

Ultrasound Determinants of Endometrial Receptivity

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Abstract

This thesis is a study of “the prognostic accuracy of endometrial patterns on ultrasound to predict implantation”. I hypothesised that endometrial pattern on ultrasound can predict and help improve the outcome of in vitro fertilisation (IVF).

I performed a systematic review and meta-analysis on the prognostic accuracy of endometrial patterns on ultrasound to predict implantation. It showed that women with triple pattern at trigger are more likely to become pregnant when compared to women with a homogenous pattern. Similarly, women with a homogenous pattern at fresh embryo transfer were more likely to become pregnant compared to women with an intermediate pattern. However, this is limited by the low to moderate quality evidence of some of the studies, and is also limited by the substantial heterogeneity of the studies and the different classification systems. (Chapter 2)

In chapter 3, in review of the literature and from day to day scanning and along with my own observation, I developed the “endometrial pattern recognition” classification system. I tested the reliability of the old classification system. I taught and introduced the new classification system and tested its reliability. The old classification system showed substantial agreement between observers while the new classification system showed moderate to substantial agreement.

In chapter 4, I performed a “prognostic accuracy study” of endometrial patterns on ultrasound to predict implantation using our novel classification system. The results of this showed that women with a homogenous pattern at egg collection were less likely to become pregnant when compared to women with a triple pattern. It also showed that women with late intermediate pattern at egg collection were less likely to become pregnant when compared to women with a triple pattern (clinical pregnancy OR 0.65, 95% CI 0.21 to 1.98). These results are statistically significant with a p value of <0.05. However, there are some limitations and larger studies are need to validate these results.

In chapter 5, I performed a study to explore the relationship between the endometrial pattern on ultrasound, histology and microarray. This showed that there is a positive correlation between histology and ultrasound endometrial patterns on principle component analysis plots.

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For
Mohamed Elhassan
& Awatif Elsafi
&
Dedicated to
the memory of
Mohamed Ali Elsafi

Abbreviations

2D	Two Dimensional
3D	Three Dimensional
AFC	Antral Follicle Count
AMH	Anti Mullerian Hormone
ANOVA	Analysis of Variance
BMI	Body Mass Index
cAMP	3',5'-cyclic adenosine monophosphate
cDNA	Complementary Deoxyribonucleic Acid
CHARMS	Checklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies
CI	Confidence interval
COVID-19	Coronavirus disease of 2019
CP	Clinical Pregnancy
CPR	Clinical Pregnancy Rate
E2	Oestradiol
ECM	Extracellular Matrix
Endo-Scratch	Endometrial Scratch
ERA	Endometrial Receptivity Array
ESHRE	European Society of Human Reproduction and Embryology
ET	Embryo Transfer
EV	Endometrial Volume
FDR	False Discovery Rate
FSH	Follicle-stimulating hormone
g	Gram
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation

GE	General Electric
GIFT	Gamete Intrafallopian transfer
GnRH	Gonadotrophin Releasing Hormone
H&E	Haematoxylin and Eosin
hCG	Human Chorionic Gonadotropin
HE	Hiba Elhassan
HEFA	Human Fertilisation and Embryology Authority
HMG	Human Menopausal Gonadotrophin
HRT	Hormone Replacement Therapy
Hz	Hertz
ICSI	Intra-Cytoplasmic Sperm Injection
ICTRP	International Clinical Trials Portal
IETA	International Endometrial Tumour Analysis
IMS	Industrial methylated spirit
IOTA	International Ovarian Tumour Analysis
IR	Implantation Rate
IVF	In Vitro Fertilisation
kHz	Kilo Hertz
L	Litter
LH	Luteinising Hormone
M	Mean
mg	Milligram
MHz	Mega Hertz
ml	Millilitre
mm	millimetre
NA	Not Applicable
NF	Nick Fenning
NHS	National Health Service

OHSS	Ovarian Hyper stimulation Syndrome
OR	Odd Ratio
P	Progesterone
PC1	First Principal Component
PC2	Second Principal Component
PC3	Third Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PI	Pulsatility index
PICOT	Population, Index prognostic factor, Comparator prognostic factors, Outcome, Time
PIS	Participant Information Sheet
pmol	Picomole
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
QC	Quality Control
QMC	Queens Medical Centre
QUIPS	Quality In Prognosis Studies
R&D	Research and Development
REC	Research Ethics Committee
rFSH	Recombinant Follicle stimulating hormone
RMA	Robust Multi-array Average
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
RPE RNA	Precipitating Elution
SAE	Serious Adverse Event
SD	Standard deviation
SPSS	Statistical package for the Social Sciences

TAUS	Transabdominal ultrasound scan
TVOR	Transvaginal Oocyte Retrieval
TVUS	Transvaginal Ultrasound scan
UK	United Kingdom
USA	United State of America
VS	Vide Supra
WHO	World Health Organisation
WOI	Window of Implantation
ZIFT	Zygote Intrafallopian Transfer
μL	Microliter

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Chapter 1 Background and hypothesis

1.1 Rationale

Subfertility can affect 1 in 6 couples in the UK and can cause tremendous psychological, as well as emotional and financial pressure. Globally, it has been estimated that 48 million individuals worldwide live with infertility. (Health 2022) It has been more than 40 years since the birth of Ruth Brown in 1978, the first in vitro fertilisation (IVF) baby, and despite numerous developments in techniques in assisted conceptions, endometrial receptivity remains the rate limiting step in the process.

Ultrasound is a non-invasive tool that can provide very useful information and allow assessment of endometrium. Women undergoing IVF have regular scans throughout their treatment and it seems prudent that we utilise these scans to perform a thorough examination and obtain as much information as possible to help them achieve success. Research into the predictive value of endometrial pattern however, has yielded conflicting results, meaning that many clinics opt to overlook this (Hershko-Klement and Tepper 2016). I hypothesise that endometrial pattern on ultrasound can predict the outcome of IVF. In this introductory chapter an overview about markers of endometrial receptivity will be provided.

1.2 The human endometrium

The endometrium is composed of multiple layers and is a dynamic organ which sits above the myometrium. It is made up of a functional layer and a basal layer. Every month during menstruation, the functional layer cells are shed from the basal layer. The basal layer remains intact, affixed to the myometrium, and acts as a base for endometrial regeneration. Several different cell types comprise the endometrium including glandular epithelial cells and luminal cells, stromal fibroblastic cells, blood vessels and immunocompetent cells. Throughout the menstrual cycle, the activity, structure, numbers, and function of these cells changes, and they then change again during pregnancy. (Diedrich et al. 2007)

1.3 The endometrial cycle

1.3.1 Proliferative phase

During the proliferative phase, the main feature of the endometrial tissue is angiogenesis and active proliferation, while suppressing apoptotic factors. This is to ensure nourishment of the developing new tissue, and is underlied by the action of oestrogen. During this phase of the cycle, it is crucial for the endometrium to develop adequately. This is so that there is synchronisation of the maturation process which is needed for implantation during the endometrium's secretory phase. (Hoozemans et al. 2004)

This encourages the proliferation of cells and shows an indirect effect by exciting the expression of steroid receptors such as the progesterone receptor (PR), which is necessary for the action of progesterone during the secretory phase. (Strowitzki et al. 2006)

1.3.2 The secretory phase

During the late secretory phase of the menstrual cycle in humans, the morphological changes that are characteristic of decidualization take place independently of conception. All the endometrium's cell types are affected, with the functionally distinct tissue being infiltrated by large numbers of immune cells, and characteristic endometrial cell differentiation taking place. Progesterone, which induces cell differentiation and suppresses proliferation, is this second half of the menstrual cycle's major player, and at least partially acts via the progesterone type A receptor (PR). (Wang et al. 2018; Bergeron 2000). Moreover, PR A behaves as a repressor of PR B, which is the most transcriptionally active receptor. PR B is reduced in the glandular epithelial cells, but is still present in the stroma (Lessey 2000b). Therefore, during the implantation window, the action of progesterone is most likely through PR B in the stromal compartment. (Strowitzki et al. 2006)

1.3.3 Decidualisation

Interestingly, in contrast to most mammals, decidualisation of the human endometrium does not require embryo implantation. Decidualisation is "the transformation of

endometrial stromal fibroblasts into specialized secretory decidual cells that provide a nutritive and immunoprivileged matrix essential for embryo implantation and placental development”(Gellersen et al. 2018). It is thought that this process is driven by the postovulatory rise in progesterone levels and increasing local cyclic adenosine monophosphate production (cyclic AMP, or 3',5'-cyclic adenosine monophosphate) which is a second messenger important in many biological processes. In response to falling progesterone levels, spontaneous decidualization causes menstrual shedding and cyclic regeneration of the endometrium (Gellersen et al. 2018).

1.3.4 Implantation

The process of implantation is a complex process involving synchronized intricate coordination between the embryo and the endometrium. It can be divided into three stages: apposition, adhesion and invasion (Enders 1967; Achache and Revel 2006).

During the blastocyst apposition, the trophoblast cells will adhere to the receptive endometrial epithelium. Subsequently it will anchor the endometrial basal lamina and stromal extracellular matrix (ECM). When the embryo-endometrial lineage is achieved it can no longer be dislocated by uterine flushing. The next stage of this process is invasive blastocyst penetration.

Surprisingly, while the embryo can implant in different human tissues, in the endometrium this phenomenon is time limited between days 20 and 24 of a regular

menstrual cycle (day LH+7 to LH+11). This time period is known as the window of implantation (WOI) (Psychoyos 1973; Achache and Revel 2006).

During the window of implantation (WOI), the human endometrium is primed for blastocyst attachment, given that it has acquired an accurate morphological and functional state initiated by ovarian steroid hormones (Achache and Revel 2006; Finn and Martin 1974; Yoshinaga 1988; Paria et al. 2002).

Implantation involves a complex sequence of signaling events requiring a large variety of mediators including adhesion molecules, cytokines, lipids growth factors and others (Achache and Revel 2006; Simón, Martín, and Pellicer 2000; Lessey et al. 1992).

Endometrial receptivity requires both the acquisition of adhesions ligands as well as the loss of any inhibitory components that can act as a barrier to the embryo.(Aplin 2000).

1.4 Histological changes in the endometrium

The fluctuation in hormonal levels during the menstrual cycle is accompanied with changes in the endometrium layer in the uterus. This can be designated into 6 phases:

1.4.1 Early Proliferative Phase

During the early proliferative phase, the endometrium is usually less than 2 mm in thickness. Proliferation of basal and epithelial cells persisting in the lower uterine and cornual segments results in the restoration of the luminal epithelium by day 5 of the menstrual cycle. At that time, mitotic activity is seen in both the stroma and glandular epithelium. The glands are narrow during the early proliferative phase, straight, and tubular, lined with low columnar cells that have round nuclei located near the cell base.(Jerome F. Strauss 2018)

1.4.2 Late Proliferative Phase

The endometrium thickens in this phase as a result of glandular hyperplasia and an increase in stromal extracellular matrix. Near the endometrial surface the glands are widely separated, and are more crowded and tortuous deeper into the endometrium. The glandular epithelial cells grow vertically and become pseudostratified as the time of ovulation approaches. (Jerome F. Strauss 2018)

1.4.3 Early Secretory Phase

Ovulation marks the start of the endometrial cycle's secretory phase, although it should be noted that the endometrial luminal and glandular epithelial cells also show secretory activity during the proliferative phase. Mitotic activity in epithelial and stromal cells is restricted to the first 3 days after ovulation and is rarely seen later in the cycle.

Glandular epithelial cells and stromal cells' nuclei develop heterochromatin in the early secretory phase. (Jerome F. Strauss 2018)

1.4.4 Midsecretory Phase

A characteristic feature of this phase of the cycle is the development of the spiral arteries. These vessels become increasingly coiled, as they lengthen more rapidly than the endometrium thickens. In the midsecretory and late secretory phases the endometrial glands are tortuous. Their secretory activity reaches a maximum around 6 days after ovulation, as reflected by loss of vacuoles from the epithelial cell cytoplasm. (Jerome F. Strauss 2018)

An ordered spherical stack of interdigitating tubules, the nucleolar channel system, appears transiently in the nucleoli of approximately 5% to 10% of the secretory phase epithelial cells between day 16 and 24. This system forms in response to progesterone and is an ultrastructural hallmark of the secretory phase during the anticipated time of implantation. (Jerome F. Strauss 2018)

1.4.5 Premenstrual and Menstrual Phase

The main histologic features of the premenstrual phase are degradation of the stromal reticular network, which is catalyzed by MMPs; mononuclear and polymorphonuclear leukocyte infiltration of the stroma; and the endometrial glands' "secretory exhaustion", whose epithelial cells now show basal nuclei. Morphologic changes in granular

lymphocytes' nuclei, including pyknosis and karyorrhexis suggesting apoptosis, have been proposed to be some of the first events presaging menses; these changes occur before the breakdown of extracellular matrix and leukocyte infiltration. In the glandular epithelium, the nucleolar channel system and giant mitochondria characteristic of the early and midsecretory phases disappear. The endometrium shrinks before menstruation, partly as a result of diminished secretory activity and the breakdown of extracellular matrix. (Jerome F. Strauss 2018)

1.4.6 Menstruation

Menstruation, caused mainly by progesterone withdrawal, signifies a failure to achieve pregnancy and the need to expel the specialized uterine lining that results from spontaneous decidualization. The uniqueness of this process is underlined by the fact that, although circulating progesterone and estrogen levels decline with corpus luteum regression in nonfertile cycles in all mammals, menstruation appears almost exclusively in humans and some Old World primates. (Jerome F. Strauss 2018)

Spiral arterioles supply the functional zone of the human endometrium. Spiral arteries, in contradistinction to the radial and basal arteries that feed them, are highly sensitive to steroid hormones. The classic studies of Markee that observed the structure of autologous endometrial transplants into the anterior eye chamber suggested that vasoconstriction of the arterioles and spiral arteries which created an ischemic phase precedes the onset of menstrual bleeding by 4 to 24 hours. It has been proposed that

bleeding occurs after the arterioles and arteries relax, leading to hypoxia or reperfusion injury. (Jerome F. Strauss 2018)

1.5 Ovarian stimulation cycle: physiology and IVF stimulation protocols

Controlled ovarian stimulation (COS) is a pharmacological therapy with the objective of provoking the development of multiple ovarian follicles. This means that mature oocytes may be retrieved. (Palinska-Rudzka and Mathur 2023)

In the normal menstrual cycle, from a cohort of early antral follicles, a dominant follicle is selected. A reduction in follicle stimulating hormone (FSH) concentrations below a certain threshold leads to the degeneration of the remaining antral follicles, whilst the dominant follicle, due to its higher sensitivity to FSH, continues to grow. (Palinska-Rudzka and Mathur 2023)

Administration of exogenous FSH in an IVF cycle helps to maintain FSH level above threshold, expanding the window of time for recruitment of follicles, and encouraging the simultaneous growth of multiple follicles. One of the concerns associated with this method is that the large number of growing follicles leads to markedly elevated oestradiol levels and can result in a premature endogenous LH surge. (Palinska-Rudzka and Mathur 2023)

Therefore, to inhibit ovulation, it is necessary to desensitize the pituitary, either by coadministration of a GnRH antagonist, or GnRH agonist. Providing LH activity either

in the form of an induced endogenous LH surge through the use of a GnRH agonist, or human chorionic gonadotrophin (hCG), achieves final oocyte maturation. (Palinska-Rudzka and Mathur 2023)

1.5.1 Pituitary suppression regimen

1.5.1.1 Long GnRH agonist protocol

During the first week of use, initial administration of GnRH agonist either by a nasal spray or subcutaneous/depot injection, causes a 'flare up effect', leading to increased secretion of LH and FSH. Continued administration in agonist treatment cycles results in downregulation of the pituitary, and prevents the LH surge. (Palinska-Rudzka and Mathur 2023)

An undesired effect associated with early use, includes the formation of functional cysts. To reduce this, GnRH agonist treatment is typically started in the mid-luteal phase of the menstrual cycle. Within the next two weeks, menstrual bleeding is observed. Transvaginal ultrasound showing quiescent ovaries and a thin endometrium, and low serum oestradiol concentration confirms pituitary suppression. Ovarian stimulation with daily gonadotrophin injections is initiated once downregulation is confirmed. To assess the recruitment and growth of follicles, ultrasound monitoring is required. Once an appropriate number of follicles have reached 17–18 mm in size, a final hCG trigger is given, before the oocytes are retrieved. (Palinska-Rudzka and Mathur 2023)

For many years, the predominant regimen used in the ART setting was the GnRH agonist protocol. The benefit of this protocol is a good control of the treatment cycle. However, this regimen is less commonly used now due to the longer duration of treatment, higher cumulative doses of gonadotrophins needed and increased risk of Ovarian Hyperstimulation Syndrome (OHSS) in comparison to the antagonist protocol. (Palinska-Rudzka and Mathur 2023)

1.5.1.2 Short GnRH agonist protocol

In this protocol, GnRH agonist administration is started in the early follicular phase, between day 1–2 of the menstrual cycle, and maintained until the day of oocyte maturation trigger. Gonadotrophin injections are started the day of, or day following the first GnRH agonist administration. Alternatively, GnRH agonist is used for the first 3 days of the follicular phase only, and is stopped once gonadotrophins injections are introduced. This is known as the ultrashort protocol. (Palinska-Rudzka and Mathur 2023)

In this regime, the initial flare-up effect from administration of GnRH agonist is used to provoke follicular development. This protocol was designed to support follicular recruitment in women who were anticipated to have a poor response, but studies have failed to show any significant advantages of using this regime. Some of the concerns surrounding the use of this regime include ‘rescuing’ the corpus luteum from previous menstrual cycle, resulting in high progesterone levels at the offset of the treatment cycle. (Palinska-Rudzka and Mathur 2023)

1.5.1.3 GnRH antagonist protocol

In the early follicular phase of the menstrual cycle, gonadotrophins are administered, followed by treatment with GnRH antagonist to prevent the LH surge.

Converse to the GnRH agonist, the GnRH antagonist prompts an immediate reversible suppression of the pituitary, with no flare effect, resulting in a drop in FSH/LH serum concentration within 8 hours of administration. In this protocol, GnRH antagonist is initiated on the 6th day of gonadotrophin stimulation (fixed administration) or when the leading follicle is 14mm in size (flexible administration), and continued daily. Gonadotropin injections are maintained till the day of the trigger. (Palinska-Rudzka and Mathur 2023)

In unselected patient populations, pregnancy rates for GnRH antagonist IVF protocols and long GnRH agonist protocols are comparable. The main advantages of GnRH antagonist regimen are a shorter duration and reduced risk of OHSS, as well as a reduced treatment burden. Patients are less likely to experience side effects that are associated with GnRH agonist use such as formation of functional cysts, and menopausal symptoms. Moreover, treatment cost may be reduced, as often lower cumulative gonadotrophin doses are used in comparison to the agonist protocol. (Palinska-Rudzka and Mathur 2023)

1.5.1.4 Ovulation trigger

Once the follicles have reached the sufficient sizes, the next treatment step is to ensure oocyte maturation. Typically, the presence of three 17-18mm diameter follicles is considered adequate for administration of ovulation trigger.

Human chorionic gonadotrophin is frequently used as a triggering agent, due to its molecular similarities to LH and binding to the same receptor-LHCGR. There are two main preparations: recombinant hCG or urinary hCG which have comparable clinical outcomes.

After hCG is administered, granulosa cells undergo a luteinization process which involves enlargement of cells, and greater progesterone production. Moreover, the surrogate LH surge results in resumption of meiotic maturation of oocytes and the attainment of second metaphase. It is recommended that HCG administration is completed 36 hours before the oocyte retrieval procedure. (Palinska-Rudzka and Mathur 2023)

1.5.1.5 Types of gonadotrophin

There are two main types of preparations: recombinant and urinary gonadotropin.

In the 1960s, the use of human menopausal gonadotrophin (hMG) began, when preparations were first extracted from post menopausal women's urine. Menotrophins'

initial use (composed of equal amounts of LH and FSH), was restricted due to its needing intramuscular administration, and that a large amount of contaminant proteins it contained caused adverse reactions. Since then, the development of highly purified urinary FSH without LH activity, and highly purified hMG has occurred. (Palinska-Rudzka and Mathur 2023)

Recombinant FSH (rFSH) has been developed following the advances in molecular technology. The Chinese hamster ovary is the cell line used for this.

The produced human rFSH contains minimal amounts of contaminating proteins, is homogenous and is void of LH. The widely used preparations of rFSH are follitropin alfa and follitropin beta. Alternative recombinant follitropins have been developed and tested including, corifollitropin, which is a long acting form, and has the objective of reducing the number of injections in an IVF treatment cycle. Of late, a new fully recombinant FSH, which stems from a human retinal cell line- follitropin delta- has been introduced. (Palinska-Rudzka and Mathur 2023)

In chapter 4, the different protocols used at Nurture fertility will be outlined.

1.5.2 Endometrial development under controlled hormonal stimulation

There is a plethora of evidence that high oestradiol (E₂) levels following ovarian stimulation limit endometrial maturation and may negatively influence embryo

implantation. E₂ levels above 2500 pg/ml in clinical studies in an oocyte donation programme were seen to result in a markedly reduced implantation rate, although the quality of embryo was not affected (Simón et al. 1998). Moreover, there was found to be significantly improved pregnancy rates if a step-down regimen of gonadotrophin stimulation leading to reduced E₂ levels, was compared with a standard stimulation protocol (Simón et al. 1998). However, no negative impact of ovarian stimulation on embryo quality or pregnancy rate could be found in patients with supraphysiological E₂ levels in a large retrospective, comparative study between IVF/embryo transfer patients and recipients of donated oocytes (Levi et al. 2001)

The possible impact of hormonal stimulation on endometrial development is not limited to the WOI. A similar pattern of endometrial development throughout the menstrual cycle has been described in a large number of carefully conducted studies, all based on histomorphological endometrial dating. (Gellersen et al. 2018)

1.5.3 Gonadotrophin and GnRH agonist/antagonist protocols

The majority of studies were conducted in a standard GnRH analogue/gonadotrophin protocol.

Premature secretory changes were found (Marchini et al. 1991) in the late proliferative phase. Endometrial maturation is generally advanced by up to 2–4 days (Ubaldi et al.

1997; Lass et al. 1998) during the periovulatory period, with a reduced staining for progesterone and oestrogen receptors (Bourgain and Devroey 2003).

An advanced pattern in HMG/HCG cycles was also seen in studies of the early post-ovulatory phase (Garcia et al. 1984). Later studies however, have not shown any difference between natural controls and stimulated cycles in this particular cycle phase (Palinska-Rudzka and Mathur 2023).

In stimulated cycles in the luteal phase, data on endometrial morphology are contradictory. Stromal-glandular dissynchrony along with an advanced pattern is mostly revealed in mid-luteal biopsies (Noci et al. 1997; Meyer et al. 1999; Basir et al. 2001). Compared with controls, retarded emptying of secretory materials and a reduced size of endometrial glands is seen seven days after hCG (Basir et al. 2001). Reduced endometrial receptivity is seen in association with small gland volume (Rogers 1995). In IVF cycles seven days after ovulation, (Lukassen et al. 2004), however, could not demonstrate an increased endometrial advancement when compared with natural controls. Hormonal stimulation was seen to affect the CD56 cell ratio towards improved implantation conditions when CD56 cell groups with positive or negative implantation impact were compared (Lukassen et al. 2004). Different effects of varying E2 levels may at least partially explain the different findings. Diminished endometrial alterations were seen in moderate responders, when compared with high responders (Basir et al. 2001).

Important for a regular endometrial development seems to be luteal support. Irrespective of the type of luteal support, studies of IVF cycles with luteal support revealed normal in-phase histology (Bourgain and Devroey 2003; Ragni et al. 1999; Palinska-Rudzka and Mathur 2023)

Normal endometrial morphology is seen in the late luteal phase (Balasch et al. 1990) because of different stimulation protocols it is difficult to interpret data.

