. The full manuscripts were assessed and data was extracted with pre-defined spreadsheets. A second author (I.G.) verified extracted data against the full manuscripts. Any disagreement was resolved through discussion, with a plan to involve a third author (J.C.) if the disagreement persisted.

The Newcastle–Ottawa Scale (Wells et al.) for observational studies was employed to assess the selection of cohorts, the comparability of study design and the adequacy of outcome assessment and follow-up. The scale uses a stars system to award the highest quality studies up to nine stars.

Primary and secondary outcomes

The included studies reported various endometrial receptivity markers evaluated by ultrasound imaging, endometrial biopsy, endometrial fluid aspirate or hysteroscopy. The markers were described separately in the results section.

Association and accuracy data were provided in relation to clinical pregnancy (defined as intrauterine pregnancy diagnosed by the presence of a gestational sac on ultrasound scan), miscarriage (defined as clinical pregnancy loss before 24 weeks of gestation) or live birth (defined as a live born baby after 24 weeks of gestation).

Data analysis and presentation

The pooled outcome was calculated as a mean difference (MD) for markers of endometrial receptivity reported as means between study groups. If the SD was not provided, it was calculated according to the guidelines of the Cochrane Collaboration (Higgins and Green, 2011) assuming that both groups had the same variance. The Inverse Variance method was used for the calculation of MD with 95% CI under the random-effects model (DeMets, 1987) to account for the clinical heterogeneity between the study populations. Risk ratio (RR) with 95% CI was calculated for markers of endometrial receptivity reported as dichotomous variables (or relative to a cut-off value) using the Mantel–Haenszel method under the random-effects model.

Heterogeneity was explored using the χ^2 test, with significance set at $P < 0.05$. I^2 was used to quantify heterogeneity (Higgins and Thompson, 2002), with a maximum value of 40% identifying low heterogeneity, while >40% identified substantial heterogeneity. Forest plots were used for the graphical display of the results from the association meta-analyses. The square around the estimate is proportional to the weight used in meta-analysis and the horizontal line represents the 95% CI. Review Manager (RevMan) software (Version 5.3, The Cochrane Collaboration, 2014) was used for the calculation of MD and RR.

For tests with sufficient data, we plotted estimates of sensitivities and specificities from individual studies on summary receiver operating characteristics space for visual examination of heterogeneity. We used STATA statistical package to meta-analyse a pair of sensitivity and specificity from each included study by using the hierarchical summary receiver operating characteristics approach (Rutter and Gatsonis, 2001; Macaskill, 2004). This approach estimates the position and shape of the summary receiver operating characteristics curve and takes into account both within and between study variations. We fitted a two-level mixed logistic regression model, with independent binomial distributions for the true positives and true negatives conditional on the sensitivity and specificity in each study, and a bivariate normal model transforming sensitivity and specificity between studies. When all the parameters of the hierarchical summary receiver operating characteristics model could not be estimated owing to a limited number of studies, we simplified it by assuming a symmetrical shape for the curve. For meta-analysis of studies that used the same cut-off values, we used parameter estimates from the models to derive summary operating points (that is, summary sensitivities and specificities), with 95% confidence regions, and summary likelihood ratios.

Endometrial receptivity markers were grouped and reported based on the investigation that led to their measurement (ultrasound, endometrial biopsy, endometrial fluid aspirate and hysteroscopy). The methods of conception (natural, IUI and IVF with fresh or non-fresh embryo transfers) were considered as sub-groups according to the published protocol.

Accuracy measures (sensitivity, specificity, likelihood ratios for positive and negative test results, post-test probabilities) were presented in three different tables for IUI, IVF with fresh embryo transfer and IVF with non-fresh (frozen–thawed or donated embryos with or without endometrial preparation) embryo transfer, respectively. Pooled measures were presented if sufficient data were available for meta-analysis. Accuracy measures from the largest studies reporting individual endometrial receptivity markers and cut-offs were presented if data were insufficient for meta-analysis. Post-test probabilities were calculated using the likelihood ratios and a pre-test probability was defined by the overall clinical pregnancy rate of the largest study reporting on women undergoing IUI, IVF with fresh and non-fresh embryo transfer, respectively. In the absence of a gold standard diagnostic test for endometrial receptivity, we considered

clinical pregnancy as an outcome to reflect the presence of receptive endometrium.

Results

The literature search identified 36 145 articles after removal of duplicates. Titles and abstracts were screened to exclude 35 791 articles for not being relevant to the question of the present review. The full text of the remaining 354 articles was assessed for eligibility. Out of these, 191 were excluded. We included 163 studies (88 834 women) in the narrative synthesis, 96 out of which were included in the meta-analyses. Figure 1 displays the flow diagram for the selection of the studies. Figure 2 displays the summary of the main results.

The vast majority of the included studies reported on markers of endometrial receptivity in the context of IVF (138/163, 85%) and evaluated by ultrasound (120/163, 74%). The studies were conducted in 36 different countries and included a median of 124 women (range: 17–21 752). The characteristics of included studies are given in Supplementary Table SII.

The overall quality of the studies assessed using The Newcastle–Ottawa Scale was moderate. High scores were obtained for participants' selection and follow up of reported outcomes. Low scores were obtained for cohorts' comparability as confounding factors were very rarely accounted for. Few studies reported on live birth as a reproductive outcome, while clinical pregnancy was the most frequently reported outcome during follow up.

The largest IUI study (Khalil *et al.*, 2001) assessing endometrial receptivity markers included 893 women (2473 cycles) and reported an overall clinical pregnancy rate of 11.9%. The largest fresh embryo transfer IVF study (Gallos *et al.*, 2018) included 21 752 women (25 433 cycles) and reported a clinical pregnancy rate of 39.9%. For IVF with non-fresh embryo transfer, the largest study (Bu *et al.*, 2016) included 2997 women (2997 cycles) and reported a clinical pregnancy rate of 40.6%. These pre-test probabilities were used to calculate the post-test probabilities for IUI (Table I), IVF with fresh embryo transfer (Table II) and IVF with non-fresh embryo transfer (Table III), respectively.

Endometrial receptivity markers evaluated by ultrasound

Endometrial thickness

Studies measured the endometrial thickness for women undergoing IUI and IVF with fresh or non-fresh embryo transfer. Endometrial thickness was reported at various time points in relation to IUI (during ovarian stimulation, on the day of hCG injection, on the day of IUI), fresh embryo transfer (mid-luteal phase in the cycle preceding the IVF cycle, day of hCG injection, day after hCG injection, day of oocyte retrieval, day of embryo transfer) and non-fresh embryo

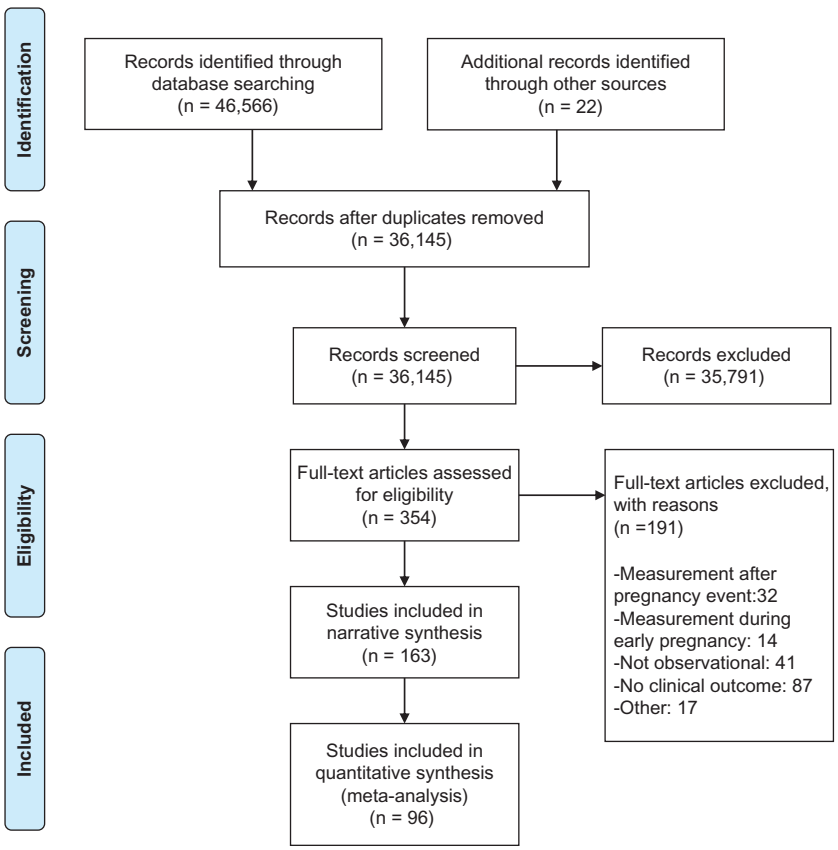

Figure 1 PRISMA flow diagram of search and selection strategy.

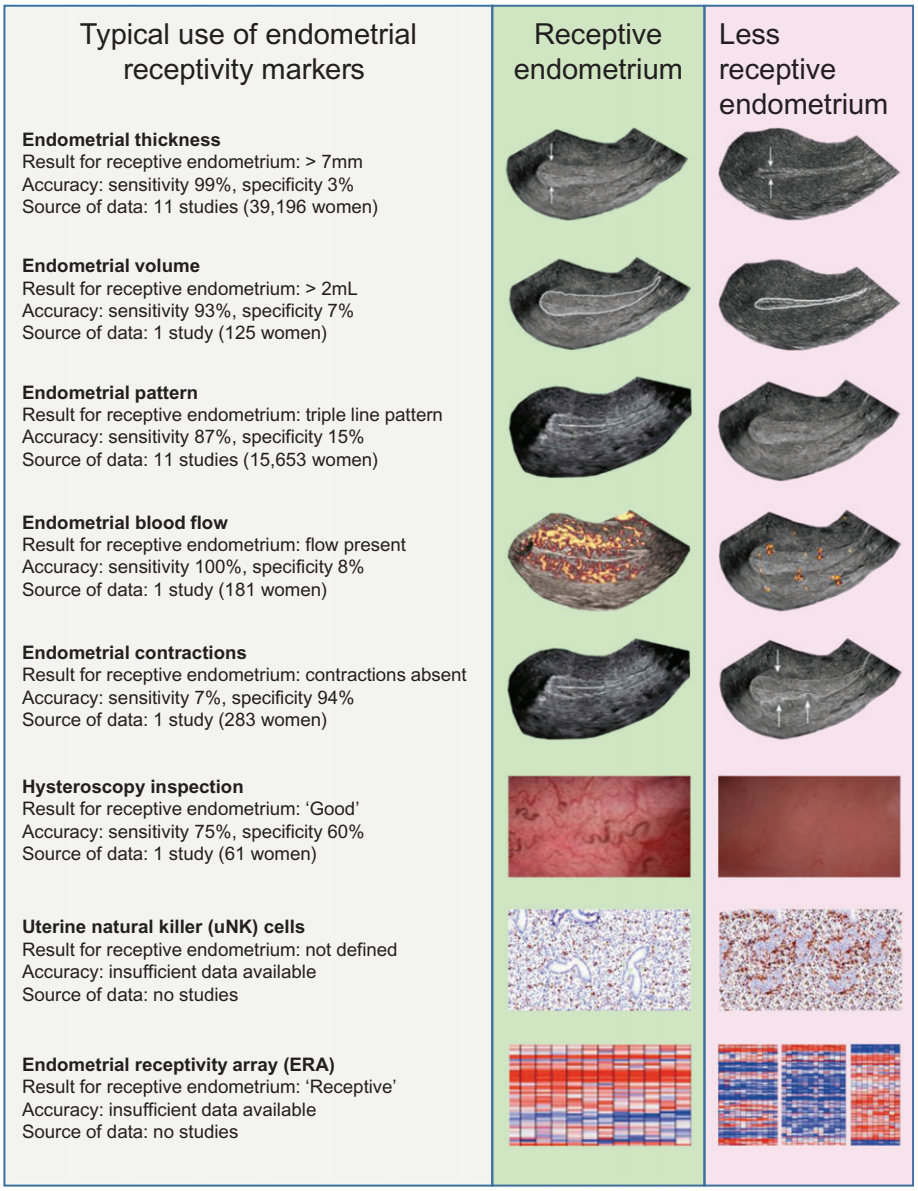

Figure 2 Summary of the main findings. The prognostic accuracy of endometrial receptivity markers for clinical pregnancy.

transfer (day of LH surge in natural cycle, day of commencing progesterone, day of embryo transfer).

Association analyses (using means): Sufficient data were available to perform meta-analysis of studies reporting the mean endometrial thickness between clinically pregnant and not pregnant women in the context of IUI and IVF with fresh and non-fresh embryo transfer.

Ten studies reported the mean endometrial thickness for women achieving clinical pregnancy versus women without a clinical pregnancy after IUI. The endometrial thickness measured on the day of the hCG injection was higher in the clinical pregnancy group compared to no clinical pregnancy group (MD, 1.16; 95% CI: 0.29–2.03; $z = 2.62$; $P < 0.0009$; six studies; 1635 cycles; substantial heterogeneity: $I^2 = 97\%$, Supplementary Fig. S1). No significant difference was observed in the endometrial thickness measured on the day of IUI between the groups (MD, 0.54; 95% CI: −0.3 to 2.5; $z = 1.58$; $P = 0.11$; four studies; 556 cycles; low heterogeneity: $I^2 = 36\%$, Supplementary Fig. S1).

Thirty-four studies reported the mean endometrial thickness for women achieving clinical pregnancy versus women without a clinical pregnancy after IVF with fresh embryo transfer. Endometrial thickness measured on the day of hCG injection was higher in the group of women who achieved a clinical pregnancy compared to women who did not (MD, 0.43; 95% CI: 0.21–0.64; $z = 3.87$; $P < 0.0001$; 20 studies; 18 690 cycles; substantial heterogeneity: $I^2 = 83\%$, Supplementary Fig. S2). No difference was observed in the mean endometrial thickness between the clinically pregnant and not pregnant groups on the day of oocyte retrieval (MD, −0.5; 95% CI: −1.29 to 0.3; $z = 1.23$; $P = 0.22$; three studies; 252 cycles; no heterogeneity: $I^2 = 0\%$,

Table I Accuracy measures for endometrial receptivity markers based on their ability to predict clinical pregnancy (CP) following IUI.

Studies (cycles)	Cut-off for positive test (should identify receptive endometrium)	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR− (95% CI)	Post-test probabilities for CP (95% CI) Based on a pre-test probability of 11.9%	
						After test if positive (%)	After test if negative (%)
Endometrial receptivity markers evaluated by ultrasound							
Endometrial thickness on the day of hCG injection to predict CP after IUI							
1 (562)	>3 mm	100 (96.03–100)	0.42 (0.05–1.53)	1 (1–1.01)	0	11.9 (11.9–12)	0
1 (562)	>4 mm	96.7 (90.67–99.31)	4.88 (3.12–7.24)	1.02 (0.97–1.06)	0.68 (0.21–2.2)	12.1 (11.6–12.5)	8.4 (2.8–22.9)
1 (562)	>5 mm	87.91 (79.4–93.81)	12.53 (9.67–15.86)	1.01 (0.92–1.09)	0.96 (0.53–1.76)	12 (11.1–12.8)	11.5 (6.7–19.2)
4 (1569)	>6 mm	91.92 (75.59–97.66)	13.75 (6.9–25.53)	1.07 (1–1.13)	0.59 (0.28–1.24)	12.6 (11.9–13.2)	7.4 (3.6–14.3)
1 (562)	>7 mm	52.75 (42–63.31)	47.98 (43.39–52.6)	1.01 (0.82–1.25)	0.98 (0.78–1.25)	12 (10–14.4)	11.7 (9.5–14.4)
1 (562)	>8 mm	30.77 (21.51–41.32)	65.61 (61.12–69.89)	0.89 (0.64–1.25)	1.06 (0.91–1.23)	10.7 (8–14.4)	12.5 (10.9–14.2)
1 (562)	>9 mm	21.98 (13.97–31.88)	79.83 (75.92–83.36)	1.09 (0.71–1.67)	0.98 (0.87–1.1)	12.8 (8.8–18.4)	11.7 (10.5–12.9)
1 (562)	>10 mm	12.09 (6.19–20.6)	90.02 (86.95–92.58)	1.21 (0.65–2.24)	0.98 (0.9–1.06)	14 (8.1–23.2)	11.7 (10.8–12.5)
1 (562)	>11 mm	5.49 (1.81–12.36)	94.06 (91.52–96.01)	0.92 (0.37–2.33)	1 (0.95–1.06)	11.1 (4.8–23.9)	11.9 (11.4–12.5)
1 (562)	>12 mm	1.1 (0.03–5.97)	98.3 (96.68–99.26)	0.65 (0.08–5.11)	1.01 (0.98–1.03)	8.1 (1.1–40.8)	12 (11.7–12.2)
Endometrial thickness on other days to predict CP after IUI							
1 (1368)	>6 mm on Day 10 of cycle	58.7 (51.22–65.89)	38.85 (36.06–41.69)	0.96 (0.84–1.09)	1.06 (0.88–1.28)	11.5 (10.2–12.8)	12.5 (10.6–14.7)
1 (100)	>7 mm on Day of IUI	41.67 (25.51–59.24)	35.94 (24.32–48.9)	0.65 (0.42–1)	1.62 (1.06–2.49)	8.1 (5.4–11.9)	18 (12.5–25.2)
1 (100)	>14 mm on Day of IUI	2.78 (0.07–14.53)	100 (94.4–100)	N/A	0.97 (0.92–1.03)	N/A	11.6 (11.1–12.2)
Endometrial pattern at various timings to predict CP after IUI							
1 (1371)	Triple line on Day 10 of cycle	82.07 (75.75–87.32)	17.61 (15.48–19.9)	1 (0.93–1.07)	1.02 (0.73–1.42)	11.9 (11.2–12.6)	12.1 (9–16.1)
5 (1525)	Triple line on day of hCG	84.36 (68.02–93.19)	27.24 (17.49–39.81)	1.16 (1.07–1.26)	0.57 (0.35–0.93)	13.5 (12.6–14.5)	7.1 (4.5–11.2)
1 (241)	Triple line on day of IUI	100 (92.75–100)	10.94 (6.9–16.23)	1.12 (1.07–1.18)	0	13.1 (12.6–13.7)	0
Other ultrasound markers on the day of IUI to predict CP after IUI							
1 (104)	Endometrial volume >2 mL	78.57 (49.2–95.34)	56.67 (45.8–67.08)	1.81 (1.26–2.6)	0.38 (0.14–1.05)	19.6 (14.5–26)	4.9 (1.9–12.4)
1 (105)	Uterine artery diastolic notch: absent	0 (0–16.11)	91.67 (83.58–96.58)	0	1.09 (1.02–1.16)	0	12.8 (12.1–13.5)
1 (241)	<4 contractions/min	61.22 (46.24–74.8)	57.29 (49.97–64.39)	1.43 (1.09–1.89)	0.68 (0.47–0.98)	16.2 (12.8–20.3)	8.4 (6–11.7)
Endometrial receptivity markers evaluated by endometrial fluid aspirate							
1 (50)	Activin A >0.04 ng/mL	76 (56.6–88.5)	100 (86.7–100)	19.8	0.25	72.8	3.3
1 (71)	Urocortin >0.321 ug/L,	60.7 (40.6–78.5)	97.7 (87.7–99.6)	26.11	0.4	77.9	5.1

LR+ = likelihood ratio of a positive test result; LR− = likelihood ratio of a negative test result.

Supplementary Fig. S2). Endometrial thickness measured on the day of fresh embryo transfer was higher in the clinical pregnancy group compared to the no pregnancy group (MD, 0.26; 95% CI, 0.02 to 0.49; $z = 2.16$; $P < 0.03$; 13 studies; 3695 cycles; substantial heterogeneity: $I^2 = 58\%$, Supplementary Fig. S2).

Eleven studies reported the mean endometrial thickness for women achieving clinical pregnancy versus women without a clinical pregnancy after IVF with non-fresh embryo transfer. Endometrial thickness measured on the day of commencing progesterone was higher in the clinical pregnancy group compared to the no clinical pregnancy group (MD, 0.46; 95% CI: 0.04–0.87; $z = 2.17$; $P < 0.03$; three studies; 2054 cycles; substantial heterogeneity: $I^2 = 91\%$, Supplementary Fig. S3). Endometrial thickness measured on the day of the non-fresh embryo transfer was similar between the groups (MD, 0.19; 95% CI: −0.57 to 0.96; $z = 0.49$; $P = 0.63$; six studies; 366 cycles; substantial heterogeneity: $I^2 = 74\%$, Supplementary Fig. S3).

Association analyses (using cut-offs): Sufficient data were available to perform meta-analysis of studies reporting various cut-offs for endometrial thickness and the corresponding clinical pregnancy rates in the context of IUI and IVF with fresh embryo transfer.

Four studies contributed data for association analyses between endometrial thicknesses cut-offs ranging from 3 to 12 mm on the day

Table II Accuracy measures for endometrial receptivity markers based on their ability to predict clinical pregnancy (CP) following IVF with fresh embryo transfer (ET).