GnRH antagonists in combination with gonadotrophin stimulation also impair the maturation of the endometrium. No premature advanced secretory transformation was detected on the day of ovulation induction (Kolibianakis et al. 2005). However, one day after the LH peak in glands and stroma, progesterone receptors were already significantly up-regulated when compared with natural cycles, suggesting an early onset of accentuated maturation. An advanced endometrial development at the day of oocyte retrieval by an average of two and a half days was reported in 55 patients undergoing ICSI, and was correlated to LH levels prior to the administration of GnRH antagonists (Kolibianakis et al. 2004). Similar results were obtained in 10 oocyte donors (Kolibianakis et al. 2004). Interestingly, there was a less pronounced advancement in the luteal phase in some patients with advanced endometrial dating of 2–3 days on the day of oocyte retrieval, highlighting the importance of early biopsy (Kolibianakis et al. 2004). No difference in advanced endometrial maturation at the day of oocyte retrieval and in the mid-luteal phase was visible when agonist and

antagonist protocols were compared (Saadat et al. 2003; Palinska-Rudzka and Mathur 2023; Bourgain and Devroey 2003).

1.6 Assessment of endometrial receptivity

Many methods have been employed to assess endometrial receptivity. These include endometrial biopsy, ultrasound assessment, molecular markers and genetic markers. In the next section, a quick overview of these methods will be given.

1.6.1 Endometrial biopsy

Endometrial biopsy and histological dating was first established by Noyes in 1950 (Noyes, Hertig, and Rock 1950). His classical criteria remained the gold standard for dating the endometrium for many years. In fertility the primary role was the assessment of the luteal phase. However, recently the clinical utility of the endometrial biopsy as a routine test has been largely abandoned due to imprecision of and inconsistency of histologic dating, which in turn is reflected in the wide diversity of opinion in the literature regarding the incidence and clinical importance of luteal phase deficiency (LPD) (Jerome F. Strauss 2018). LPD is uncommon and probably accounts for no more than 3% to 5% of the infertile population. Currently the best method for diagnosing luteal phase problems may be the presence of a shortened luteal phase, as shown by methods such as basal body temperature charting (Jerome F. Strauss 2018).

1.6.2 Natural Killer cells

Uterine natural killer (uNK) cells are the most abundant leukocytes in preimplantation endometrium (Quenby and Farquharson 2006). They can secrete cytokines that are important in angiogenesis, placental development and establishment of early pregnancy. They also have the ability to interact directly with foetal trophoblasts. Despite the limited understanding of their specific role, increased numbers have been associated with implantation failure. (Quenby and Farquharson 2006).

Quenby et al. (Quenby et al. 1999) assessed various endometrial leucocytes in women with three or more recurrent miscarriages. Higher percentages of CD4⁺, CD8⁺, CD14⁺, CD16⁺ and CD56⁺ cells were detected in women who miscarried (4/15) compared to women who had a live birth (11/15) in the subsequent pregnancy. There were no differences of CD45⁺, CD3⁺, CD22⁺, CD57⁺ or CD69⁺ between the groups. (Craciunas et al. 2019)

Michimata et al. (*Michimata et al. 2002*) assessed various endometrial leucocytes 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 and women who miscarried in the subsequent pregnancy. (Craciunas et al. 2019)

Tuckerman et al. (Tuckerman et al. 2007) assessed the percentage of stromal cells positive for CD56 in 87 women with three or more unexplained recurrent miscarriages.

Similar CD56⁺ cell counts were detected in 19 women who miscarried and 32 women who had a live birth in the subsequent pregnancy. (Craciunas et al. 2019)

Liu et al (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. (Craciunas et al. 2019)

1.6.3 Ultrasound Assessment

Ultrasound is a non-invasive modality that allows assessment of reproductive organs. Transvaginal ultrasound has been used in the assessment of endometrial receptivity. Different parameters have been utilised including thickness, pattern, volume, blood flow and contractility.

1.6.3.1 Endometrial thickness

Endometrial thickness is the most extensively investigated parameter for assessment of endometrial receptivity. Most authors might agree that endometrial thickness <7 mm is correlated with lower implantation rates, however this doesn't imply a linear correlation between the two. (Zhao et al. 2014; Bonilla-Musoles et al. 2013).

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 in a

recent meta- analysis by Craciunas et al. (MD, 0.43; 95% CI: 0.21–0.64; $z = 3.87$; $P < 0.0001$; 20 studies. (Craciunas et al. 2019)

The systematic review and meta-analysis published in 2014 supported the limited capacity of endometrial thickness to predict conception in assisted reproductive technology cycles. (Kasius et al. 2014a; Bonilla-Musoles et al. 2013)

It presented significantly lower clinical pregnancy rates among women with endometrial thickness ≤ 7 mm (odds ratio 0.42, 95% confidence interval 0.27–0.67; $p=.0003$). This was followed by a recent meta-analysis which showed overall, the predictive accuracy of endometrial thickness for clinical pregnancy was low between women who achieved a clinical pregnancy and women who did not (Craciunas et al. 2019).

1.6.3.2 Endometrial volume

Endometrial volume by 3D ultrasound has been used to assess endometrial (Kovachev et al. 2005). Kovachev et al. examined the predictive value of endometrial volume as assessed by 3-D ultrasound on the day of embryo transfer (ET) and found that a volume of < 2 ml resulted in significantly lower implantation rates, whereas an endometrial volume of > 2 ml was a positive predictor for successful ART outcome. In another study that endometrial volume decreased significantly after the administration of hCG in women who succeeded to conceive, but not in those who failed to do so (Järvelä et al. 2005; Heger, Sator, and Pietrowski 2012).

Another study showed that endometrial volume on the day of oocyte aspiration did not differ between conception and non-conception cycles confirming findings from previous studies. Mercé et al. however, have found a significantly higher endometrial volume in patients that became pregnant.

A recent meta-analysis by (Craciunas et al. 2019) reported endometrial volume for women undergoing IVF with fresh embryo transfer. There was no difference in the endometrial volume measured on the day of trigger injection between women who achieved a clinical pregnancy compared to those who did not.

1.6.3.3 Endometrial Motion/contractility

Sub endometrial contractility illustrated by endometrial “waves,” can be observed in transvaginal ultrasound (TVUS) uterine studies (Heger, Sator, and Pietrowski 2012). These waves originate from the sub-endometrial myometrium and are hormone responsive. They are not routinely measured in IVF. However the endometrial “waves” were found to display different patterns, vectors, and phasic behaviors, which were all cycle dependent (Heger, Sator, and Pietrowski 2012; IJland et al. 1997; van Gestel et al. 2003). Early follicular phase was represented by wave movements from fundus to cervix, whereas wave movements from cervix to fundus were observed in the late follicular and periovulatory phases. Because uterine contractility must involve sperm propagation and embryo transportation, and later, uterine quiescence should support embryo implantation, there is a great biologic rationale in the study and interpretation of these endometrial waves. Generally, IVF treatments were associated with more

vigorous wave activity, probably reflecting the supraphysiologic E₂ levels. Some groups have reported an inverse correlation between the magnitude of wave activity and cycle outcome (Fanchin and Ayoubi 2009; Heger, Sator, and Pietrowski 2012)

1.6.3.4 Blood flow and Doppler

Ultrasound can also provide tools for evaluation of blood supply with the use of doppler studies. Though some of the indices tested showed some value in predicting implantation (Mercé et al. 2008) receiver operating characteristic curves failed to show a true value (Ng et al. 2006; Heger, Sator, and Pietrowski 2012).

A recent meta-analysis by (Craciunas et al. 2019), showed that there is no difference between pregnant and non pregnant women on the day of trigger in terms of endometrial vascularity index (VI), (flow index FI), vascularisation flow index (VFI) and sub-endometrial VFI. Sub-endometrial FI on trigger day was higher in women who achieved clinical pregnancy while sub-endometrial FI was lower in women who achieved clinical pregnancy. Uterine artery pulsatility index (PI) and resistance index (RI) was similar between pregnant and non pregnant women on the day of trigger. A uterine artery PI of <3 measured on the day of fresh embryo transfer was associated with higher clinical pregnancy rates. (Craciunas et al. 2019)

One study (Polanski et al. 2016) correlated manual and spherical endometrial spatio-temporal image correlation vascularity indices for 127 women undergoing fresh and

frozen–thawed embryo transfers to report no difference between clinically pregnant and not pregnant women. (Craciunas et al. 2019)

The presence of endometrial blood flow on the day of trigger was associated with higher clinical pregnancy rates (RR 1.98, 95% CI: 1.37-2.86), while the presence of blood flow on embryo transfer day showed no difference.

1.6.3.5 Endometrial pattern

Research into endometrial patterns, have yielded conflicting results (Hershko-Klement and Tepper 2016), with some papers supporting the ability of ultrasound to predict successful outcome of IVF (Zhao, Zhang, and Li 2012; Chen et al. 2010; Wu et al. 2014; Zhao et al. 2014) while others are dismissive (De Geyter et al. 2000; Rashidi et al. 2005) (Detti et al. 2008). the different classification systems will be discussed in the next section. Chapter 2 will include a systematic review and meta-analysis of the endometrial patterns.

1.6.4 Other Biomarker of Endometrial Receptivity

Here are some of the other biomarkers of endometrial receptivity

1.6.4.1 Pinopodes

Pinopodes demonstrate co-existence with a member of the EGF family, the heparin-binding epidermal growth factor-like growth factor (HB-EGF), family (Stavreus-Evers

et al. 2002). A possible role of HB-EGF in the attachment process was suggested because of the presence of HB-EGF both on the surface of pinopodes, and on the inside the luminal epithelial cells, which was demonstrated on confocal microscopy experiments. Slightly preceding the expression of $\alpha_v\beta_3$ integrins and HOXA10 in the mid-secretory phase, HB-EGF expression is increased in the mid-secretory phase (Lessey 2002). During the menstrual cycle, there is a remarkable shift in HB-EGF, with maximal stromal expression in the proliferative phase and, in contrast, peak expression in glandular and luminal epithelial cells during the WOI (Leach et al. 1999).

1.6.4.2 Epidermal growth factor (EGF)

It is not fully understood what the role of the EGF system is in endometrium. There are four specific receptors through which this growth factor family acts, HER1 to HER4. Exclusively expressed in the secretory epithelium and glands are HER3 and HER4 (Ejskjaer et al. 2007). A potential implantation-promoting factor is HB-EGF which works through HER1 and HER4 (Lessey 2002). Throughout the cycle, data on EGF expression in the human endometrium are contradictory. Whereas some groups describe EGF expression in a cycle-dependent manner in the epithelial cells or in epithelium and stroma (Reis et al. 2005), others were not able to demonstrate EGF in the endometrium regardless of the cycle phase (Ejskjaer et al. 2007).

1.6.4.3 Mucins

Mucins, particularly MUC1, are candidates for selection of the site of implantation. Anti adhesive properties of a MUC1 barrier on the epithelium of the lumen impairs interactions between the embryo and the endometrium. A selected site of facilitated adhesion may be resultant from interactions with integrins and changes of MUC1 glycoforms (Krasnow et al. 1996). Moreover, the blastocyst itself may influence the most appropriate implantation site by cleaving MUC1 (very locally). In a clinical study, compared with samples from normal fertile women, women with idiopathic infertility were shown to have endometrial samples with a reduced expression of the MUC1 D9B1 epitope (Aplin, Hey, and Graham 1998). This epitope was also reduced in women using an intrauterine device as well as in women with the endometrium out of phase (LPD) (Aplin, Hey, and Graham 1998), suggesting that there are some parts of the MUC1 glycoprotein that are more important for implantation than others.

1.6.4.4 Integrins

The best-studied group of cell adhesion molecules in the endometrium are integrins. They unite heterodimeric, non-covalently bound α and β subunits. Their extracellular domain acts as a receptor for various ECM ligands such as collagen, fibronectin, and laminin (Lessey et al. 1992). $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_6\beta_4$ are the permanently expressed integrins on endometrial epithelial cells. On stromal cells, the only permanent one is

$\alpha_5\beta_1$, the fibronectin receptor. Many integrins demonstrate a cycle-dependent expression. Some evidence suggests that in the implantation process, $\alpha_v\beta_3$ is the most important integrin (Lessey et al. 1992). It appears to be stimulated by EGF and is co-localized with osteopontin (Lessey 2002). A low expression of integrin $\alpha_v\beta_3$ seems to accompany several conditions associated with infertility e.g. unexplained infertility (Lessey 1995), endometriosis (Hasty et al. 1994), luteal phase insufficiency (Lessey, Ilesanmi, et al. 1996), and the presence of hydrosalpinges (Meyer et al. 1997).

1.6.4.5 Osteopontin

An important receptor for integrins is Osteopontin, with an obvious possible function for embryonic implantation. In glandular epithelial cells Osteopontin has been detected during the mid- to late-secretory phase (Apparao et al. 2001; von Wolff et al. 2001) and in uterine secretions from the secretory phase. It has an arginine–glycine–aspartic acid binding site that can bind to the transiently expressed $\alpha_4\beta_1$ and $\alpha_v\beta_3$ integrin heterodimers present during the window of implantation (Lessey et al. 1994). Microarrays analysing the WOI have shown that when compared with both the late proliferative phase and the early secretory phase, Osteopontin is consistently up-regulated during the WOI, consolidating its role in endometrial receptivity (Horcajadas et al. 2004).

1.6.4.6 Leukaemia- inhibitory factor (LIF)

A mouse model lacking a functional leukaemia-inhibitory factor (LIF) gene has demonstrated the role of LIF in implantation; after mating with a wild-type male, the embryos of a homozygous female did not attach and implant. There remains limited data on humans however. Clinically, in contrast with fertile women, more women with idiopathic infertility have undetectable levels of LIF in their uterine flushing, and it has been shown that there are mutations in the coding region of the LIF gene in some women with infertility. Cullinan *et al.* (Cullinan et al. 1996) first described LIF and LIF receptor in human endometrium. In glandular epithelium, LIF mRNA expression is 3-fold higher compared with stroma, and is cycle dependent, with preferred expression in the secretory phase compared with the proliferative phase. (Simón et al. 1998; Lessey 2002; Bourgain and Devroey 2007)

After it hatches from the zona pellucida, the embryo expresses L-selectin (Genbacev et al. 2003). Expressed on vessel walls is L-selectin, where, with their oligosaccharide, it captures leukocyte structures and binds them after integrin activation occurs at the site where they are needed. When Genbacev *et al.* discovered that highly O-glycosylated proteins resembling the glycoproteins known to act as selectin ligands were up-regulated at the start of the WOI, a similar process was postulated. The hypothesis postulates that mucin flow transports the embryo through the uterine cavity. L-selectin binds oligosaccharides on the epithelial surface when it hatches, slows down the blastocyst and permits a firm interaction with integrins, resulting in embryo adhesion. (Genbacev et al. 2003).

1.6.4.7 Endometrial Receptivity Array (ERA)

The endometrial receptivity array (ERA) is based on analysis of expression of 238 genes that are thought to be involved in the receptivity of the endometrium to implantation. It has been suggested that the ERA test will enable the determination of a personalized window of implantation (WOI) (Mahajan 2015; Díaz-Gimeno et al. 2013). This test is done by obtaining endometrial biopsy samples on day LH+ 7 in a natural cycle or on the 6th day of progesterone supplementation during an HRT cycle. Results are expressed as pre-receptive, receptive, or post-receptive. If the result is non-receptive, the embryo replacement timing may be adjusted in a subsequent cycle enabling personalized embryo transfer (Díaz-Gimeno et al. 2013). (Bassil et al 2018) showed it does not seem to improve outcome in good prognosis patients and that larger prospective studies need to be performed.

Mahajan et al. (Mahajan 2015) showed a higher rate of non-receptive endometrium in women who suffered two or more implantation failures (22/80, 28%) compared to women who had only one implantation failure (14/93, 15%) following IVF with standard embryo transfer of good quality embryos. (Craciunas et al. 2019)

Hashimoto et al. (Hashimoto et al. 2017) performed ERA testing on 50 women with recurrent implantation failure and reported non-receptive endometrium of 24% (12/50). The clinical pregnancy rate in the subsequent personalised embryo transfer 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%). (Craciunas et al. 2019)

Díaz- Gimeno et al. (Díaz-Gimeno et al. 2017) assessed 771 women diagnosed by ERA and further stratified the receptive endometrium based on the outcome following personalised embryo transfer (biochemical pregnancy vs live birth) to identify additional transcriptomic profiles: proliferative, early pre-receptive, late pre-receptive, receptive, late receptive and post-receptive. The ongoing pregnancy rates ranged between 76.9 and 80% in the late pre-receptive and receptive signatures compared to 33.3% in the late receptive endometrium. (Craciunas et al. 2019)

1.7 Different classification systems of endometrial patterns

Firstly, an overview of the literature shows that many classification systems of endometrial patterns have been described. These can be broadly divided into a basic two grade classification system, a three-grade classification system which is the most common and a four-grade classification system. Very occasionally more than four-grades classification systems have been used. These classification systems are summarised in table 2 at the end of this section.

The most famous four grade classification system is the system first described by Smith in the 1980's. He described the endometrial pattern as “qualitative change in grey-scale appearance or reflectivity” (Sterzik et al. 1991) from A-D with A being the most favourable and D being the least favourable” (Smith et al. 1984).

- Grade A “endometrium is characterized by an increased reflectivity pattern compared with that of the myometrium and, therefore, it appears brighter on the greyscale ultrasonographic image”
- Grade B “endometrium has a reflectivity comparable to that of the myometrium. A perceptible low-echo zone occurs between the myometrium and endometrium”.
- Grade C “endometrium is characterized by a higher density of the echo. As a whole, however, the endometrium still has a poorer echo than the surrounding myometrium. They considered that this appearance represents a mild but positive response by the endometrium to increasing levels of circulating oestrogens”.
- Grade D “endometrium is characterized ultrasonically by an anechoic or black region in the presence of a prominent midline echo. This is commonly seen in the early to mid-follicular phase and represents a very poor endometrial response”. (Smith et al. 1984)

Smith hypothesised that “the increasing echogenicity of the endometrium, related to the presence of tortuous glands filled with glycogen and mucin, is supposed to be a sonographic criterion for a good endometrial response.” (Smith et al. 1984)

However, the three grade classification system (A-C or 1-3) is the most common classification system used by authors with slight variation where type A or 1 is sometimes the triple pattern and Type C or 3 is the homogenous pattern or Vice Versa.

Yoshimitsu described endometrial patterns in 1989 using the three-grade classification system. The first type has one central linear echo representing the endometrial cavity often surrounded by two linear echoes representing the myo-endometrial junctions. The second type is hypoechogenic layers surrounding the linear cavity echo, representing the growing endometrium and the last type is characterised by increasing echogenicity starting from the peripheral border of the two myo-endometrial echoes, representing differential changes of the endometrial layers. (Yoshimitsu, Nakamura, and Nakano 1989)

Another example is the classification system described by Check in the 1990's as type A-C.

- Type A: "Triple line pattern or a multi-layered endometrium in which hyperechogenic outer lines and a well-defined central echogenic line were visualised with hypo-echogenic or black areas seen between these lines"
 - Pattern B: "intermediate pattern in which the endometrium had the same echogenicity as the myometrium with a poorly defined central echogenic line"
 - Pattern C: "An entirely homogenous, echo-dense endometrium in comparison with the myometrium in which no central echogenic line could be visualised".
- (Check et al. 1993)

Similarly, Gonen and Casper also used the three-grade classification system and described the endometrial pattern as Type A-C where type A is multi-layered triple-endometrium consisting of a prominent outer and central hyperechogenic line and inner hypoechogenic or black regions, type B is an intermediate isoechogenic pattern and type C has a homogenous pattern (Gonen and Casper 1990)

Sakamoto had a slight variation in his three-grade classification system where pattern C was defined by fluid distention in the cavity as illustrated.

- Pattern A: An entirely homogenous hyperechogenic endometrium
- Pattern B: A multi-layered endometrium characterized by three hyperechogenic lines with inner hypoechogenic areas
- Pattern C: A uterine cavity distended by fluid and having a 'ring' configuration.

This is also the same system used by Zhao in 2012. Boehnlein also had a similar description of patterns in 2009:

- Pattern 1: "A multi-layered endometrium appeared as a triple line pattern in which the hyperechogenic outer lines a well-defined central echogenic line were seen with hypoechogenic or black areas between these lines".
- Pattern 2: "An intermediate endometrium appeared as an endometrial pattern that was transitioning into a non-multi-layered appearance at the myometrial and endometrial interface, but still had some elements of a well-defined central echogenic line with hypoechogenic or black areas between these lines".

- Pattern 3: “A non-multi-layered endometrium appeared as a homogenous endometrial pattern characterised by either an increased reflectivity compared with the myometrium (isoechogenic endometrium)”. (Barker et al. 2009)

Similarly, other authors utilised this three grade system with slight modifications such as Zachova, Singh and Tepper.

Interestingly however, Grunfeld used a slightly different classification system despite it being a three-grade system. He utilised the percentage of the endometrial thickness when describing patterns as follows:

- Type 1: Late proliferative (hyperechoic endometrium constituting <50 % endometrial thickness with a hyperechoic basalis and a hypoechoic functionalis)
- Type 2: early secretory (hyperechoic basalis and functionalis extending to >50% of the endometrial thickness, but not compassing the entire endometrial cavity)
- Type 3: Mid-late secretory (homogeneous hyperechoic functionalis extending from the basalis to the lumen. (Gingold et al. 2015b)

Miyara has tried a slightly different approach in 1996 where he tried to correlate endometrial patterns with histology. He also described three different patterns.

- Pattern L: multi-layered endometrium characterized by 3 hyperechogenic lines with inner hypoechogenic regions. Histology had smaller, similar-sized endometrial glands and few stromal cells
- Pattern H: Entirely homogenous hyperechogenic endometrium, histology had larger various sized glands and more stromal cells
- Pattern I: heterogenous hyperechogenic and partially hypoechogenic endometrium, histology had the largest, most variable-sized glands and many stromal cells.

He concluded “that differences in sonographic texture may be related to the histological and morphometrical findings” (Miyara et al. 1996)

The most simplistic classification system however is the two-grade classification system which has been described by few authors. Fujimoto and Ohno have utilised this system in 1998 and described the patterns as:

- Group A: triple line pattern or multi-layered endometrium in which hyperechogenic outer lines and well defined central echogenic line were visualised with hypoechogenic or black areas seen between lines
- Group B: an echo pattern in which the endometrium had the same echogenicity as the myometrium, homogenous and echo echo-dense in comparison with the myometrium. (Ohno and Fujimoto 1998)

Sher also used a similar system in 1991 where he described endometrial patterns as Grade I or II. Grade I is characterised by homogenous echogenicity of the endometrium whereas grade II is characterised by an outer peripheral layer of dense echogenicity surrounding a central sonolucent area, visualised using a transverse axis (Sher et al. 1991). It is not surprising that this system has been described by many authors with different variations such as triple or homogenous, triple pattern or no triple etc. However, given the simplistic nature of this classification system, the limitations it has in describing the full range of patterns and consequently its significance, is obvious.

It is worth noting that all the above classification systems are very subjective and hence have a big degree of inter-observer variability. Fanchin (Fanchin et al. 2000b) tried to solve this issue by using objective computer assisted measurement in 2000, and dividing the echogenicity into 5 different scales: <30%, 31-40%, 41-50%, 51-60% and 61-70%. The pregnancy rates were reported to fall progressively and significantly from the low-echogenicity group to the high-echogenicity group.