Studies (cycles)	Cut-off for positive test (should identify receptive endometrium)	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR− (95% CI)	Post-test probabilities for CP (95% CI) Based on a pre-test probability of 39.9%	
						After test if positive (%)	After test if negative (%)
Endometrial receptivity markers evaluated by ultrasound							
Endometrial thickness on the day of hCG injection to predict CP after fresh ET							
4 (28 868)	>6 mm	99.63 (98.75–99.89)	0.98 (0.32–3)	1 (1–1.01)	0.38 (0.18–0.77)	39.9 (39.9–40.1)	20.1 (10.7–33.8)
11 (39 196)	>7 mm	98.82 (98.2–99.23)	2.73 (1.72–4.31)	1.01 (1–1.02)	0.43 (0.35–0.53)	40.1 (39.9–40.4)	22.2 (18.9–26)
10 (37 238)	>8 mm	94.77 (91.75–96.72)	10.16 (5.51–17.98)	1.05 (1.01–1.1)	0.51 (0.42–0.64)	41.1 (40.1–42.2)	25.3 (21.8–29.8)
7 (35 733)	>9 mm	87.79 (84.07–90.74)	17.75 (12.75–24.16)	1.07 (1.03–1.1)	0.69 (0.64–0.74)	41.5 (40.6–42.2)	31.4 (29.8–32.9)
9 (35 568)	>10 mm	68.29 (57.68–77.28)	41.1 (29.45–53.84)	1.16 (1.07–1.25)	0.77 (0.72–0.83)	43.5 (41.5–45.4)	33.8 (32.3–35.5)
6 (34 776)	>11 mm	56.61 (49.42–63.53)	53.72 (43.7–63.44)	1.22 (1.1–1.36)	0.81 (0.76–0.86)	44.7 (42.2–47.4)	35 (33.5–36.3)
9 (35 449)	>12 mm	30.19 (20.41–42.18)	78.92 (65.46–88.09)	1.43 (1.17–1.76)	0.88 (0.85–0.92)	48.7 (43.7–53.9)	36.9 (36.1–37.9)
6 (34 776)	>13 mm	23.77 (17.76–31.03)	82.62 (74.11–88.76)	1.37 (1.15–1.63)	0.92 (0.9–0.95)	47.6 (43.3–62)	37.9 (37.4–38.7)
15 (42 163)	>14 mm	9.08 (6.28–12.95)	92.78 (89.78–94.95)	1.26 (1.09–1.45)	0.98 (0.97–1)	45.5 (42–49)	39.4 (39.2–39.9)
1 (25 433)	>15 mm	9.4 (8.84–9.98)	92.14 (91.7–92.56)	1.2 (1.1–1.3)	0.98 (0.98–0.99)	44.3 (42.2–46.3)	39.4 (39.4–39.7)
1 (25 433)	>16 mm	5.11 (4.69–5.55)	95.59 (95.25–95.91)	1.16 (1.04–1.29)	0.99 (0.99–1)	43.5 (40.8–46.1)	39.7 (39.7–39.9)
1 (25 433)	>17 mm	2.68 (2.37–3.01)	97.79 (97.55–98.02)	1.21 (1.04–1.42)	1 (0.99–1)	44.5 (40.8–48.5)	39.9 (39.7–39.9)
Endometrial thickness on the day of ET to predict CP after fresh ET							
1 (1228)	>7 mm	99.75 (98.62–99.99)	0.36 (0.07–1.06)	1 (0.99–1.01)	0.68 (0.07–6.56)	39.9 (39.7–40.1)	31.1 (4.4–81.3)
1 (1228)	>8 mm	98.76 (97.12–99.59)	2.54 (1.58–3.86)	1.01 (1–1.03)	0.49 (0.19–1.29)	40.1 (39.9–40.6)	24.5 (11.2–46.1)
1 (1228)	>9 mm	91.29 (88.1–93.86)	11.14 (9.07–13.48)	1.03 (0.99–1.07)	0.78 (0.54–1.13)	40.6 (39.7–41.5)	34.1 (26.4–42.9)
1 (1228)	>10 mm	76.62 (72.17–80.67)	28.81 (25.74–32.03)	1.08 (1–1.15)	0.81 (0.66–1)	41.8 (39.9–43.3)	35 (30.5–39.9)
1 (1228)	>11 mm	53.48 (48.47–58.44)	56.17 (52.71–59.59)	1.22 (1.08–1.38)	0.83 (0.73–0.93)	44.7 (41.8–47.8)	35.5 (32.6–38.2)
1 (1228)	>12 mm	34.08 (29.45–38.94)	74.46 (71.34–77.4)	1.33 (1.12–1.60)	0.89 (0.82–0.96)	46.9 (42.6–51.5)	37.1 (35.2–38.9)
1 (1228)	>13 mm	24.36 (19.7–29.51)	88.01 (85.6–90.15)	2.03 (1.55–2.66)	0.86 (0.8–0.92)	57.4 (50.7–63.8)	36.3 (34.7–37.9)
1 (1228)	>14 mm	7.96 (5.51–11.5)	94.43 (92.64–95.89)	1.43 (0.93–2.21)	0.97 (0.94–1.01)	48.7 (38.2–59.5)	39.2 (38.4–40.1)
1 (1228)	>15 mm	3.23 (1.73–5.47)	98.06 (96.87–98.89)	1.67 (0.81–3.44)	0.99 (0.97–1.01)	52.6 (35–69.5)	39.7 (39.2–40.1)
1 (1228)	>16 mm	0.75 (0.15–2.17)	99.39 (98.59–99.8)	1.23 (0.3–5.13)	1 (0.99–1.01)	45 (16.6–77.3)	39.9 (39.7–40.1)
Endometrial pattern at various timings to predict CP after fresh ET							
11 (15 653)	Triple line on day of hCG	86.93 (81.02–91.2)	14.83 (7.93–26.05)	1.02 (0.97–1.07)	0.88 (0.69–1.13)	40.4 (39.2–41.5)	36.9 (31.4–42.9)
6 (778)	Triple line on day of ET	69.59 (34.82–90.74)	35.43 (16.63–60.15)	1.08 (0.92–1.26)	0.86 (0.55–1.34)	41.8 (37.9–45.5)	36.3 (26.7–47.1)
Endometrial volume at various timings to predict CP after fresh ET							
1 (103)	>2 mL on day of hCG	93.33 (81.73–98.6)	6.9 (1.91–16.73)	1 (0.9–1.11)	0.97 (0.23–4.1)	39.9 (37.4–42.4)	39.2 (13.2–73.1)
1 (103)	>4 mL on day of hCG	68.89 (53.35–81.83)	44.83 (31.74–58.46)	1.25 (0.92–1.69)	0.69 (0.41–1.17)	45.4 (37.9–52.9)	31.4 (21.4–43.7)
1 (125)	>2 mL on day of ET	93.5	22.2	1.2	0.29	44.3	16.1
1 (125)	>2.5 mL on day of ET	90.3	35.8	1.41	0.27	48.3	15.2
Uterine artery PI at various timings to predict CP after fresh ET							
1 (112)	<3 on day of hCG	100 (90.51–100)	2.67 (0.32–9.30)	1.03 (0.99–1.07)	0	40.6 (39.7–41.5)	0
1 (174)	<3 on day of ET	91.94 (82.17–97.33)	26.79 (18.86–35.98)	1.26 (1.1–1.44)	0.3 (0.12–0.74)	45.5 (42.2–48.9)	16.6 (7.4–32.9)
Uterine artery protodiastolic notch at various timings to predict CP after fresh ET							
1 (96)	Absent mid-luteal before ET cycle	31.03 (15.28–50.83)	71.64 (59.31–81.99)	1.09 (0.56–2.12)	0.96 (0.72–1.28)	42 (27.1–58.5)	38.9 (32.3–45.9)
1 (112)	Absent on day of hCG	78.38 (61.79–90.17)	42.67 (31.31–54.62)	1.37 (1.06–1.77)	0.51 (0.26–0.99)	47.6 (41.3–54)	25.3 (14.7–39.7)
1 (178)	Absent on day of ET	7.46 (2.47–16.56)	99.1 (95.08–99.98)	8.28 (0.99–69.4)	0.93 (0.87–1)	84.6 (39.7–97.9)	38.2 (36.6–39.9)

Continued

Table II *Continued*

Studies (cycles)	Cut-off for positive test (should identify receptive endometrium)	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR− (95% CI)	Post-test probabilities for CP (95% CI) Based on a pre-test probability of 39.9%	
						After test if positive (%)	After test if negative (%)
Endometrial blood flow at various timings to predict CP after fresh ET							
1 (96)	Present mid-luteal before ET cycle	79.31 (60.28–92.01)	55.22 (42.58–67.4)	1.77 (1.28–2.45)	0.37 (0.18–0.79)	54 (45.9–61.9)	19.7 (10.7–34.4)
1 (181)	Present on day of hCG	100 (94.87–100)	8.11 (3.77–14.83)	1.09 (1.03–1.15)	0	42 (40.6–43.3)	0
1 (623)	Present on day of ET	36.16 (29.08–43.70)	84.3 (80.59–87.56)	2.3 (1.72–3.08)	0.76 (0.67–0.85)	60.4 (53.3–67.2)	33.5 (30.8–36.1)
Uterine contractions on the day of ET to predict CP after fresh ET							
1 (283)	Absent	6.72 (2.95–12.82)	93.9 (89.07–97.04)	1.1 (0.45–2.71)	0.99 (0.93–1.06)	42.2 (23–64.3)	39.7 (38.2–41.3)
1 (220)	<3 contractions/min	39.44 (28.03–51.75)	83.22 (76.24–88.84)	2.35 (1.48–3.72)	0.73 (0.6–0.89)	60.9 (49.6–71.2)	32.6 (28.5–37.1)
1 (220)	<4 contractions/min	71.83 (59.9–81.87)	65.1 (56.87–72.72)	2.06 (1.58–2.68)	0.43 (0.29–0.64)	57.8 (51.2–64)	22.2 (16.1–29.8)
1 (220)	<5 contractions/min	85.92 (75.62–93.03)	42.95 (34.88–51.31)	1.51 (1.27–1.78)	0.33 (0.18–0.6)	50.1 (45.7–54.2)	18 (10.7–28.5)
Endometrial receptivity markers evaluated by endometrial biopsy							
1 (69)	BLC6 ≤1.4	55 (31.53–76.94)	87.76 (75.23–95.37)	4.49 (1.92–10.49)	0.51 (0.31–0.84)	74.9 (56–87.4)	25.3 (17.1–35.8)
1 (52)	α-Inhibin >1.26	64 (47–78)	68 (46–85)	2.02 (0.99–4.10)	0.53 (0.31–0.92)	57.3 (39.7–73.1)	26 (17.1–37.9)
1 (52)	β-Glycan >1.22	67 (50–80)	74 (51–88)	2.53 (1.15–5.58)	0.45 (0.26–0.79)	62.7 (43.3–78.7)	23 (14.7–34.4)
1 (66)	Luminal αvβ3 >0.7	85.71 (67.33–95.97)	28.95 (15.42–45.9)	1.21 (0.94–1.55)	0.49 (0.18–1.39)	44.5 (38.4–50.7)	24.5 (10.7–48)
1 (56)	L-selectin ligand: high	68.18 (45.13–86.14)	61.76 (43.56–77.83)	1.78 (1.07–2.98)	0.52 (0.26–1)	54.2 (41.5–66.4)	25.7 (14.7–39.9)
1 (122)	Aromatase P450 <8.3	93.75 (79.19–99.23)	21.11 (13.21–30.99)	1.19 (1.03–1.37)	0.3 (0.07–1.2)	44.1 (40.6–47.6)	16.6 (4.4–44.3)
1 (49)	Glandular VEGF-A >6	60 (26.24–87.84)	87.18 (72.57–95.7)	4.68 (1.79–12.25)	0.46 (0.21–0.99)	75.7 (54.3–89.1)	23.4 (12.2–39.7)
Endometrial receptivity markers evaluated by endometrial fluid aspirate							
1 (109)	hDP 200 <100 mU/mg	92.86 (66.13–99.82)	17.89 (10.78–27.1)	1.13 (0.95–1.34)	0.4 (0.06–2.77)	42.9 (38.7–47.1)	21 (3.8–64.8)
1 (109)	hDP 200 <1000 mU/mg	57.14 (28.86–82.34)	69.47 (59.18–78.51)	1.87 (1.08–3.23)	0.62 (0.33–1.15)	55.4 (41.8–68.2)	29.2 (18–43.3)
1 (109)	hDP 200 <10000 mU/mg	28.57 (8.39–58.1)	95.79 (89.57–98.84)	6.79 (1.91–24.1)	0.75 (0.53–1.04)	81.8 (55.9–94.1)	33.2 (26–40.8)
1 (133)	IL-18 <12.5 pg/mL	85.71 (71.46–94.57)	35.16 (25.44–45.88)	1.32 (1.09–1.61)	0.41 (0.18–0.9)	46.7 (42–51.7)	21.4 (10.7–37.4)
Endometrial receptivity markers evaluated by hysteroscopy							
1 (61)	'Good' endometrium	75 (47.62–92.73)	60 (44.33–74.3)	1.88 (1.19–2.96)	0.42 (0.17–1.01)	55.5 (44.1–66.3)	21.8 (10.1–40.1)
1 (75)	Endometrial blood flow >29 mL/min/100 g	71.43 (47.82–88.72)	61.11 (46.88–74.08)	1.84 (1.19–2.82)	0.47 (0.23–0.95)	55 (44.1–65.2)	23.8 (13.2–38.7)

LR+ = likelihood ratio of a positive test result; LR− = likelihood ratio of a negative test result.

of the hCG injection and clinical pregnancy following IUI. The most used cut-off was 6 mm. There was no difference in clinical pregnancy after IUI between women who had an endometrial thickness higher than 6 mm compared to women with a thinner than 6 mm endometrium on the day of hCG injection (RR, 1.19; 95% CI: 0.82–1.71; $z = 0.92$; $P = 0.36$; four studies; 1569 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S4). No difference was observed for any of the other endometrial thickness cut-offs (Supplementary Fig. S4).

Nineteen studies contributed data for association analyses between endometrial thicknesses cut-offs ranging from 6 to 17 mm on the day of the hCG injection and clinical pregnancy following IVF with fresh embryo transfer. There was a positive association between clinical pregnancy and higher endometrial thickness for every cut-off. The measure of association decreased gradually from the 6 mm cut-off (RR, 1.85; 95% CI: 1.28–2.67; $z = 3.28$; $P < 0.001$; four studies; 30 361 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S5) to the 17 mm cut-off (RR, 1.14; 95% CI, 1.06 to 1.24; $z = 3.38$; $P < 0.0007$; five studies; 30 793 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S5). Figure 3 summarizes the pooled outcomes shown in Supplementary Fig. S5.

Accuracy analyses (using cut-offs): Sufficient data were available to perform test accuracy meta-analysis of studies reporting various cut-offs for endometrial thickness and the corresponding clinical pregnancy rate in the context of IUI and IVF with fresh embryo transfer.

Four studies provided clinical pregnancy data in relation to the 6 mm cut-off for endometrial thickness as measured on the day of hCG injection in women undergoing IUI. The sensitivity was 91.9% and the specificity was 13.8% (four studies, 1569 cycles).

Table III Accuracy measures for endometrial receptivity markers based on their ability to predict clinical pregnancy (CP) following IVF with non-fresh embryo transfer (ET).

Studies (cycles)	Cut-off for positive test (should identify receptive endometrium)	Sensitivity (95% CI)	Specificity (95% CI)	LR+ (95% CI)	LR− (95% CI)	Post-test probabilities for CP (95% CI) Based on a pre-test probability of 40.6%	
						After test if positive (%)	After test if negative (%)
Endometrial receptivity markers evaluated by ultrasound							
Endometrial thickness on the day of progesterone start to predict CP after non-fresh ET							
1 (1512)	>6 mm	99.21 (98.44–99.66)	2.98 (1.68–4.87)	1.02 (1.02–1.04)	0.27 (0.11–0.62)	41.1 (41.1–41.5)	15.6 (7–29.8)
1 (1512)	>7 mm	96.13 (94.75–97.24)	6.16 (4.23–8.63)	1.02 (1–1.05)	0.63 (0.4–0.99)	41.1 (40.6–41.8)	30.1 (21.5–40.4)
1 (1512)	>8 mm	80.67 (78.1–83.07)	24.65 (20.94–28.66)	1.07 (1.01–1.14)	0.78 (0.64–0.96)	42.2 (40.8–43.8)	34.8 (30.4–39.6)
1 (1512)	>9 mm	41.53 (38.46–44.64)	63.22 (58.84–67.45)	1.13 (0.99–1.29)	0.92 (0.85–1.01)	43.6 (40.4–46.9)	38.6 (36.7–40.8)
1 (1512)	>10 mm	22 (19.48–24.69)	82.11 (78.47–85.36)	1.23 (0.99–1.53)	0.95 (0.9–1)	45.7 (40.4–51.1)	39.4 (38.1–40.6)
1 (1512)	>11 mm	9.81 (8.05–11.82)	91.85 (89.1–94.09)	1.2 (0.85–1.7)	0.98 (0.95–1.01)	45.1 (36.7–53.7)	40.1 (39.4–40.8)
1 (1512)	>12 mm	5.15 (3.87–6.7)	95.83 (93.69–97.4)	1.23 (0.75–2.03)	0.99 (0.97–1.01)	45.7 (33.9–58.1)	40.4 (39.9–40.8)
1 (1512)	>13 mm	2.68 (1.77–3.87)	97.42 (95.62–98.62)	1.04 (0.54–1.99)	1 (0.98–1.02)	41.5 (27–57.6)	40.6 (40.1–41.1)
1 (1512)	>14 mm	0.89 (0.41–1.69)	99.2 (97.98–99.78)	1.12 (0.35–3.62)	1 (0.99–1.01)	43.4 (19.3–71.2)	40.6 (40.4–40.8)
Endometrial thickness on the day of ET to predict CP after non-fresh ET							
1 (737)	>6 mm	92.76 (88.51–95.81)	7.36 (5.26–9.97)	1 (0.96–1.05)	0.98 (0.56–1.73)	40.6 (39.6–41.8)	40.1 (27.7–54.2)
1 (236)	>7 mm	89.29 (82.03–94.34)	9.68 (5.1–16.29)	0.99 (0.91–1.08)	1.11 (0.52–2.36)	40.4 (38.3–42.5)	43.1 (26.2–61.7)
1 (2997)	>8 mm	89.49 (87.63–91.16)	14.33 (12.74–16.05)	1.04 (1.02–1.07)	0.73 (0.6–0.89)	41.5 (41.1–42.2)	33.3 (29.1–37.8)
1 (737)	>9 mm	33.94 (27.72–40.59)	69.38 (65.20–73.33)	1.11 (0.88–1.39)	0.95 (0.85–1.06)	43.1 (37.6–48.7)	39.4 (36.7–42)
1 (737)	>10 mm	14.93 (10.51–20.33)	80.43 (76.74–83.76)	0.76 (0.53–1.09)	1.06 (0.99–1.13)	34.2 (26.6–42.7)	42 (40.4–43.6)
1 (236)	>11 mm	14.29 (8.39–22.16)	81.45 (73.48–87.86)	0.77 (0.43–1.38)	1.05 (0.94–1.18)	34.5 (22.7–48.5)	41.8 (39.1–44.6)
1 (236)	>12 mm	6.25 (2.55–12.45)	87.9 (80.83–93.07)	0.52 (0.22–1.22)	1.07 (0.98–1.16)	26.2 (13.1–45.5)	42.2 (40.1–44.2)
1 (2997)	>14 mm	8.87 (7.33–10.61)	92.69 (91.38–93.86)	1.21 (0.95–1.55)	0.98 (0.96–1)	45.3 (39.4–51.4)	40.1 (39.6–40.6)
Endometrial pattern at various timings to predict CP after non-fresh ET							
1 (100)	Triple line on day of ovulation	100 (89.72–100)	9.09 (3.41–18.74)	1.1 (1.02–1.19)	0	42.9 (41.1–44.9)	0
1 (2244)	Triple line on day before commencing Progesterone	83.77 (81.04–86.25)	18.02 (16.07–20.11)	1.02 (0.98–1.06)	0.9 (0.74–1.09)	41.1 (40.1–42)	38.1 (33.6–42.7)
1 (1512)	Triple line on day of commencing Progesterone	61.65 (58.56–64.66)	44.53 (40.13–49)	1.11 (1.01–1.22)	0.86 (0.76–0.98)	43.1 (40.8–45.5)	37 (34.2–40.1)
1 (236)	Triple line on day of ET	91.96 (85.29–96.26)	11.29 (6.31–18.22)	1.04 (0.95–1.13)	0.71 (0.32–1.58)	41.5 (39.4–43.6)	32.7 (17.9–51.9)
Endometrial volume to predict CP after non-fresh ET							
1 (40)	>3.2 mL on day of ET	80 (28.36–99.49)	77.14 (59.86–89.58)	3.5 (1.65–7.41)	0.26 (0.04–1.51)	70.5 (53–83.5)	15.1 (2.7–50.8)
Endometrial receptivity markers evaluated by endometrial biopsy							
1 (126)	Pinopode score > −26.48	83	45	1.51	0.38	50.8	20.6

LR+ = likelihood ratio of a positive test result; LR− = likelihood ratio of a negative test result.

Nineteen studies provided clinical pregnancy data in relation to endometrial thicknesses cut-offs ranging between 6 and 17 mm as measured on the day of hCG injection for women undergoing IVF with fresh embryo transfer. Sufficient data were available for test accuracy meta-analysis of endometrial thickness cut-offs ranging from 6 to 14 mm.

Overall, the predictive accuracy of endometrial thickness for clinical pregnancy was low, as the hierarchical summary receiver operating characteristic (HSROC) curve for all studies and all endometrial thicknesses cut-offs shows no discrimination between women who achieved a clinical pregnancy and women who did not (area under the HSROC: 0.57 [95% CI: 0.52–0.61], Fig. 4).