Table 1. 1 Summary of Endometrial Patterns' Classification systems

Classification system	Authors	Summary
Four grade classification system	Smith et al	Grade A-D, grade A is most favourable
Three grade classification system	Yoshimitsu et al	Grade 1-3, Grade 3 increased echogenicity
	Check et al	Grade A-C (Triple to homogenous)
	Gonen et al Sakamoto et al, also used by Zhao et al	Very similar (triple to homogenous)

	Boehnlein, also utilised by other authors with slight variation (Zachova, Singh and Tepper) Grunfeld et al Miyara et al	Slight variation (Grade A is homogenous, B is triple, C fluid filled cavity) Grade 1-3 (triple, intermediate, homogenous) Slight variation, utilised % of endometrial thickness Type 1: Late proliferative, 2 is early secretory, 3 is mid late secretory Utilised histology, Pattern L, H and I
Two grade system	Sher et al and many authors	Triple, homogenous or triple and no triple etc
Objective computer assisted measurement	Fanchin et al	The echogenicity divided into 5 different scales as shown below: <30%, 31-40%, 41-50%, 51-60% and 61-70%

1.8 Summary and hypothesis

In summary endometrial receptivity, is responsible for approximately two-thirds of implantation failures, whereas the embryo itself is responsible for only one-third of these failures (Simón et al. 1998; Ledee-Bataille et al. 2002)

Ultrasound is non-invasive tool that can provide very useful information and allow assessment of endometrium. Research into endometrial patterns, have yielded conflicting results (Hershko-Klement and Tepper 2016), with some papers supporting the ability of ultrasound to predict successful outcome of IVF (Zhao, Zhang, and Li

2012; Chen et al. 2010; Wu et al. 2014; Zhao et al. 2014) while others are dismissive (De Geyter et al. 2000; Rashidi et al. 2005) (Detti et al. 2008). We believe this can be explained by many factors including the heterogeneity of the studies, different methodology, variable classification systems used and inter and intra-observer variability. There have also been developments in ultrasound quality since many of these studies were performed.

We hypothesise that endometrial pattern on ultrasound can predict the outcome of assisted conception (IVF/ICSI).

If we succeed in identifying cycles where success is low or negligible, we can defer embryo transfer for future cycles. This will save valuable embryos and reduce parental emotional distress. The research will demonstrate the utility of ultrasound assessment of the endometrium to predict and improve IVF outcomes. Future research studies may be able to use ultrasound appearance as a surrogate for successful implantation.

The Human Fertilisation and Embryo Authority (HFEA) reports IVF/ICSI success rate between 29% in under 35-year-old women to 2% in women over 44 years old. Improved predictors of IVF/ICSI success will reduce parental stress and save money

In order to test my hypothesis, the following objectives will be addressed:

1.9 Pre-Covid aims/objectives

1. To perform a systemic review on endometrial patterns as predictor of successful endometrial pattern on ultrasound to predict implantation in IVF/ICSI. (Chapter 2)
2. Conduct a reliability study on reporting of ultrasound endometrial patterns and measure the inter and intra observer variability. (Chapter 3)
3. Conduct a prognostic accuracy study on endometrial pattern as predictor of IVF/ICSI success. (Chapter 4)
4. Assess the correlation between histology, genetic markers of endometrial receptivity and endometrial patterns on ultrasound. (Chapter 5)
5. Produce a reliable a novel classification system of endometrium patterns. (chapter 1, 3 and 5)

1.10 COVID 19 impact and post COVID 19 aims and objectives

The main impact of the COVID-19 pandemic was on recruitment/ data collection and subsequently analysis as both the university and the IVF unit closed for few months during the COVID 19 pandemic. For the main study (Exploratory analysis of the prognostic accuracy of endometrial patterns on ultrasound to predict implantation), the aim was to have 200 cycles (100 fresh and 100 frozen), 191/200 cycles were achieved instead. This included 116 fresh cycles and 75 frozen cycles.

For the biopsy study (the relationship between endometrial pattern on ultrasound, histology and microarray in endometrial biopsies), the initial target was to recruit 50

women however this later had to be modified to 30 women instead due to COVID 19 disruption. Furthermore there was significant delay in processing the endometrial biopsy samples for micro-Array analysis. I performed the RNA extraction after the university reopened. Those samples for Micro-array analysis were sent to Sutton Bonington, which further delayed the processing of these samples. The final results were only received in September 2021.

The updated post COVID 19 aims and objectives are listed below

1. To perform a systemic review on endometrial patterns as predictor of successful endometrial pattern on ultrasound to predict implantation in IVF/ICSI. (Chapter 2)
2. To simplify ultrasound assessment by developing a novel classification system (chapter 3).
3. Conduct a reliability study on reporting of ultrasound endometrial patterns and measure the inter and intra observer variability. (Chapter 3)
 - To assess the interrater reliability for the current classification system between different operators.
 - To assess the interrater reliability for the “new pattern recognition” classification system between different operators.
 - To assess the intra rater reliability for the current classification system for the same operator.
 - To assess the intra rater reliability for the “new pattern recognition” for the same operator.
4. Conduct a prognostic accuracy study on endometrial pattern as predictor of IVF/ICSI success. (Chapter 4)

- To investigate whether endometrial pattern on ultrasound can predict the outcome of IVF or ICSI.
 - To identify whether test characteristics vary with fresh or frozen cycles.
 - To assess whether rate of change of endometrial pattern between the different scan points has an impact on the success rate.
 - To investigate whether other ultrasound parameters such as endometrial Doppler and endometrial thickness can affect/predict IVF/ICSI success rate.
 - To assess whether other parameters such as hormonal profile has an impact on the success rate.
4. Assess the correlation between histology, genetic markers of endometrial receptivity and endometrial patterns on ultrasound. (Chapter 5)

Chapter 2 Endometrial ultrasound pattern to predict pregnancy in women undergoing embryo transfer; a systematic review and meta-analysis

2.1 Background

The process of embryo implantation requires good harmony between the endometrium and blastocyst. (Singh et al. 2011; Kader et al. 2016). Many factors can impact the success of assisted conception, but endometrial receptivity and embryo quality are the key players in this process.

Different ultrasound parameters have been utilised to assess endometrial receptivity. Endometrial pattern can provide valuable information which can help decipher the window of implantation as it reflects the hormonal status as well as the histological maturation. However, research into the predictive value of endometrial pattern has yielded conflicting results (Hershko-Klement and Tepper 2016). The discrepancy may relate to the use of different classification systems, the timing of the assessment, the different treatment protocols used, and the population studied.

In this review, the aim is to investigate whether endometrial pattern on ultrasound can predict the successful pregnancy in women undergoing embryo transfer. This review might also help guide treatment protocols with regards to optimising the timing of embryo transfer and ultimately in improving the success rate of assisted conception.

2.2 Methods

This is a systematic review of prognostic factor studies. The methods described in the Cochrane Handbook for Systematic Reviews of prognostic studies were followed. (Deeks et al. 2011; Macaskill et al. 2010; Moher et al. 2009). This review has been registered on Prospero (CRD42021219015).

2.2.1 Criteria for considering studies for this review

The study selection used the PICOT (Population, Index prognostic factor, Comparator prognostic factors, Outcome, Time) framework. The PICOT was developed based on the Eligibility criteria stated below.

2.2.1.1 Eligibility criteria

2.2.1.1.1 Inclusion Criteria

- Women undergoing in vitro fertilisation/ intracytoplasmic sperm injection IVF/ICSI.
- Age of participants at least 18 years with no upper limit.
- Test clearly defined as endometrial pattern on transvaginal ultrasound.
- Primary outcome clearly defined as clinical pregnancy (Intrauterine pregnancy with foetal heart on ultrasound).
- Secondary outcomes live birth can be included if available.

- Studies where 2x2 tables can be generated from raw data where the test is endometrial pattern, and reference standard is clinical pregnancy as defined by intrauterine pregnancy with foetal heart seen on ultrasound.

2.2.1.1.2 Exclusion Criteria

- Intrauterine insemination, ovulation induction, zygote intrafallopian tube transfer (ZIFT), gamete intrafallopian tube transfer (GIFT) and oocyte or embryo donation cycles.
- Reference standard other than clinical pregnancy. Studies where the outcome was biochemical pregnancy only were excluded.
- No raw data or inability to generate 2x2 or 3x2 tables from the data given.
- Animal studies, book chapters, reviews, interventional studies, case reports, conference abstracts, letters and editorials.

2.2.1.2 Types of studies

Cohort or case-controlled studies evaluating the pregnancy outcomes of women who received either fresh or frozen embryos during IVF or ICSI were included. Studies that used intrauterine insemination were not included. Studies written in any language other than English were excluded. Review papers, case reports, interventional studies, animal studies, books, and book chapters were excluded.

2.2.1.3 Participants

Two populations were studied. Women who underwent fresh embryo transfer or women who underwent frozen embryo transfer during IVF or ICSI. Studies where a participant was involved in more than one cycle were also included. Women who underwent ovulation induction, intrauterine insemination, ZIFT, GIFT as they did not undergo uterine embryo transfer. Oocyte or embryo donation were also excluded to reduce the risk of introducing bias. Prognostic factor

Studies that assessed the endometrial pattern on transvaginal ultrasound during IVF/ICSI cycles as the prognostic factor to predict pregnancy outcome were included. It was expected that the classification systems of endometrial patterns would vary across literature. Therefore, studies that used either a three-grade system (e.g. triple, intermediate, homogenous) or two grade system (e.g. triple, homogenous) were included. Studies that used more than the three-grade system such as Fanchin et al. (Fanchin et al. 2000b) were incorporated into either 2 or 3 grade systems if appropriate. Table 2.1 illustrates some of the different classification systems used in the literature.

Table 2.1 Example of the different endometrial pattern classification systems.

Classification system	Authors	Summary
Four grade classification system	(Smith et al. 1984)	Grade A-D,
Three grade classification system	(Yoshimitsu et al. 1987) (Check et al. 1991) (Gonen and Casper 1990) Sakamoto et al, also used by (Zhao, Zhang, and Li 2012) (Barker et al. 2009), also utilised by other authors with slight variation (Zachova, Singh and Tepper) (Singh et al. 2011) (Grunfeld 1996) (Miyara et al. 1996)	Grade 1-3, Grade 3 increased echogenicity Grade A-C (Triple to homogenous) Very similar (triple to homogenous) Slight variation (Grade A is homogenous, B is triple, C fluid filled cavity) Grade 1-3 (triple, intermediate, homogenous) Slight variation, utilised % of endometrial thickness Type 1: Late proliferative Type 2: Early secretory Type 3: Mid late secretory Utilised histology, Pattern L, H and I
Two grade system	(Sher et al. 1991) and many authors	Triple, homogenous or triple and no triple etc
Objective computer assisted measurement	(Fanchin et al. 1999)	The echogenicity divided into different scales as shown below: <30%, 31-40%, 41-50%, 51- 60%, 61-70% & >70%

The predicative accuracy of each pattern was measured on the day of final follicular maturation (trigger), egg collection, and embryo transfer for fresh cycles. Trigger refers to the ovulation trigger used once the follicles have reached the sufficient sizes to ensure oocyte maturation. Typically, the presence of three 17-18mm diameter follicles is considered adequate for administration of ovulation trigger.

Human chorionic gonadotrophin is frequently used as a triggering agent to promote oocyte maturation and is usually administered 36 hours before the oocyte retrieval procedure. (Palinska-Rudzka and Mathur 2023)

For frozen cycles, the predictive accuracy of endometrial pattern was measured on the day when initiation of luteal support (progesterone) was started and the day of embryo transfer.

2.2.1.4 Comparator prognostic factors

Comparator prognostic factors were not included in this review.

2.2.1.5 Type of outcome measures

2.2.1.5.1 *Primary outcome*

The primary outcome is clinical pregnancy defined as intrauterine pregnancy with foetal cardiac activity on ultrasound. Included studies should have provided a clear definition of the clinical pregnancy. Ideally the primary outcome should be live birth, however a scoping review showed there is insufficient data (only 3 studies reported on this) and hence clinical pregnancy was selected instead. Unfortunately the outcomes were measured per cycle and not per women as recommended by Cochrane as this is how it was reported by most of the studies in the literature.

2.2.1.5.2 Secondary outcome

Live birth was stated as the secondary outcome. Studies reporting only biochemical pregnancy were excluded. Miscarriage data was not included as chromosomal and genetic factors are likely to have a bigger role in causing miscarriages than endometrial receptivity.

2.2.1.6 Timing

As the outcomes were defined as clinical pregnancy and live birth, the time setting was the duration of the pregnancy.

2.2.2 Search methods for identification of studies

Electronic searches were used to identify eligible studies. Full searches were developed where strategies were based on the scoping searches, expert advice, and consultation with the help of librarians. No date restriction was applied for the search; however, Language restrictions were applied and studies only in English were included. The bibliographic databases searched were MEDLINE, MEDLINE In Process and Embase via Ovid, Cochrane (Wiley) CENTRAL, Science Citation Index (Web of Science), CINAHL (EBSCO) with search strategies adapted for each database as required. Searches for ongoing studies utilised the ISRCTN Registry, UK Clinical Trials Gateway, World Health Organisation (WHO) International Clinical Trials Portal (ICTRP) and ClinicalTrials.gov. Specialists abstract and conference proceeding

resources were found via the British Library's ZETOC and Web of Science Conference Proceedings Citation Index. The search was carried out on 19 November 2020 and then updated on 5 April 2022. Search strategies for each electronic resource are listed in Appendix 1. The search was updated in 2022 to include any new studies which were eligible and to ensure that the review is up to date.

2.2.3 Data collection and analysis

2.2.3.1 Selection of studies

The articles were retrieved from each search database and saved into the research information systems (.ris) format. They were then imported to EndNote X9 for collation and removal of duplicates. After removal of duplicates the articles were transferred into Abstrakt (Rathbone, Hoffmann and Glasziou 2015), an online screening tool for screening of eligible studies. Two reviewers independently screened the titles and abstracts of all studies identified by the search strategy and assessed studies for inclusion using the pre-specified inclusion criteria stated earlier. The full texts of potentially relevant studies were then accessed and screened by two reviewers to confirm their eligibility for inclusion in the review. A third reviewer resolved any disagreement when needed (Moher et al. 2009).

2.2.3.2 Data extraction and management

The data extraction form was developed from the adapted Checklist for critical Appraisal and data extraction for systematic Reviews of prediction Modelling Studies (CHARMS) checklist (Moons et al. 2014). Data extraction was performed by at least two review authors who independently used the standardised data extraction form.

The form was piloted using four of the included studies before it was finalised. It included general information about the study (authors' names, date of publication, country where the study conducted, title, and type of the study), study characteristics (aims, study design, study duration, ethics obtained). Other details included participant description (Population Description, participants age, sample size), treatment received (type of assisted reproduction technique used either IVF, ICSI or both, number of cycles received, and protocol used in treatment, number of and age of embryos if they were specified, grade of embryo used), prognostic factor (definition of endometrial pattern classification system used, other interventions or measurements e.g., hormonal assessment), timing of prognostic factor measurement (pattern measured at trigger, egg collection, initiation of progesterone, or at embryo transfer), outcomes to be predicted (clinical pregnancy/live birth and definitions used), results (tables of results used include both tables for clinical pregnancy as well as live birth if available, and tables of raw data and information about confounders, handling of missing data, and statistical analysis used in the study..

2.2.3.3 Assessment of methodological quality

To assess the risk of bias and the overall quality of the included studies, the Quality In Prognosis Studies (QUIPS) tool (Hayden et al. 2013) was used. Two review authors independently assessed the risk of bias for each study using the QUIPS tool's following domains: study participation; study attrition; prognostic factor measurement; outcome measurement; Study Confounding; and statistical analyses. Each domain was considered to have a low risk of bias, moderate risk of bias, or high risk of bias according to the overall assessment of the items within the domain.

Formal assessment of reporting bias was not undertaken due to the current uncertainty about how to assess this in prognostic study reviews (Macaskill et al. 2010).

In all cases, the analyses were performed according to the recommendations of Cochrane as stated in the Cochrane statistical package Review Manager 5.4.1 for synthesising the data (Deeks et al. 2011).

2.2.3.4 Data synthesis & Statistical analysis

The odd ratio or adjusted odd ratio with 95% confidence interval (CI) for the outcomes were calculated using the Cochrane statistical package Review Manager 5.4.1 (Deeks et al. 2011). The random effect model was used due to the heterogeneity in the

populations, treatment protocols and the endometrial pattern classification systems. The results were reported as forest plots for the meta-analysis.

Subgroup analysis was performed based on the population, women undergoing fresh IVF/ICSI embryo transfer and women undergoing frozen IVF/ICSI embryo transfer. The subgroups were further subdivided based on the type of endometrial pattern classification system (two grade or three grade classification system) and the timing of the test (trigger, egg collection, initiation of luteal support or embryo transfer).

In all cases, we performed analyses according to the recommendations of Cochrane and will use the Cochrane statistical package Review Manager 5 for synthesising the data (Deeks et al. 2011).

2.2.3.5 Investigation of heterogeneity

Heterogeneity was examined between studies by visually inspecting the Forest plot using (I^2) statistics. An I^2 greater than 50% was considered to be moderate heterogeneity; an I^2 greater than 80% was considered to be considerable heterogeneity (Deeks et al. 2011).

Tau² values from the meta-analyses were calculated to be able to make a more robust judgment on the degree of statistical heterogeneity.

2.2.3.6 Assessment of reporting bias

We did not undertake any formal assessment of reporting bias due to current uncertainty about how to assess this in prognostic study reviews (Macaskill et al. 2010).

2.3 Results

There were 3254 studies identified through the literature search after duplicates were removed. Titles and abstracts were screened and 3016 studies were excluded. The full text screening of 238 articles identified 33 eligible studies which were included in the systematic review and meta-analysis. Figure 2.1 shows the total numbers of retrieved references and the numbers of included and excluded studies reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al. 2009).

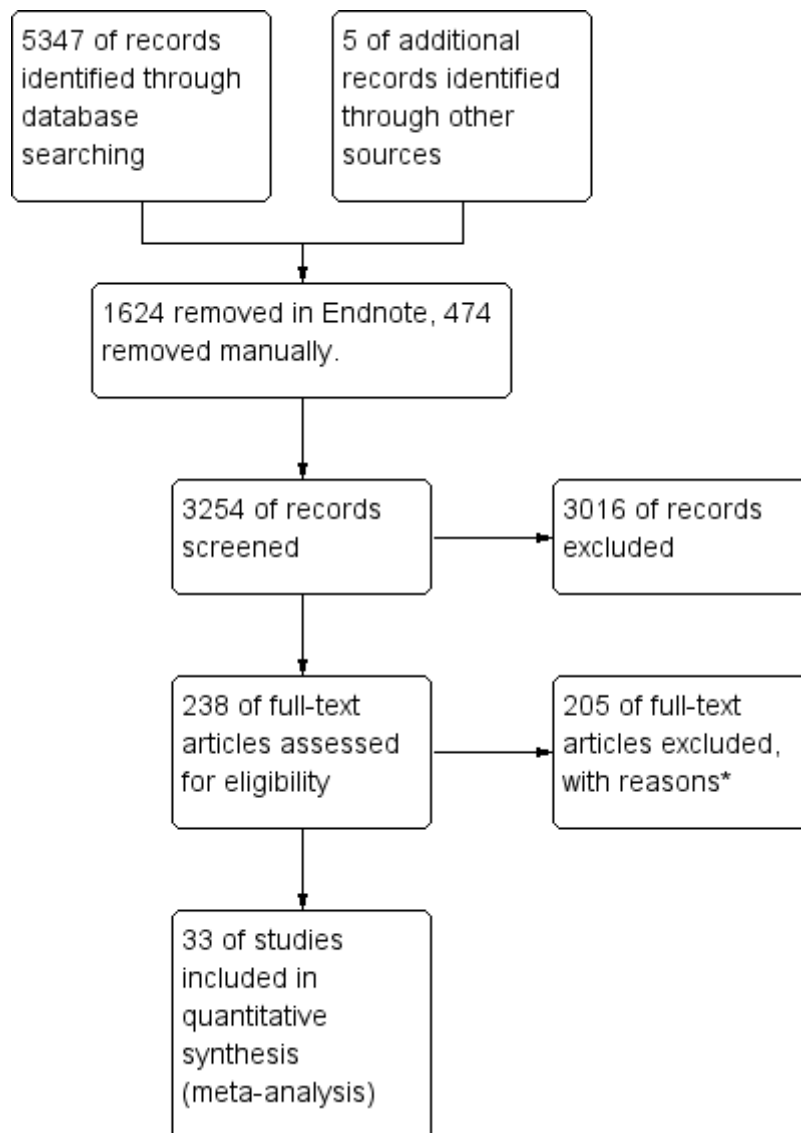


Figure 2.1 Study flow diagram according to PRISMA

*The main reasons for exclusion are: Reference standard not clinical pregnancy eg biochemical pregnancy, endometrial pattern not assessed against clinical pregnancy or live birth, not IVF/ICSI cycles eg IUI or ovulation induction, interventional study where impact of intervention/drug is assessed on endometrial pattern, comparison of endometrial pattern between IVF patients and normal cycling women, endometrial pattern not assessed, oocyte donation cycles, endometrial pattern not defined, no data provided, review articles or editorials.

2.3.1 Selected studies

There were 33 eligible studies which included observational, prospective and retrospective cohort studies. Appendix 2 provides a summary of all included studies.

The overall quality of the studies were assessed using the QUIPS tool (Hayden et al. 2013). Studies were classed at low risk of bias if they demonstrated adequate participation and good description of the population baseline characteristics, inclusion and exclusion criteria, recruitment period and no attrition. A good description of the endometrial pattern classification system was required and all the endometrial patterns were to have been identified via transvaginal ultrasound along with a clear description of the ultrasounds' timing. The primary outcome needed to be clearly defined as clinical pregnancy with intrauterine gestational sac and foetal heart visualised on ultrasound. A good description of the statistical analysis and reporting was needed and confounding factors addressed with no selective reporting of results. Studies were classed as being at high risk of bias if there was a poor description of the study population and method of recruitment, if the endometrial pattern classification system used was poorly described, and where data was missing without explanation. Studies were still included if they mentioned 'clinical pregnancy' as the outcome but would be classed as at risk of bias if they failed to provide clear definition of 'clinical pregnancy'. Studies were also classed as being at high risk of bias if they failed to mention or address confounding factors and where there was a poor description of statistical

methods used and selective reporting of results. “Studies are assessed as at unclear risk of bias when too few details are available to make a judgement of ‘high’ or ‘low’ risk; when the risk of bias is genuinely unknown despite sufficient information about the conduct; or when an entry is not relevant to a study” (Deeks et al. 2011). Figure 2.2 below demonstrates the risk of bias graph for the studies.

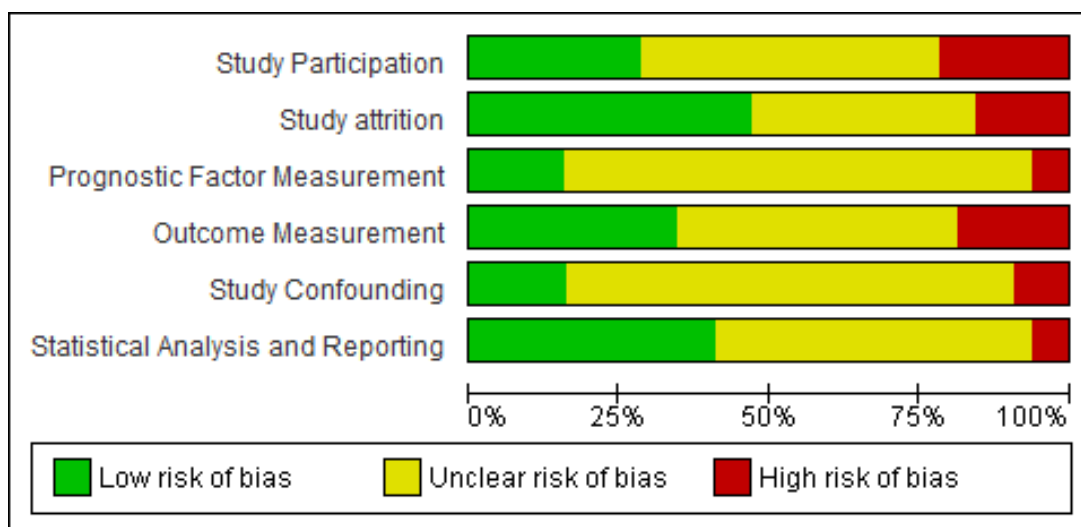


Figure 2.2 Risk of bias graph

2.3.2 Endometrial pattern in women undergoing embryo transfer

The majority of the studies used either the two grade classification system of endometrial pattern with slight variations and the three grade classification systems.