The 6 mm cut-off (four studies, 28 868 cycles) had the highest sensitivity (99.6%) and the lowest specificity (0.98%). The 14 mm cut-off (15 studies, 42 163 cycles) had the lowest sensitivity (9.1%) and the highest specificity (92.8%). There was a gradual decrease in sensitivity

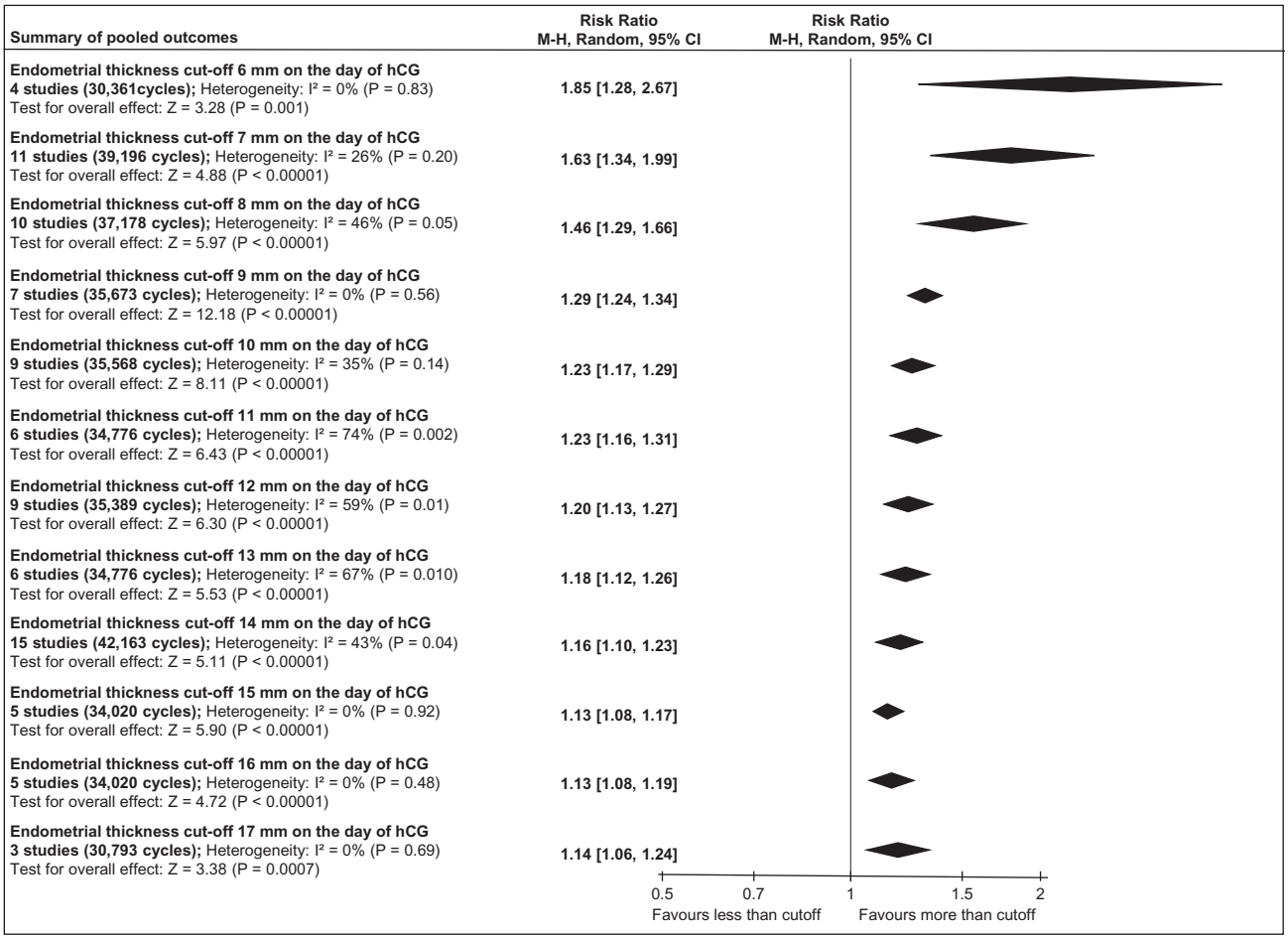

Figure 3 Summary of association between endometrial thickness (ET) cut-offs on the day of hCG injection and clinical pregnancy (CP) for women undergoing IVF with fresh embryo transfer.

and increase in specificity from the 6 mm cut-off to the 14 mm cut-off resulting in area under the receiver operator curve (AUC), ranging between 0.49 and 0.74 (Supplementary Fig. S6).

Insufficient data were available to perform test accuracy meta-analysis based on live birth as an outcome. Live birth accuracy data from the largest study (Gallos *et al.*, 2018) was similar to clinical pregnancy accuracy data.

Endometrial volume

Studies measured the endometrial volume for women undergoing IUI and IVF with fresh or non-fresh embryo transfer. Endometrial volume has been reported on the day of IUI, at various time points in relation to IVF with fresh embryo transfer (day of hCG injection, day of oocyte retrieval, day of embryo transfer), and on the day of non-fresh embryo transfer.

Association analyses (using means): Sufficient data were available to perform meta-analysis of studies reporting the mean endometrial volume between clinical pregnancy and no clinical pregnancy groups in the context of IUI and IVF with fresh and non-fresh embryo transfer.

Four studies reported the endometrial volume for women undergoing IUI. The endometrial volume measured on the day of IUI was higher for women who achieved a clinical pregnancy compared to those who did not (MD, 0.63; 95% CI: 0.03–1.23; $z = 2.05$; $P < 0.04$; four studies; 550 cycles; low heterogeneity: $I^2 = 35\%$, Supplementary Fig. S7).

Eight studies reported endometrial volume for women undergoing IVF with fresh embryo transfer. There was no difference in the endometrial volume measured on the day of hCG injection between women who achieved a clinical pregnancy compared to those who did not (MD, 0.49; 95% CI: −0.23 to 1.2; $z = 1.34$; $P = 0.18$; five studies; 943 cycles; substantial heterogeneity: $I^2 = 69\%$, Supplementary Fig. S8). No difference was also observed between the groups when the endometrial volume was measured on the day of fresh embryo transfer (MD, 0.34; 95% CI: −0.17 to 0.86; $z = 1.31$; $P = 0.19$; three studies; 652 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S8).

Accuracy analyses (using cut-offs): Insufficient data were available to perform meta-analysis of studies reporting various cut-offs for endometrial volume and the corresponding clinical pregnancy rate.

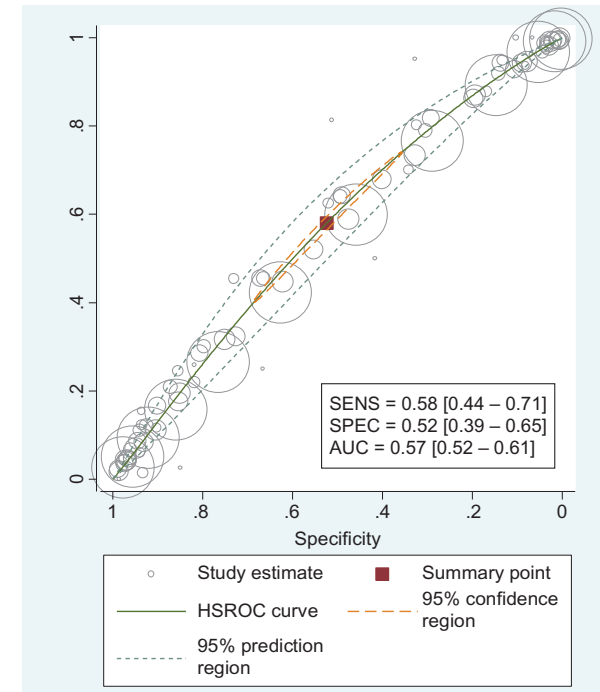

Figure 4 Hierarchical summary receiver operating characteristic curve (HSROC) for endometrial thickness prediction of clinical pregnancy for all cut-off values and all studies reporting on endometrial thickness measurements on the day of hCG injection. Circle size reflects the sample size of the studies. SENS = sensitivity; SPEC = specificity; AUC = area under the curve.

One study (Zollner *et al.*, 2003a) reported clinical pregnancy data following IUI based on the cut-off of 2 mL for endometrial volume as measured on the day of IUI. The sensitivity was 78.6% and the specificity was 56.7% (Table I).

Aboulghar *et al.* (2005) reported clinical pregnancy data following IVF with fresh embryo transfer based on two cut-offs for endometrial volume as measured on the day of hCG injection. The cut-off of 2 mL offered a sensitivity of 93.3% and a specificity of 6.9%, while the cut-off of 4 mL offered a sensitivity of 68.9% and a specificity of 44.8% (Table II).

One study (Zollner *et al.*, 2003b) reported clinical pregnancy data following IVF with fresh embryo transfer based on two cut-offs for endometrial volume as measured on the day of the embryo transfer. The cut-off of 2 mL offered a sensitivity of 93.5% and a specificity of 22.2%, while the cut-off of 2.5 mL offered a sensitivity of 90.3% and a specificity of 35.8% (Table II).

One study (Zollner *et al.*, 2012) reported clinical pregnancy data following IVF with frozen–thawed embryo transfer based on the cut-off of 3.2 mL for endometrial volume as measured on the day of the embryo transfer. The sensitivity was 80% and the specificity was 77.1% (Table III).

Endometrial pattern

Studies assessed the endometrial pattern for women undergoing IUI and IVF with fresh or non-fresh embryo transfer. Endometrial pattern has been reported at various time points in relation to IUI (Day 10 of cycle, day of hCG injection, day of IUI), fresh embryo transfer (luteal phase prior to IVF cycle, day of hCG injection, day after hCG injection, day of oocyte retrieval, day of embryo transfer) and non-fresh embryo transfer (day of donor ovulation, day before commencing progesterone, day of commencing progesterone, day of embryo transfer).

Association analyses: Sufficient data were available to perform meta-analysis of studies reporting the endometrial pattern in the context of IUI and IVF with fresh and non-fresh embryo transfer.

Eight studies reported clinical pregnancy in relation to the endometrial pattern in women undergoing IUI. Triple line pattern assessed on the day of hCG injection was associated with higher clinical pregnancy rates (RR, 1.45; 95% CI: 1.08–1.95; $z = 2.49$; $P < 0.01$; five studies; 1 525 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S9). Triple line pattern assessed on the day of IUI was also associated with higher clinical pregnancy rates (RR, 3.21; 95% CI: 1.35–7.61; $z = 2.64$; $P < 0.008$; three studies; 445 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S9).

Twenty studies reported clinical pregnancy in relation to the endometrial pattern in women undergoing IVF with fresh embryo transfer. There were similar clinical pregnancy rates between women with triple line pattern and women without triple line pattern assessed on the day of hCG injection (RR, 1.05; 95% CI: 0.91–1.22; $z = 0.73$; $P = 0.47$; 11 studies; 15 653 cycles; substantial heterogeneity: $I^2 = 58\%$, Supplementary Fig. S10). Clinical pregnancy rates were also similar between women with triple line pattern and women without triple line pattern assessed on the day after hCG (RR, 2.19; 95% CI: 0.92–5.22; $z = 1.78$; $P = 0.08$; three studies; 719 cycles; substantial heterogeneity: $I^2 = 69\%$, Supplementary Fig. S10). There were also similar clinical pregnancy rates between women with triple line pattern and women without triple line pattern assessed on the day of embryo transfer (RR, 1.02; 95% CI: 0.75–1.4; $z = 0.13$; $P = 0.89$; six studies; 778 cycles; low heterogeneity: $I^2 = 32\%$, Supplementary Fig. S10).

Five studies reported clinical pregnancy in relation to the endometrial pattern for women undergoing IVF with non-fresh embryo transfer. There were similar clinical pregnancy rates between women with triple line pattern and women without triple line pattern assessed on the day of commencing progesterone (RR, 1.78; 95% CI: 0.96–3.29; $z = 1.85$; $P = 0.06$; three studies; 1 870 cycles; substantial heterogeneity: $I^2 = 90\%$, Supplementary Fig. S11).

Accuracy analyses: Sufficient data were available to perform meta-analysis of studies reporting endometrial pattern and the corresponding clinical pregnancy rates for women undergoing IUI and IVF with fresh embryo transfer.

Five studies reported clinical pregnancy data in relation to triple line pattern assessed on the day of hCG injection for women undergoing IUI. The sensitivity was 84.4% and the specificity was 27.2% (five studies, 1525 cycles).

Eleven studies reported clinical pregnancy data in relation to triple line pattern assessed on the day of hCG injection for women undergoing IVF with fresh embryo transfer. The sensitivity was 86.9% and the specificity was 14.8% (11 studies, 15 653 cycles).

Six studies reported clinical pregnancy data in relation to triple line pattern assessed on the day of embryo transfer for women undergoing IVF with fresh embryo transfer. The sensitivity was 69.6% and the specificity was 35.4% (six studies, 778 cycles).

Doppler signals

Studies measured various Doppler indices for women undergoing IUI and IVF with fresh or non-fresh embryo transfer. The measurements were acquired at various time points in relation to IUI (day of hCG injection, day of IUI) and IVF with fresh embryo transfer (day of hCG injection, day of oocyte retrieval, day of embryo transfer), and on the day of non-fresh embryo transfer.

Association analyses (using means): Three studies reported insufficient data to perform meta-analysis of mean Doppler indices between clinical pregnancy and no pregnancy groups in the context of IUI. Riad and Hak (2014) evaluated 90 women undergoing IUI and found lower pulsatility index (PI) and lower resistance index (RI) of the subendometrial blood flow on the day of hCG injection in women who achieved a clinical pregnancy compared to those who did not. Kim *et al.* (2010) evaluated 106 women undergoing IUI and reported higher endometrial vascularity index (VI), flow index (FI) and vascularization flow index (VFI) scores on the day of IUI in women who achieved a clinical pregnancy compared to women who did not. No difference was observed between the groups in subendometrial VI, FI and VFI scores or uterine artery PI, RI, systolic/diastolic (S/D) ratio. Engels *et al.* (2011) evaluated 79 consecutive IUI cycles and reported higher subendometrial FI on the day of hCG injection in women who achieved a clinical pregnancy compared to women who did not become pregnant. No difference was observed in the subendometrial VI or VFI.

Twenty-two studies reported various mean Doppler indices between women who achieved a clinical pregnancy following IVF with fresh embryo transfer and women who did not.

Endometrial VI, measured on the day of hCG injection, was similar between the groups (MD, −0.68; 95% CI: −3.00 to 1.63; $z = 0.58$; $P = 0.56$; four studies; 840 cycles; substantial heterogeneity: $I^2 = 88\%$, Supplementary Fig. S12). When measured on the day of fresh embryo transfer, endometrial VI was higher in women who achieved a clinical pregnancy compared to those who did not (MD, 0.96; 95% CI: 0.06–1.86; $z = 2.08$; $P < 0.04$; two studies; 527 cycles; substantial heterogeneity: $I^2 = 82\%$, Supplementary Fig. S12).

Endometrial FI, measured on the day of hCG injection, was similar between the groups (MD, 0.9; 95% CI: −1.76 to 3.57; $z = 0.67$; $P = 0.51$; three studies; 805 cycles; substantial heterogeneity: $I^2 = 91\%$, Supplementary Fig. S13). Similar results were obtained on the day of the fresh embryo transfer (MD, 2.83; 95% CI: −8.5 to 14.15; $z = 0.49$; $P = 0.62$; two studies; 527 cycles; substantial heterogeneity: $I^2 = 97\%$, Supplementary Fig. S13).

Endometrial VFI, measured on the day of hCG injection, was similar between clinically pregnant and not pregnant women (MD, 1.02; 95% CI: −0.92 to 2.97; $z = 1.02$; $P = 0.3$; three studies; 805 cycles; substantial heterogeneity: $I^2 = 79\%$, Supplementary Fig. S14). Higher endometrial VFI measured on the day of the fresh embryo transfer was observed in women who achieved a clinical pregnancy (MD, 0.21; 95% CI: 0.09–0.33; $z = 3.43$; $P < 0006$; two studies; 527 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S14).

Subendometrial VI, measured on the day of hCG injection, was lower in women who achieved a clinical pregnancy compared to women who did not (MD, −1.71; 95% CI: −3.11 to −0.3; $z = 2.38$; $P < 02$; three studies; 763 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S15). No differences between the groups were observed in subendometrial VI measured on the day of fresh embryo transfer (MD, −0.03; 95% CI: −0.42 to 0.37; $z = 0.13$; $P = 0.9$; two studies; 527 cycles; low heterogeneity: $I^2 = 7\%$, Supplementary Fig. S15).

Subendometrial FI, measured on the day of hCG injection, was higher in women who achieved a clinical pregnancy compared to women who did not (MD, 0.76; 95% CI: 0.22–1.3; $z = 2.74$; $P < 0.006$; two studies; 728 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S16). No differences between the groups were observed in the subendometrial FI measured on the day of fresh embryo transfer (MD, 0.6; 95% CI, −1.77 to 2.97; $z = 0.5$; $P = 0.62$; three studies; 616 cycles; substantial heterogeneity: $I^2 = 75\%$, Supplementary Fig. S16).

Subendometrial VFI, measured on the day of hCG injection was similar between clinically pregnant and not pregnant women (MD, −0.35; 95% CI: −0.81 to 0.12; $z = 1.47$; $P = 0.14$; two studies; 728 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S17). No difference between the groups was observed in the subendometrial VFI measured on the day of the fresh embryo transfer (MD, −0.01; 95% CI: −0.19 to 0.18; $z = 0.05$; $P = 0.96$; two studies; 527 cycles; substantial heterogeneity: $I^2 = 61\%$, Supplementary Fig. S17).

Uterine artery PI, measured on the day of hCG injection, was similar between women who achieved a clinical pregnancy and those who did not (MD, −0.01; 95% CI: −0.14 to 0.12; $z = 0.14$; $P = 0.89$; three studies; 227 cycles; substantial heterogeneity: $I^2 = 59\%$, Supplementary Fig. S18). No difference between the groups was observed in uterine artery PI measured on the day of oocyte retrieval (MD, 0.04; 95% CI: −0.12 to 0.2; $z = 0.49$; $P = 0.62$; two studies; 99 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S18). Similar uterine artery PIs were measured on the day of fresh embryo transfer between clinically pregnant and not pregnant women (MD, −0.07; 95% CI, −0.19 to 0.12; $z = 1.1$; $P = 0.27$; seven studies; 1 487 cycles; substantial heterogeneity: $I^2 = 72\%$, Supplementary Fig. S18).

Uterine artery RI, measured on the day of fresh embryo transfer, was similar between clinically pregnant and not pregnant women (MD, −0.01; 95% CI: −0.03 to 0; $z = 1.72$; $P = 0.09$; four studies; 1318 cycles; substantial heterogeneity: $I^2 = 67\%$, Supplementary Fig. S19).

Three studies reported mean Doppler indices in relation to clinical pregnancy following IVF with frozen–thawed embryo transfer. Data were insufficient for meta-analysis. One study (Son *et al.*, 2014) assessed 70 women on the day of embryo transfer and reported similar uterine artery PIs and RIs and subendometrial RIs and PIs between clinically pregnant and not pregnant women. One study (Nandi *et al.*, 2014) assessed 45 women at various times in relation to the embryo transfer and found no differences in endometrial VI, FI and VFI between women who achieved a clinical pregnancy and those who did not. One study (Polanski *et al.*, 2016) correlated manual and spherical endometrial spatio-temporal image correlation (STIC) vascularity indices for 127 women undergoing fresh and frozen–thawed embryo transfers to report no difference between clinically pregnant and not pregnant women.

Association analyses (using cut-offs): Sufficient data were available for meta-analyses in the context of IVF with fresh embryo transfer.

The presence of endometrial blood flow on the day of hCG injection was associated with higher clinical pregnancy rates (RR, 1.98; 95% CI: 1.37–2.86; $z = 3.63$; $P < 0.0003$; three studies; 393 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S20). The presence of endometrial blood flow on the day of fresh embryo transfer was not associated with clinical pregnancy (RR, 1.82; 95% CI: 0.98–3.37; $z = 1.91$; $P = 0.06$; three studies; 945 cycles; substantial heterogeneity: $I^2 = 79\%$, Supplementary Fig. S20).

A uterine artery PI of <3 measured on the day of fresh embryo transfer was associated with higher clinical pregnancy rates (RR, 3.07; 95% CI,: 1.54–6.12; $z = 3.18$; $P < 0.001$; three studies; 400 cycles; no heterogeneity: $I^2 = 0\%$, Supplementary Fig. S21).

Accuracy analyses: Insufficient data were available to perform accuracy meta-analysis of studies reporting Doppler indices and the corresponding clinical pregnancy rates. Accuracy measurements from the largest study reporting each Doppler index and cut-off value are presented in Tables I–III.

Endometrial wave-like activity

Six studies assessed the relation between endometrial wave-like activity and pregnancy outcomes in natural cycles, IUI and IVF with fresh and frozen–thawed embryo transfer. Data were insufficient for meta-analysis.

IJland *et al.* (1997) recruited 33 couples with unexplained infertility and assessed the endometrial activity throughout the menstrual cycle using ultrasound recordings for 3–15 min. Women who conceived (9/33, 27%) during the study cycle had lower endometrial wave-like activity compared to women who conceived in later cycles or those who never conceived.

Kim *et al.* (2014) evaluated the endometrial activity for 3 min on the day of IUI for 241 cycles. Women who achieved a clinical pregnancy (49/241, 20.3%) displayed reduced endometrial activity overall, but had a higher cervico-fundal movement rate.

Swierkowski-Blanchard *et al.* (2017) recorded 5 min of uterine activity for 100 women undergoing IUI. Women with clinical pregnancy following IUI (18/100, 18%) were more likely to have low frequency and high intensity uterine contractions compared to women who failed to conceive.

Chung *et al.* (2017) evaluated the changing pattern of uterine contractions in 286 women undergoing IVF with fresh embryo transfer. Ultrasound recordings were acquired 5 min before, 5 min after and 60 min after the embryo transfer. There was no difference in uterine contractility 5 min before the embryo transfer between the clinically pregnant and not pregnant groups; however, the contraction frequency measured 5 min after the embryo transfer was reduced in women who achieved a clinical pregnancy.

Fanchin *et al.* (1998) monitored the uterine activity for 5 min just before fresh embryo transfer in 220 cycles. A stepwise decrease in clinical and ongoing pregnancy rates occurred from fewer than 3 contractions/min to more than 5 contractions/min.

Zhu *et al.* (2014) evaluated the uterine peristaltic wave frequency before 292 fresh and frozen–thawed embryo transfers. The clinical pregnancy rate was the highest when fewer than 2 waves/min were observed, and it decreased significantly for women with more than 3 waves/min.

Endometrial receptivity markers evaluated by endometrial biopsy

Histology and cytology

Studies correlated histological appearances and cytological compartments of the endometrium in the context of natural conception and IVF. Data were insufficient for meta-analysis.

Three studies used Noyes *et al.*'s (1950) histological criteria for endometrial dating in women with unexplained infertility. The endometrial biopsies were performed in the mid-luteal phase of an ovulatory cycle.

Driessen *et al.* (1980) divided 232 infertile women into four groups based on endometrial dating: 'no delay of the secretory phase, a secretory phase with a delay of 2 days, a secretory delay of 3 days or more, and an endometrium which could not be dated because of inadequate material'. No differences were reported in pregnancy rates within two years between the four groups.

Balasch *et al.* (1992) evaluated 1492 endometrial biopsies taken from 1055 women diagnosed with unexplained infertility. Authors reported no association between histological endometrial adequacy in the cycle of conception or in previous cycles and the outcome of pregnancy.

Klentzeris *et al.* (1992) divided 47 women based on endometrial dating into 'in phase' or 'retarded' endometrium and assessed their pregnancy outcomes in the following 3 years. Women with 'in phase' endometrium were more likely to become pregnant (18/36, 50%) following IVF compared to women with 'retarded' endometrium (1/11, 9%).

Three studies evaluated the association between uterine natural killer (uNK) cells and the outcome of subsequent pregnancies in women who suffered unexplained recurrent miscarriage. The endometrial biopsies were timed in the window of implantation dated in relation to the LH surge or confirmed by histological criteria.

Tuckerman *et al.* (2007) assessed the percentage of stromal cells positive for CD56 in women with three or more unexplained recurrent miscarriages ($n = 87$). Similar $CD56^+$ cell counts were observed in 19 women who miscarried (mean 9.6%, range: 1.7–25.0%) and 32 women who had a live birth (mean 13.3%, range: 1.1–41.4%) in the subsequent pregnancy.

Quenby *et al.* (1999) assessed various endometrial leucocytes in 22 women with three or more unexplained recurrent miscarriages, out of which 15 obtained a subsequent pregnancy completed by miscarriage or live birth. Higher percentages of $CD4^+$, $CD8^+$, $CD14^+$, $CD16^+$ and $CD56^+$ cells were observed in women who miscarried (4/15) compared to women who achieved a live birth (11/15) in the subsequent pregnancy. There were no differences of $CD45^+$, $CD3^+$, $CD22^+$, $CD57^+$ or $CD69^+$ between the groups.

Michimata *et al.* (2002) assessed various endometrial leucocyte subsets in 17 women who suffered two or more recurrent miscarriages. No difference in $CD45^+$, $CD56^+$, $CD16^+$, $CD20^+$, $CD3^+$ or $CD8^+$ cells were observed between women who achieved a live birth (11/17, 65%) and women who miscarried (6/17, 35%) in the subsequent pregnancy.

Liu *et al.* (2014) combined the histological criteria with uNK count to predict the fate of future pregnancies in 83 women diagnosed with recurrent miscarriage or recurrent implantation failure. No correlation

was observed between uNK count and subsequent pregnancy outcome. 'Retarded' endometrium was associated with a higher miscarriage rate (13/19, 68%) compared to 'in phase' endometrium (23/64, 35%). Combining uNK count and histological dating increased their individual prognostic value.

Two studies correlated pinopode formation with subsequent pregnancy outcomes. Pantos *et al.* (2004) assessed the pinopode formation in a mock cycle for 46 women scheduled to undergo IVF with donated oocytes. The embryo transfer was then timed in relation to previous cycle's pinopode formation. Higher clinical pregnancy (76.47 versus 33.33%) and live birth (67.64 versus 25%) rates were observed in women with delayed embryo transfer as directed by pinopode formation compared to women with standard embryo transfer.

Jin *et al.* (2017) reported a custom scoring system for the pinopode formation in 126 women undergoing frozen–thawed embryo transfer. Pinopode index scores higher than −26.48 were associated with higher clinical pregnancy rates compared to lower scores. This pinopode index score cut-off had 83% sensitivity and 45% specificity for clinical pregnancy (Table III).

Endometrial receptivity array

Endometrial receptivity array (ERA) is a molecular diagnostic test based on microarray technology that classifies endometrial biopsies into receptive, prereceptive or proliferative based on the expression of 238 selected genes (Díaz-Gimeno *et al.*, 2011). Women then undergo personalized embryo transfer (pET) where the frozen–thawed embryo transfer is timed according to the receptive status as identified by ERA. Five studies reported clinical outcomes following the use of ERA and pET in women with previous unsuccessful embryo transfers. Meta-analysis was not performed due to clinical and methodological heterogeneity in patient populations (number of previously failed cycles), reported comparisons and unit of analysis (per couple or per cycle).

One study (Ruiz-Alonso *et al.*, 2013) assessed the endometrial receptivity in 85 women scheduled to undergo frozen–thawed embryo transfer in natural or hormonally prepared cycles. ERA test identified a higher rate of non-receptive endometrium in women with recurrent implantation failure (22/85, 25.9%) compared to women without recurrent implantation failure (3/25, 12%). Women diagnosed with non-receptive endometrium on the initial ERA test achieved a pregnancy rate of 50% (four out eight women with follow-up data) after pET.

One study (Ruiz-Alonso *et al.*, 2014) reported on 17 women who failed to achieve ongoing pregnancies following standard embryo transfer on a day in which the endometrium was diagnosed as non-receptive (pre- or post-receptive) by the ERA test. The same 17 women underwent a total of 20 subsequent pET based on ERA result and 53% (9/17) reached the stage of ongoing pregnancy.

Mahajan (2015) reported a higher rate of non-receptive endometrium in women who suffered two or more implantation failures (22/80, 28%) compared to women who suffered only one implantation failure (14/93, 15%) following IVF with standard embryo transfer of good quality embryos. The use of pET led to similar ongoing pregnancy rates between women diagnosed with non-receptive endometrium (20/48, 42%) compared to women diagnosed with receptive endometrium (8/18, 44.5%) on the initial ERA test.

Hashimoto *et al.* (2017) performed ERA testing on 50 women with recurrent implantation failure and reported non-receptive endometrium at a rate of 24% (12/50). The clinical pregnancy rate in the subsequent pET was higher in women with non-receptive endometrium based on the ERA test (5/10, 50%) compared to women with standard embryo transfer (12/34, 35.3%).

Tan *et al.* (2018) assessed endometrial receptivity in 88 women and reported an overall non-receptive rate of 44.3% (39/88). The rate of non-receptive endometrium was 37.5% (18/48) in women with at least one failed frozen–thawed euploid embryo transfer. Ongoing pregnancy rates following the subsequent embryo transfer were similar between women who had a receptive endometrium (50.9%) and those who were non-receptive and required pET (51.6%).

Díaz-Gimeno *et al.* (2017) analysed 771 women diagnosed by ERA and further stratified the receptive endometrium based on the outcome following pET (biochemical pregnancy vs live birth) to identity additional transcriptomic profiles: proliferative, early prereceptive, late prereceptive, receptive, late receptive and post-receptive. The ongoing pregnancy rates ranged between 76.9 and 80% in the late prereceptive and receptive signatures compared to 33.3% in the late receptive endometrium.

Other molecular markers

Various individual molecular markers have been investigated by studies with sample sizes ranging from 20 to 122. Data were insufficient for meta-analyses and none of the markers were further developed as diagnostic tests.

Five studies (Thomas *et al.*, 2003; Brosens *et al.*, 2004; Wang *et al.*, 2008; Almquist *et al.*, 2017; Silveira *et al.*, 2017) reported clinical pregnancy in relation to expression levels of BLC6, aromatase P450, α-inhibin and β-glycan, integrins and L-selectin ligand, respectively. Accuracy measures in relation to reported cut-offs were presented in and Table II.

Ten studies (Rizk *et al.*, 1992; Damario *et al.*, 2001; Jinno *et al.*, 2001; Shamonki *et al.*, 2006; Foulk *et al.*, 2007; Serafini *et al.*, 2008, 2009; Seo *et al.*, 2011; Maia-Filho *et al.*, 2015; Krylova *et al.*, 2016) compared subsequently pregnant versus not pregnant women based on mean measurements of various integrins, L-selectin ligand, VEGF, matrix metalloproteinases and E-cadherin expression, alpha-2 PEG, hCG-LH receptor, LIF, macrophage colony-stimulating factor, HOXA-10 and vascular endothelial growth factor A. No convincing evidence for clinical use emerged from these studies.

Endometrial receptivity markers evaluated by endometrial fluid aspirate

Studies correlated endometrial receptivity markers from endometrial fluid aspirate with pregnancy outcomes following IUI or IVF. Data were insufficient for meta-analysis and none of the markers were further developed into diagnostic tests.

Four studies (Halperin *et al.*, 1995; Lédée-Bataille *et al.*, 2004; Florio *et al.*, 2008, 2010) provided clinical pregnancy data based on cut-offs for urocortin, activin A, human decidua-associated protein (hDP) and interleukin-18. Their accuracy measures are presented in Tables I and II.

Six studies (Lédée-Bataille et al., 2002; Gillott et al., 2008; Boomsma et al., 2009a,b; Bentin-Ley et al., 2011; Rahiminejad et al., 2015, 2016) evaluated the mean levels of various cytokines, glycodelin, isoforms of leucine-rich alpha2-glycoprotein, LIF and TNF, interleukin-1β, TNF-α, interferon gamma-induced protein 10 and monocyte chemoattractant protein between various outcome groups following fertility treatments. No convincing evidence for clinical use emerged from these studies.

Endometrial receptivity markers evaluated by hysteroscopy

The mid-luteal endometrium was classified as 'good' based on the ring type aspect of the glandular openings and presence of well-developed varicose-like vessels during hysteroscopic assessment (Inafuku, 1992). Four studies reported pregnancy outcomes following the assessment of endometrial receptivity by hysteroscopy in the mid-luteal phase of a natural cycle. Data were insufficient for meta-analysis.

Li et al. (2010) evaluated 79 ovulatory infertile women and reported 'poor' mid-luteal endometrium at a rate of 67.1% (53/79). The clinical pregnancy rate following fertility treatment (ovulation induction, IUI or IVF) was higher in women with 'good' endometrium (14/26, 53.9%) compared to women with 'poor' endometrium (14/53, 26.4%).

Masamoto et al. (2000) evaluated 160 ovulatory infertile women and reported 'poor' midsecretory endometrium at a rate of 61.3% (98/160). The miscarriage rate was higher in women with 'poor' endometrium (33/98, 33.7%) compared to women with 'good' endometrium (9/62, 14.5%).

Sakumoto et al. (1992) investigated 61 women prior to IVF and reported 'poor' mid-luteal endometrium at a rate of 50.8% (31/61). The pregnancy rate after IVF was higher in women with 'good' endometrium (12/30, 40%) compared to women with 'poor' endometrium (4/31, 13%).

Santi et al. (2012) assessed the endometrium of 162 infertile women and reported a 33% (54/162) rate of 'poor' endometrium. The pregnancy rate after fertility treatments was higher in women with 'good' endometrium (47/108, 43.5%) compared to women with 'poor' endometrium (13/54, 24%).

Jinno et al. (2001) used the hysteroscopic approach to measure the endometrial blood flow between luteal days 4 and 6 in 75 women scheduled to undergo IVF. The cut-off endometrial blood flow of 29 mL/min/100 g of tissue had a sensitivity of 71.4% and a specificity of 61.1% for clinical pregnancy (Table II).

Discussion

Successful implantation involves complex interactions between the embryo and the endometrium. Receptivity and selectivity are two intrinsic functions of the endometrium that facilitate the recognition of a high quality embryo and nurture its development into a normal foetus. Embryos with reduced potential to develop into a normal foetus are declined implantation, allowing the woman to preserve her resources for the next menstrual cycles. Embryos account for one-third of implantation failures, while suboptimal endometrial receptivity and altered embryo–endometrial dialogue are responsible for the remaining two-thirds (Fig. 5).

The present review identified a large variety of endometrial receptivity markers correlated with clinical outcome data in the context of natural conception, IUI and IVF with fresh or non-fresh embryo transfer. The markers were evaluated by ultrasound, endometrial biopsy, endometrial fluid aspirate and hysteroscopy. The overall quality of the studies was moderate due to low scores obtained for cohort comparability as confounding factors were very rarely accounted for. Associations were identified between clinical pregnancy and various endometrial receptivity markers (endometrial thickness, endometrial pattern, Doppler indices, endometrial wave-like activity and various molecules); however, their poor ability to predict clinical pregnancy (Tables I–III) prevents them from being used as diagnostic tests of endometrial receptivity.

Endometrial thickness was the most commonly investigated marker of endometrial receptivity. The pooled data from association studies revealed no clinically significant difference in endometrial thickness between pregnant and non-pregnant women following IUI and IVF. In addition, high quality evidence from accuracy studies pooled in our meta-analysis revealed a poor ability to predict clinical pregnancy. Two recent meta-analyses of endometrial thickness during IUI reported no evidence for an association (Weiss et al., 2017) or uncertain association based on the mean endometrial thickness (Gadalla et al., 2018). The sROC curve calculated in a previous meta-analysis shows that endometrial thickness does not discriminate between cases that achieved a clinical pregnancy following IVF and cases that did not (Kasius et al., 2014).

Other markers of endometrial receptivity measured by ultrasound (endometrial volume, endometrial pattern, Doppler signals, wave-like activity) were supported by low to very low quality of evidence, due to bias (no adjustment for important prognostic factors), inconsistency (significant heterogeneity) and imprecision (caused by the small number of participants and events). A recent meta-analysis reported

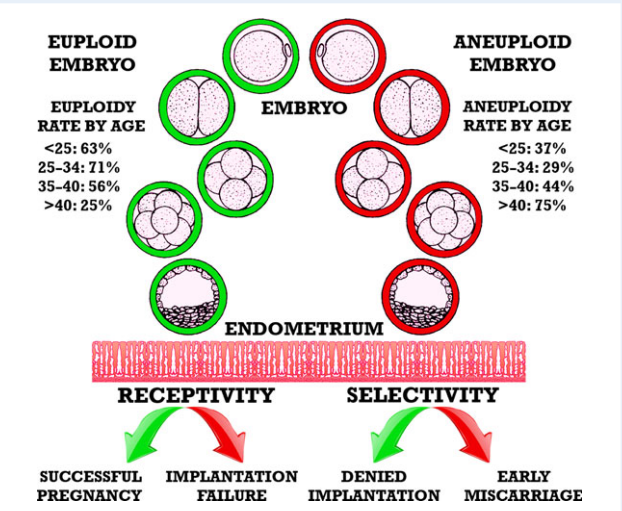

Figure 5 The fate of pregnancy depending on the quality of the embryo and the intrinsic functions of the endometrium (receptivity and selectivity).

statistically significant associations between various Doppler signals and pregnancy rates; however, their clinical relevance remains uncertain and further studies were advised (Wang *et al.*, 2018).

The major limiting factor for the quality of the evidence supporting markers of endometrial receptivity measured by endometrial biopsy, endometrial fluid aspirate or hysteroscopy was imprecision. Most of the markers were investigated by small single studies leading to uncertainty regarding reproducibility, true effect and clinical value.

The groundwork in transcriptomic characterization of the endometrial cycle (Ponnampalam *et al.*, 2004; Talbi *et al.*, 2006) culminated with the development of the ERA diagnostic test for endometrial receptivity. Studies reported promising results following the use of ERA testing coupled with pET as an intervention to address non-receptive endometrium; however, insufficient data were available to compare the outcomes following embryo transfer in receptive versus non-receptive endometrium as assessed by ERA. Additional information about its clinical value will become available with the publication of the ongoing randomized controlled trial (ClinicalTrials.gov Identifier: NCT01954758). ER Map/ER Grade is a new endometrial receptivity test based on the expression of 184 genes involved in endometrial proliferation and maternal immune response associated to embryonic implantation (Enciso *et al.*, 2018). Studies have not yet evaluated its clinical value.

Means are not useful for endometrial receptivity

Endometrial thickness was the most investigated marker of endometrial receptivity and has become a classic example to demonstrate limited benefit of means in the context of endometrial receptivity. The mean endometrial thickness difference between women who achieved a clinical pregnancy and women who did not ranged from only −0.5 to 1.16 mm at various times during IUI and IVF with fresh or non-fresh embryo transfers. Despite this small difference being statistically significant on few occasions, it is unlikely to be considered a clinically significant difference given the inter-observer variation of 1.5 mm (Karlsson *et al.*, 1994).

Given the similar mean endometrial thicknesses in the clinically pregnant and not pregnant women, one may assume that endometrial thickness is not associated with clinical pregnancy and not an useful test. However, association analyses based on cut-off measures identified significant associations between thicker endometrium and higher pregnancy rates for every cut-off. Furthermore, there may be a biological gradient with the strongest association identified for the 6 mm cut-off (RR 1.85, 95% CI: 1.28–2.67 in favour of thicker than 6 mm endometrium).

Impaired endometrial receptivity may be characterized by extreme values of a continuous endometrial receptivity marker which may have a low incidence (e.g. the incidence of thinner than 6 mm endometrium on the day of hCG injection was 0.33%). Means, which report the average across the whole cohort population, may fail to account for important findings at the extreme levels of the range of observations.

Limitations

In the absence of a gold standard diagnostic test for endometrial receptivity, we considered clinical pregnancy as a proxy outcome to confirm receptive endometrium. However the absence of a clinical pregnancy may be a consequence of embryo quality (aneuploidy or poor implantation potential) or other factors (for example, abnormal endometrial microbiome, structural uterine defects or systemic maternal conditions) and may not necessarily reflect the absence of endometrial receptivity. This may underestimate the accuracy of the biomarkers we reviewed.

Insufficient data were available to explore the sources of substantial heterogeneity between the studies pooled in the meta-analyses. Various ultrasound scanning machines, measurement techniques, classification systems and tissue/sample processing protocols were used by individual researchers. Studies included diverse populations and lacked adequate details related to known sources of heterogeneity (infertility duration, stage of embryo at transfer, embryo quality, number of transferred embryos or number of previous failed cycles).

It was not feasible to contact authors for further clarifications due to the large number of included studies published over several decades. Only studies published in full manuscript were included, while studies published as abstracts might have reported on additional markers of endometrial receptivity.

Strengths

This is the first systematic review to summarize the clinical value of existing endometrial receptivity markers. We have performed both association and accuracy analyses to enable comparisons between various endometrial receptivity markers.

We have conducted a very broad literature search to give an accurate overview of the current progress in the diagnosis of endometrial receptivity. This allowed the inclusion of 163 studies reporting on more than 40 markers of endometrial receptivity correlated with subsequent pregnancy outcomes.

Various endometrial receptivity markers were analysed and reported according to the context (natural conception, IUI, IVF with fresh or non-fresh embryo transfer) and timing of measurement (before the start of treatment cycle, at various times during ovarian stimulation or on the day of oocyte retrieval, IUI or embryo transfer, etc.) to account for some potential sources of heterogeneity.

Implications for clinical practice

None of the endometrial receptivity markers included in the present review has sufficient discriminatory value to act as a diagnostic test for endometrial receptivity based on their ability to predict clinical pregnancy. The post-test probabilities presented in Tables I–III may be used in clinical practice to manage couples' expectations during fertility treatments. Further data relevant to the clinical value of the modern molecular tests of endometrial receptivity (ERA, ER Map/ER Grade) are awaited.

Implications for further research

The time has come to reconsider the classical definition for the window of implantation as a time frame of maximal endometrial receptivity surrounded by refractory endometrium. Endometrial receptivity appears to be a continuous variable reflected in the molecular changes triggered by ovulation and progesterone exposure.Various levels of endometrial receptivity exist within the window of

implantation as identified by different transcriptomic signatures coupled with different pregnancy outcomes (Díaz-Gimeno et al., 2017). The transition from non-receptive endometrium to increasing levels of endometrial receptivity that reach a maximal receptivity followed by decreasing levels of endometrial receptivity has also been suggested by the poor pregnancy outcomes associated with late implantation (Wilcox et al., 1999; Jukic et al., 2011; Asvold et al., 2014).

Quantifying endometrial receptivity by endometrial biopsy postpones the completion of fertility treatment due to the invasiveness of the procedure. Endometrial fluid aspirate is less invasive and may be performed before embryo transfer without affecting the pregnancy outcome in a negative way (van der Gaast et al., 2003; Boomsma et al., 2009a,b). Furthermore, endometrial fluid aspirate analysis correlates with endometrial biopsy results (Vilella et al., 2017).

Single molecule testing may not be sufficient to describe the complexity of endometrial receptivity and transcriptomic profiles may be more reliable (Zhang et al., 2013). The dynamic, cyclic nature of the endometrium suggests that it may be difficult, if not impossible, to reliably assess endometrial function on the basis of a single test (i.e. a snapshot) given, for example, how dynamic the uNK cells are from one cycle to the next (Brighton et al., 2017). Next-generation sequencing and various omics- techniques offer an unprecedented opportunity to investigate novel endometrial receptivity markers.

Further research of continuous endometrial receptivity markers should avoid comparing study groups by means alone and should aim to identify cut-off levels that provide maximum accuracy measures. Cumulative pregnancy rates may be a more robust way to evaluate the efficacy of an endometrial test, considering the high incidence of failures that may either be iatrogenic or embryonic in origins.

Increasing value and reducing waste in endometrial receptivity research

Chalmers and Glasziou (2009) estimated that 85% of research funding was being avoidably wasted across the clinical, health services and basic science research. Their article was followed by the publication of the 'Research: increasing value, reducing waste' series in The Lancet. The series included five articles describing ways to increase the value and reduce the waste at various levels in biomedical research: priorities setting, design and conduct, regulation and management, accessibility and reporting. Future studies in the field of endometrial receptivity may benefit directly from recommendations related to design and conduct (Ioannidis et al., 2014) and reporting (Glasziou et al., 2014).

In terms of study design and conduct, a detailed protocol published prior to starting a study will improve the overall poor documentation of endometrial research. Reproducibility of research suffers from the lack of details in study design, starting with population selection, continuing with the measurement of the proposed endometrial receptivity marker, and ending with reporting of results. Most novel endometrial receptivity markers will not allow accurate power or sample size calculations; however, some rational design calculations should be done at least for foreseeable variables at the time of study design (i.e. anticipated overall clinical pregnancy rate). Prospective longitudinal cohort studies may be most appropriate for investigating prognostic markers of endometrial receptivity, while the multicentre approach may address some of the biases induced by small, single-centre studies.

The final report of the study should be based on the pre-published protocol. Deviations from the protocol do not automatically lessen the quality of the study as long as they are accounted for and explained. Several reporting standards exist to facilitate the transparency of reports (STROBE (Vandenbroucke et al., 2007) for observational studies, STARD (Cohen et al., 2016) for diagnostic studies). Future study publications should consider reporting on measures that allow comparison to previously published research in order to integrate the new findings in the overall context.