Two grade: Triple and no triple or triple/trilaminar and homogenous or multi-layered and homogenous etc.

Three grade: Triple, intermediate, homogenous or late proliferative, early secretory and homogenous or three layer, two layer and homogenous or trilaminar, iso-echogenic and hyperechogenic etc.

There was one study (Fanchin et al. 2000a) which measured the extent of hyperechogenic transformation of the endometrium over the endometrial thickness.

For the purpose of the review, it was combined into three grades as follows:

- <30% (for this study classified as Triple Line)
- 31%– 40% (for this study classified as Triple Line)
- 41%–50% (for this study classified as Intermediate)
- 51%–60% (for this study classified as Intermediate)
- 61%–70% (for this study classified as Homogeneous)
- >70% (for this study classified as Homogeneous)

The three grade classification system for all the studies was analysed as

- Triple vs homogenous
- Triple vs intermediate
- Intermediate vs homogenous

The results were analysed separately for fresh and frozen cycles.

2.3.2.1 Fresh embryo transfer cycles

2.3.2.1.1 Assessment of endometrial pattern at trigger

There were 24 studies that reported clinical pregnancy outcomes (19048 cycles) at trigger for the comparison between triple and homogenous pattern. 10 studies used the three grade classification system and 14 studies used the two grade classification system. Triple line pattern assessed on the day of trigger is associated with higher clinical pregnancy rates (OR 1.89 95% CI:1.39, 2.58), with substantial heterogeneity $I^2=74\%$ in comparison to homogenous pattern as illustrated in figure 2.3. Figure 2.3 illustrates the comparison between triple pattern and homogenous pattern at trigger where the outcome is clinical pregnancy.

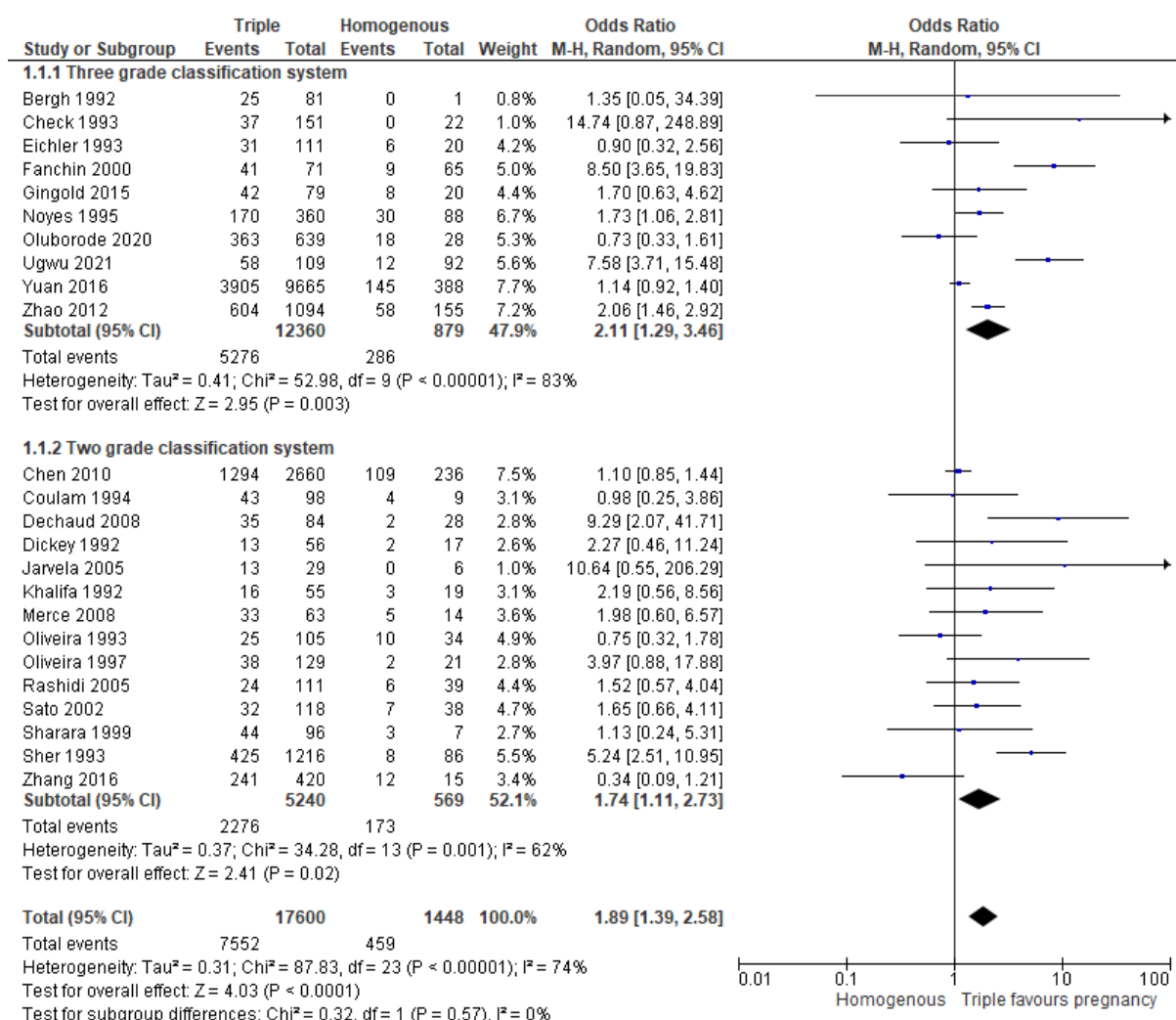


Figure 2.3 Forest plot of comparison of triple vs homogenous at trigger, outcome: Clinical pregnancy.

Subgroup analysis comparing women with triple pattern to intermediate pattern did not show any significant difference. Figure 2.4 below illustrates the comparison between triple and intermediate pattern at trigger where the outcome is clinical pregnancy.

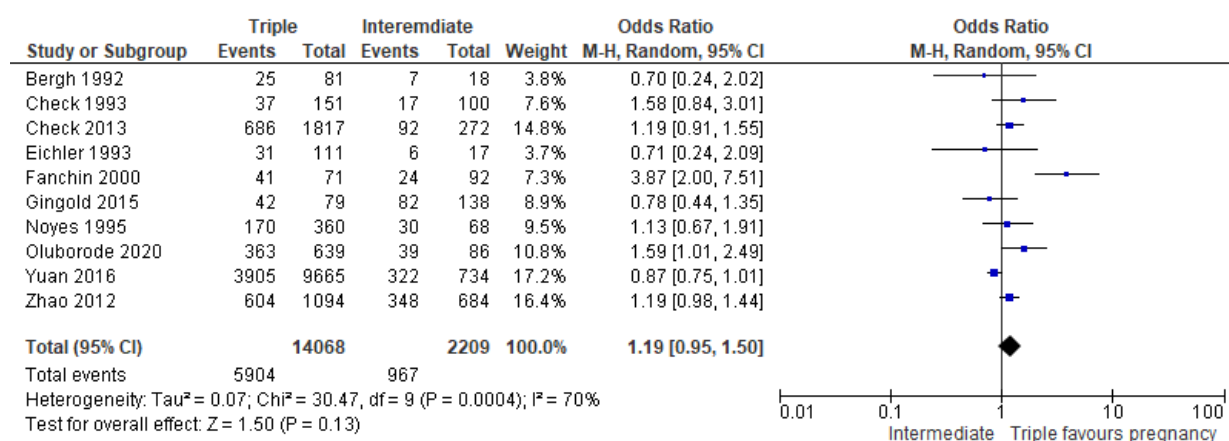


Figure 2.4 Forest plot of comparison: Triple vs Intermediate at trigger, outcome: Clinical pregnancy.

Women with intermediate pattern were more likely to become pregnant in comparison to women with homogenous pattern at trigger. Figure 2.5 below illustrates the comparison between intermediate pattern and homogenous pattern at trigger where the outcome is clinical pregnancy.

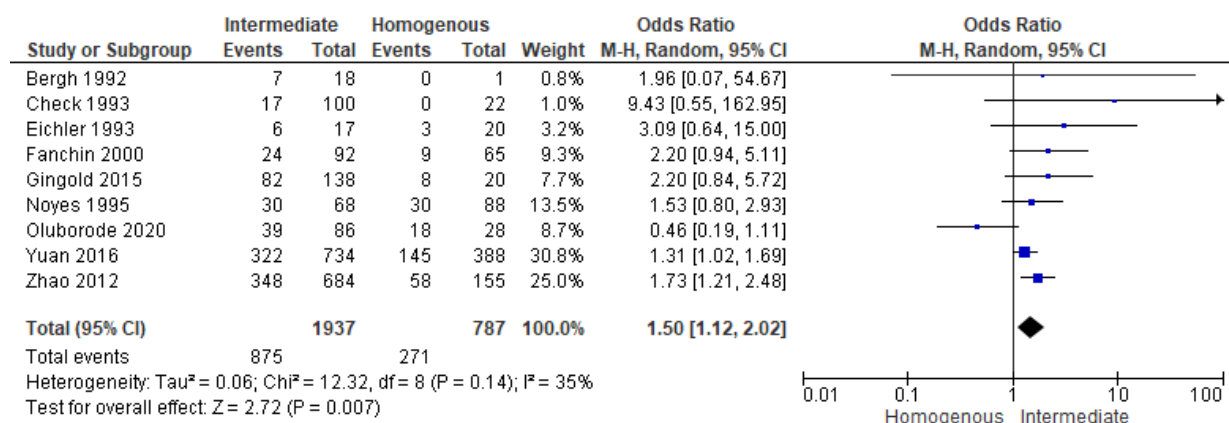


Figure 2.5 Forest plot of comparison: Intermediate vs Homogenous at trigger, outcome: Clinical pregnancy.

There were only 3 studies that reported live birth outcomes for endometrial pattern at trigger (2032 cycles). The largest study is from Sher et al. (Sher, Salem, et al. 1993) in 1322 fresh cycles. They reported outcomes from 1302 cycles. They used the two grade classification system. In their conclusion, they reported a direct correlation between the quality of the late proliferative phase endometrium and the likelihood of subsequent implantation following IVF/ET (Sher, Dodge, et al. 1993).

Dickey et al also used the two grade classification system and 200 cycles (75 IVF, 25 Gamete intrafallopian transfer and 32 tubal embryo transfer). For the purpose of this review, only the IVF cycles were included. It is a small study but their findings indicated that endometrial pattern and thickness are associated with fecundity after IVF (Dickey et al. 1992).

Oluborode et al (Oluborode et al. 2020) used the three-classification system; triple, intermediate, and homogenous. It is a retrospective study and they assessed 778 fresh IVF/ICSI cycles. They concluded that endometrial pattern does not appear to significantly affect live birth in assisted conception (Oluborode et al. 2020).

Figure 2.6 below illustrates the comparison between the different endometrial patterns (triple vs homogenous, triple vs intermediate and intermediate vs homogenous) at trigger where the outcome is live birth.

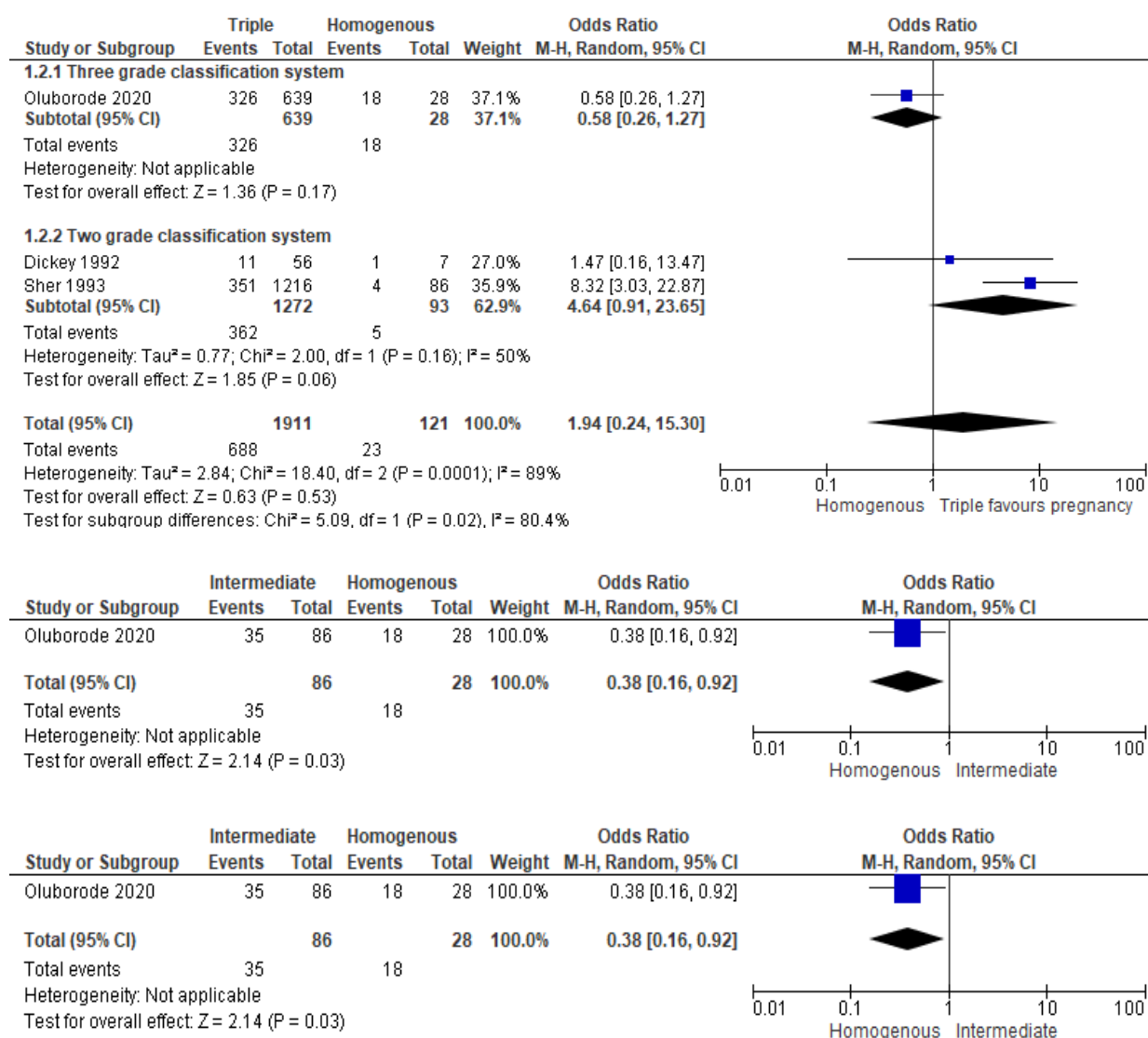


Figure 2.6 Forest plot of comparison: 1 triple vs homogenous, 2 triple vs Intermediate and 3 intermediate vs homogenous at trigger, outcome: Live birth.

2.3.2.1.2 Assessment of endometrial pattern at egg collection

There were 5 studies that reported clinical pregnancy outcomes (383 cycles) at egg collection for the comparison between triple pattern and homogenous. One study used

the three grade classification system and 4 studies used the two grade classification system. There were no studies that reported on live birth outcomes at egg collection. The results were not statistically significant. Figure 2.7 illustrates the comparison between triple pattern and homogenous pattern at egg collection and the comparison between triple pattern and intermediate pattern where the outcome is clinical pregnancy.

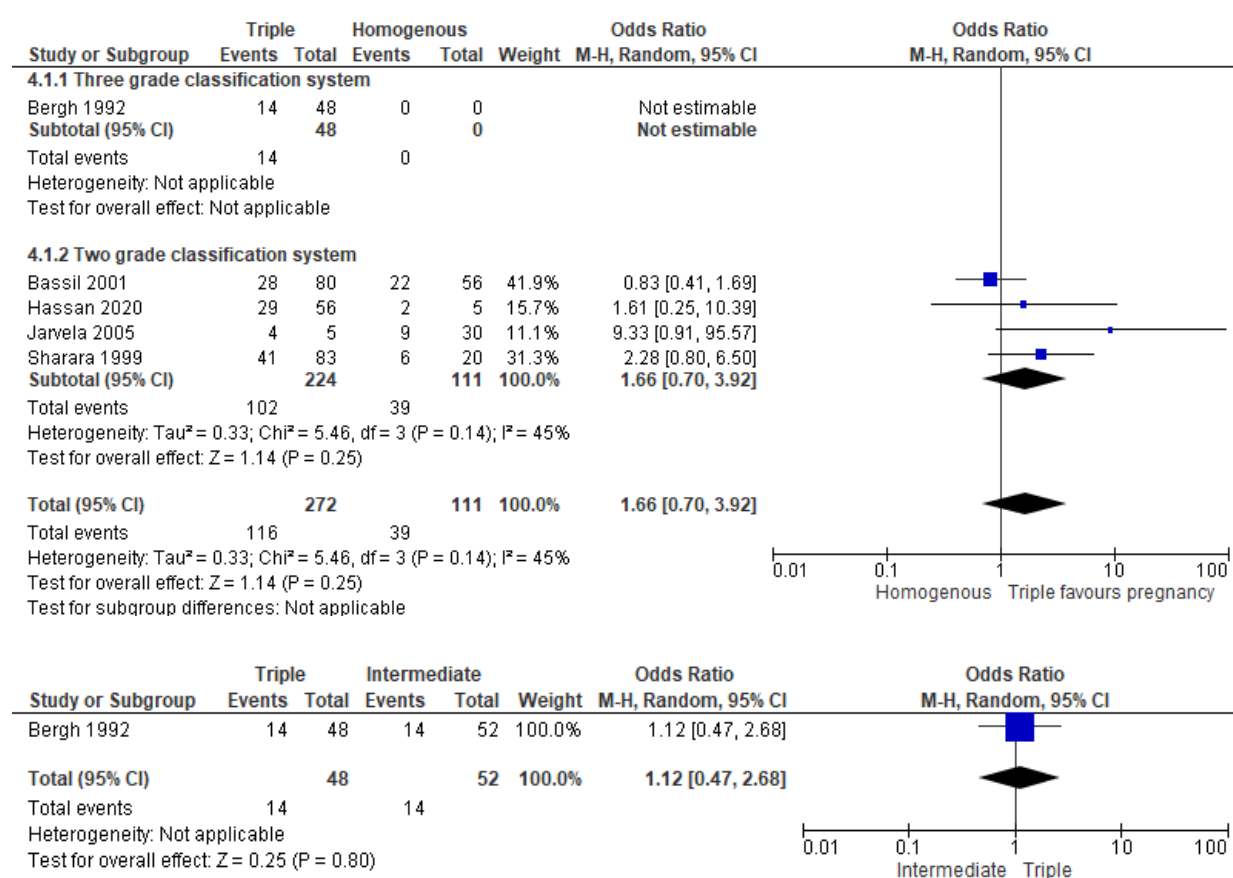


Figure 2.7 Forest plot of comparison: Triple vs homogenous at egg collection and triple vs intermediate, outcome: Clinical pregnancy.

2.3.2.1.3 Assessment of endometrial pattern at embryo transfer

There were 7 studies that reported clinical pregnancy outcomes (777 cycles) at embryo transfer for the comparison between triple pattern and homogenous. Three studies used the three grade classification system and 4 studies used the two grade classification system. There were no studies that reported on live birth outcomes at embryo transfer. Although studies that used the three grade classification system showed that homogenous pattern favoured pregnancy compared to triple pattern at embryo transfer (OR 0.24 95% CI 0.07, 0.89), the overall result was not statistically significant when combining the three grade classification system with the 2 grade classification system (OR 0.59 95% CI 0.30, 1.19). Figure 2.8 illustrates the comparison between triple pattern and homogenous pattern at embryo transfer where the outcome is clinical pregnancy.

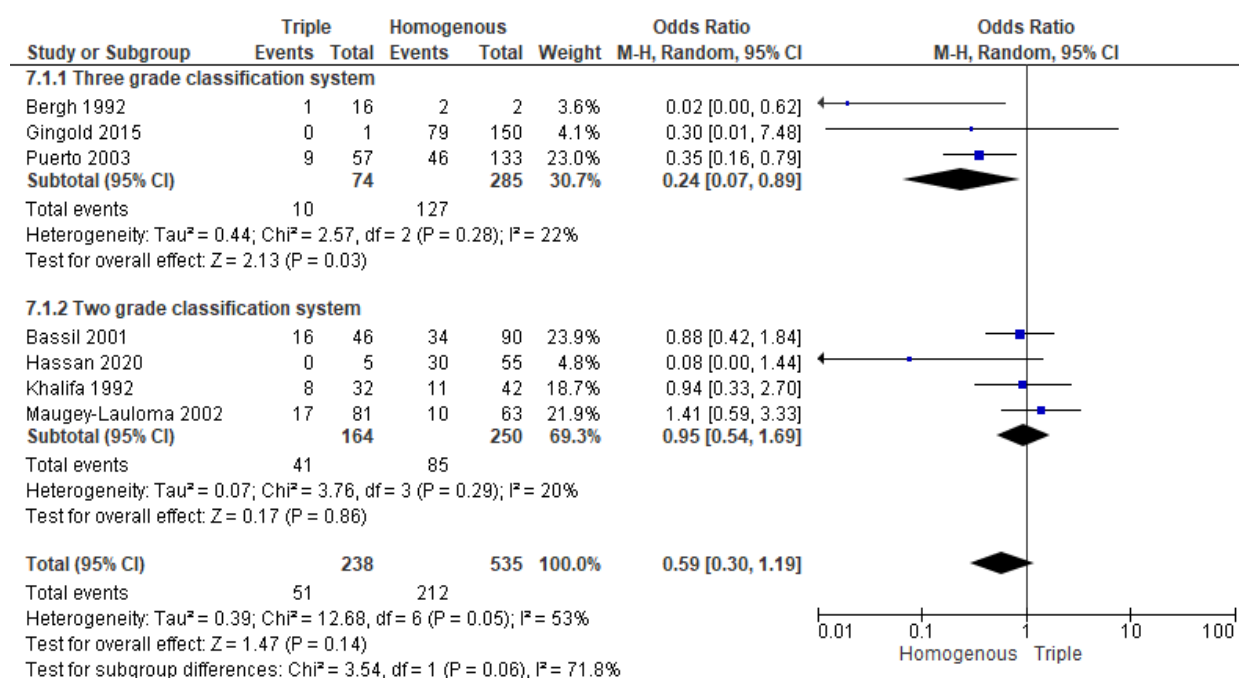


Figure 2.8 Forest plot of comparison: Triple vs Homogenous at embryo transfer, outcome: Clinical pregnancy.

There was no significant difference between triple and intermediate pattern at embryo transfer. Women with homogenous pattern at embryo transfer were more likely to have clinical pregnancy (OR 0.53 95% CI 0.31, 0.92). Figure 2.9 below illustrates the comparison between triple pattern and intermediate pattern at embryo transfer and the comparison between intermediate and homogenous pattern where the outcome is clinical pregnancy.