Supplementary data

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

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Authors' roles

L.C. designed the study, ran the literature search, extracted data, performed the association analyses and drafted the article. I.G. contributed to the literature search, checked extracted data, performed accuracy analyses and critically revised the article. J.C., T.B., S.Q. and J.J.B. interpreted the data and critically revised the article. A.C. contributed to the study design, interpreted the data and critically revised the article. All authors approve the final version of the article.

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Conflicts of interest

None of the authors have any conflict of interest related to this publication.

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CHAPTER FOUR:

TARGET PRODUCT PROFILE FOR AN ENDOMETRIAL RECEPTIVITY TEST: WOMEN'S PERSPECTIVE

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Target Product Profile for an endometrial receptivity test: women's perspective

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ABSTRACT

Objective: To assess the women's views in relation to the characteristics of an endometrial receptivity test in the context of recurrent miscarriage with an overarching aim to guide the development of a Target Product Profile (TPP) based on minimum acceptable ("worst-case") and ideal ("best-case") features.
Study design: This was a descriptive cross-sectional study involving a total of 131 women who answered questions related to the development of an endometrial receptivity test between December 2017 and May 2018. Women attending the recurrent miscarriage clinic at the Tommy's National Centre for Miscarriage Research in Birmingham, United Kingdom, were invited to participate. Referral criteria included two or more miscarriages irrespective of the timing in relation to successful pregnancies. The 'best-case' (ideal) and 'worst-case' (minimum acceptable) thresholds were arbitrary set to satisfy at least 80% and 40% of responders, respectively.
Results: The ideal endometrial receptivity test should be indicated after two miscarriages to comply with the wish of 80.9% (106 women) of responders. It should be performed in a window of three to four days within the menstrual cycle (93.2%; 122 women) and results should be available within one to two days (87.7%; 115 women). Invasiveness of testing should not extend beyond a vaginal examination (85.4%; 112 women). Repeating the test should not be required more than twice (96.1%; 125 women) and the results should remain useful for at least six menstrual cycles (89.3%; 117 women). The importance score given for the endometrium was weakly associated with the willingness to pay for testing; however, there was no evidence to suggest this correlation was different from 0 (Kendall's tau = 0.1101765, z = 1.4327, p-value = 0.1519; Spearman's rho = 0.1268444, S = 327136, p-value = 0.1488).
Conclusions: Women understand the important role the endometrium plays for a successful pregnancy and they have specific views in relation to the indication, timing and invasiveness of testing, need for test repetition, validity of results and costs of testing.

1 Introduction

Endometrial selectivity and receptivity are two opposite concepts introduced to define the endometrium as a bio filter of embryo quality and a nurturing environment [1]. Selectivity is a fundamental encoded function of the endometrium to recognise and reject embryos with reduced development potential. In contrast, receptivity supports the endometrium to provide a prime environment for embryo growth and placenta development.

Impaired endometrial selectivity and receptivity balance has a negative effect on the embryo-endometrial cross-talk [2] leading to implantation failure. It is particularly relevant in the context of recurrent miscarriage unexplained by standard investigations such as thrombophilia screen, thyroid function testing, pelvic ultrasound and cytogenetic testing of the products of conception, and recurrent assisted reproduction failure following the transfer of high quality blastocysts.

Endometrial receptivity can be evaluated by ultrasound and hysteroscopic assessment, Doppler blood flow assessment, endometrial biopsy histology or molecular profiling from endometrial fluid aspirate. A recent systematic review [3] identified associations between clinical pregnancy and various endometrial receptivity markers (endometrial thickness, endometrial pattern, Doppler indices, endometrial wave-like activity and various

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molecules); however, their poor prediction of clinical pregnancy limits their use in clinical practice and further research should focus on identifying new markers of endometrial receptivity.

A Target Product Profile (TPP) is a set of both minimum acceptable ("worst-case") and ideal ("best-case") features of a medical product (diagnostic test, vaccine, medicine or intervention) developed with an aim to identify the critical attributes of a product before development begins, to ensure that the final product responds to the needs of the end-users [4]. Organisations such as the World Health Organization (WHO), the United Nations Children's Fund (UNICEF) and the Drugs for Neglected Diseases initiative (DNDi), have developed TPPs that have served to guide industries in their own product development process [5].

TPPs can evaluate potential pitfalls and create modification plans at all stages of the clinical development process. They allow researchers to review study design and increase collaboration across different groups of stake holders. One example of successful TPPs is the case of tuberculosis diagnostics [6]. Using the list of nine potential TPPs supported by the World Health Organization, participants at a tuberculosis Modelling and Analysis Consortium (TB MAC) meeting conducted a priority-setting exercise to identify the highest priority tests for TPP development and investment in research and development [7].

The objective of our study was to evaluate patients' perspective on endometrial receptivity diagnostics in order to inform future research by incorporating the findings into a TPP for an endometrial receptivity test.

2 METHODS

2.1 Setting

The study was performed in the setting of the Tommy's National Centre for Miscarriage Research in Birmingham, United Kingdom (UK). This is a tertiary care centre attended by patients from all over the UK following referral from General Practitioners (GPs) or secondary care hospitals within the National Health Service (NHS). The objectives of the Centre are to provide medical care for couples who suffered recurrent miscarriages, in the form of counselling, investigations and treatments, and to expand the understanding of miscarriage pathophysiology by undertaking relevant research projects with an overarching aim of reducing the number of miscarriages.

All the standard investigations and treatments are cost-free for the patients, while the research projects are funded by Tommy's Charity or by other funding bodies such as the National Institute for Health Research (NIHR).

2.2 Study population and design

This is a descriptive cross-sectional study comprising of one data collection point for each participant. The study population consisted of women attending Tommy's Recurrent Miscarriage Clinic between December 2017 and May 2018. Referral criteria include two or more miscarriages irrespective of: gestation, timing in relation to other pregnancies, maternal age, mode of conception (natural or fertility treatment), ethnicity or country of residence within the UK. Women were invited to fill in the anonymised questionnaire in the waiting room prior to their clinic appointment. One member of the research team was always available to answer any queries.

2.3 Questionnaire design

A mixed group of nine doctors, specialist nurses and lay persons contributed to the design of the questionnaire. This was then piloted on 16 patients in Tommy's Recurrent Miscarriage Clinic. Pilot participants were asked to comment on the clarity of the questions, their relevance in relation to an endometrial test and the length of the questionnaire in relation to the time available prior to their consultation.

The feedback from pilot participants and from the Ethics Committee was incorporated in the final version of the questionnaire which included eight questions displayed on one A4 page with simple layout (Supplementary file 1).

Question 1 assessed women's perception on the importance of the endometrium for a successful pregnancy. Question 2 assessed their view on the eligibility for testing depending on the number of miscarriages suffered. Questions 3-7 assessed important characteristics of an endometrial test such as the timescale for the availability of results, how strict the timing of the test should be within the endometrial cycle, how invasive the test should be, the need for repeat testing for accurate results, the length of time for test result validity and usefulness. Question 8 assessed the perceived value of an endometrial test outside an NHS funded context.

2.4 Data analysis

Questionnaires were numbered prior to being handed out to account for their return. Data from each questionnaire was entered into an electronic database by two of the study authors.

Responses from Questions 1, 2 and 6 were aggregated as follows: (i) For Question 1, all the responses scoring 7 or less were assigned to a single group called "Importance score of 7 or less" representing women who did not consider the role of endometrium as being of high importance for a successful pregnancy; (ii) For Questions 2 and 6, all the responses scoring 4 or more were aggregated together and assigned to "More than three miscarriages" and "Repeat more than three times" groups respectively. The Questionnaire database was then used to extract descriptive statistical measures such as counts and percentages for each Question, and correlation tests (Spearman's rho and Kendall's tau statistics) were performed to measure the significance of associations between Question 8 and Questions 1-7. The following classification was employed to determine the level of association: -1/+1 indicate perfect negative or positive correlation; 0.7 to 0.9 – strong association; 0.4 to 0.6 – moderate association; 0.1 to 0.3 – weak; 0 – no association. All data analyses were carried out using R 3.6.0 – a language for statistical programming and visualisation [8]. Data visualisation was performed using *ggplot2* package [9].

In the absence of any standards or previous recommendations in the field of reproductive medicine, the 'best-case' and 'worst-case' thresholds were arbitrary set to satisfy at least 80% and 40% of responders, respectively.

2.5 Ethical approval

The study was sponsored by the University of Birmingham. It was funded by Tommy's Charity and approved by West Midlands – South Birmingham Research Ethics Committee (reference 17/WM/0382).

3 Results

The questionnaire return rate was 87.9% (131 out of 149 women invited to participate). All the participants responded to every question of the questionnaire with the exception of one participant who has not responded to question 6 about the need to repeat the test.

3.1 Importance of endometrium for successful pregnancy

The endometrium was given an importance score of 10 by 98 women (74.8%), score of 9 by 8 women (6.1%), score of 8 by 16

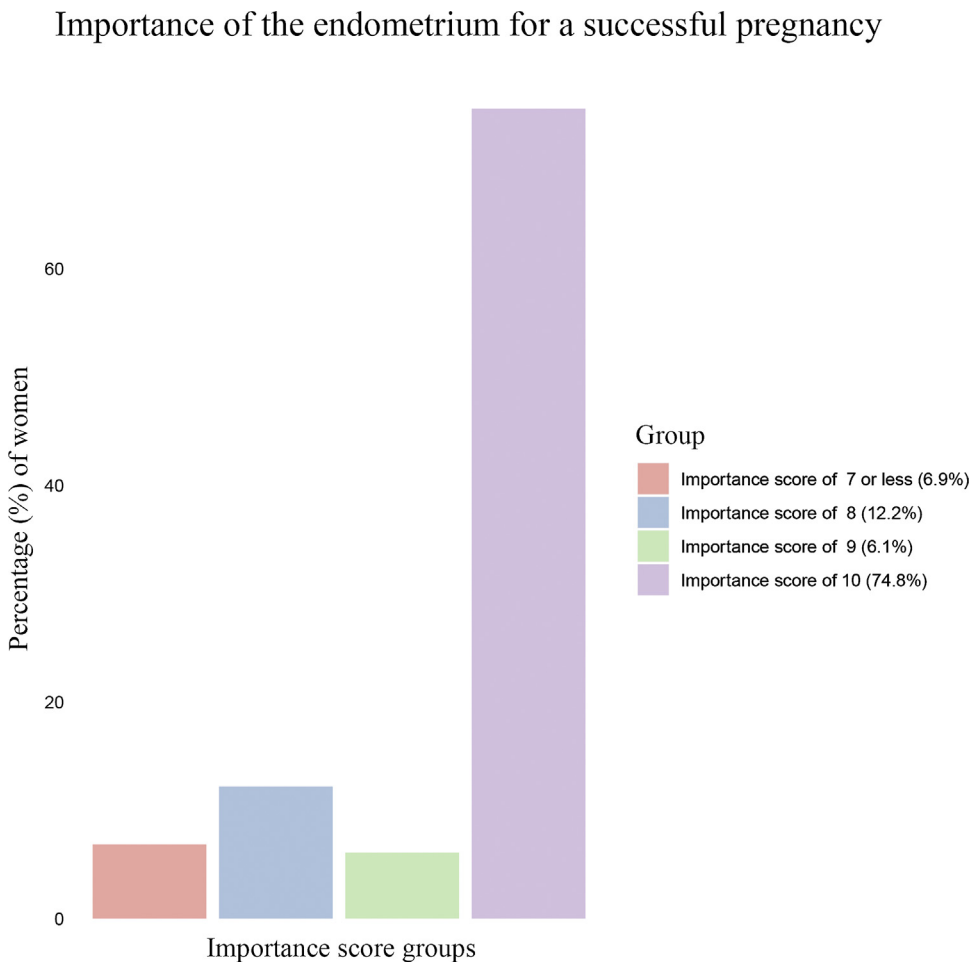

Fig. 1. Importance of the endometrium for a successful pregnancy.

women (12.2%), while 9 women (6.9%) considered the endometrium's importance score of 7 or less (Fig. 1).

3.2 Indication for endometrial testing

Endometrial testing was considered appropriate after the first miscarriage by 25 women (19.1%), after the second miscarriage by 68 women (51.9%), after the third miscarriage by 26 women (19.8%), while 12 women (9.2%) would consider testing the endometrium after four or more miscarriages (Fig. 2).

3.3 Results availability, timing, invasiveness, repetition, validity

Table 1 displays women's responses in relation to important characteristics of an endometrial test.

3.4 Cost of testing

Paying for an endometrial test was not considered by 13 women (9.9%); 49 women (37.4%) would pay up to £100, 32 women (24.4%) would pay £100-200, 29 women (22.1%) would pay £200-500, 5 women (3.8%) would pay £500-1000 and 3 women (2.3%) would pay £1000-2000.

A weak association was identified between the importance score given for the endometrium in achieving a successful pregnancy (Q1) and the willingness to pay for testing; however, there was no evidence to suggest that this correlation was different from 0 (Kendall's tau = 0.1101765, z = 1.4327, p-value = 0.1519; Spearman's rho = 0.1268444, S = 327136, p-value = 0.1488). No associations were identified between the willingness to pay and the indication for endometrial testing (Q2) or the characteristics of the endometrial test (Q3-7) (Table 2).

3.5 Target product profile

Based on women's responses to the questionnaire we identified minimum acceptable ("worst-case") and ideal ("best-case") features of an endometrial test. These are presented in Table 3.

4 Discussion

The vast majority of women understand the important role the endometrium plays in a successful pregnancy. This was highlighted by the high importance scores (8, 9 and 10) allocated by the responders to the role of the endometrium as explored in question 1.

The ideal endometrial test should be performed after the second miscarriage which is in keeping with the definition of recurrent miscarriage according to the European Society of Human Reproduction and Embryology (ESHRE) [10] and the American Society for Reproductive Medicine [11]. The results should become available within one to two days from testing and the timing of the test should not be stricter than three to four specific days within the menstrual cycle which may correspond to the window of

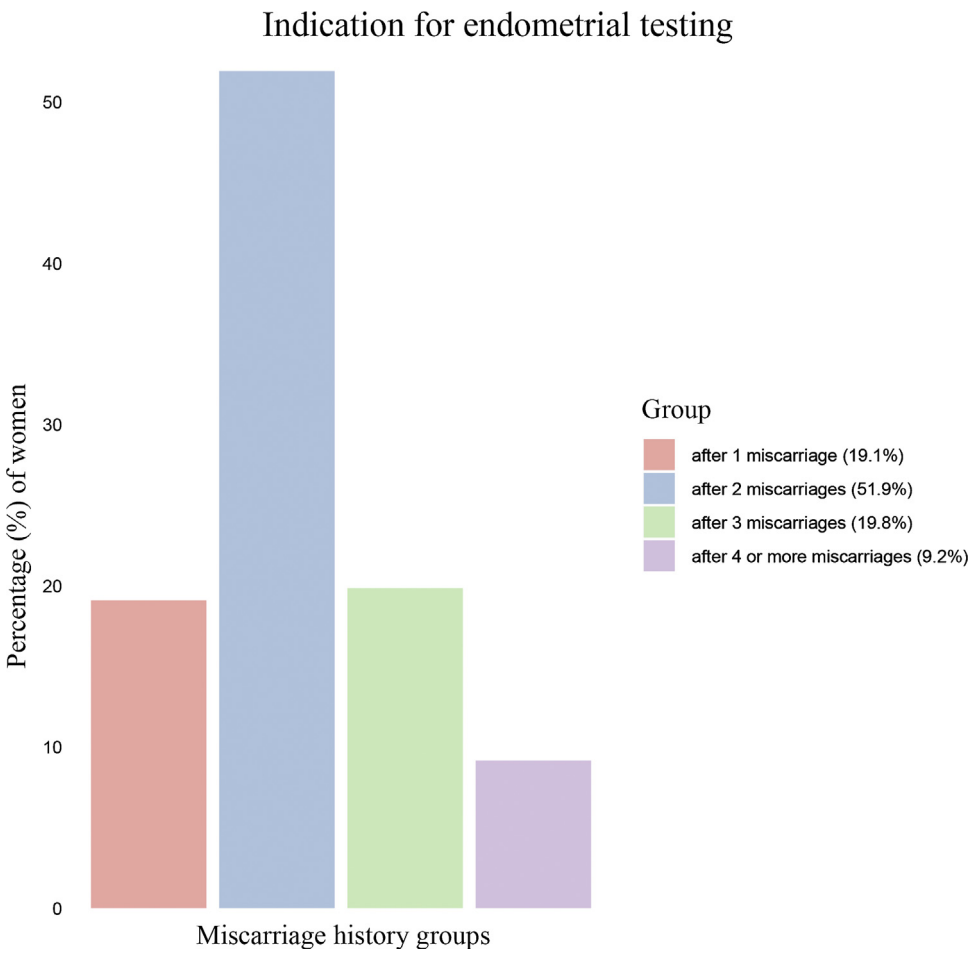

Fig. 2. Indication for endometrial testing.

Table 1
Women's responses to questions 3-7.

Characteristic	Response	Number (%)
Availability of results	On the same day	16 (12.2)
	Within 1-2 days	24 (18.3)
	Within 1-2 weeks	30 (22.9)
	Within the same month	24 (18.3)
	Anytime	37 (28.2)
Strictness of test timing	Exact day and hour	44 (33.6)
	Exact day	42 (32.1)
	Within 3-4 days	36 (27.5)
	Within 10 days	9 (6.9)
Invasiveness of testing	Blood sample	19 (14.5)
	Sample of vaginal discharge	13 (9.9)
	Endometrial biopsy	29 (22.1)
	Hysteroscopy	70 (53.4)
Need for test repetition	Only once	5 (3.8)
	Twice	46 (35.4)
	Three times	48 (36.9)
	Four times or more	31 (23.8)
Validity of the result	Very next menstrual cycle	41 (31.3)
	2-3 menstrual cycles	52 (39.7)
	Up to 6 menstrual cycles	24 (18.3)
	Up to 12 menstrual cycles	5 (3.8)
	More than 12 menstrual cycles	9 (6.9)

implantation. In terms of invasiveness, ideal endometrial testing should not require more than vaginal examinations to collect swab samples. Obtaining an accurate result should not require testing more than twice and the utility of the result should extend to at least six menstrual cycles. In order to be affordable, the endometrial test should cost less than £100.

As minimum acceptable features, the endometrial test could be offered after the third miscarriage which is in accordance with the Royal College of Obstetricians and Gynaecologists' (RCOG) [12] definition for recurrent miscarriage. The results could become available by the end of the month of testing and the timing of the test could be as strict as one particular day in the menstrual cycle. In terms of invasiveness, the test could involve as much as a hysteroscopy to obtain images or samples. Obtaining an accurate result could require testing up to three times as long as the result would be relevant for the next two to three menstrual cycles. The highest acceptable price could be up to £100-200.

Given the weak association identified between the importance score given for the endometrium in achieving a successful pregnancy and the willingness to pay for testing in the absence of evidence to suggest that this correlation was different from 0 we believe the strength of the conclusions are not affected.

4.1 Limitations

The 'best-case' and 'worst-case' features were identified based on responses from unselected women who suffered recurrent miscarriages. While the results are true for this particular group of participants, extrapolation of data to other clinical scenarios requiring endometrial evaluation such as unexplained infertility or assisted reproduction may require caution.

This was an anonymised survey of women's opinions in a recurrent miscarriage clinic and no socio-economic or clinical data

Table 2
Associations between responses to questions 1-7 (indication for testing and important characteristics for an endometrial test) and responses to question 8 (willingness to pay).

	Question 8 – Willingness to pay for an endometrial test	
	Kendall's rank correlation tau	Spearman's rank correlation rho
Question 1 – Importance of the endometrium	z = 1.4327, p-value = 0.1519 tau = 0.1101765 weak association	S = 327136, p-value = 0.1488 rho = 0.1268444 weak association
Question 2 - Indication for testing	z = -1.1364, p-value = 0.2558 tau = -0.08478676 no association	S = 411400, p-value = 0.2652 rho = -0.09805168 no association
Question 3 - Availability of results	z = -0.14898, p-value = 0.8816 tau = -0.01070172 no association	S = 380820, p-value = 0.8521 rho = -0.01644586 no association
Question 4 - Strictness of test timing	z = -0.12419, p-value = 0.9012 tau = -0.009205226 no association	S = 374940, p-value = 0.9931 rho = -0.0007587061 no association
Question 5 - Invasiveness of testing	z = -0.093294, p-value = 0.9257 tau = -0.00697485 no association	S = 375410, p-value = 0.9818 rho = -0.002011407 no association
Question 6 - Need for test repetition	z = -0.028919, p-value = 0.9769 tau = -0.002169637 no association	S = 366820, p-value = 0.9835 rho = -0.001835503 no association
Question 7 - Validity of the result	z = 0.22756, p-value = 0.82 tau = 0.01678856 no association	S = 364880, p-value = 0.7673 rho = 0.02609664 no association

Table 3
Women's perspective on minimum acceptable and ideal targets for an endometrial receptivity test.

Property	Ideal target	Minimum acceptable target
When should the test be indicated?	After two or more miscarriages	After three or more miscarriages
When should results be available?	Within 1-2 days	Within the same month
How strict should the timing be?	To an exact window of 3-4 days within the menstrual cycle	To an exact day within the menstrual cycle
How invasive should the test be?	Not more than having a vaginal swab	Not more than having a hysteroscopy
How many repetitions should the test require?	Not more than two	Not more than three
For how long should the test result be useful?	Up to six menstrual cycles	At least 2-3 menstrual cycles
How much should the test cost?	Up to £100	£100-200

were available. Analyses were not adjusted based on clinical characteristics such as age, number of previous miscarriages, or other aspects that could influence their responses. It may be hypothesised that women of older age, those with higher socio-economic status or those who suffered a higher order miscarriage might be more inclined to accept an endometrial test with suboptimal characteristics.

4.2 Wider implications

The NHS covers the cost of evidence based investigations for recurrent miscarriage such as acquired thrombophilia screening, pelvic ultrasound, cytogenetics and parental karyotype according to the RCOG guideline. The RCOG considers the diagnosis of recurrent miscarriage only after three or more consecutive miscarriages which is stricter than ESHRE and ASRM definitions. This denies investigations for couples who suffered only two or three non-consecutive miscarriages, while the desire to be investigated sooner is reflected in the responses to question two related to the indication of endometrial testing.

The concept of endometrial receptivity testing and its applicability are different for women who suffered recurrent miscarriages compared to those who are due to undergo embryo transfer as part of assisted reproduction. If we accept endometrial receptivity as a constant feature of the endometrium that is unchanged from a menstrual cycle to another, testing it for these two populations would have similar implications based on either a normal (receptive) or abnormal (non-receptive) result.

However, recent research suggests that endometrial development extends beyond the length of a single menstrual cycle [13]. This may involve a cascade of events that are linked from one menstrual cycle to another with potential implications for endometrial receptivity's cycle to cycle variation. In these circumstances, testing for endometrial receptivity in a single cycle may deem the result impractical in the absence of an overall understanding of endometrial receptivity cyclicity. In this scenario, a woman due to undergo embryo transfer would be interested in the exact phase of her endometrial receptivity at the time of transfer, with less importance given to the future cycles. On the other hand, a woman who suffered recurrent miscarriages would be more interested in her overall receptivity, irrespective of the phase linked to her current menstrual cycle.

This will also have implications on the invasiveness of endometrial testing. While vaginal swabs or endometrial fluid aspirates are safe to be performed during the IVF cycle [14,15], hysteroscopy or endometrial biopsy may have negative effects on the outcome of the embryo transfer [16].

The future steps in designing a TPP for an endometrial receptivity test should involve clinicians, researchers and stakeholders to integrate women's wishes with what is possible, useful and cost effective. For instance, the number of test repetitions needed to define the inter-cycle variation of endometrial receptivity may be more important than the quick turnaround of results as long as they remain useful for a longer period following the test.

5 Conclusion

This study shows that women understand the important role the endometrium plays in a successful pregnancy. They would like to be tested after two miscarriages with an aim to predict their endometrial receptivity for at least two to three future menstrual cycles. The invasiveness of testing does not appear to be a limiting factor with the majority of women considering hysteroscopy acceptable. Testing window should be at least one day wide and the results should become available during the month of testing. The majority of women would pay up to £100 for endometrial testing even if the NHS covers most of the evidence based investigations.

Authors' roles

Study design: LC, AC. Ethical, HRA and R&D approvals: LC. Data collection: LC, OP, JC. Data management: LC, OP. Data analysis: JZ. Data interpretation: LC, AC. Manuscript drafting: LC. Final manuscript approval: LC, OP, JC, JZ, AC.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CHAPTER FIVE:

THE TRANSCRIPTOMIC PROFILE OF ENDOMETRIAL RECEPTIVITY IN RECURRENT MISCARRIAGE

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Full length article

The transcriptomic profile of endometrial receptivity in recurrent miscarriage

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ABSTRACT

Objective: To characterise the endometrial transcriptomic profiles of women who suffered recurrent miscarriage and to set the foundation for the development of an endometrial receptivity test that could predict the fate of subsequent pregnancies.

Study design: This was a prospective multicentre cohort study performed at the Tommy's National Centre for Miscarriage Research in Birmingham, Saint Mary's Hospital in Manchester and Royal Devon & Exeter Hospital, United Kingdom. The study was conducted between December 2017 and December 2019. Endometrial biopsies were obtained during the window of implantation from 24 women aged 18–35 years, who were not pregnant and regularly menstruating, diagnosed with unexplained recurrent miscarriage by standard investigations as per the ESHRE guidelines. Exclusion criteria included risk factors such as smoking, obesity or hyperprolactinemia. The RNA transcripts abundances were quantified using *Kallisto*. R packages *tximport* and *DESeq2* were used to summarize count estimates at the gene level and to analyse the differential gene expression.

Results: Women who suffered four or more miscarriages had 19 differently expressed genes after adjustment for multiple comparisons. They were related to biological processes such as immunity (*HLA-DMA*, *CCR8*, *ALOX5*), energy production (*ATP12A*), hormone secretion (*CGA*), adhesion (*CHAD*, *ADGRF2*, *AQP5*, *TBCD*, *CTNND1*, *NKD2*) and cell proliferation (*NCCRP1*). Based on 421 differently expressed genes, women who achieved a subsequent live birth displayed an enrichment of processes related to the regulation of cell structure and proliferation, and a depletion of processes related to immunity, trans-membrane transport and coagulation.

Conclusions: Women in the extreme miscarriage cohort had a distinctive endometrial transcriptomic signature compared to women with low order miscarriages. There was a partial overlap with the transcriptome of asynchronous endometrium suggesting the endometrial factor to be a different entity in the context of recurrent miscarriage. Women who achieved a live birth in their subsequent pregnancy displayed an enrichment of genes related to the regulation of cell structure and proliferation, while women who suffered a subsequent miscarriage displayed an enrichment of genes related to immunity, trans-membrane transport and coagulation.

Introduction

Miscarriage represents the spontaneous demise of a pregnancy before the fetus reaches viability. It is a common complication affecting 25 % of clinically recognised pregnancies, while 50 % cease development at pre-clinical stages through implantation failure or biochemical miscarriage [1]. The vast majority of miscarriages occur in the first trimester, with fewer than 10 % occurring in the second trimester of pregnancy [2]. Up to 5 % of women suffer recurrent miscarriage defined as 2−3 or more (depending on the defining organisation) miscarriages leading to physical, emotional and financial consequences for women and their families, doctors and health services [3,4].

It has been hypothesized that the largest single cause of failed pregnancy is an error of implantation, the rate of which may be as

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high as 78 % in humans [5]. Establishment of successful pregnancy depends upon implantation, involving complex interactions between the endometrium and the blastocyst. It is well accepted that the window of implantation is a narrow time frame with maximal endometrial receptivity, surrounded by a refractory endometrial status [6,7].

Endometrial receptivity and the features of the window of implantation have been the focus of continuous research for over eight decades, since Rock and Bartlett [8] described the histological characteristics of the endometrium around the time of implantation; however, current clinical practice lacks an accurate test of endometrial receptivity to allow the prediction of successful pregnancy [9].

Transcriptomics refer to the comprehensive analysis of the complete set of RNA transcripts that are produced by the genome in a biological specimen and holds promise to inform the practice of precision medicine. Over twenty studies have already investigated the endometrial transcriptome during natural and stimulated cycles to enable the characterisation of up- and down-regulated genes during the window of implantation [10]. The efforts of developing clinical tools have culminated with the commercialisation of diagnostic tests for endometrial receptivity such as the Win-Test [11], Endometrial Receptivity Array [12], Endometrial Receptivity Map [13] and ERPeak Endometrial Receptivity Test [14]. However, none of the studies have targeted women who suffered recurrent miscarriage and their applicability is limited to the context of assisted reproduction with personalised embryo transfer.

The objective of this study was to characterise the endometrial transcriptomic profiles of women who suffered recurrent miscarriage and to set the foundation for the development of an endometrial receptivity test that could predict the fate of subsequent pregnancies.

Methods

Setting

The study was performed in the setting of the Tommy's National Centre for Miscarriage Research in Birmingham, United Kingdom (UK) in collaboration with Saint Mary's Hospital in Manchester, Royal Devon & Exeter NHS Foundation Trust, and Genomics Birmingham.

Tommy's National Centre for Miscarriage Research is a tertiary care centre attended by patients from all over the UK following referral from General Practitioners (GPs) or secondary care hospitals within the National Health Service (NHS). It provides medical care for couples who suffered recurrent miscarriages, in the form of counselling, investigations and treatments, and expands the understanding of miscarriage pathophysiology by undertaking relevant research projects with an overarching aim of reducing the number of miscarriages.

Study population and design

This was a prospective multicentre cohort study conducted between December 2017 and November 2019. Women attending the recurrent miscarriage clinics at the recruiting sites were invited to participate if they were diagnosed with unexplained recurrent miscarriage based on strict criteria. An endometrial biopsy was obtained during the window of implantation timed based on the luteinising hormone (LH) surge. The transcriptomic profiles of the endometrial samples were then analysed according to the protocol.

Inclusion and exclusion criteria

The aim of the strict inclusion and exclusion criteria was to identify women with a narrow phenotype at high risk of suffering from an undiagnosed endometrial cause for their recurrent miscarriages by excluding other known causes and risk factors for miscarriage. Women were aged between 18 and 35 years and suffered two or more miscarriages in the first trimester of pregnancy. They underwent routine investigations such as full blood count, lupus anticoagulant, anticardiolipin antibodies, beta-2 glycoprotein 1 antibodies, thyroid function testing, thyroid peroxidase (TPO) antibodies, and pelvic ultrasound. Women and their partners underwent parental karyotyping if they had a history of five or more miscarriages. Additional investigations were performed to exclude other pathologies on a case by case basis. For instance, women with breast symptoms such as discharge had a prolactin test to exclude hyperprolactinemia, while women with inter-pregnancy interval over one year had a follicle-stimulating hormone (FSH) test to exclude significantly low ovarian reserve.

Women were excluded if they had risk factors for miscarriage such as body mass index (BMI) over 35, irregular menstrual cycles, polycystic ovarian syndrome (PCOS), endometriosis, smoking or heavy drinking, or if the miscarriages occurred following fertility treatments. Due to the nature of the study, we excluded women who were pregnant and those who participated in other interventional studies at the time of the planned endometrial biopsy.

Timing and processing of the endometrial biopsy sample

Women were provided with urine LH kits (One Step® Ovulation Test, AI DE Diagnostic Co. Ltd.) and instructed to select a menstrual cycle at least three months following any hormonal treatment or pregnancy. They were advised on the importance of avoiding a pregnancy in the study cycle by abstaining from sexual intercourse or by using condoms.

The endometrial biopsy was timed in the 7–11 days window following the LH surge which correlates with the window of implantation. Women underwent a pelvic ultrasound to measure the endometrial thickness on the day of the procedure. The endometrial sample was obtained using an endometrial suction curette (Pipelle®, CooperSurgical, Inc.) following the removal of the cervical mucus. The endometrial sample was placed in a sterile cryogenic vial containing RNA*later* (Thermo Fisher®) and transported on ice to be stored at −80 °C until recruitment was complete.

RNA was extracted using RNeasy Plus Micro Kit (QIAGEN). Samples were submitted in tubes and the concentration checked on RNA HS Qubit assay, and the quality checked on a RNA Tape on the TapeStation system (Agilent). All libraries were normalised to 10 nM, pooled and an additional AmpureXP bead clean up performed on this pool and eluted in 25 ul of resuspension buffer. RNA sequencing (RNA-seq) was performed using Illumina NextSeq 500 High throughput technology and outputs were mapped to Genome Reference Consortium Human Build 38 (GRCh38.p13).

Data analysis

Two main comparisons were predefined in the protocol based on the number of previous miscarriages and based on the outcome of the subsequent pregnancy. Firstly (a), the endometrial transcriptomic profiles of women who suffered low order miscarriages were compared with those of women who suffered high order miscarriages. It is well accepted that sporadic embryo aneuploidy is the most common cause of miscarriage; however, the frequency of normal embryonic karyotypes significantly increases with the number of previous abortions with a cut-off at four to five miscarriages [15]. This implies the existence of a potential persistent cause other than embryo aneuploidy for women who suffer high order miscarriages and thus defining the extreme recurrent miscarriage group.

Secondly (b), women who conceived within one year following the endometrial sampling were followed up until the end of their subsequent pregnancy. The endometrial transcriptomic profiles of women who achieved a live birth were compared with those of women who suffered another miscarriage.

FASTQ files were downloaded from Basespace. In total there were two runs with four lanes per run across each sample. Firstly, we merged lanes per sample and then we merged runs per sample to have two paired-end FASTQ files that describe a sample. We then used *Kallisto* [16] program to quantify abundances of transcripts from bulk RNA-seq samples. *Kallisto* uses pseudoalignment in order to rapidly determine the compatibility of reads with targets. The main quantification/abundances are reported in transcripts per million (TPMs) and as estimated counts.

Kallisto provides estimates of transcript level counts, thus we used *tximport* [17] R package to summarize count estimates at the gene level. To discover quantitative changes in gene expression levels between experimental groups given in (a) and (b) settings, we have used statistical testing. Therefore differential gene expression analysis was performed using *DESeq2* package in R that determines whether mean expression levels of different sample categories are significantly different [18]. For this we first pre-filtered low count genes and kept rows that had at least 10 reads in total. We have used default settings with Benjamini-Hochberg adjustment for multiple testing problem, which provides an adjusted p value for each gene. We have set a fraction of 10 % (adjusted p value <0.1) false positives to be acceptable threshold. Significant genes were then sorted by *log2* fold change to get the significant genes with the strongest down- or up-regulation.

AmiGO [19] and GeneTrail [20] were used to explore gene ontologies and to perform enrichment analyses, respectively.

Ethical approval

The study was sponsored by the University of Birmingham. It was funded by Tommy's Charity and approved by West Midlands – South Birmingham Research Ethics Committee (reference 17/WM/0382). The protocol was registered on ClinicalTrials.gov (NCT03442335). Participants provided written consent to the use of their biological samples before participation.

Results

We obtained endometrial samples from 24 women with a mean age of 30 years. They suffered a number of miscarriages ranging from two to eleven. Eighteen women conceived within twelve months from the endometrial biopsy. Participants' characteristics are presented in Table 1.

Low order miscarriage versus high order miscarriage

The endometrial transcriptomic profiles of women who suffered four or more miscarriages (extreme cohort) were different compared to women who suffered two or three miscarriages (control group) (Supplementary Table 1).

The four up-regulated genes (*ALOX5*, *NKD2*, *CPNE8*, and *NUDT10*) are involved in processes such as folate metabolism and eicosanoid synthesis, cell signalling and regulation of intracellular trafficking. The *log2* fold change for up-regulated genes ranged from 0.8 for *NUDT10* to 3.49 for *ALOX5*.

The fourteen down-regulated genes (*CTNND1*, *TBCD*, *HLA-DMA*, *HIST1H3E*, *GAD1*, *ZNF773*, *CCR8*, *AQP5*, *NCCRP1*, *ADGRF2*, *CHAD*, *CGA*, *ATP12A*, and *HLA-DMA*) are involved in processes such as cell adhesion, immune response, DNA and proteins structure, and transmembrane transport. The *log2* fold change for down-regulated genes ranged from -5.35 for *HLA-DMA* to -0.34 for *CTNND1*.

When the threshold to define the extreme cohort was set at five recurrent miscarriages, the transcriptomic profiles differed by two genes that were up-regulated in the high order miscarriage group. *MTND6P4* is a pseudogene and *NPL* regulates cellular concentrations of sialic acid.

Live birth versus miscarriage in subsequent pregnancy

The endometrial transcriptomic profiles of women who achieved a live birth were different compared to women who suffered a miscarriage in the subsequent pregnancy following endometrial biopsy. There were 164 up-regulated and 257 down-regulated genes in the endometrial samples obtained from women who achieved a live birth (Supplementary Table 2). Fig. 1 displays the heatmap of the 20 most up-regulated and the 20 most down-regulated genes plotted against pregnancy outcome.

Over-representation analysis of up-regulated genes identified enrichment in five molecular functions, twelve biological processes, and nine cellular components (Supplementary Table 3). Over-representation analysis of down-regulated genes identified enrichment in eight molecular functions, 77 biological processes, and 30 cellular components (Supplementary Table 4). Supplementary Figs. 1 and 2 display the volcano plots of enrichment results for up-regulated and down-regulated genes, respectively.

Discussion

Endometrial transcriptomics have been widely investigated over the last decade with the primary interest being the identification of specific transcriptomic signatures that may diagnose the receptive function and improve the effectiveness of reproductive treatments [10]. Various transcriptomics based tools are being used in clinical practice in conjunction with personalised embryo transfer; however, none of the available diagnostic tests are applicable to women who aim to conceive naturally.

We have reported the results of the first endometrial transcriptomics study applied in the context of unexplained recurrent miscarriage in order to characterise the endometrial signature of the extreme miscarriage cohort. Additionally, we aimed to set the foundations of an endometrial receptivity test that could predict the fate of subsequent pregnancies by comparing the endometrial signature of women who achieved a live birth with those who suffered another miscarriage.

Table 1
Characteristics of women undergoing endometrial biopsy and transcriptomics analysis. RM = recurrent miscarriage; LH = day of luteinising hormone surge; N = number.

	2–3 RM (N = 9)	≥4 RM (N = 15)	Live birth (N = 11)	Miscarriage (N = 7)
Mean age (years)	31.8	28.9	29.5	29.8
Mean BMI (Kg/m^2)	27.8	24.4	24.9	28.2
Median biopsy day	LH + 8	LH + 8	LH + 8	LH + 8
Mean endometrial thickness (mm)	9.6	9.5	9.8	9.9
Median duration to conception (cycles)	–	–	2	2

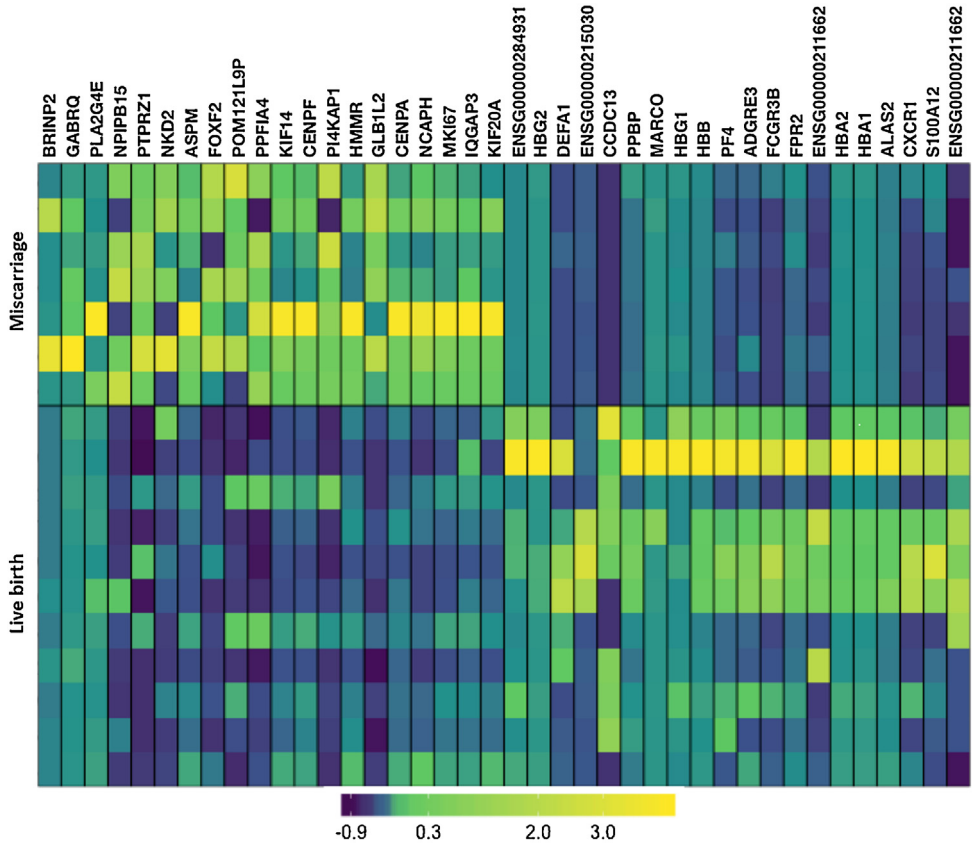

Fig. 1. Heatmap of the 20 most up-regulated and the 20 most down-regulated genes in the mid-secretory endometrium of women who suffered recurrent miscarriage based on the outcome of the subsequent pregnancy. The top rows represent women who suffered another miscarriage, the bottom rows represent women who achieved a live birth.

The extreme miscarriage cohort

The threshold for suspecting an endometrial factor in the extreme unexplained recurrent miscarriage cohort appears to be four miscarriages. Previous research by Ogasawara [15] identified a steep increase in the frequency of miscarriages from non-chromosomal causes in women who have suffered four or five miscarriages. We have reported on 19 differentially expressed genes in the endometrium of women who suffered four or more miscarriages as opposed to one gene and one pseudogene when the threshold was set at five miscarriages.

Previous RNA-seq based endometrial transcriptomic studies identified a 1:1 ratio of up- and down-regulated genes between the pre-receptive and receptive endometrium [21,22]. They analysed serial endometrial biopsies from healthy women and identified 2372 and 3297, respectively, protein coding genes that were differentially expressed. The ratio of up- and down-regulated genes in the present extreme miscarriage cohort was 1:3 and only 6 out of 19 differentially expressed genes were previously reported (*NUDT10*, *GAD1*, *AQP5*, *NCCRP1*, *ADGRF2*, and *ATP12A*). This may suggests that recurrent miscarriage due to a potential endometrial factor does not overlap with asynchronous endometrium and it is likely to represent a different entity.

Predicting the fate of subsequent pregnancies

Investigating the risk of recurrence has been identified as a miscarriage research priority by a recent James Lind Alliance priority setting partnership [23]. We have followed women up until the completion of their subsequent pregnancy and we have compared the endometrial transcriptomic signatures based on the outcome of the pregnancy. The endometrium of women who achieved a live birth had 421 genes that were differentially expressed compared to women who suffered another miscarriage. Ninety-two of these genes have been previously reported by Hu [21] and 114 by Sigurgeirsson [22] with an overlap of 65 genes (Supplementary Table 5).

This set of genes could be used as a training dataset for an algorithm aimed at predicting the fate of subsequent pregnancies. Further research should provide a validation dataset to assess the prediction of outcomes based on the fitted model [24].

Implications for translational medicine

Gene function analysis identified a broad range of molecular functions, biological processes and cellular components linked to the differentially expressed genes based on the outcome of subsequent pregnancies. They may serve as potential targets for future interventions aimed at reducing the risk of subsequent miscarriage.

For instance, biological processes involved in the regulation of cell structure and proliferation (regulation of cell cycle, cell division, regulation of mitotic cell cycle, chromosome organization, microtubule-based process, regulation of cell cycle process, nuclear chromosome segregation, sister chromatid segregation, microtubule cytoskeleton organization, microtubule cytoskeleton organization involved in mitosis, chromosome segregation, mitotic cytokinesis) appear to be enriched in women who subsequently achieve a live birth. Similar observations were made by Huang [25] who investigated the mid-luteal endometrial transcriptomes of women who suffered recurrent miscarriage and recurrent implantation failure in comparison to fertile controls.