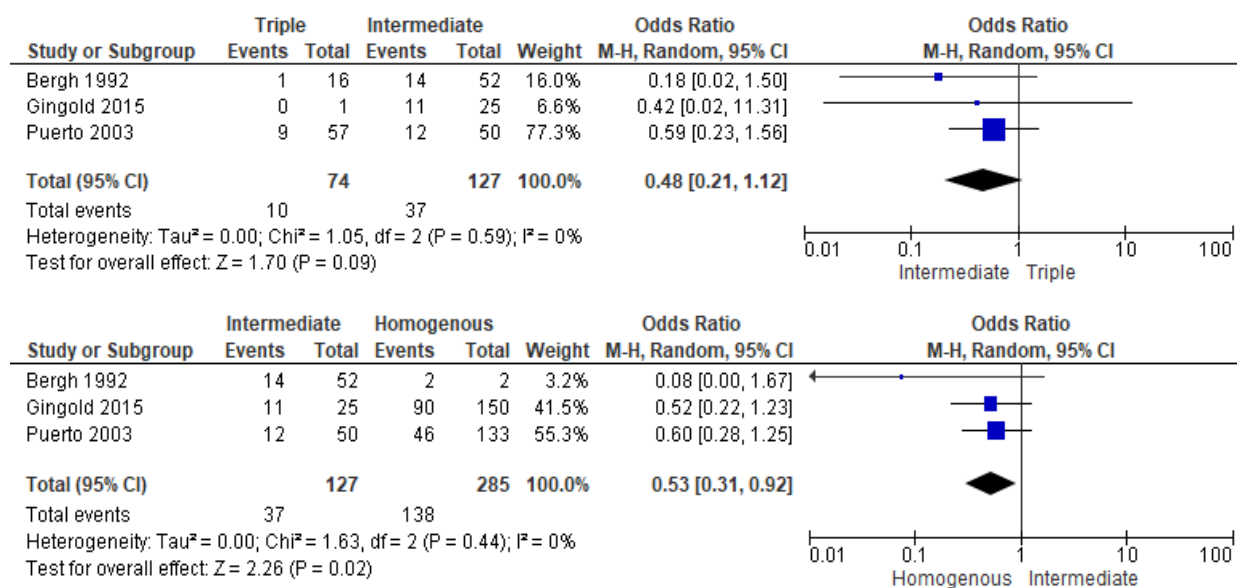


Figure 2.9 Forest plot of comparison: Triple vs intermediate at embryo transfer and intermediate pattern vs homogenous pattern, outcome: Clinical pregnancy.

2.3.2.2 Frozen embryo transfer cycles

2.3.2.2.1 Assessment of endometrial pattern on the day of progesterone initiation

There were 4 studies that reported on that reported clinical pregnancy outcomes (3530 cycles) on the day of progesterone initiation for the comparison between triple and homogenous pattern. Two studies used the three grade classification system and three studies used the two grade classification system. The overall odds ratio comparing triple pattern with homogenous pattern (OR 1.30 95%CI 1.11, 1.51) is as illustrated in figure 2.10 below. Figure 2.10 illustrates the comparison between triple

pattern and homogenous pattern on the day of progesterone initiation where the outcome is clinical pregnancy.

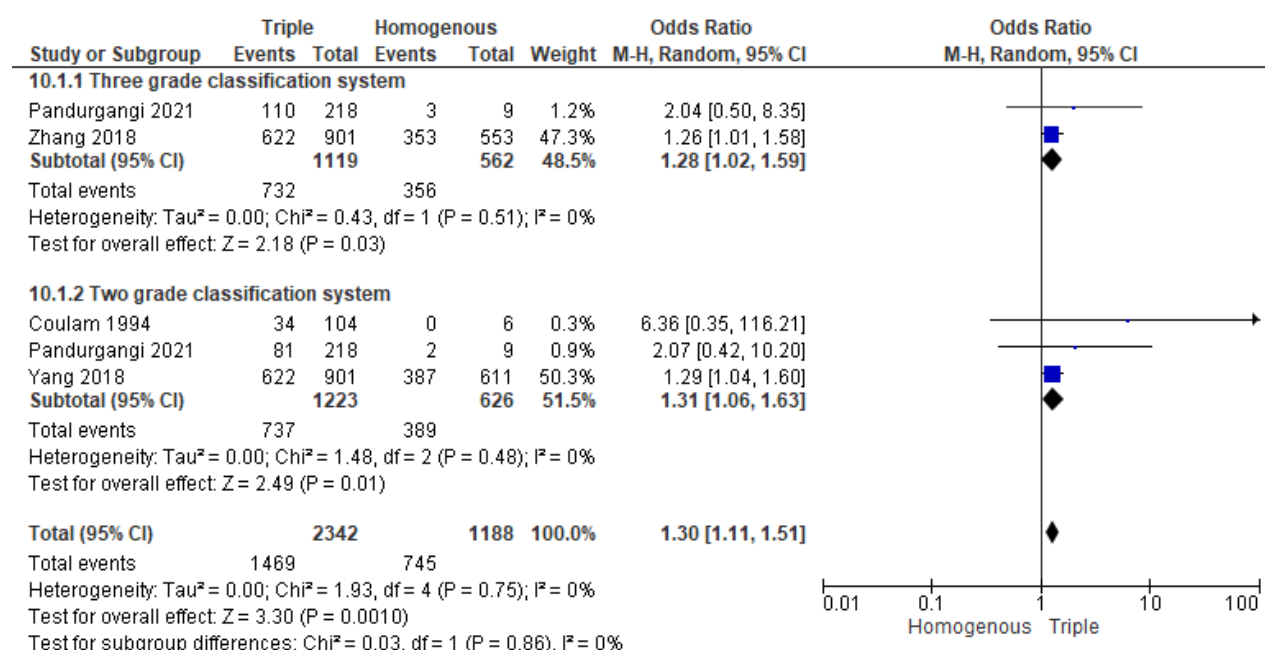


Figure 2.10 Forest plot of comparison: 10 Triple vs Homogenous at day of progesterone initiation, outcome: 10.1 Clinical pregnancy

The results were not statistically significant when comparing triple pattern with intermediate pattern. This was also the case for the comparison between intermediate pattern and homogenous pattern as illustrated in figure 2.11 below. Figure 2.11 illustrates the comparison between triple pattern and intermediate pattern on the day of progesterone initiation and the comparison between intermediate and homogenous pattern where the outcome is clinical pregnancy.

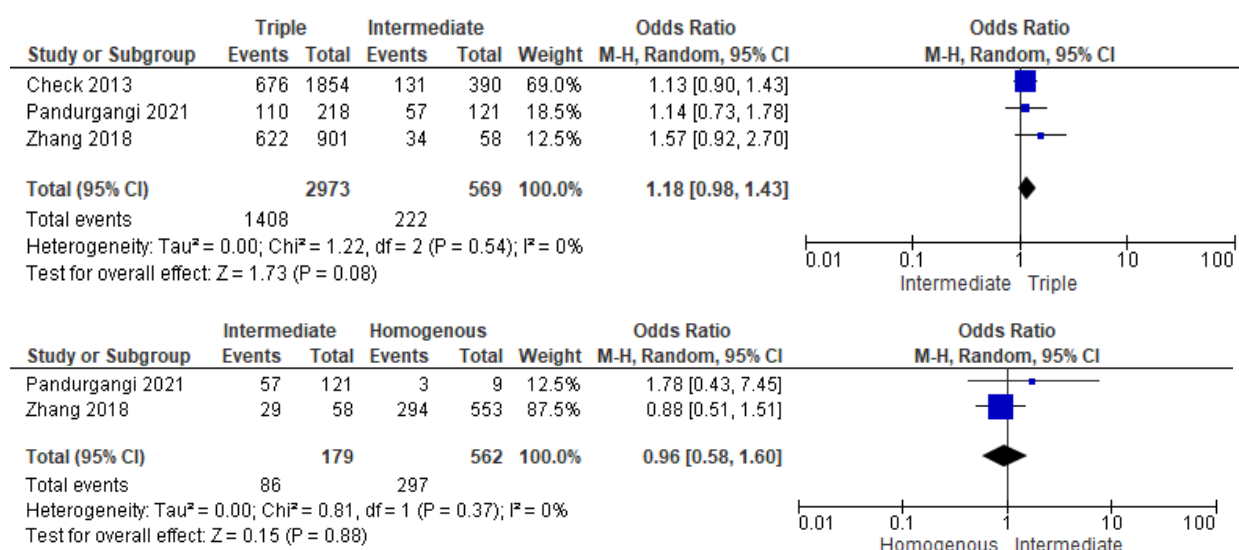


Figure 2.11 Forest plot of comparison: Triple vs intermediate and Intermediate vs homogenous at day of progesterone initiation, outcome: Clinical pregnancy.

There were only 3 studies that reported live birth outcomes for endometrial pattern on the day of progesterone initiation. Zhang et al. retrospectively analysed 1512 frozen embryo transfer cycles. They used the three grade classification system of triple intermediate and homogenous in their assessment. They concluded that endometrial pattern did not contribute significantly to live birth outcomes (Zhang et al. 2018).

Pandurgangi et al prospectively assessed endometrial pattern in 348 women undergoing frozen embryo transfer (Pandurangi et al. 2021). They used the three grade endometrial pattern in their assessment. They noted higher pregnancy rates with triple and intermediate pattern however it was not statistically significant.

Check et al. retrospectively assessed 2244 frozen cycles (Check et al. 2013). Although they used the three grade classification system for their assessment of endometrial patterns, they only included triple and intermediate pattern as they cancelled embryo transfer for women with homogenous pattern. They found no significant differences between the triple pattern and intermediate pattern.

There is another study that reported live birth outcomes for frozen cycles but they recorded endometrial pattern 1-2 days prior to the initiation of progesterone and hence were not included in this analysis. Oluborode et al. retrospectively evaluated 256 frozen cycles and found no significant difference between endometrial patterns in determining clinical pregnancy or live births.. However, results from their study at trigger were included in the meta-analysis of fresh IVF cycles. The strength of this study is that they reported both clinical pregnancy and live birth outcomes and tried to address confounding factors with adjusted odd ratios. However, the main limitation was that it was retrospective and there were missing data (endometrial pattern was not recorded for 64 cycles) and the pattern was recorded by different operators and therefore subject to inter-observer variation.

Figure 2.12 illustrates the comparison between the different endometrial patterns on the day of progesterone initiation where the outcome is clinical pregnancy.

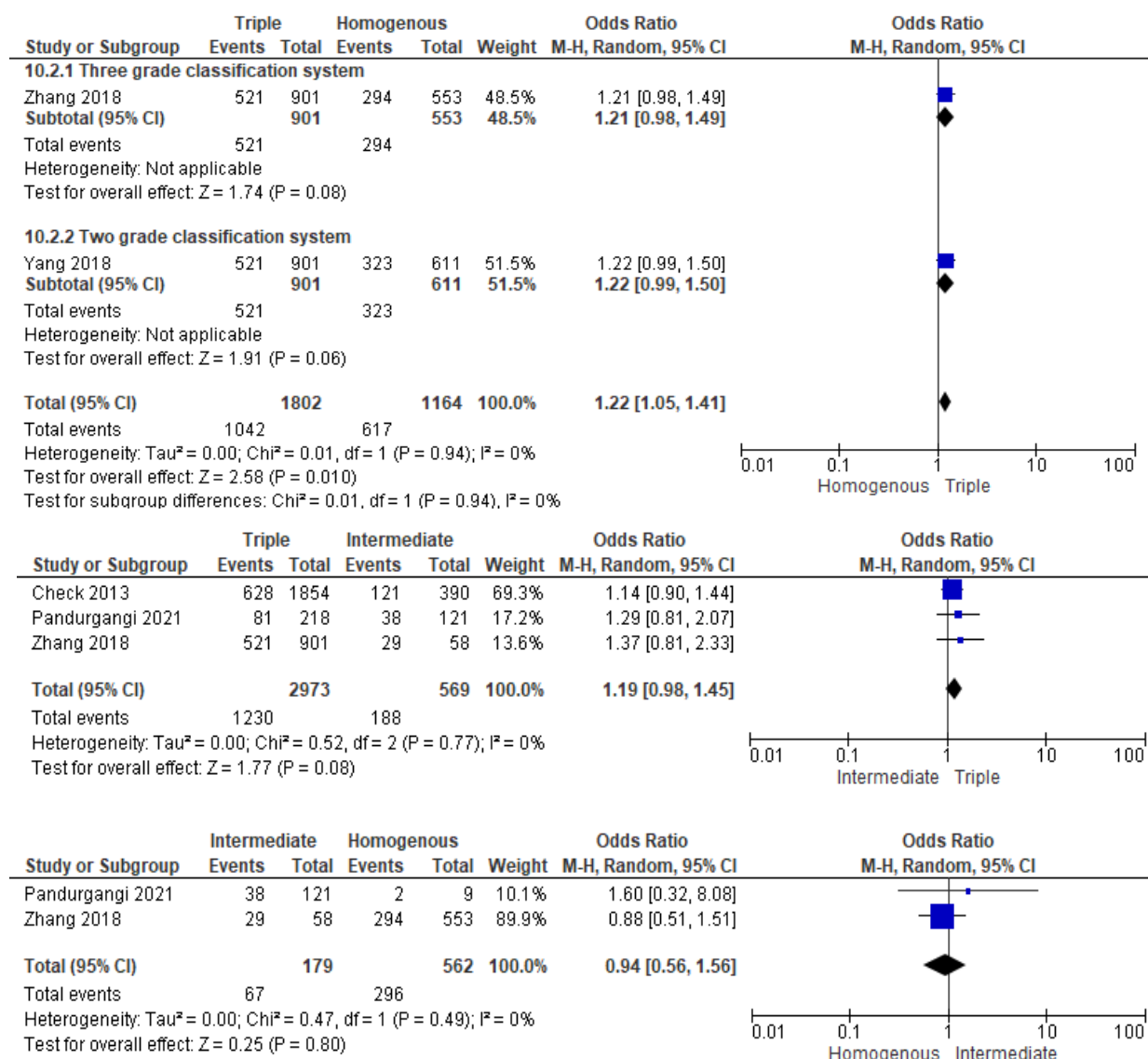


Figure 2.12 Forest plot of comparison: Triple vs homogenous, Triple vs intermediate and Intermediate vs homogenous at day of progesterone initiation, outcome: Live birth.

2.3.2.2.2 Assessment of endometrial pattern on the day of frozen embryo transfer

There were only two studies that reported endometrial patterns on the day of frozen embryo transfer along with clinical pregnancy outcomes (196 cycles). There were no studies that reported live birth outcomes..

Gingold et al performed a retrospective cohort study looking at 277 women where a total of 476 euploid embryos were transferred into 277 patients over the course of 356 IVF cycles. This included 180 frozen cycles in 152 patients. They classified the endometrial pattern as type 1 late proliferative, type 2 early secretory and type 3 mid-late secretory. They concluded that although in fresh cycles endometrial pattern seems to favour pregnancy, in frozen cycles there were no detectable differences between implantation rates and clinical pregnancy rates between type 2-3 (early secretory and mid-late secretory) endometrial patterns. They also suggested that decreased implantation rates observed in patients with homogeneous pattern at trigger day compared with those with intermediate pattern suggest that the premature luteinisation caused asynchrony between the blastocyst and the endometrium, leading to implantation failure (Gingold et al. 2015a).

Zachova et al prospectively analysed 30 women in natural cycle frozen embryo transfer. The endometrial pattern was classified as hyperechogenic and hypoechogenic. They concluded that the triple line pattern of the endometrium at the

time of frozen embryo transfer was the only sign that predisposed pregnancy. However, the small sample size is a major limitation (Zackova et al. 2009).

Figure 2.13 below illustrates the comparison between the different endometrial patterns on the day of frozen embryo transfer where the outcome is clinical pregnancy.

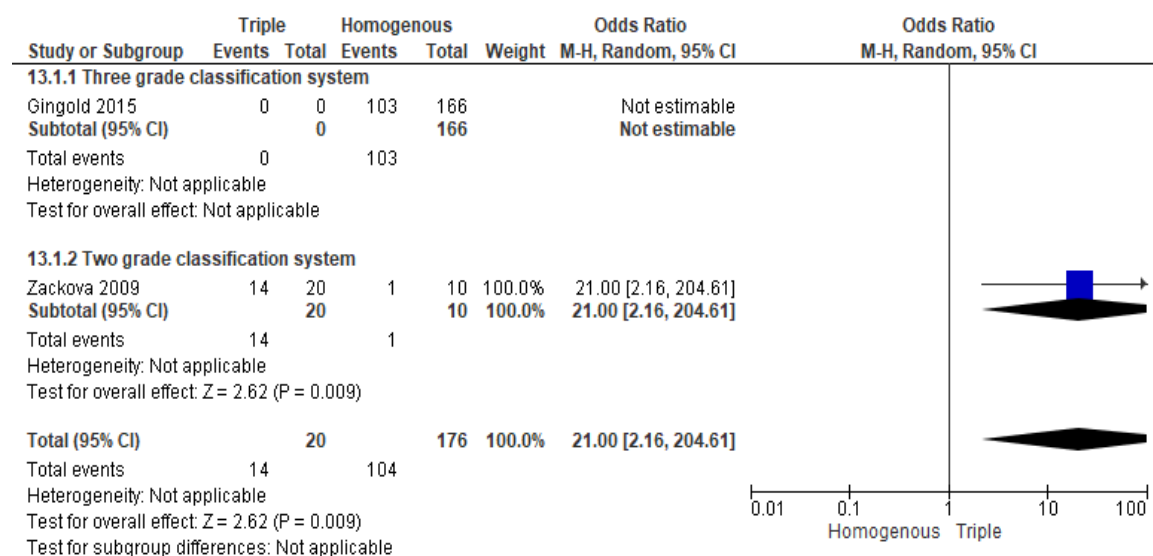


Figure 2.13 Forest plot of comparison: Triple vs Homogenous at frozen embryo transfer, outcome: Clinical pregnancy.

2.4 Discussion

The process of implantation is complex and involves an intricate synchronized coordination between the endometrium and embryo. (Enders 1967; Achache and Revel 2006).

Endometrial thickness is the most extensively investigated parameter of endometrial receptivity. In a meta-analysis by Kasius et al. (2014b), their calculated summary receiver operating characteristics (sROC) curve showed that endometrial thickness was not a discriminator between cases that achieved a clinical pregnancy and cases that did not achieve pregnancy following IVF. Craciunas et al also showed that there was a poor ability to predict clinical pregnancy from the studies pooled in their meta-analysis (Craciunas et al. 2019).

Despite this, many fertility centres will use endometrial thickness as a standard to guide treatment protocols and decide whether to transfer embryos or not. However, research into the predictive value of endometrial pattern has yielded conflicting results and is therefore not utilised by many clinics.

It is thought that the endometrial pattern can provide valuable information which can help decipher the window of implantation as it reflects the hormonal status, as well as the histological maturation. The changes in oestrogen to progesterone ratio may affect endometrial structure and pattern, and thus may affect how the endometrium prepares for implantation (Bourgain and Devroey 2003; Smith et al. 1989; Macklon and Fauser 2000). It is therefore crucial to assess the predictive accuracy of endometrial patterns as some endometria might be poorly receptive despite normal thickness, while some seemingly thin endometria might be physiologically normal and receptive. The timing of these assessments is also vital.

To test endometrial pattern, and in the absence of a gold standard diagnostic test for endometrial receptivity, we considered clinical pregnancy as a surrogate marker to confirm receptive endometrium. Live birth was included; however it is seldom reported in the literature. There were only 3 studies that reported outcomes of live birth. We only included studies for women undergoing IVF/ICSI with their own oocytes/ embryos to avoid potential bias secondary to changes caused by the interaction between the endometrium and “foreign” or donated ovum/ embryo.

When comparing the endometrial pattern classification systems, the three grade system was not reduced into the two grade classification . This was in order to lessen bias and potential inaccuracy of reporting the results. One of the major limitations faced in this systematic review was the breadth of different classification systems used in the studies reviewed, which resulted in an inability to compare like with like. Many studies used the two grade classification system which provides very limited information about the endometrium, and where important information is missed in the transitional changes of endometrial pattern between the different phases from proliferative, early secretory, mid to late secretory. This is in addition to the already existent limitations associated with endometrial pattern including the different classification systems, variability of different ultrasound machines, inter-observer variability as well as intra-observer observation.

Most studies have investigated endometrial pattern at trigger point (24/33 studies in this meta-analysis). The results of this review show that women with triple pattern at

trigger are more likely to become pregnant when compared to women with a homogenous pattern (OR 1.89 95% CI:1.39, 2.58). This might be due to premature changes of proliferative endometrium to secretory endometrium, leading to asynchrony between the embryo and endometrium, which then results in implantation failure. Killick et al suggested that elevated progesterone levels towards the end of the follicular phase may result in a premature evolution of a proliferative endometrium to a secretory endometrium. This, on ultrasound, appears as a homogenous hyperechoic (unilinear) endometrial pattern (Killick, 2007; Mahajan, 2015).

Similarly, women with a homogenous pattern at fresh embryo transfer were more likely to become pregnant compared to women with an intermediate pattern (OR 0.53 95% CI 0.31, 0.92). This could be explained by the delayed endometrial development ("immature endometrium") and also results in asynchrony between the embryo and endometrium, leading to implantation failure. However, the results are limited by the paucity of the studies, as only 3 studies were included (412 cycles).

The findings from this review are in contrast to a very comprehensive review on endometrial markers of receptivity by Craciunas et al. They analysed the results from 20 studies investigating endometrial pattern, where 11/20 studies assessed endometrial pattern at trigger. However, they classified endometrial pattern as triple or not triple, where intermediate pattern is combined with either triple or homogenous pattern. This may have acted as a limiting factor to their results. (Craciunas et al. 2019).

There were a number of limitations faced in this review. These included the heterogeneity of the studies and their different methodology, as well as the poor quality of some of the studies. Another major limitation was with regards to the different classification systems used and the fact that assumptions had to be made about the classification systems, such as assuming that the three grade classification systems used in different studies were equivalent. The limitations were compounded by not being able to account for inter-observer variation within one classification system, let alone the many classification systems used. For the sake of the review, some of the classification systems also needed to be pooled together, for example, the 6 grade classification system was pooled into three. A lot of the studies did not account for the grade of embryos, number of embryos transferred and gestations of embryos, and at what stage the embryos were transferred (day two, or day five). This was all important information that could not be formally accounted for, further limiting the results of this review. Moreover, it was not possible to adjust for the different protocols used in the studies, and the different populations used. Moreover, the ultrasound and imaging quality have improved over the years. It was not possible to account or adjust for much of the missing information that was not provided in the studies. Whilst every attempt was made to gather as much information as possible, it was not feasible or practical to contact the authors in each study, given that some of these studies were published numerous decades ago.

This is the largest systematic review and meta-analysis focusing solely on endometrial pattern as a marker of endometrial receptivity in women undergoing IVF/ICSI. There

is one other comprehensive systematic review on markers of endometrial receptivity published by Craciunas et al. which reported on 20 studies on endometrial pattern.

2.5 Conclusion

The results from this review suggest that endometrial pattern can provide useful information about endometrial receptivity. However, this is limited by the low to moderate quality evidence of some of the studies and is also limited by the substantial heterogeneity of the studies and the different classification systems used. The IETA has produced a consensus statement on terms, definitions and measurements that may be used to describe the sonographic features of the endometrium and uterine cavity on gray-scale sonography, colour flow imaging and sonohysterography (Leone et al. 2010).

Perhaps it is time we do the same with the different endometrial pattern classifications systems used in assessing the endometrium in fertility and reproductive medicine. A consensus on terms and definitions for a classification of endometrial pattern followed by the production of well-designed prospective studies and adequately powered randomised controlled trials would place us in good stead for determining whether endometrial pattern on ultrasound can be used to predict and improve outcome in assisted conception.