On the contrary, biological processes involved in immunity (immune system process, myeloid leukocyte activation, neutrophil

activation involved in immune response, neutrophil degranulation neutrophil activation, leukocyte degranulation, leukocyte activation, leukocyte migration, immune response-activating signal transduction, immune response-regulating signaling pathway, immune response-activating cell surface receptor signaling pathway, immune response, activation of immune response, innate immune response, immune response-regulating cell surface receptor signaling pathway, granulocyte chemotaxis, leukocyte chemotaxis), trans-membrane transport (vesicle-mediated transport, regulated exocytosis, exocytosis, negative regulation of execution phase of apoptosis, secretion by cell) and coagulation (coagulation, blood coagulation, hemostasis) appear to be enriched in women who subsequently miscarry. Similar observations were made by Lédée [26] who investigated the mid-luteal endometrial transcriptomes of women who suffered recurrent miscarriage and recurrent implantation failure in comparison to fertile controls.

Strengths and limitations

The primary strength of our study is the narrow phenotype of the included women. It is well accepted that miscarriage is multifactorial and selection of participants is essential in interpreting study results [27]. We were particularly stringent with the inclusion criteria to exclude known causes and risk factors for miscarriage and thus enabling the selection of women with a potential endometrial factor identifiable through transcriptomic analysis. This counterbalances the relative small sample size (N = 24 women with recurrent miscarriage and 18 subsequent conceptions) and improves statistical relevance.

The biopsies were timed accurately using LH kits aiming for the days with the highest endometrial receptivity within the window of implantation. The endometrium changes very rapidly around the time of implantation and improperly timed biopsies could introduce a significant source of variance to the results [25].

We used RNA-seq rather than micro-array to analyse the endometrial tissue. Earlier studies investigating endometrial transcriptomics used predominantly micro-array [10] which profiles predefined transcripts through hybridization while RNA-seq allows for full sequencing of the whole transcriptome facilitating the acquisition of additional informative data for mechanistic investigations or biomarker discovery.

The control group for the first comparison included women who had low order miscarriages (two or three). One might argue that they are part of the interest group; however, we assumed that the extreme phenotype is defined by high order unexplained miscarriages, while women in the control group were more likely to have had a chromosomal cause for their low order miscarriages [15]. Selecting known fertile or parous women might have induced bias due to the potential endometrial changes caused by the normal pregnancies.

Endometrial transcriptomics testing complies with the wishes of women expressed in a recent survey for the development of a target product profile for endometrial testing [28]. It may be performed in a window of three to four days of the menstrual cycle, results may become available within one to two days from sample collection, and the invasiveness does not extend beyond a vaginal examination. Further research should clarify the reproducibility and the length of time for the validity of results.

Conclusion

We have performed a high-throughput transcriptomic analysis of mid-secretory endometrial samples from women who suffered unexplained recurrent miscarriages. Women in the extreme miscarriage cohort had a distinctive endometrial transcriptomic signature compared to women with low order miscarriages. There was a partial overlap with the transcriptome of asynchronous endometrium suggesting the endometrial factor to be a different entity in the context of recurrent miscarriage.

Women who achieved a live birth in their subsequent pregnancy displayed an enrichment of genes related to the regulation of cell structure and proliferation, while women who suffered a subsequent miscarriage displayed an enrichment of genes related to immunity, trans-membrane transport and coagulation.

Authors' roles

Study design: LC, AC. Ethical, HRA and R&D approvals: LC. Data collection: LC, OP, JC, MC. Data management: LC, OP. Samples processing, data analysis and interpretation: JZ, LC. Manuscript drafting: LC. Final manuscript approval: LC, OP, JC, MC, JZ, AC.

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The study was funded by Tommy's Charity and there are no competing interests.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2021.04.041.

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CHAPTER SIX:

THE METABOLOMIC PROFILE OF ENDOMETRIAL RECEPTIVITY IN RECURRENT MISCARRIAGE

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1. Introduction

Miscarriage is defined as the spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age according to The International Glossary on Infertility and Fertility Care [1]. It is the most common complication of pregnancy affecting 25% of clinically recognised conceptions, while 50% fail to continue development past pre-clinical stages through implantation failure or biochemical miscarriage [2]. Up to 5% of women suffer recurrent miscarriage defined as two or more miscarriages leading to physical, emotional and financial consequences for women and their families, doctors and health services [1, 3, 4].

Embryonic chromosome abnormalities are the most common cause of first trimester miscarriage. In addition, maternal endocrine (diabetes, thyroid disease), immunological (lupus), thrombophilic or anatomical (uterine polyp, fibroid, septum) conditions may trigger miscarriages and could account for half of the miscarriages [5]. However, the remaining half of the miscarriages do not have a cause identifiable with the current clinical diagnostic tests.

It has been hypothesised the largest single cause of failed pregnancy to be an error of implantation, the rate of which may be as high as 78% in humans [6]. Establishment of successful pregnancy depends upon implantation, involving complex interactions between the endometrium and the blastocyst. It is well accepted that the implantation window is a narrow time frame with maximal endometrial receptivity, surrounded by a refractory endometrial status [7]. Endometrial receptivity and the features of the window of implantation have been the focus of continuous research for over eight decades; however, current clinical practice lacks an accurate test of endometrial receptivity to allow the prediction of successful pregnancy [8].

Metabolomics refer to the comprehensive analysis of metabolites in a biological specimen and holds promise to inform the practice of precision medicine. Current

metabolomic technologies are capable of precise analyses of hundreds to thousands of metabolites to enable detailed characterization of metabolic phenotypes [9]. They are more informative than genomics, transcriptomics, or proteomics, because they denote the final products of the cell metabolism and are closer to the functional phenotype [10].

Metabolomics studies can be divided into discovery or hypothesis generating and targeted or hypothesis testing. On the one hand, discovery studies enable the (semi)-quantitative (global) detection of a wide range of metabolites and data acquisition without *a priori* knowledge of the biologically interesting metabolites. They provide a phenotypic readout comprising of hundreds to thousands of metabolites requiring identification post data acquisition [11]. On the other hand, targeted or semi-targeted studies rely on the quantification of a smaller number of (related) metabolites whose identity is already known [12].

In the context of human reproduction, metabolomic studies have focused on the assessment of oocyte quality and embryo viability in vitro [13, 14], and diagnosis of endometriosis [15]. Lipidomics, a subset of metabolomics, have been used to characterise the prostaglandin expression in human endometrium [16].

The objective of this study was to characterise the endometrial metabolomic profiles of women who suffered recurrent miscarriage using discovery metabolomics and to set the foundation for the development of an endometrial receptivity test.

2. Materials and methods

2.1 Setting

The study was performed in the setting of the Tommy's National Centre for Miscarriage Research in Birmingham, United Kingdom (UK) in collaboration with Saint

Mary's Hospital in Manchester, Royal Devon & Exeter NHS Foundation Trust, and Phenome Centre Birmingham.

Tommy's National Centre for Miscarriage Research is a tertiary care centre attended by patients from all over the UK following referral from General Practitioners (GPs) or secondary care hospitals within the National Health Service (NHS). It provides medical care for couples who suffered recurrent miscarriages, in the form of counselling, investigations and treatments, and expands the understanding of miscarriage pathophysiology by undertaking relevant research projects with an overarching aim of reducing the number of miscarriages.

Phenome Centre Birmingham offers a complete collaborative service to academic, industry, and government partners, providing expertise and advice in metabolic phenotyping studies from conception and experimental design through data acquisition to data analysis and biological interpretation.

2.2 Study population and design

This was a prospective multicentre cohort study conducted between December 2017 and November 2019. Women attending the recurrent miscarriage clinics at the recruiting sites were invited to participate if they were diagnosed with unexplained recurrent miscarriage based on strict criteria. An endometrial biopsy was obtained during the window of implantation timed based on the luteinising hormone (LH) surge. The metabolomic profiles of the endometrial samples were then analysed according to the protocol.

2.3 Inclusion and exclusion criteria

As previously described [17], the aim of the strict inclusion and exclusion criteria was to identify women with a narrow phenotype at high risk of suffering from an undiagnosed endometrial cause for their recurrent miscarriages by excluding other known causes and risk factors for miscarriage. Women were aged between 18 and 35 years and suffered two or more

miscarriages in the first trimester of pregnancy. They underwent routine investigations such as full blood count, lupus anticoagulant, anticardiolipin antibodies, beta-2 glycoprotein 1 antibodies, thyroid function testing, thyroid peroxidase (TPO) antibodies, and pelvic ultrasound. Women and their partners underwent parental karyotyping if they had a history of five or more miscarriages. Additional investigations were performed to exclude other pathologies on a case-by-case basis. For instance, women with breast symptoms such as discharge had a prolactin test to exclude hyperprolactinemia, while women with inter-pregnancy interval over one year had a follicle-stimulating hormone (FSH) test to exclude significantly low ovarian reserve.

Women were excluded if they had risk factors for miscarriage such as body mass index (BMI) over 35, irregular menstrual cycles, polycystic ovarian syndrome (PCOS), endometriosis, smoking or heavy drinking, or if the miscarriages occurred following fertility treatments. Due to the nature of the study, we excluded women who were pregnant and those who participated in other interventional studies at the time of the planned endometrial biopsy.

2.4 Timing and processing of the endometrial biopsy sample

Women were provided with urine LH kits (One Step® Ovulation Test, AI DE Diagnostic Co. Ltd.) and instructed to select a menstrual cycle at least three months following any hormonal treatment or pregnancy. They were advised on the importance of avoiding a pregnancy in the study cycle by abstaining from sexual intercourse or by using condoms.

The endometrial biopsy was timed in the 7 to 11 days window following the LH surge which correlates with the window of implantation. Women underwent a pelvic ultrasound to measure the endometrial thickness on the day of the procedure. The endometrial sample was obtained using an endometrial suction curette (Pipelle®, CooperSurgical, Inc.) following the

removal of the cervical mucus. The endometrial sample was placed in a sterile cryogenic vial and transported on ice to be stored at -80 °C.

The metabolite composition and relative concentrations of samples were analysed applying ultra-high performance liquid chromatography-mass spectrometry to investigate water-soluble and lipid metabolites. Raw data were processed applying XCMS and statistical analysis was applied using the software MetaboAnalyst. Metabolite annotation was performed using the software PUTMEDID_LCMS.

2.5 Data analysis

Two main comparisons were predefined in the protocol based on the number of previous miscarriages and based on the outcome of the subsequent pregnancy. Firstly, the endometrial metabolomic profiles of women who suffered low order miscarriages were compared with those of women who suffered high order miscarriages. It is well accepted that sporadic embryo aneuploidy is the most common cause of miscarriage; however, the frequency of normal embryonic karyotypes significantly increases with the number of previous abortions with a cut-off at four to five miscarriages [18]. This implies the existence of a potential persistent cause other than embryo aneuploidy for women who suffer high order miscarriages and thus defining the extreme recurrent miscarriage group.

Secondly, women who conceived within one year following the endometrial sampling were followed up until the end of their subsequent pregnancy. The endometrial metabolomic profiles of women who achieved a live birth were compared with those of women who suffered another miscarriage.

3. Results

We obtained endometrial samples from 24 women with a mean age of 30 years. They suffered a number of miscarriages ranging from two to eleven. Eighteen women conceived

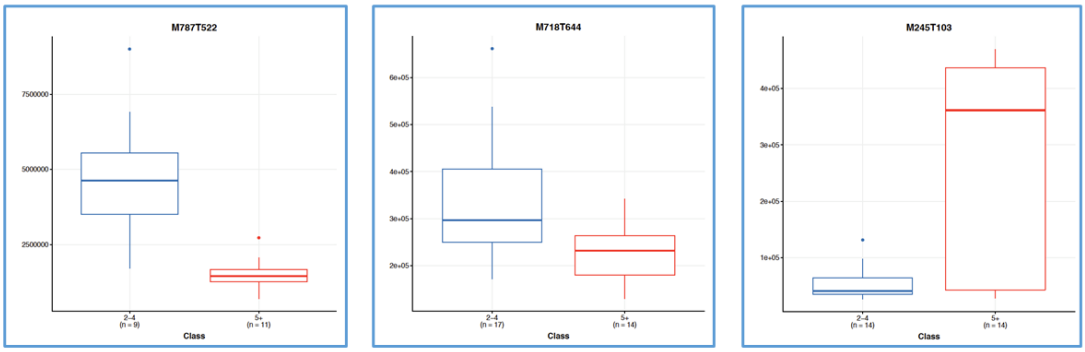
within twelve months from the endometrial biopsy. Processing and analysis of all samples was feasible and results account for all the data. Participants' characteristics are presented in Table 1.

Table 1: Characteristics of women undergoing endometrial biopsy and metabolomics analysis. RM = recurrent miscarriage; LH = day of luteinising hormone surge; N = number.

	2-4 RM (N=17)	**≥5 RM (N=7)**	**Live birth (N=11)**	**Miscarriage (N=7)**
Mean age (years)	30.3	29.4	29.5	29.8
Mean BMI (Kg/m^2)	26.7	23.3	24.9	28.2
Median biopsy day	LH + 8	LH + 8	LH + 8	LH + 8
Mean endometrial thickness (mm)	9.4	9.9	9.8	9.9
Median duration to conception (cycles)	-	-	2	2

3.1 Low order miscarriage (two to four) versus high order miscarriage (five or more)

There was increased lipolysis of triacylglycerol (TAGs) and diacylglycerols (DAGs) to release fatty acids such as tetradecadienoic acid at higher order miscarriages compared to lower order miscarriages (Figure 1).



Triacylglyceride (48:2) | Diacylglyceride (44:1) | Tetradecadienoic acid

Figure 1: Box plots of triacylglyceride(48:2), diacylglyceride(44:1) and tetradecadienoic acid levels based on the number of previous miscarriages (2-4 versus 5 or more).

There was mitochondrial dysfunction and reduced medium-chain fatty acid beta-oxidation to synthesis ATP at higher order miscarriages compared to lower order miscarriages (Figure 2).

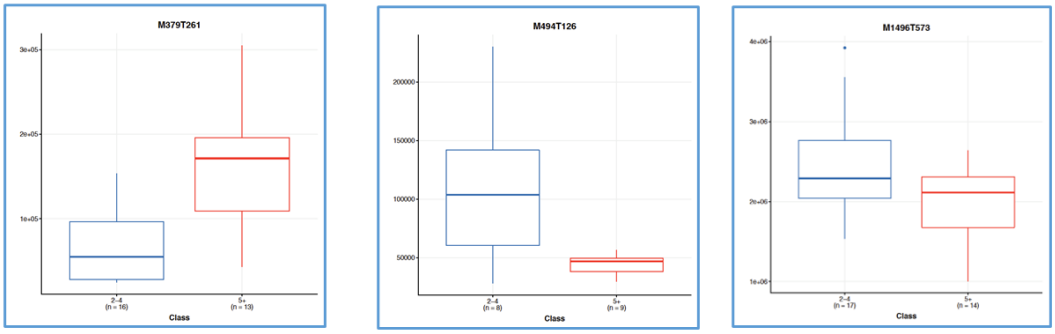


Figure 2: Box plots of decanoylcarnitine, stearoylcarnitine and cardiolipin(76:12) levels based on the number of previous miscarriages (2-4 versus 5 or more).

The oxidised form of redox-active flavin co-factors are more prevalent and purine and pentose phosphate pathway (PPP) metabolites are reduced in concentration at higher order compared to lower order miscarriages. Heme was decreased and heme degradation products were increased at higher order miscarriages.

Oxidised form of redox-active NADP co-factors are present at higher concentration and tryptophan metabolites are present at reduced concentration in higher order compared to lower order miscarriages.

3.2 Live birth versus miscarriage in subsequent pregnancy

The metabolic balance between cholesterol and cholesterol sulfate was perturbed in subsequent live birth versus miscarriage comparison (Figure 3).

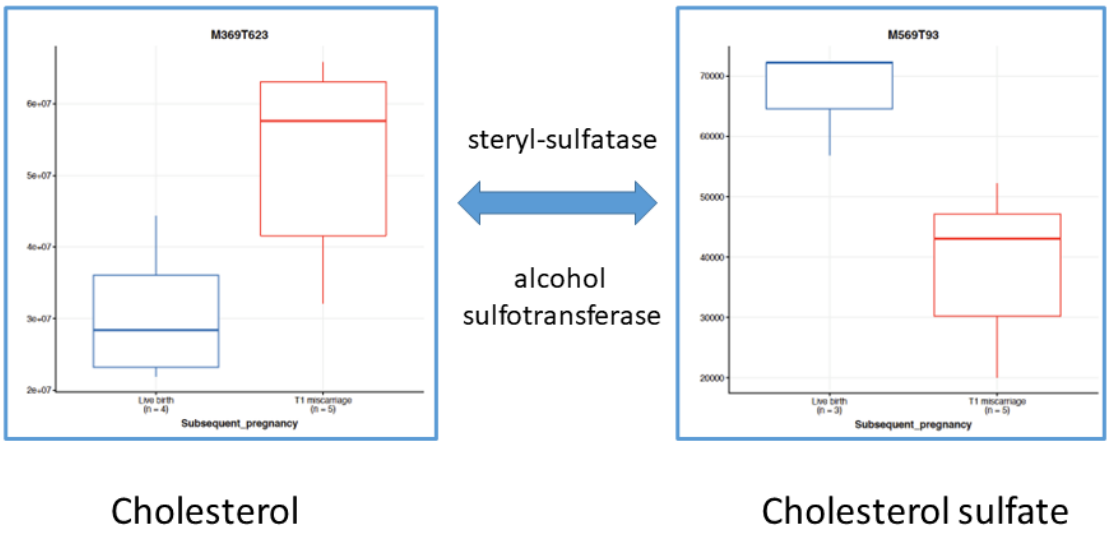


Figure 3: Box plots of cholesterol and cholesterol sulphate levels in subsequent live birth versus miscarriage.

There was decreased lipolysis of TAGs and increased lipolysis of DAGs to release fatty acids such as decenedioic acid in subsequent live birth compared to miscarriage (Figure 4).

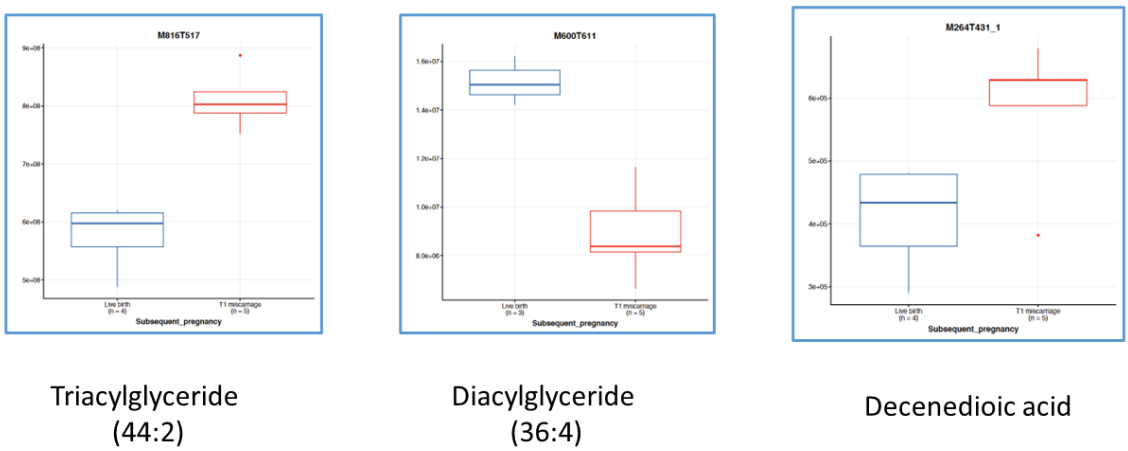


Figure 4: Box plots of triacylglyceride(44:2), diacylglyceride(36:4) and decenedioic acid levels in subsequent live birth compared to miscarriage.

There was mitochondrial dysfunction and reduced medium-chain fatty acid beta-oxidation to synthesis ATP in subsequent live birth compared to miscarriage (Figure 5).

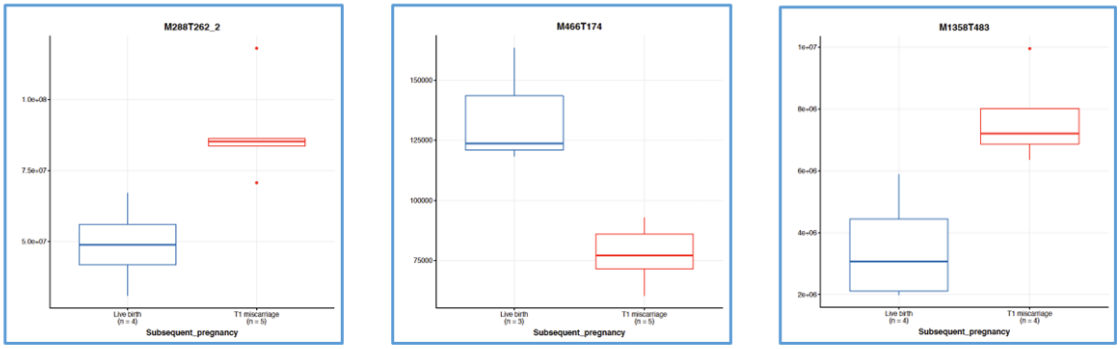


Figure 5: Box plots of octanoylcarnitine, stearoylcarnitine and cardiolipin(60:4) levels in subsequent live birth compared to miscarriage.

4. Discussion

Various studies attempted to assess endometrial receptivity based on the levels of single molecules such as integrins [19], vascular endothelial growth factor (VEGF) [20], matrix metalloproteinases and E-cadherin expression [21], hCG-LH receptor [22], leukemia inhibitory factor (LIF) [23], or L-selectin ligand [24]; however, the emerging evidence was not convincing based on their poor ability to predict clinical pregnancy [8]. Single molecule testing is unlikely to be successful in describing the complexity of endometrial receptivity [25] given the dynamic and cyclic nature of the endometrial changes [26].