Chapter 3 Study on the inter-rater and intra-rater reliability of endometrial patterns on ultrasound

3.1 Background

Endometrial pattern on ultrasound can provide useful information about endometrial receptivity, however, research into its clinical utility to predict implantation has yielded conflicting results (Hershko-Klement and Tepper 2016). Inter-rater and intra-rater variability might be one of the contributing factors to this result. It would therefore be difficult to accurately assess whether endometrial pattern can predict the outcome of IVF without initially establishing the reliability of the classification system. We will therefore assess for inter and intra observer variation and check for reliability and reproducibility of the classification system (Raine-Fenning et al. 2002; Raine-Fenning, Campbell, Clewes, Kendall, et al. 2003; Raine-Fenning, Campbell, Clewes, and Johnson 2003)

Reliability relates to the consistency of a measure. Inter-rater and intra-rater reliability are aspects of test validity. Inter-rater reliability is the degree of agreement among different raters. It is a score of how much homogeneity, or consensus, there is in the ratings given by various judges. In contrast, intra-rater reliability is a score of the consistency in ratings given by the same person across multiple instances. There are different statistical methods to assess reliability such as: the intraclass correlation

coefficient (ρ), Cohen's kappa coefficient (κ), and Fleiss' kappa. (Donner and Eliasziw 1987). There is evidence to suggest inter-rater as well as intra-variability with ultrasound, there it is important to conduct reliability studies.

3.1 The development of the “endometrial pattern recognition” classification system

In assessing the literature, my day to day scanning experience and own observation from scanning with different operators, a few issues arose with the existing classification systems. Firstly was the problem of high inter-rater variability. Secondly was the problem of misclassification of triple pattern. Thirdly was the complexity of some classification systems, limiting their clinical applicability and use. Finally, was the variability of pattern type definitions- ie a “triple” pattern in one study may differ to a “triple” pattern in another study, all of which have been demonstrated in the now complete systematic review.

I sought to tackle each of these issues in creating this endometrial pattern recognition classification system.

To address the issue of high inter rater variability, a simplified classification system which could be more easily adhered to by operators was needed.

The IETA has produced a consensus statement on terms, definitions and measurements that may be used to describe the sonographic features of the

endometrium and uterine cavity on gray-scale sonography, colour flow imaging and sonohysterography (Leone et al. 2010). I tried modelling their work while developing the “pattern recognition” classification system.

The endometrial pattern classification system was initially developed as a 5 grade classification system see appendix 3. However as my scanning progressed, it became clear that the system would be too cumbersome for clinical applicability. To ensure a more practical yet comprehensive classification system, the classification system was then refined and developed into the 4 grade classification system. This was agreed by NF see appendix 3.

The endometrium becomes more hyperechogenic on ultrasound as it transitions from proliferative to secretory phase until it becomes completely hyperechogenic in the late secretory phase. The echogenicity represents oedema. The midline echo (white line the middle) represents the interface between two layers; the basal and functional layer of the endometrium.

Fig 3.1-3.4 illustrates the novel “pattern recognition classification” system.

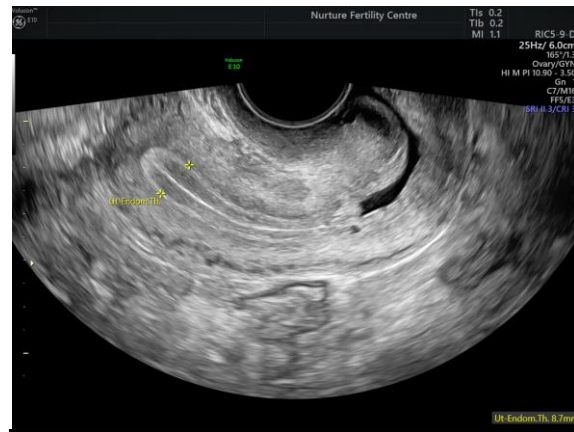
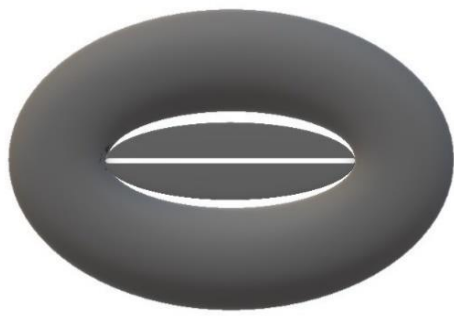


Figure 3. 1: Triple pattern: Hypo-echogenic endometrium with thin hyperechogenic outer border which is less than 1/3 of the total endometrial thickness typically seen in proliferative phase.

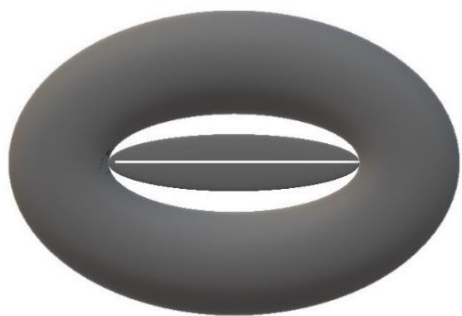


Figure 3.2: Early intermediate pattern, hyperechogenic area (white line on outside) is $\geq 1/3$ of the total endometrial thickness but $< 2/3$ of the total endometrial thickness. This is normally seen in late proliferative/early secretory phase

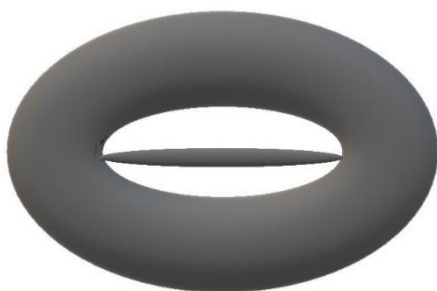


Figure 3.3: Late intermediate pattern, endometrium almost completely homogenous with the exception of the central hypoechoic area. (Hyperechogenic area occupying more than $2/3$ of the total endometrial thickness. This is seen in mid secretory phase.



Figure 3.4: Homogenous pattern, endometrium completely hyperechogenic, effect of progesterone can be seen. This is seen in mid to late secretory phase.

3.2 Objectives

1. To assess the interrater reliability for the current classification system between different operators.
2. To assess the interrater reliability for the “new pattern recognition” classification system between different operators.
3. To assess the intra rater reliability for the current classification system for the same operator.
4. To assess the intra rater reliability for the “new pattern recognition” for the same operator.

3.2.1 Methods

3.2.2 Ethical Approval

Ethical approval was obtained from Faculty of Medicine & Health Sciences Research Ethics Committee, Nottingham University Hospitals. Reference Number 87-1808.

3.2.2.1 Initial stage to test the reliability of the current classification system

Cine loops of endometrial patterns were collected during the study to assess for endometrial pattern. The cine loops were collected over few months at various stages of the IVF treatment to represent the different endometrial patterns. Cine loops were used instead of still images as a “still” images can change the classification, depending on the quality of image. Cine loops are also more realistic, reflecting everyday scanning which is a dynamic process. The images chosen were initially

assessed and agreed with almost perfect agreement ($k=0.9$) between 2 raters, myself (HE) and (NF), one of whom is an expert scanner with over 25 years of scanning experience. This was repeated a year later to ensure consistency and produced similar results ($k=0.9$). Care was taken to choose cineloops which were representative of real, every-day scanning with various machines and various image quality, but where the pattern can clearly be defined.

In this study, 30 cineloops were used. These were duplicated and randomly assigned as 60 cineloops and assessed via an online survey. This was done to be able to assess intra-rater variability (allowing comparison of each cineloop and its duplicate by each observer). The cineloop was edited using “video editor” software to reduce its length from around 20 seconds to about 5 seconds ensuring the whole endometrium is included and the pattern can be defined in this shorter cineloop. This was done to improve compliance with completing the survey ensuring that it is not too lengthy.

The cineloops were assessed through an online survey. The online survey was created using the Microsoft software “Forms”. A link was sent to the different raters and they were asked to watch the cineloops (each video lasting a few seconds). They were asked to grade the pattern as either triple, intermediate or homogenous (current classification system). As the cineloops were randomly assigned and dispersed into 60 videos, most operators were unaware about the duplication of the cineloop and they treated each cineloop as a separate video. This allowed blinding

while testing for intra-rater variability. All the cineloops were completely anonymised in keeping with the general data protection regulation (GDPR) Act 2018.

3.2.2.2 Second stage to test the reliability of the “new pattern recognition” classification system

Once all responses were received, training regarding the new pattern recognition system was provided through power point presentation, explaining the concept and demonstrating some cineloops, and included a short quiz at the end. The training was short and only lasted 20-30 minutes, given the heavy clinical schedule. After a period of 3 months, the 30 duplicated cineloops were assessed through an online survey. The online survey was created using the Microsoft software “Forms”. A new link was sent to the same raters and they were asked to watch the cineloops (each video lasting a few seconds). They were again asked to grade the pattern as triple, early intermediate, late intermediate or homogenous (New classification system).

All the cineloops were completely anonymised in keeping with the general data protection regulation (GDPR) Act 2018.

3.2.1 Sample size calculation

It was not possible to do a sample size calculation for Fleiss kappa. However, a sample size estimate using the statistical software N-query has shown that a sample size of 22 (images) will give 80% power to detect Cohen kappa agreement of 0.6 or better.

We extrapolated from that and used 60 images (cineloops) instead to account for the increased number of raters and groups, and to increase the power.

3.2.2 Data synthesis and statistical analysis

The results were collated in SPSS. To test for intra- rater variability we used Cohen Kappa.

Cohen's kappa coefficient (κ) is a statistical analysis which measures inter-rater agreement for qualitative (categorical) items. It is generally thought to be a more robust measure than simple percent agreement calculation, as κ takes into account the possibility of the agreement occurring by chance. Cohen Kappa is normally used to assess the level of agreement among two raters. Table 3.1 shows the interpretation of kappa value.

Table 3. 1: Cohen kappa interpretation.

Cohen's kappa coefficient	Interpretation
< 0	Poor agreement
0.01 – 0.20	Slight agreement
0.21 – 0.40	Fair agreement
0.41 – 0.60	Moderate agreement
0.61 – 0.80	Substantial agreement
0.81 – 1.00	Almost perfect agreement

On the other hand, Fleiss' kappa (named after Joseph L. Fleiss) is a statistical measure for assessing the reliability of agreement between two or more raters when assigning categorical ratings to several items or classifying items. The measure calculates the degree of agreement in classification, over that which would be expected by chance.

To test for inter-rater variability we used Fleiss Kappa. The Fleiss kappa function was not available in SPSS and Stata SE 15 was used to provide Fleiss kappa results.

While designing the study, and on discussion with a statistician, it was advised that the new classification system would need to be combined into a 3 grade classification system to be able to compare it to the old classification system. Fleiss kappa has its limitation as it can overestimate or underestimate agreement, and introducing another category would increase the margin of error. It was prospectively decided to combine pattern 1 and 2 together.

3.3 Results

3.3.1 Reliability of the current three grade classification system

12 raters responded to the initial survey 15 (80% response rate).

The raters were personnel who routinely perform ultrasound scans for fertility treatment assessments, with varying level of scanning experience. This included

experienced consultants, clinical fellows, sonographers, and fertility nurses. The experience of scanning ranged from 1 year to 30 years. After the completion of the study, a further 2 observers replied to the initial survey of the old classification system, but as they did not respond to the second survey, they were excluded. Their exclusion did not affect the overall kappa value.

Each observer was asked to rate themselves as either beginner, intermediate, experienced or expert according to years of experience and how they rated their scanning skill. Table 3.2 shows participants level of experience.

Table 3. 2: Participants level of experience

Level of experience	N (%)
Beginer	1 (8.3)
Intermediate	3 (25)
Experienced	5 (41.7)
Expert	3 (25)

3.3.1.1 Inter-rater variability of the current three grade classification system

We calculated the Fleiss Kappa using Stata 15 SE software to determine the inter-rater agreement for the current three grade classification system. The overall $K=0.61$

3.3.1.2 Intra-rater variability of the current three grade classification system

The intra-rater variability ranged from substantial to almost perfect agreement.

. Each cineloop was classified according to either the “current” endometrial pattern classification system or the “new pattern recognition endometrial classification system” by 2 observers, one of whom has > 25 years scanning experience (NF). Although not testing for validity, % correct column donates the expected value (marked against the assigned pattern by NF and HE) to achieve $k=1$. Table 3.3 illustrates the intra-rater variability between the different observers.

Table 3. 3: Intra-rater variability of the current three grade classification system for participants

Observer	Cohen Kappa	p value	% correct
Observer 1	0.94	<0.001	86.7
Observer 2	0.94	<0.001	66.7
Observer 3	0.83	<0.001	66.7
Observer 4	0.69	<0.001	66.7
Observer 5	0.84	<0.001	76.7
Observer 6	0.73	<0.001	86.7
Observer 7	0.79	<0.001	73.3
Observer 8	0.95	<0.001	78.3
Observer 9	0.75	<0.001	90
Observer 10	0.70	<0.001	81
Observer 11	0.65	<0.001	70
Observer 12	0.89	<0.001	83.3
% correct refers to accuracy against the control pattern (how many correct out of 60)			

3.3.2 Reliability of the new 4 grade classification system

3.3.2.1 Inter-rater variability of the new classification system

We calculated the Fleiss Kappa using Stata 15 SE software to determine the inter-rater agreement for the new four grade classification system. $K=0.64$ when combining

pattern 1 and 2 together as prospectively decided. Out of interest and although not comparable we calculated the kappa for the four grade classification system. $K=0.52$ moderate agreement.

3.3.2.2 Intra-rater variability of the “new pattern recognition” classification system

The intra-rater variability varied substantially between the different observers from $K=0.4$ to 0.94 .

Table 3. 4: Inter-rater variability of the “new pattern recognition” classification system for participants

	Combined (Pattern 1+2)			Four grade classification system		
Observer	Cohen Kappa	p value	% correct	Cohen kappa	P value	% correct
Observer 1	0.89	<0.001	98	0.78	<0.001	93
Observer 2	0.40	<0.001	67	0.49	<0.001	58
Observer 3	0.83	<0.001	92	0.68	<0.001	85
Observer 4	0.69	<0.001	68	0.57	<0.001	53
Observer 5	0.79	<0.001	75	0.57	<0.001	75
Observer 6	0.56	<0.001	85	0.59	<0.001	83
Observer 7	0.89	<0.001	93	0.68	<0.001	80
Observer 8	0.65	<0.001	80	0.58	<0.001	60
Observer 9	0.84	<0.001	97	0.82	<0.001	95
Observer 10	0.84	<0.001	73	0.57	<0.001	56
Observer 11	0.94	<0.001	93	0.86	<0.001	86
Observer 12	0.89	<0.001	95	0.67	<0.001	91
% correct refers to accuracy against the control pattern (how many correct out of 60 cine loops) Green highlight represents expert and experienced observers  Red highlight represents beginner and intermediate observers 						

Table 3.5 summarises the results of the “current” and “new pattern recognition” classification system

Table 3. 5:Summary of the results illustrating Cohen Kappa of the “current” and “new pattern recognition” classification system

	Current classification system		New classification system combined (pattern 1+2)		New classification system (4 grade)	
Observer	Cohen Kappa	% correct	Cohen kappa	% correct	Cohen Kappa	% correct
Observer 1	0.94	87	0.89	98	0.78	93
Observer 2	0.94	67	0.40	67	0.49	58
Observer 3	0.83	67	0.83	92	0.68	85
Observer 4	0.69	67	0.69	68	0.57	53
Observer 5	0.84	77	0.79	75	0.57	75
Observer 6	0.73	87	0.56	85	0.59	83
Observer 7	0.79	73	0.89	93	0.68	80
Observer 8	0.95	78	0.65	80	0.58	60
Observer 9	0.75	90	0.84	97	0.82	95
Observer 10	0.70	81	0.84	73	0.57	56
Observer 11	0.65	70	0.94	93	0.86	86
Observer 12	0.89	83	0.89	95	0.67	91
% correct refers to accuracy against the control pattern (how many correct out of 60 cine loops) Green highlight represents expert and experienced observers  Red highlight represents beginner and intermediate observers 						

3.4 Discussion

The inter-rater variability for the old three grade classification system shows substantial agreement between observers. Intra-rater variability also showed good intra-rater consistency.

The most accurate pattern was homogenous, however, triple pattern seemed to be overrepresented. It seems that whenever there is a midline echo, the pattern is described as triple regardless of the appearance, and this is problematic as the midline echo can persist well into the secretory phase.

One of the limitations encountered was that it was not possible to directly compare the old classification system with the new “pattern recognition” classification system as they are “unequal”- one had four grades of classification, the other three grades. Fleiss kappa measures agreement of observers regardless of “accuracy”. It can also sometimes overestimate or underestimate without providing clinically meaningful information. To be able to more directly compare the old classification with the new “pattern recognition” system, a decision was therefore taken to combine the triple pattern and early intermediate pattern. This is because there was an overlap between them, as triple pattern signifies proliferative phase and early intermediate signifies the transition from late proliferative phase to early secretory phase.

The new classification system showed moderate (using 4 grade classification) to substantial (combining triple and early intermediate) agreement between raters. However, there was substantial intra-rater variability between observers. Three raters (25%) achieved moderate agreement while 9 (75%) had substantial to almost perfect agreement. This suggests that with further training perhaps less experienced scanners can achieve higher agreement.

There is an increasing recognition of the role of “pattern recognition” classification systems. One of the limitation of this study is that it looked at reliability of analysis of endometrial pattern and not acquisition which is likely to increase variability. Ideally, we wanted to assess this but due to technical difficulties, this was not achievable. However having a cineloop rather than a “still image” produced a more reliable classification. Although some studies have looked at image acquisition in different conditions for example endometrial cancer, as far as we are aware, none reported on image acquisition in endometrial pattern. Wong et al looked at the reliability of pattern recognition of endometrial cancer and conducted a reliability study between 2 raters. They were able to show 90% accuracies by ultrasound in differentiating between benign and malignant lesions. (Wong et al. 2021)

A further limitation encountered was surrounding the lengthy period of time taken for an adequate number of observers to respond to both the first and second surveys- in total it took more than a year for observers to respond to both surveys. This may have been due to some of the observers being discouraged from participating, as each survey contained 60 cineloop clips. It was necessary to have a high number of cineloops in each survey, as this was mandated in testing intra observer reliability. Although it was not planned, it was not possible to commence a second phase (providing further training and then running a third and fourth survey), prior to receipt of responses from the first and second survey. This provided a further limitation. In future, shifting a focus to interobserver reliability only would allow a reduction in the number of cineloops required, and hopefully optimising the response rate.

Wary of the length of the survey and so as not to discourage participant response, care was taken when editing the cineloops to strike the right balance between having an adequate amount of time for responders to be able to classify the pattern, and not making the survey too long. In spite of this, the cineloop time duration may have inadvertently been too short, reducing the quality of the test. Further possible directions for the study in future include expanding the study to participants outside of Nottingham, to obtain a greater number of participants with greater breadth and diversity of experience.

3.4.1 Conclusion

The results show a moderate to substantial agreement in the endometrial pattern classification system. Despite limitation, the data suggests that with greater training, better accuracy and agreement can be achieved.

Chapter 4 Exploratory analysis of the prognostic accuracy of endometrial patterns on ultrasound to predict implantation

4.1 Background

The process of embryo implantation is a critical step for achieving pregnancy, requiring a coordination of complex interactions between the blastocyst and endometrium (Bonilla-Musoles et al. 2013). Many factors can impact the success of assisted conception, but endometrial receptivity and embryo quality are the key players in this process.

Ultrasound is a non-invasive tool which is used to assess the endometrium. While endometrial thickness has been extensively studied, research utilising all ultrasound parameters such as sonographic patterns, has either been sparse or yielded conflicting results (Hershko-Klement and Tepper 2016). Some papers support the ability of ultrasound to predict successful outcome of IVF or ICSI (Zhao, Zhang, and Li 2012; Chen et al. 2010; Wu et al. 2014; Zhao et al. 2014) while others are dismissive (De Geyter et al. 2000; Rashidi et al. 2005; Detti et al. 2008). This can be explained by different study designs, variable classification systems used, and scans done on different cycle days, utilising different grades of embryos, as well as inter observer variation.

Other factors that may have contributed to the heterogeneity of the studies and possibly the conflicting results include ultrasound assessment being different days and in different cycles (fresh vs frozen), with different numbers of embryos at different stages of development (for example day 2 embryo versus blastocyst).

4.2 Study aims and objectives

4.2.1 Primary objective

To investigate whether endometrial pattern on ultrasound can predict the outcome of IVF or ICSI.

My hypothesis is that endometrial pattern on ultrasound can predict the outcome of IVF or ICSI.

4.2.2 Secondary objectives

1. To identify whether test characteristics vary with fresh or frozen cycles.
2. To assess whether rate of change of endometrial pattern between the different scan points has an impact on the success rate.
3. To investigate whether other ultrasound parameters such as endometrial Doppler and endometrial thickness can affect/predict IVF/ICSI success rate.

4. To assess whether other parameters such as hormonal profile has an impact on the success rate.
5. To simplify ultrasound assessment by developing a novel classification system.

4.3 Materials and Methods

4.3.1 Study design

This is a prospective observational single-site study that took place at Nurture Fertility (Nottingham, UK) between September 2018 - September 2021. The study looked at two main groups: women who had fresh embryo transfer and women who had frozen embryo transfer. The study was designed around routine patients' visits (see protocol and visits' schedule).

The protocol has been prospectively registered at trials.gov with pre-defined primary and secondary outcomes, and clear inclusion and exclusion criteria. The registration number is NCT03860636.

4.3.2 Ethical Approval

Ethical approval was obtained from the Faculty of Medicine & Health Sciences Research Ethics Committee, Nottingham University Hospitals. Reference Number 87-1808.

4.3.3 Study participants

4.3.3.1 Participants recruitment

Patients were invited to participate in the study via posters at Nurture fertility, the research web page, being approached directly in clinic or electronically via portal messages. Information leaflets were posted or sent electronically either via email, or the website portal. Consent forms were signed in the clinic once patients were happy to participate. They needed to have read the information leaflet at least 24 hours prior to signing the consent forms.

4.3.3.2 Inclusion Criteria

- Females over 18 years old undergoing IVF or ICSI at Nurture Fertility.
- Usage of own eggs or donated eggs.
- Informed consent.

4.3.3.3 Exclusion Criteria

- **At recruitment:**
 - Known pathology affecting the endometrial cavity (polyp, submucosal fibroid)
 - Septate uterus or more complex congenital uterine anomaly.

- **Exclusion from analysis after initial recruitment:**

- Failure of fertilisation/blast formation- all participants who do not achieve at least one embryo ready for transfer.
- Inability to obtain a clear image of endometrial pattern for any reason, for example, pathology and uterine position. For example, if at one point in time a scan showed an unclear endometrial pattern, the scan with the unclear pattern would be excluded, but the other scans with a clear endometrial pattern from this patient at other time points would be recorded and analysed.
- New pathology affecting the endometrial cavity (polyp, submucosal fibroid)

4.3.3.4 Sample size calculation

Sample size calculation was difficult due to lack of sufficient information from the literature to be able to calculate this accurately, as well as heterogeneity of the existing studies. However, we have calculated the sample size of women to give reasonable precision in our estimate of the proportion of women who have various endometrial patterns. For example, if the observed rate of triple pattern is 40%, a sample size of 200 cycles will give 95% CI of 33-47.

4.3.4 Protocol and visits' schedule

4.3.4.1 Standard care

Routine clinical care pathways were followed, and patients had standard long protocol treatment, antagonist protocol, short flare protocol or natural cycle.

The standard long protocol at Nurture Fertility involved down regulation with gonadotrophin releasing hormone (GnRH) analogue (Busrelin, Aventis Pharma, Kent, UK) which was given as either a 300µg nasal spray three times a day, or as a 0.5ml once daily subcutaneous injection. Patients were advised to start downregulation 7 days prior to their expected first day of the menstrual period (day 21 in a lady who has a regular 28-day cycle). They were then expected to have a withdrawal bleed within the next two weeks. The women were advised to attend for a scan once they had a bleed to confirm downregulation. At the scan, downregulation was confirmed if the endometrium thickness was <5 mm and the ovaries were “quiet” with no follicles larger than 10 mm. Additionally, oestradiol level should be <200pmol/l. Once downregulation was confirmed, they started ovarian stimulation with either Human Menopausal Gonadotrophin (HMG, Menopur, Ferring UK) or recombinant follicle stimulating hormone (FSH) (Gonal F, Merck Serono, UK), and the first day of starting the ovarian stimulation drugs was regarded as day 1 of the treatment cycle.

Patients attended for a scan around day 9-10 of stimulation to monitor ovarian response by a process known as follicular tracking. They attended regularly for scans

to assess follicular growth and monitoring of the oestradiol level every 1-3 days until they were ready to have egg retrieval. The dose was adjusted to the rate and size of follicular growth for each patient.

The antagonist protocol was given to patients who were at high risk of developing ovarian hyper stimulation syndrome (OHSS) (a life-threatening condition) such as women with high anti mullerian hormone (AMH >67.9) or antral follicle count (AFC >40), or those with polycystic ovarian syndrome or previous history of ovarian hyper stimulation. There was no downregulation with the antagonist protocol and instead patients started the ovarian stimulation drugs (HMG or rFSH) on the second day of their menstrual cycle at a lower dose. Follicular tracking commenced on day 7 and once the leading follicle developed (≥ 11 mm or oestradiol > 1000 pmol/L) a GnRH antagonist, cetrorelix 0.25 mg daily (Cetrotide, Merck Serono, UK), was given subcutaneously to control follicular growth.

Occasionally, the short flare protocol was used for patients who were poor responders and involved giving GnRH on the first day of the menstrual cycle. Ovarian stimulation was then commenced on day 2 with either HMG or rFSH. Follicular tracking commenced from day 5 of gonadotrophin administration.

Natural cycle IVF was also used for patients with poor ovarian reserve (AFC < 5 or AMH < 3) or according to patient preference. It involved patients calling on their first day of the menstrual cycle. There were no ovarian stimulation drugs involved. Follicular tracking commenced on day 5-6 and once the leading follicle developed, a

GnRH antagonist, cetrorelix (Cetrotide, Merck Serono, UK), was given subcutaneously to control follicular growth.

The follow up regime for all the aforementioned protocols was the same. Follicular tracking was continued every 1-3 days depending on follicular development until they were ready to have egg retrieval in theatre.

Criteria for egg retrieval included having 3 follicles >20 mm with a corresponding increase in oestradiol level. Once they had reached theatre criteria for egg retrieval, the patients were advised to take their trigger injection, recombinant human chorionic gonadotropin (hCG) (Ovitrelle 250 IU to trigger oocyte maturation), later that day. Egg collection was performed 36-38 hours after the hCG injection. This was performed under sedation and transvaginal ultrasound guidance. An embryologist assessed the follicular fluid to check for oocytes. The oocytes were then mixed with the sperm sample which was pre-washed and prepared earlier that day (for those who undergoing IVF), or sperm was directly injected into the oocyte (for those who undergoing ICSI); the decision to do IVF or ICSI depended on the quality of the sperm sample. The patients were asked to start progesterone pessaries (Cyclogest, Alparma, UK) for luteal support, and embryo transfer was then performed at blastocyst stage about 5 days later.

Embryo transfer was performed under transabdominal ultrasound guidance. Embryos were transferred at blastocyst stage and were graded by the embryologist. Initially at the beginning of the study, embryos were graded from grade A-C, cavitating &

compacting according to (Richardson et al. 2015) (see appendix 3). This was later changed to grade A-F and cleavage, (see appendix 4). Grade A/B were considered top quality embryos. Cycles were cancelled and did not progress to embryo transfer if the endometrium was deemed too thin (<8mm) in fresh cycles. Failure of fertilisation or blast formation resulted in cycle cancellation as no embryo's were available for transfer.

The patients were advised to do a pregnancy test about 14 days after embryo transfer and to call in with the result. If they had a positive pregnancy test, they were asked to attend for a transvaginal ultrasound scan 5-6 weeks after egg collection to confirm the viability of the pregnancy. If the pregnancy test was negative, they were booked for a review consultation.

Frozen embryo transfer was performed for patients who were at high risk of developing ovarian hyperstimulation syndrome (OHSS) or patients who had frozen embryos left from a previous treatment cycle. It involved downregulation with Busrelin, and having a confirmatory downregulation scan. Once downregulation was confirmed they started hormonal replacement therapy (HRT) which consisted of oestradiol valerate tablets 4mg once daily from day 1 to day 5. This was increased to 4mg twice daily from day 6 to day 9, and further increased to 4mg three times daily from day 10 to day 14. Some patients were on standard HRT dose, while others were started on double dose, for example if they had a thin endometrium in previous cycles. The patients were asked to attend for a scan on day 15 to confirm that the endometrial thickness was >7 mm.

This was known as the coordination scan. If the endometrial thickness was $>7\text{mm}$, then the embryologist decided on their embryo transfer day. The patients were normally asked to start the progesterone pessaries (Cyclogest, Alpharma, UK) for luteal support on the same day, or a day later depending on workload, and they attended 5 days later for embryo transfer. Some patients had progesterone injections (Lubion, Pharmasure) instead of the pessaries, or in addition to progesterone pessaries. The follow up after embryo transfer was the same for both fresh and frozen cycle. If the endometrium failed to reach 7mm , the cycle would be cancelled. Women who had >20 eggs collected are deemed to be at high risk of ovarian hyperstimulation syndrome and their eggs would be frozen and the cycle would be cancelled. Occasionally women with thin endometrium might be allowed to progress to embryo transfer after counselling if they are prone to have thin endometrium and multiple attempts at previous cycles have failed to achieve thickness $\geq 7\text{mm}$. In these cases, a lower threshold with their optimal thickness would be accepted instead.

For each patient, the treatment cycle was identified by the date of the last menstrual period or the first day of ovarian stimulation if they were on the long protocol, or the first day of hormonal replacement therapy if they were having frozen embryo transfer.

4.3.4.2 Study protocol

Patients had transvaginal ultrasound scans and blood tests (for oestradiol and progesterone) at different time points per treatment cycle depending on whether they were undergoing fresh embryo transfer or frozen embryo transfer.

For patients undergoing fresh embryo transfer they had a transvaginal ultrasound scan and blood tests (for oestradiol and progesterone) at three time points (Figure 4.1):

- The day they received their hCG trigger ± 1 day, (as there were no extra visits, data was collected on the day they attended for their scan and deemed suitable for trigger, which could be on the same day or the next day). Progesterone was collected for the study.
- The day of transvaginal oocyte retrieval (TVOR)/ egg collection (both oestradiol and progesterone were collected for the study)
- The day of embryo transfer (ET). A transvaginal ultrasound pre-embryo transfer was performed (this is an extra scan as ET is normally performed under transabdominal scan). Oestradiol and progesterone were collected for the study.

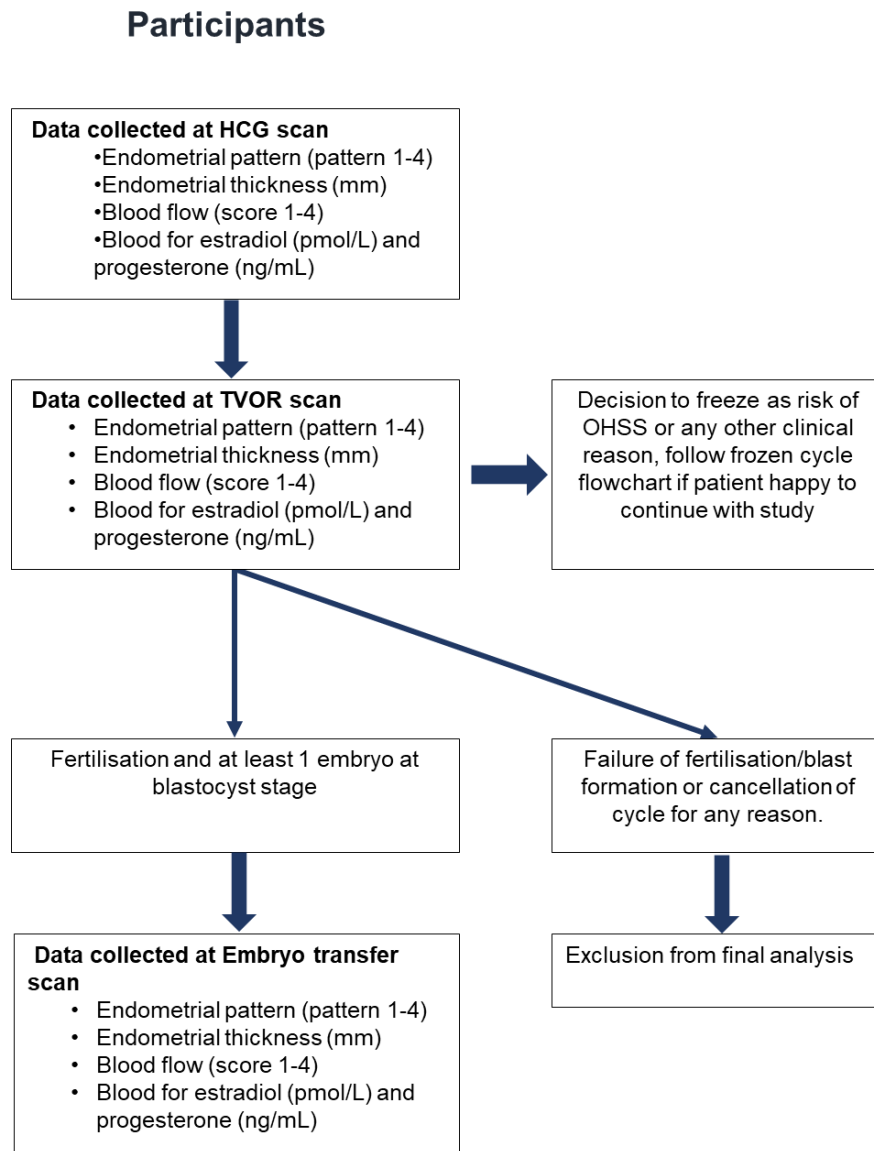


Figure 4.1 Flow chart of Fresh cycle

For patients undergoing frozen embryo transfer, ultrasound assessment and bloods tests were carried at two time points (Figure 4.2):

- Day 15, this is also the same day they initiated taking progesterone +/-1 day. Blood is collected for oestradiol and progesterone level for the study.

- At embryo transfer. A transvaginal ultrasound pre-embryo transfer was performed (this is an extra scan as ET is normally performed under transabdominal scan). Oestradiol and progesterone were collected for the study.

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Participants

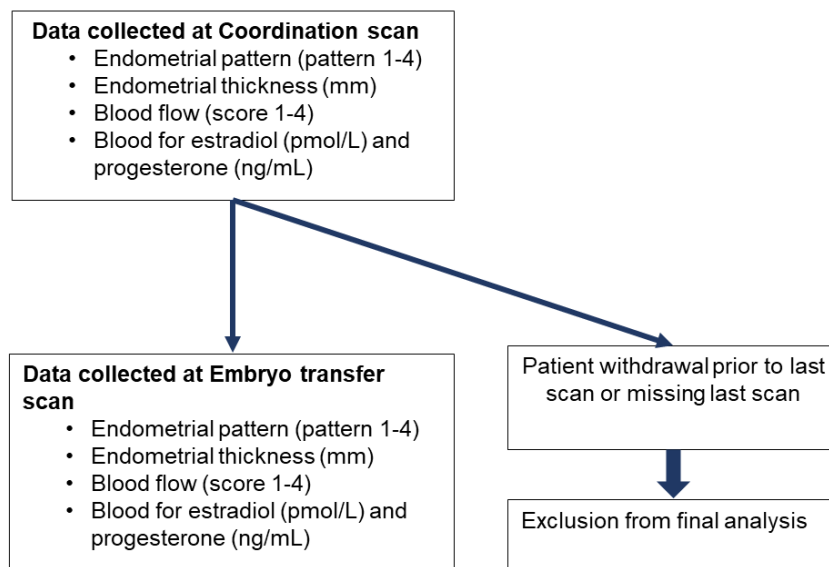


Figure 4.2 Flow chart for frozen cycle

The embryo transfer scan was the only extra scan in the study. Embryo transfer is performed under transabdominal ultrasound guidance with a full bladder while endometrial pattern assessment for the study was performed using transvaginal ultrasound. Women were therefore asked to attend thirty minutes prior to their

scheduled time to have the research scan. The following was assessed during the transvaginal ultrasound scan:

1. Endometrial patterns using the “new pattern recognition classification system” (4 grades see appendix 1)
 - a. Pattern 1: Triple
 - b. Pattern 2: Early intermediate
 - c. Pattern 3: Late intermediate
 - d. Pattern 4: Homogenous
2. Endometrial thickness
3. Endometrial blood flow using the International Ovarian Tumor Analysis (IOTA) setting and scoring system (score 1-4), as explained in appendix 4.

Endometrial thickness was obtained in the sagittal plane and measured at the thickest part in millimetres. The images of endometrial patterns were also recorded and stored as pictures and short 5-20 seconds videos (appendix 3). These were analysed by a single operator (HE).

4.3.5 Study variables and data source

4.3.5.1 Test

- Endometrial patterns on ultrasound at trigger point (hCG injection).

- Endometrial patterns on ultrasound at oocyte retrieval (egg collection).
- Endometrial patterns on ultrasound at embryo transfer.

4.3.5.2 Primary outcome (Reference standard)

Clinical pregnancy is defined as intrauterine pregnancy with foetal heart visualised on transvaginal ultrasound scan (TVUS) detected at any point following embryo transfer (routinely performed around 6-7 weeks gestation at Nurture).

4.3.5.3 Secondary outcomes

- Live birth is defined as delivery of a live foetus after 24 weeks gestation.
- Biochemical pregnancy is defined as having a positive urinary pregnancy test two weeks after embryo transfer.
- Implantation is defined as the presence of an intrauterine gestational sac (one or multiple) on transvaginal ultrasound with or without foetal heart present at 6 weeks gestation and regardless of how many embryos were transferred.

4.3.6 Image acquisition

Transvaginal ultrasound (TVUS) was performed using a Voluson E8 or E10 (GE healthcare) ultrasound machine. The transducer 5-9MHz was used.

Images were obtained in the sagittal plane, and an effort was made to obtain the best view/image. I tried to create pre-sets for obtaining an endometrial pattern, however, this was not possible due to variation among patients which affected the quality of images, therefore it was decided that it would be best to optimise each image for each patient individually.

4.3.7 Ultrasound settings

4.3.7.1 Two-dimensional (2D) ultrasound settings

The power was set to 100%. Focus was set to 5 cm deep but that was adjusted when needed, depending on the patient. Tissue harmonics started as high, and were adjusted to mid, low or off depending on best image obtained. Gain was adjusted for the best image possible with maximal differentiation between the layers. Finally, dynamic contrast was adjusted to obtain the best image.

4.3.7.2 Power Doppler settings

The power Doppler setting was based on IOTA setting and scoring system. (Leone et al. 2010), (see appendix 4)

The colour and power Doppler box included the endometrium with the surrounding myometrium. Magnification and settings were adjusted to ensure maximal sensitivity for blood flow.

Ultrasound frequency at least 5.0 MHz,

Pulse repetition frequency 0.3–0.9 kHz,

Wall filter 30–50 Hz,

Colour power Doppler gain was reduced until all colour artefacts disappeared.

4.3.8 Data collection

All data was collated in a Microsoft access database sheet. A single operator (HE) analysed all the ultrasound images to reduce inter-observer variation.

The recording of endometrial pattern was carried out by the investigator (HE) prospectively while being blind to the outcome of IVF/ICSI.

4.3.9 Statistics and data analysis

Statistical analysis of the results was performed using the Statistical package for the Social Sciences (SPSS, Chicago, IL, USA) software.

The distribution of the data was checked for normality using a normal probability plot. The statistical significance of the data was analysed using the relevant statistical techniques.

The primary analysis measured the pregnancy rates for each of the four endometrial patterns. The odds ratio and 95% confidence interval for each of the endometrial patterns was calculated. Chi squared test (χ^2) was used to generate the p values and where there were small numbers, fisher exact test was used instead. Triple pattern was used as the reference pattern when calculating the odd ratios. When there was no triple pattern for example at embryo transfer, early intermediate was used as the reference pattern instead. Where zeros caused problems with computation of the odds ratio or its standard error, 0.5 was added to all cells (a, b, c, d) (Pagano & Gauvreau, 2000; Deeks & Higgins, 2010) and an online software, (Medcalc Software Ltd, accessed 2022) was used to calculate the odds ratios. Fisher exact test was performed instead of chi squared test when there were small numbers (count <5).

Exploratory analysis was performed as there was insufficient data to perform logistic regression.

McNamar test was used to assess the change in endometrial pattern between the different time points.

To assess the impact of hormonal profile on the success rate, oestradiol to progesterone ratio was computed. This was then converted into categorical data by dividing into tertile groups and chi squared tests were performed for clinical pregnancy and live birth. Odds ratio and 95% confidence interval were calculated at each of the time points.

To assess the impact of endometrial thickness on the success rate, endometrial thickness was measured at each of the timelines. The data was converted to categorical data by dividing into tertile groups and chi squared tests were performed for clinical pregnancy and secondary live birth. Odds ratio and 95% confidence interval were calculated at each of the time points.

To assess the impact of endometrial Doppler flow on the success rate, chi squared tests were performed for clinical pregnancy and live birth. Odds ratio and 95% confidence interval were calculated at each of the time points.

To assess the relationship between hormonal profile and endometrial pattern, the Levene test was performed to test for homogeneity in the first instance and if not significant (p value >0.05) one way ANOVA test was then used to assess the relationship between hormonal profile and endometrial pattern.

Three subgroup analysis was performed, with the following groups:

1. Type of embryo transfer (fresh or frozen).
2. Single or multiple embryos transferred.
3. Grade of embryo- good quality (A/B), or lesser grade. Initially at the beginning of the study embryo were graded from grade A-C, cavitating & compacting according to (Richardson et al. 2015) (see appendix 3). This was later changed to grade A-F and cleavage, (see appendix 4). For this study,

subgroup analysis of top-quality embryos was performed. Grade A-B from either classification systems were considered as top-quality embryos.

The results were summarised and represented graphically where appropriate.

4.3.9.1 Deviation from protocol and handling of missing data

At the beginning of the study, I tried to test for endometrial contractility, however, this required having a long cineloop which was not achievable with the GE Voluson machines due to limited buffer size. This was therefore abandoned.

While developing the protocol and on discussion with statisticians, it was decided that imputation methods would not be helpful in dealing with missing data. Instead, it was decided where there was significant missing data, these would be better excluded from the study. To reduce the risk of bias, where there were significant missing data (for example 2/3 fresh cycle scans were missed), these were excluded from the study. For frozen cycles, if there was no embryo transfer scan, cases were excluded from analysis.

Endometrial volume was measured, however as there was significant missing data >50% for various reasons (not measured with pattern, two faulty probes at different times) this was excluded from the analysis as per protocol.

Cases where there was failure of fertilisation or cycle cancellation were excluded from analysis as per eligibility criteria. Over-recruitment was carried out in anticipation of this.

4.4 Results

4.4.1 Participants and recruitment

Initially, 193 women expressed interest to take part in the study. 173 women signed the consent form, some of those women were found to be ineligible after their pre-treatment scan showed congenital abnormalities. Two women fell pregnant spontaneously after signing the consent form and prior to starting treatment. Some women changed their mind regarding treatment or decided to have treatment elsewhere, while some women delayed their treatment for after the COVID pandemic. 230 cycle scans (around 540 scans) were performed. Of those only 155 women undergoing 191 cycles were eligible to be included in the final analysis. The main reason for exclusion as per eligibility criteria was failure to proceed to embryo transfer due to cycle cancellation, either because of risk of ovarian hyperstimulation or failure of fertilisation/ blast formation, or thin endometrium. 116 fresh cycles and 75 frozen cycles were included in the final analysis. The total number of scans analysed was 467 scans. Figure 4.3 summarises the process of recruitment, the reasons for exclusion from the study, number of participants, cycles, and scans at each stage.

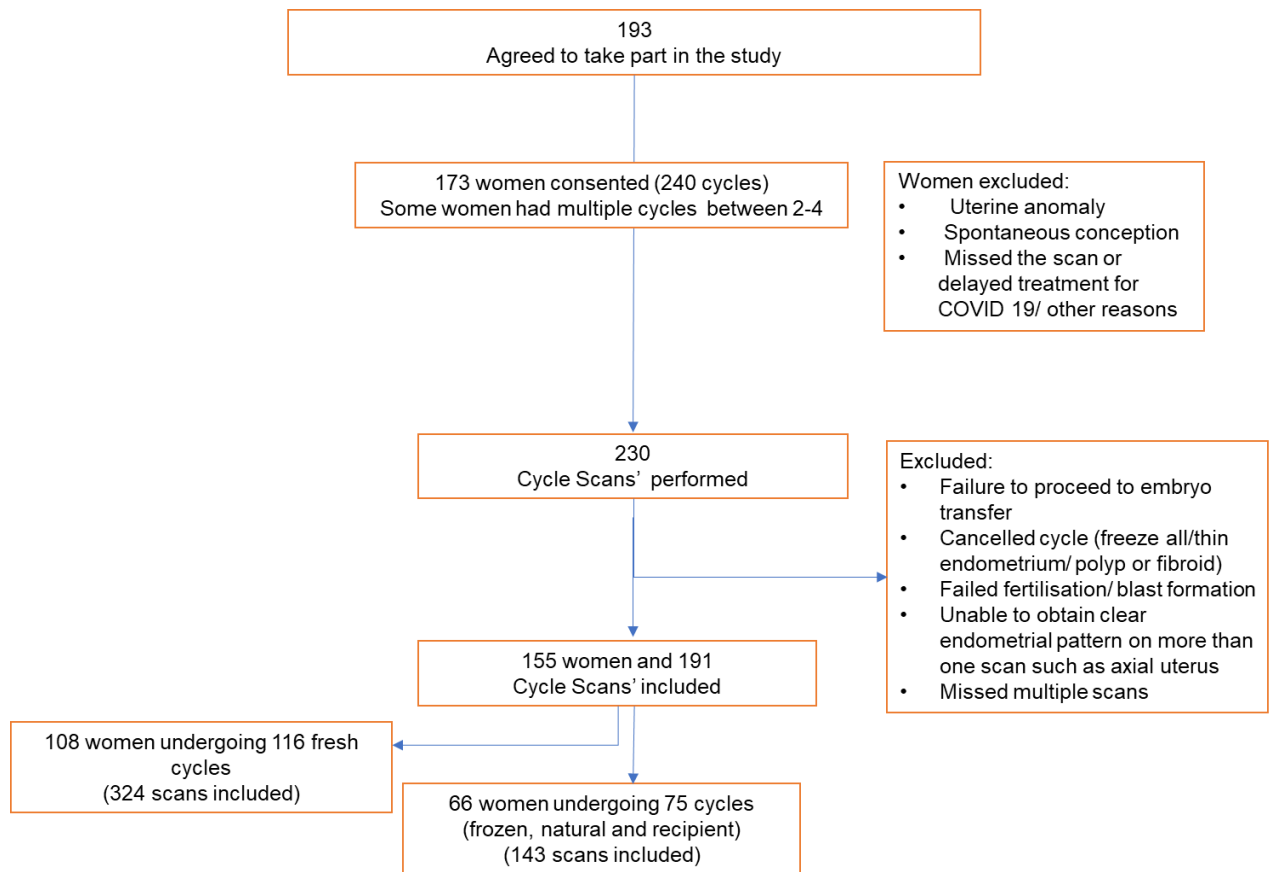


Figure 4.3 Flow chart of recruitment

4.4.1.1 Participants characteristics

The age of the participants ranged from 23 - 44 years ($M \pm SD = 34.4.8$). Other characteristics of the participants are summarised in Table 4.1.