We have conducted the first endometrial metabolomics discovery study to investigate endometrial receptivity using ultra-high performance liquid chromatography-mass spectrometry in the context of recurrent miscarriage. We identified metabolic perturbations

associated with the observation of having suffered high order miscarriages and with achieving a live birth in the subsequent pregnancy.

Lipolysis is the biochemical pathway responsible for the mobilisation of stored energy from cytosolic lipid droplets by the catabolic hydrolysis of triacylglycerols (TAGs) to diacylglycerols (DAGs), monoacylglycerol (MAG) and glycerol [27]. Hormone-sensitive lipase (HSL) is rate-limiting for DAGs catabolism within the TAGs hydrolysis cascade [28]. DAGs stimulate resident macrophages to produce prostaglandins [29] with potential negative effects for a pregnancy [30]. The results of the present study suggested that midluteal phase endometrium of women who suffered higher order miscarriage display fatty acid metabolic perturbations towards increased lipolysis when compared to women who suffered lower order miscarriages. In addition, women who achieved a live birth in their subsequent pregnancy displayed an increased DAGs lipolysis compared to women who suffered another miscarriage.

Mitochondria are known to be the *powerhouses of the cell* due to their central role in energy production [31]. Additionally, they participate in other pathways such as cellular signalling [32], apoptosis [33], regulation of cellular metabolism [34], steroid synthesis [35] and hormonal signalling [36]. Cardiolipin (CL) is the signature phospholipid of energy-transducing membranes and plays a fundamental role in mitochondrial respiration and energy production in addition to its structural purpose in the architecture and morphology of the mitochondrial membranes [37]. The mitochondrial inner membrane lacks an acyl-CoA transporter which means that the acyl group is transferred to the shuttle molecule carnitine for translocation into the matrix for subsequent β-oxidation [38].

We found differences in how fatty acids are transported into mitochondria for ATP synthesis. Larger fatty acids such as stearoylcarnitine with 16 carbon atoms seem to be lower

in concentration in women with higher order miscarriages than those with lower order miscarriages. Smaller fatty acids such as decanoylcarnitine, which is an 8-carbon fatty acid are not transported into the mitochondria in women with higher order miscarriage as well as in those with lower order miscarriages.

Another metabolomic difference between those with higher and lower order miscarriages related to metabolites involved in creating oxidative stress. In particular, women with higher order miscarriage had lower levels of flavin adenine dinucleotide (FAD) than those with lower order miscarriage. We also found higher levels of flavin mononucleotide in women with higher order miscarriage. Both of these findings suggest that there may be higher mitochondrial release of reactive oxygen species in those with higher order miscarriage.

In the comparison based on the fate of the subsequent pregnancy, women who achieved a live birth associated less release of free fatty acids when compared to those who suffered another miscarriage. The differences in cholesterol storage metabolites suggests that women who achieve a live birth store cholesterol as cholesterol sulphate rather than pure cholesterol when compared to women who suffered another miscarriage.

4.1 Strengths and limitations

The major strength of our study is the narrow phenotype of the included participants assured by the strict diagnosis of unexplained recurrent miscarriage by using standard clinical investigations and by excluding women with known risk factors for miscarriage. This enriched the study population by increasing the chance of having suffered miscarriages due to an undiagnosed endometrial problem. This counterbalances the main limitation represented by the relatively small sample size (N = 24 women with recurrent miscarriage and 18 subsequent conceptions) and improves clinical relevance.

We have further reduced the heterogeneity of the endometrial samples by performing biopsies at the time of highest endometrial receptivity within the window of implantation as timed accurately using LH kits. The rapid endometrial changes of the endometrium around the time of implantation are known to introduce a significant source of variance [39].

Endometrial metabolomics testing conforms to the requests of women expressed in a recent survey for the development of a target product profile for endometrial testing [40]. It may be performed in a window of three to four days of the menstrual cycle in the mid luteal phase when receptivity is expected to be the highest. Metabolomics results may become available within one to two days from sample collection, and the invasiveness does not extend beyond performing a Pipelle endometrial biopsy during a speculum vaginal examination. Further research should clarify the reproducibility of the metabolomics analysis and the length of time for the validity of results.

While we excluded the known maternal causes for miscarriage, we were unable to perform cytogenetic analysis on all products of conception due to insufficient samples or absence of induction in the case of low order miscarriage. However, based on the Ogasawara study [18], we would expect aneuploid miscarriage to occur more frequently in the control group and that would not reduce the strength of the evidence for the metabolomics analyses.

The choice of the control group for the first comparison could have been replaced by either women who have not had any pregnancies or women who have achieved live births in the past while accepting the risk of bias due to potential long term endometrial changes caused by normal pregnancies. Our control group included women who suffered low order miscarriages based on the assumption that higher order miscarriages are less likely to have occurred due to aneuploidy as previously suggested [18].

We have performed an untargeted metabolomics discovery study involving a large number of metabolites without adjusting for multiple testing in order to allow the identification of pathways highlighted by alterations in several molecules to increase the plausibility of the conclusions. Further studies should aim to validate these findings by applying targeted metabolomics to a new cohort of women.

5. Conclusions

Various metabolic perturbations are associated with observation of increased numbers of miscarriages. They relate to fatty acid metabolism including increased lipolysis and decreased medium chain fatty acid beta-oxidation, poorer mitochondrial health, and redox-active co-factors which are present at higher oxidative levels.

Other metabolic perturbations are associated with observation of live birth following miscarriages. They relate to perturbed cholesterol – cholesterol sulphate metabolism, fatty acid metabolism including increased diacylglyceride lipolysis and decreased medium chain fatty acid beta-oxidation, and improved mitochondrial health.

These discovery studies have implicated a small number of metabolic pathways and biological functions which are biologically important in miscarriage mechanisms. Further targeted metabolomics validation studies are required to characterise the extent of these differences and to interrogate their mechanisms.

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CHAPTER SEVEN:

GENERAL DISCUSSION

1. Rationale

Reproductive failure in the form of recurrent implantation failure and recurrent miscarriage is common and affects the vast majority of conceptions, with fewer than one third of conceptions progressing successfully to a healthy live birth [1]. The endometrium plays a crucial role in implantation failure through suboptimal endometrial receptivity and impaired embryo-endometrial cross-talk [2].

Endometrial receptivity has been the focus of extensive research over the last eight decades since Noyes described the histological features of endometrial dating in 1950 [3]. Over 50 markers of endometrial receptivity measured by ultrasound, endometrial biopsy or fluid aspirate, and hysteroscopy have been described and proposed for use in clinical practice; however, none of them have proven sufficient discriminatory precision to differentiate between a subsequent successful pregnancy and a negative outcome.

Endometrial thickness is the most common marker used to assess endometrial receptivity due to its reduced cost, increased feasibility due to availability of ultrasound machines, patient acceptance and non-interference with fertility treatment. A threshold of <6 or <7 mm (varying between clinics) is used to imply endometrial non-receptivity in order to

guide the cancellation of embryo transfer as part of IVF treatment. Whilst clinical pregnancy is less likely at the extreme of thin endometrium, the finding is relatively rare (<1.5%) [4]. In addition, thicker endometrium is not more useful due to its low accuracy in predicting successful pregnancy.

At the opposite side lay the modern markers of endometrial receptivity based on transcriptomics. Their cost ranges between hundreds and thousands of Pounds, they involve a degree of pain associated with endometrial biopsy, which subsequently means they cannot be applied in a treatment cycle due to negative interference with success rates. They rely on the assumption that endometrial development is out of synch with blastocyst development and propose to identify the ideal time of embryo transfer based on the transcriptomic signature of receptive endometrium. At least seven companies have commercialised transcriptomic based tests of endometrial receptivity such as ERA (IGENOMIX), Win-Test (INSERM), ERPeak (Cooper Surgical), ERMap (IGLS), YK-ERT (Yikon), beREADY (beREADY), BioER (Bioarray) in the recent years, whilst none of them have been supported by robust evidence of clinical effectiveness.

Table 1: Summary of findings from work packages (WP) 1-5. hCG: human chorionic gonadotrophin; ET: embryo transfer; CP: clinical pregnancy; LB: live birth; RCT: randomised controlled trial; IU: international unit; IUI: intrauterine insemination; USS: ultrasound scan

	Aim	Population	Intervention / Test	Outcome	Study design	Summary of results
WP 1	To quantify the effect of intrauterine hCG administration before ET	Women undergoing ET	Intrauterine hCG	CP, LB, miscarriage, adverse events	Meta-analysis of RCTs	17 RCTs including 4751 women Increased LB and CP in women undergoing cleavage ET with ≥500IU hCG No difference in LB and CP in women undergoing blastocyst ET with ≥500IU hCG No evidence of difference in adverse events including miscarriage
WP 2	To examine the evidence supporting the use of endometrial receptivity markers as prognostic factors for CP	Women undergoing IUI, fresh or frozen ET	USS, endometrial biopsy or fluid aspirate, hysteroscopy	Association and accuracy based on prediction of CP	Meta-analysis of prospective observational studies	7mm endometrial thickness: sensitivity 99%, specificity 3% 2ml endometrial volume: sensitivity 93%, specificity 7% triple line pattern: 87% sensitivity, 15% specificity endometrial blood flow: sensitivity 100%, specificity 8% absent contractions: sensitivity 7%, specificity 94% 'good' hysteroscopy: sensitivity 75%, specificity 60%
WP 3	To evaluate women's perspective on endometrial receptivity diagnostics	Women who suffered recurrent miscarriages	Questionnaire as part of a target product profile	Minimum acceptable and ideal features of an endometrial receptivity test	Survey	Ideal features: indicated after two miscarriages, performed in a window of three to four days within the menstrual cycle, results available within one to two days, invasiveness not beyond a vaginal examination, repeating the test not required more than twice, results useful for at least six menstrual cycles
WP 4	To characterise the endometrial transcriptomic profiles of women who suffered recurrent miscarriages	Women who suffered low and high order miscarriages	Endometrial transcriptomics	Transcriptomic signatures	Prospective cohort	19 differently expressed genes in women who suffered high order versus low order miscarriages 421 differently expressed genes in women who suffered another miscarriage versus those achieving a live birth in the subsequent pregnancy
WP 5	To characterise the endometrial metabolomic profiles of women who suffered recurrent miscarriage	Women who suffered low and high order miscarriages	Endometrial metabolomics	Metabolomic signatures	Prospective cohort	Increased number of miscarriages associate perturbations in the fatty acid metabolism and poorer mitochondrial health. Subsequent live birth associates perturbed cholesterol – cholesterol sulphate metabolism, fatty acid metabolism and improved mitochondrial health.

The overarching aim of the present research was to understand endometrial receptivity and explore the development of potential endometrial receptivity tests that are cost-effective and may be implemented in clinical practice. Five work packages with specific objectives were developed for the core of this research thesis.

2. What is the effect of intrauterine hCG administration before embryo transfer?

The aim of this work package was to identify an intervention that could improve reproductive outcomes by modulating endometrial receptivity. Based on previously published work, intrauterine hCG was suitable for further investigation of its effect on the outcomes of assisted reproduction. Intrauterine hCG administration before embryo transfer has been proposed as an intervention to increase success rates following IVF treatment through the hormone's modulation of endometrial receptivity. The present Cochrane review and meta-analysis of seventeen RCTs found moderate quality evidence that women undergoing cleavage-stage transfer using an IC-hCG dose ≥ 500 IU have an improved live birth rate.

Three previous systematic reviews [5-7] focusing on clinical pregnancy as the primary outcome found similar results; however, the strength of their results was limited by suboptimal methodology and inclusion of fewer studies (five, eight, and six, respectively).

The present meta-analysis was the first study of intrauterine hCG to identify embryo stage at transfer (cleavage vs blastocyst) and dose of hCG (<500 IU vs ≥500 IU) as critical sources of heterogeneity and recommended future studies to account for the different treatment effects based on these variables.

It is unclear why intrauterine hCG appears to be beneficial only for cleavage stage transfers and not for blastocyst transfers. One could speculate that cleavage embryos benefit from a few extra days in the uterine cavity before they need to start implantation, while blastocyst embryos require an optimal endometrium ready at the time of embryo transfer. Furthermore, the embryo-endometrial dialogue might be impaired by the extended culture of embryos to blastocyst stage due to the absence of continuous feedback between embryo and endometrial development *in vivo*. Administration of intrauterine hCG a few days before blastocyst transfer is unable to mitigate this limitation due to the non-exposure of the endometrium to the embryo development markers in case of *in vitro* culture.

3. What is the evidence supporting the use of endometrial receptivity markers?

Subsequent to identifying intrauterine hCG as a beneficial intervention prior to embryo transfer based on its positive effect on clinical pregnancy and live birth rates, we

sought to identify markers of endometrial receptivity that could be used for quantifying changes in endometrial receptivity. This would enable direct measurement of effects from various interventions aimed at endometrial receptivity as opposed to using pregnancy outcomes as surrogate outcomes. In addition, they could be used to assess the natural variation of endometrial receptivity through a natural or stimulated cycle. Through a comprehensive systematic review and over 20 meta-analyses we highlighted over 50 markers of endometrial receptivity as measured by ultrasound, endometrial biopsy or fluid aspirate, and hysteroscopy. Associations were identified between clinical pregnancy and various endometrial receptivity markers (endometrial thickness, endometrial pattern, Doppler indices, endometrial wave-like activity and various molecules); however, their poor ability to predict clinical pregnancy prevents them from being used as diagnostic tests of endometrial receptivity [8].

We recommended against the use of means for variables used in endometrial receptivity research. Impaired endometrial receptivity may be characterized by extreme values of a continuous endometrial receptivity marker which may have a low incidence (e.g., the incidence of thinner than 6 mm endometrium on the day of hCG injection was 0.33%).

Means, which report the average across the whole cohort population, may fail to account for important findings at the extreme levels of the range of observations.

4. What are women's views in relation to endometrial receptivity testing?

Target Product Profiles (TPPs) are sets of both minimum acceptable ("worst-case") and ideal ("best-case") features of a medical product (diagnostic test, vaccine, medicine or intervention) developed with an aim to identify the critical attributes of a product before development begins, to ensure that the final product responds to the needs of the end-users [9]. TPPs are not commonly used in the field of Obstetrics and Gynaecology, less so in the field of Reproductive Medicine and could be one of the reasons behind the long list of controversial add-ons [10].

We conducted the first stage of defining a TPP for endometrial receptivity testing by surveying 131 women who suffered recurrent miscarriages. They assigned high importance to endometrial receptivity for a successful pregnancy and expressed clear views in relation to the indication, availability, timing, invasiveness, repetition, validity, and cost of testing.

5. Can OMICS technologies be used for endometrial receptivity testing?

In the absence of a robust marker of endometrial receptivity identified through the systematic search of the literature, we wished to explore the potential of developing a new test of endometrial receptivity based on modern -OMICS techniques. Previous epidemiological studies identified a negative association between the number of recurrent miscarriages and the incidence of aneuploidy in the products of conception with a cut-off at four to five miscarriages [11] suggesting that higher order miscarriages are caused by a persistent cause other than spontaneous aneuploidy. We were able to confirm this theory by demonstrating the existence of an organic cause at the level of the endometrium through the use of -OMICS technologies. We applied transcriptomics and metabolomics to endometrial samples obtained from a highly selected cohort of women who suffered unexplained recurrent miscarriages.

We reported on 19 differentially expressed genes in the endometrium of women who suffered four or more miscarriages [12]. In addition, the endometrium of women who achieved a live birth had 421 genes that were differentially expressed compared to women who suffered another miscarriage. Ninety-two of these genes have been previously reported by Hu [13] and 114 by Sigurgeirsson [14] with an overlap of 65 genes.

Through the first metabolomics study applied to endometrial receptivity, we identified metabolic perturbations associated with the observation of having suffered high order miscarriages [15]. They relate to fatty acid metabolism including increased lipolysis and decreased medium chain fatty acid beta-oxidation, poorer mitochondrial health, and redox-active co-factors which are present at higher oxidative levels.

Other metabolic perturbations are associated with observation of live birth following miscarriages. They relate to perturbed cholesterol – cholesterol sulphate metabolism, fatty acid metabolism including increased diacylglyceride lipolysis and decreased medium chain fatty acid beta-oxidation, and improved mitochondrial health.

6. Strengths and limitations

The present body of work has several strengths. Overall, it employed a mixture of research methodologies including secondary data analyses (systematic reviews, meta-analyses of effects of interventions and tests accuracy), surveys of focus groups, and prospective cohorts with interventional component. The succession of chapters was cost efficient as it prioritised hypotheses verifiable through secondary data analyses and surveys which are less cost intense in order to generate the hypotheses for the subsequent chapters based on costly

OMICS technologies. Every aspect of the project was conducted according to the pre-published protocol.

At a chapter level, the secondary analyses followed robust Cochrane methodology and were based on a broad systematic search designed with input from an Information Specialist. There were no language filters which facilitated the inclusion of studies conducted across the globe and published in various languages to increase the generalisability of the results.

The survey was designed by a multidisciplinary team including doctors, nurses, and scientists and it incorporated feedback from patients following the pilot stage. It is one of the first projects to address women's views for the development of a Target Product Profile in the field of Reproductive Medicine.

The prospective cohort studies used state of the art -OMICS technologies through collaborations with the internationally renowned Genomics Birmingham and Phenome Centre Birmingham. Eligibility criteria were particularly narrow to enrich the cohort of women who were likely to have suffered recurrent miscarriages due to endometrial causes by excluding all known causes and risk factors for miscarriage.

Our studies also had some limitations. Bias was mitigated in the meta-analyses by adopting standardised methodology and by including a large number of studies; however, the quality and the strength of the meta-analysed outcomes are only as good as the data provided by the primary publications.

The survey was anonymised which means analyses were not adjusted based on clinical characteristics such as age, number of previous miscarriages, or other aspects that could influence women's responses. It only included women who suffered recurrent miscarriages which reduces the generalisability to the population of women who suffered reproductive failure through other contexts such as recurrent implantation failure.

The strict eligibility criteria for the cohort studies led to the inclusion of a relatively low number of participants despite recruiting multiple study sites through the National Institute for Health and Care Research (NIHR) Clinical Research Network (CRN). The control group included women who suffered low order miscarriages based on the assumption that higher order miscarriages are less likely to have occurred due to aneuploidy. This could have been replaced by either women who have not had any pregnancies or women who have

achieved live births in the past while accepting the risk of bias due to potential long term endometrial changes caused by normal pregnancies.

7. Future research

The findings of the intrauterine hCG review should provide a strong foundation for funding and conducting further high-quality RCTs focusing on subgroups (cleavage vs blastocyst, fresh vs frozen/thawed, single vs two or more embryo transfers, cause of subfertility, dose and timing of IC-hCG) to identify the groups of women who would benefit the most from this intervention. Live birth rate must be the primary outcome while blinding throughout the treatment cycle and during embryo transfer may reduce potential performance bias (adjusting ovarian stimulation doses; deciding the timing of maturation triggering, oocyte retrieval, and technique during embryo transfer, respectively).

Endometrial receptivity appears to be a continuous variable reflected in the molecular changes triggered by ovulation and progesterone exposure with various levels of endometrial receptivity existing within the window of implantation. Further research should avoid attempting to quantify endometrial receptivity by endometrial biopsy due to the need to postpone the completion of fertility treatment following endometrial injury. Endometrial fluid

aspirate analysis correlates with endometrial biopsy results and should be used to develop a point of care test allowing for immediate decision within the treatment cycle.

Gene function and metabolite analyses identified a broad range of molecular functions, biological processes and cellular components linked to the differentially expressed genes based on the outcome of subsequent pregnancies. They may serve as potential targets for future interventions aimed at reducing the risk of subsequent miscarriage. In addition, our datasets may be used for algorithms of a training phase for a new test of endometrial receptivity with findings to be validated in a larger cohort.

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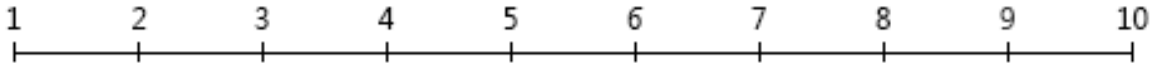
Appendix 1: Questionnaire used for Work package 3.

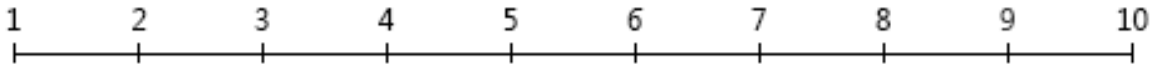
We would be grateful if you could fill in the form below. If anything is not clear, please ask a member of the clinical team. Thank you.

1. **How important do you consider the lining of the womb in ensuring a healthy pregnancy?**

(1 is low importance, 10 is high importance)



2. **If we could develop a test for the readiness of the womb lining to accept a pregnancy, after how many miscarriages should the test be performed?**



3. **Would you consider the test to be worthwhile if the results were available:**

On the same day ☐ In 1-2 days ☐ In 1-2 Weeks ☐ In the same month ☐ Anytime ☐

4. **Would you be willing to take the test if it was necessary to strictly control the timing:**

To the exact day and hour ☐ To the exact day ☐ Within 3-4 days ☐ Within 10 days ☐

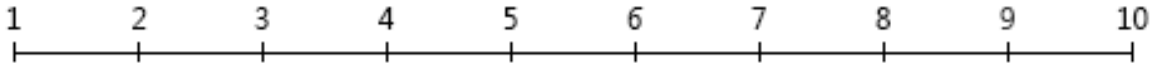
5. **Would you be willing to take the test if it involved:**

Providing a blood sample ☐ Providing a sample of vaginal discharge ☐

Providing a sample of tissue from the womb, taken with a narrow plastic tube passed through the neck of the womb ☐

Undergoing a hysteroscopy (passing a narrow camera through the neck of the womb to look inside) ☐

6. **How many times would you be willing to repeat the test in order to be sure the result is accurate?**



7. **Would you be willing to take the test if it was valid for:**

Only the very next menstrual cycle ☐ 2-3 menstrual cycles ☐ Up to 6 menstrual cycles ☐
Up to 12 menstrual cycles ☐ More than 12 menstrual cycles ☐

8. **If the test was not available within the NHS context, how much would you be willing to pay for it?**

Up to £100 ☐ £100-200 ☐ £200-500 ☐ £500-1000 ☐ £1000-2000 ☐
I would not be willing to pay ☐