Table 4.1 Participants' demographics & characteristics

	Median (interquartile range)
AFC	22 (19)
AMH	18.8 (19.95)
BMI	23.7 (5.26)
Duration of subfertility (months)	36 (24)
Parity	1
No of previous miscarriages	0
No of previous ectopic	0
No of previous terminations	0
Ethnic Background of participants	
Ethnicity	Number (%)
Caucasian	138 (89%)
Asian	14 (9%)
Black	1 (1%)
Mixed/other	2 (1%)
<i>N=155</i> <i>AFC= Antral follicle count, AMH= Anti mullerian Hormone, BMI= Body Mass Index.</i>	

4.4.1.2 Main causes of subfertility

The most common cause of subfertility was unexplained, followed by male factors. Male factors included male causes of subfertility, as well as when there was no male partner (same sex couples or single ladies). Table 4.2 below illustrates the different causes of subfertility.

Table 4.2 Main causes of subfertility

Cause of subfertility	Number (%)
Unexplained	62 (40%)
Male factors total	45 (29%)
No male partner	5 (3%)
Ovulatory	23 (15%)
Tubal factors	20 (13%)
Endometriosis	5 (3%)

4.4.2 Analysis of results

4.4.2.1 Fresh cycles

4.4.2.1.1 Primary outcomes for Fresh cycles

At egg collection, women with homogenous pattern at egg collection were 95% less likely to become pregnant when compared to women with triple pattern. Women with late intermediate pattern at egg collection were 35% less likely to become pregnant when compared to women with triple pattern. This result is statistically significant with a p value of 0.009.

At embryo transfer, there was no triple pattern at embryo transfer. Only 2 women had early intermediate pattern and both of them did get pregnant. Endometrial pattern was recorded both on a transvaginal scan and abdominal scan. Although only 35 women were recorded as having homogenous pattern on transvaginal scan, the majority of patterns were recorded as homogenous on transabdominal scan, suggesting that

transvaginal scan provides more accurate information. For statistical analysis only endometrial patterns on transvaginal scan were used. The odds of having a clinical pregnancy with homogenous pattern at embryo transfer was three times higher than an early intermediate pattern. Similarly, women with a late intermediate pattern were 6 times more likely to become pregnant than women with an early intermediate pattern at embryo transfer. This result is almost significant with p value of 0.051, however the confidence interval is wide for both of these results (given that there were only 2 women with early intermediate pattern at embryo transfer). Table 4.3 below summarizes the different odds ratios for clinical pregnancy related to the endometrial pattern at trigger, egg collection and embryo transfer.

Table 4.3 Summary of the odds ratio for clinical pregnancy related to the endometrial pattern at the different time points in fresh cycles

Endometrial Pattern at trigger					
Classification	Number	Number of clinical pregnancy (%)	OR	95% CI	*p-value
Triple	67	37 (55%)			0.18
Early Intermediate	34	14 (41%)	0.57	0.25 to 1.31	
Late Intermediate	8	2 (25%)	0.27	0.05 to 1.44	
Homogenous	1	0	0.27	0.01 to 6.90	
Total	110				
Endometrial Pattern at egg collection					
Classification	Number	Number of clinical pregnancy (%)	OR	95% CI	*p-value
Triple	19	10 (52 %)			0.009
Early Intermediate	43	26 (60%)	1.38	0.46 to 4.08	
Late Intermediate	38	16 (42%)	0.65	0.21 to 1.98	
Homogenous	8	0	0.05	0.0027 to 1.05	
Total	108				
Endometrial Pattern at Embryo transfer					
Classification	Number	Number of clinical pregnancy (%)	OR	95% CI	*p-value
Triple	0	0	NA	NA	
Early Intermediate	2	0			0.051
Late Intermediate	69	39 (57%)	6.48	0.30 to 139.90	
Homogenous	35	13 (37%)	3	0.13 to 67.30	
Total	106				
<i>*p value based on Fisher Exact test</i> <i>Triple pattern is the reference pattern at trigger & egg collection. Early intermediate is the reference pattern at embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.2.1.2 Secondary outcomes

4.4.2.1.2.1 Live birth outcomes

There was no triple pattern at embryo transfer. Only 2 women had an early intermediate pattern and none of them had a live birth. The odds of having a live birth with a homogenous pattern at embryo transfer is almost 2 times higher than early intermediate. Table 4.4 below summarises the odds ratio for live birth related to the endometrial pattern.

Only one lady had a homogenous pattern at trigger and she did not achieve a live birth. The odds of live birth for homogenous pattern was less than half OR (0.44) when compared to triple pattern. However, this result is not statistically significant.

In comparison, the odds of achieving live birth with homogenous pattern at egg collection is much lower compared to triple pattern.

Table 4.4 Summary of the odds ratio for a Live birth related to the endometrial pattern in fresh cycles

Endometrial Pattern at trigger					
Classification	Number	Live birth (%)	OR	95% CI	*p-value
Triple	67	29 (43%)			0.313
Early Intermediate	34	13 (38%)	0.81	0.35 to 1.8	
Late Intermediate	8	1 (13%)	0.19	0.02 to 1.60	
Homogenous	1	0	0.44	0.02 to 11.07	
Total	110				
Endometrial Pattern at egg collection					
Classification	Number	Live birth (%)	OR	95% CI	*p-value
Triple	19	10 (53%)			0.047
Early Intermediate	43	19 (44%)	0.71	0.24 to 2.11	
Late Intermediate	38	13 (34%)	0.47	0.15 to 1.44	
Homogenous	8	0	0.05	0.0027 to 1.05	
Total	108				
Endometrial Pattern at Embryo transfer					
Classification	Number	Live birth (%)	OR	95% CI	*p-value
Triple	0	0	NA	NA	
Early Intermediate	2	0			0.031
Late Intermediate	69	34 (49%)	4.86	0.23 to 104.92	
Homogenous	35	9 (26%)	1.79	0.08 to 40.82	
Total	106				
<i>*p value based on Fisher Exact test</i> <i>Triple pattern is the reference pattern at trigger & egg collection. Early intermediate is the reference pattern at embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.2.1.2.2 *Biochemical pregnancy outcomes*

The odds of having a positive pregnancy test with a homogenous pattern at embryo transfer is almost 4 times more likely when compared with early intermediate. Similarly, the odds of a positive pregnancy test with late intermediate pattern at embryo transfer is 12 times more likely when compared with early intermediate. Although these results are statistically significant with a low p value (0.001), the confidence interval for both is wide given that only 2/100 women had an early intermediate pattern at embryo transfer, therefore these results need to be treated with caution. One patient had a homogenous pattern at trigger point. She had a positive pregnancy test, however, sadly, this did not result in a clinical pregnancy.

At egg collection, although it appears that women with triple and early intermediate patterns have higher odds of getting a positive pregnancy test when compared to late intermediate and homogenous patterns, the results are not statistically significant.

Table 4.5 below summarises the rate and odds ratio for biochemical pregnancy related to the endometrial pattern.

Table 4.5 Summary of the odds ratio for biochemical pregnancy related to the endometrial pattern in fresh cycles

Endometrial Pattern at trigger					
Classification	Number	Number of biochemical pregnancy (%)	OR	95% CI	*p-value
Triple	67	45 (67%)			0.060
Early Intermediate	34	18 (53%)	0.55	0.23 to 1.28	
Late Intermediate	8	2 (25%)	0.16	0.030 to 0.87	
Homogenous	1	1 (100%)	1.48	0.06 to 37.89	
Total	110				
Endometrial Pattern at egg collection					
Classification	Number	Number of biochemical pregnancy (%)	OR	95% CI	*p-value
Triple	19	12 (63%)			0.107
Early Intermediate	43	30 (70%)	1.35	0.43 to 4.20	
Late Intermediate	38	21 (55%)	0.72	0.23 to 2.23	
Homogenous	8	2 (25%)	0.19	0.03 to 1.24	
Total	108				
Endometrial Pattern at Embryo transfer					
Classification	Number	Number of biochemical pregnancy (%)	OR	95% CI	*p-value
Triple	0	0	NA	NA	
Early Intermediate	2	0			0.001
Late Intermediate	69	49 (71%)	12.07	0.56 to 262.62	
Homogenous	35	14 (40%)	3.37	0.15 to 75.50	
Total	106				
<i>*p value based on Fisher Exact test</i> <i>Triple pattern is the reference pattern at trigger & egg collection. Early intermediate is the reference pattern at embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.2.1.2.3 *Implantation outcomes*

Implantation was statistically significant for endometrial pattern at trigger point with a p value of 0.03. The odds of implantation with an early intermediate pattern is less than half (0.42) when compared to a triple pattern. This is statistically significant with a small confidence interval. Although the odds of implantation appear to be higher with a homogenous pattern, this is based on only one patient, which is reflected in the wide confidence interval.

At egg collection, the odds of implantation with an early intermediate and triple pattern appear to be very similar, and much higher than the homogenous or late intermediate pattern. However, the results were not statistically significant.

At embryo transfer, the odds of implantation with a homogenous pattern is three times higher than an early intermediate pattern, and almost nine fold higher when comparing the late intermediate pattern with an early intermediate pattern. Although the result is statistically significant with p value of 0.01, only 2 women had an early intermediate pattern at embryo transfer, which was reflected in the wide confidence interval. Table 4.6 below summarises the rate and odds ratio for Implantation related to the endometrial pattern.

Table 4.6 Summary of the odds ratio for Implantation related to the endometrial pattern in fresh cycles

Endometrial Pattern at trigger					
Classification	Number	Implantation (%)	OR	95% CI	*p-value
Triple	67	42 (63%)			0.033
Early Intermediate	34	14 (41%)	0.42	0.18 to 0.97	
Late Intermediate	8	2 (25%)	0.20	0.04 to 1.06	
Homogenous	1	1 (100%)	1.80	0.07 to 45.87	
Total	110				
Endometrial Pattern at egg collection					
Classification	Number	Implantation (%)	OR	95% CI	*p-value
Triple	19	12 (63%)			0.082
Early Intermediate	43	28 (65%)	1.09	0.354 to 3.35	
Late Intermediate	38	17 (45%)	0.47	0.152 to 1.46	
Homogenous	8	2 (25%)	0.19	0.03 to 1.24	
Total	108				
Endometrial Pattern at Embryo transfer					
Classification	Number	Implantation (%)	OR	95% CI	*p-value
Triple	0	0	NA	NA	
Early Intermediate	2	0			0.012
Late Intermediate	63	44 (64%)	8.73	0.40 to 188.94	
Homogenous	35	14 (40%)	3.37	0.15 to 75.50	
Total	106				
<i>*p value based on Fisher Exact test</i> <i>Triple pattern is the reference pattern at trigger & egg collection. Early intermediate is the reference pattern at embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.2.2 Subgroup analysis for fresh cycles

4.4.2.2.1 *Single blastocyst transfer (top-quality embryo)*

Subgroup analysis was performed for fresh cycles where a single top-quality blastocyst was transferred. Grade A or B embryos were considered top quality (see appendix 3). The analysis was performed for the primary outcome of clinical pregnancy and the secondary outcome of a live birth at the three time points (trigger, egg collection, embryo transfer)

4.4.2.2.1.1 *Clinical pregnancy outcomes for top-quality blastocyst*

Clinical pregnancy was calculated for women who had a single embryo transfer with a top quality blastocyst (either grade A or B). Only 76/116 cycles had single embryo transfer and of those, only 63 had a top quality embryo transferred. The results were not statistically significant. Table 4.7 below summarises the rate and odds ratio for clinical pregnancy outcomes for single top-quality embryo related to the endometrial pattern.

Table 4.7 Summary of the odds ratio for clinical pregnancy outcomes of single top-quality blastocyst related to the endometrial pattern in fresh cycles

Endometrial Pattern at trigger					
Classification	Number	Clinical pregnancy (%)	OR	95% CI	*p-value
Triple	37	21 (57%)			0.442
Early Intermediate	17	8 (47%)	0.68	0.214 to 2.146	
Late Intermediate	4	1 (25%)	0.25	0.024 to 2.676	
Homogenous	1	0	0.26	0.001 to 6.693	
Total	59				
Endometrial Pattern at egg collection					
Classification	Number	Clinical pregnancy (%)	OR	95% CI	*p-value
Triple	10	6 (60%)			0.314
Early Intermediate	24	14 (58%)	0.933	0.208 to 4.196	
Late Intermediate	21	10 (48%)	0.606	0.132 to 2.793	
Homogenous	3	0	0.099	0.004 to 2.420	
Total	58				
Endometrial Pattern at Embryo transfer					
Classification	Number	Clinical pregnancy (%)	OR	95% CI	*p-value
Triple	0	0	NA	NA	
Early Intermediate	1	0			0.338
Late Intermediate	38	22 (58%)	4.091	0.156 to 106.89	
Homogenous	20	9 (45%)	2.478	0.090 to 68.140	
Total	59				
*p value based on Fisher Exact test					
OR= Odd ratio, CI= Confidence interval, NA= Not applicable					

4.4.2.2.1.2 *Live birth for top quality blastocyst*

Live birth was calculated for women who had a single embryo transfer with a top quality blastocyst, either grade A or B. Only 76/116 cycles had a single embryo transfer, and of those only 63 had a top-quality embryo transferred.

The odds of having a live birth with a late intermediate pattern at embryo transfer was almost 4 times more likely when compared to an early intermediate pattern. However, the results were not statistically significant. Table 4.8 below summarises the rate and odds ratio for live birth outcomes for a single top-quality embryo in relation to the endometrial pattern.

Table 4.8 Summary of the odds ratio for live birth outcomes of single top-quality blastocyst in relation to the endometrial pattern in fresh cycles

Endometrial Pattern at trigger					
Classification	Number	Live birth (%)	OR	95% CI	*p-value
Triple	37	18 (49%)			0.242
Early Intermediate	17	8 (47%)	0.938	0.297 to 2.963	
Late Intermediate	4	0	0.117	0.006 to 2.329	
Homogenous	1	0	0.371	0.014 to 9.725	
Total	59				
Endometrial Pattern at egg collection					
Classification	Number	Live birth (%)	OR	95% CI	*p-value
Triple	10	6 (60%)			0.417
Early Intermediate	24	11 (46%)	0.564	0.126 to 2.524	
Late Intermediate	21	9 (43%)	0.500	0.108 to 2.314	
Homogenous	3	0	0.099	0.004 to 2.421	
Total	58				
Endometrial Pattern at Embryo transfer					
Classification	Number	Live birth (%)	OR	95% CI	*p-value
Triple	0	0	NA	NA	
Early Intermediate	1	0			0.076
Late Intermediate	38	21 (55%)	3.685	0.141 to 96.230	
Homogenous	20	6 (30%)	1.134	0.048 to 37.636	
Total	59	0			
<i>*p value based on Fisher Exact test</i> <i>Triple pattern is the reference pattern at trigger & egg collection. Early intermediate is the reference pattern at embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.3 Secondary objectives

4.4.3.1 Change in endometrial pattern

Sequential change in endometrial pattern during the different time lines was investigated using the McNamar test.

4.4.3.2 Change in endometrial pattern from trigger to egg collection

104/116 cycles had both endometrial patterns measured at trigger and egg collection consequentially. McNamar test demonstrated that there was a change in endometrial pattern between the different timelines. 30 women had triple pattern at trigger which changed to early intermediate at egg collection, 13 women had their pattern change from triple at trigger to late intermediate at egg collection and 2 women had their pattern change from triple at trigger to a homogenous pattern at egg collection. This change is statistically significant with p value of < 0.001 . Table 4.9 below summarises the change in endometrial pattern from trigger to egg collection.

Table 4.9 Change in endometrial pattern from trigger to egg collection

		Triple	Early Intermediate	Late Intermediate	Homogenous	Total
Endometrial pattern at Trigger	Triple	17	30	13	2	62
	Early Intermediate	0	11	19	3	33
	Late Intermediate	0	2	5	1	8
	Homogenous	0	0	0	1	1
Total		17	43	37	7	104
<i>McNamar Bowker test = 62.67, degree of freedom 6, p value <0.001</i>						

4.4.3.3 Change in endometrial pattern between egg collection and embryo transfer

99/116 had their endometrial pattern measured sequentially between egg collection and embryo transfer. McNamar test requires symmetrical data/groups between the two timelines. None of the women had a triple pattern at embryo transfer, and therefore a p value could not be generated using the McNamar test. Table 4.10 below summarises the change in endometrial pattern from egg collection to embryo transfer.

Table 4.10 Change in endometrial pattern from egg collection to embryo transfer

		Early Intermediate	Late Intermediate	Homogenous	Total
Endometrial pattern at egg collection	Triple	1	14	2	17
	Early Intermediate	1	30	9	40
	Late Intermediate	0	22	13	35
	Homogenous	0	0	7	7
Total		2	66	31	99

4.4.4 Endometrial thickness

Endometrial thickness was measured at trigger, egg collection and embryo transfer. The data was converted to categorical data by dividing into tertile groups and chi squared tests were performed for primary (clinical pregnancy) and secondary outcomes (live birth).

Endometrial thickness is routinely recorded at trigger and embryo transfer, therefore I have almost complete dataset for these two timelines. However, endometrial thickness is not routinely recorded at egg collection. There is some missing data, as initially some clinicians forgot to save a still image of endometrial thickness however this was later corrected. 99/108 endometrial thickness was recoded at egg collection.

There were a couple of women with endometrium thickness less than 7 mm at trigger however their endometrial thickness has increased to ≥ 7 mm by the time of embryo transfer and they were allowed to proceed with embryo transfer.

The results show that increasing endometrial thickness is associated with increasing clinical pregnancy rates (Table 4.11) as well as increasing live birth rates (see Table 4.12). This is demonstrated across the different timelines as well (trigger, egg collection and embryo transfer), although these results were not statistically significant. Table 4.11 below demonstrates the clinical pregnancy outcomes for endometrial thickness at the different timelines.

Table 4.11 Clinical pregnancy outcomes for endometrial thickness in fresh cycles

Endometrial Thickness at trigger					
Endometrial thickness (mm)	Number	Clinical pregnancy (%)	OR	95% CI	p-value
6.3-9.3	41	15 (37%)			0.062
9.31-11.60	37	17 (46%)	1.47	0.60-3.65	
11.61-20	38	24 (63%)	2.97	1.19-7.42	
Total	116				
Endometrial Thickness at egg collection					
Endometrial thickness (mm)	Number	Clinical pregnancy (%)	OR	95% CI	p-value
6.3-8.9	33	13 (39%)			0.371
8.91-10.90	33	15 (46%)	1.28	0.482-3.410	
10.91-19.3	33	19 (58%)	2.09	0.782-5.574	
Total	99				
Endometrial Thickness at Embryo transfer					
Endometrial thickness (mm)	Number	Clinical pregnancy (%)	OR	95% CI	p-value
7.5-9.70	39	17 (44%)			0.740
9.71-11.90	39	20 (51%)	1.36	0.56-3.32	
11.91-22	37	19 (51%)	1.37	0.55-3.37	
Total	115				
<i>OR= Odd ratio, CI= Confidence interval</i> <i>6.3-9.3 was the reference thickness group at trigger, 6.3-8.9 was the reference thickness group at egg collection, 7.5-9.70 was the reference thickness group at embryo transfer</i>					

Table 4.12 below demonstrates the live birth outcomes for endometrial thickness at the different timelines

Table 4.12 Live birth outcomes for endometrial thickness in fresh cycles

Endometrial Thickness at trigger					
Endometrial thickness (mm)	Number	Live birth (%)	OR	95% CI	p-value
6.3-9.3	41	12 (29%)			0.107
9.31-11.60	37	14 (38%)	1.47	0.57-3.79	
11.61-20	38	20 (53%)	2.69	1.06-6.79	
Total	116				
Endometrial Thickness at egg collection					
Endometrial thickness (mm)	Number	Live birth (%)	OR	95% CI	p-value
6.3-8.9	33	9 (27%)			0.151
8.91-10.90	33	12 (36%)	1.52	0.536-4.328	
10.91-19.3	33	17 (51%)	2.83	1.015-7.906	
Total	99				
Endometrial Thickness at Embryo transfer					
Endometrial thickness (mm)	Number	Live birth (%)	OR	95% CI	p-value
7.5-9.70	39	12 (31%)			0.35
9.71-11.90	39	17 (44%)	1.74	0.69-4.40	
11.91-22	37	17 (46%)	1.91	0.75-4.89	
Total	115				
<i>OR= Odd ratio, CI= Confidence interval</i> <i>6.3-9.3 was the reference thickness group at trigger, 6.3-8.9 was the reference thickness group at egg collection, 7.5-9.70 was the reference thickness group at embryo transfer</i>					

4.4.5 Endometrial Doppler

The Doppler flow was scored from 1-4 according to the IOTA scoring system (Leone et al. 2010). The results were not statistically significant for endometrial Doppler. Table 4.13 summarises clinical pregnancy outcomes for endometrial Doppler at the different timelines.

Table 4.13 Clinical pregnancy outcomes for endometrial Doppler in fresh cycles

Endometrial Doppler at trigger					
Doppler score	Number	Clinical pregnancy (%)	OR	95% CI	p-value
Score 1	0	0	NA	NA	
Score 2	10	6 (60%)			0.730
Score 3	34	19 (56%)	0.844	0.201-3.546	
Score 4	10	4 (40%)	0.444	0.074-2.660	
Total	54				
Endometrial Doppler at egg collection					
Doppler score	Number	Clinical pregnancy (%)	OR	95% CI	*p-value
Score 1	1	1 (100%)			0.869
Score 2	30	15 (50%)	0.333	0.013-8.834	
Score 3	64	30 (47%)	0.295	0.012-7.506	
Score 4	2	1 (50%)	0.333	0.007-16.800	
Total	97				
Endometrial Doppler at Embryo transfer					
Doppler score	Number	Clinical pregnancy (%)	OR	95% CI	*p-value
Score 1	2	1 (50%)			0.942
Score 2	41	20 (49%)	0.952	0.056-16.279	
Score 3	39	21 (54%)	1.167	0.068-20.016	
Score 4	13	7 (54%)	1.167	0.059-22.937	
Total	95				
<i>*p value based on Fisher Exact test</i> <i>Score 2 was the reference score at trigger, Score 1 was the reference score at egg collection and embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

Table 4.14 below summarises the live birth outcomes for endometrial Doppler at the different timelines.

Table 4.14 Live birth outcomes for endometrial Doppler in fresh cycles

Endometrial Doppler at trigger					
Doppler score	Number	Live birth (%)	OR	95% CI	*p-value
Score 1	0	0	NA	NA	
Score 2	10	6 (60%)			0.730
Score 3	34	15 (44%)	0.526	0.125-2.210	
Score 4	10	4 (40%)	0.444	0.074-2.660	
Total	54				
Endometrial Doppler at egg collection					
Doppler score	Number	Live birth (%)	OR	95% CI	*p-value
Score 1	1	1 (100%)			0.491
Score 2	30	12 (40%)	0.225	0.009-5.986	
Score 3	64	24 (38%)	0.202	0.008-5.147	
Score 4	2	0 (0%)	0.067	0.001-5.494	
Total	97				
Endometrial Doppler at Embryo transfer					
Doppler score	Number	Live birth (%)	OR	95% CI	*p-value
Score 1	2	1 (50%)			0.873
Score 2	41	17 (41%)	0.708	0.041-12.131	
Score 3	39	16 (41%)	0.696	0.040-11.958	
Score 4	13	7 (54%)	1.167	0.059-22.937	
Total	95				
<i>*p value based on Fisher Exact test</i> Score 2 was the reference score at trigger, Score 1 was the reference score at egg collection and embryo transfer. OR= Odd ratio, CI= Confidence interval, NA= Not applicable					

4.4.5.1 Hormonal profile for fresh cycles

Oestradiol and progesterone levels were measured at trigger, egg collection and embryo transfer. Unfortunately, at the beginning of the study the clinic was in the process of changing the existing analyser to a Rosch analyser which has led to having some missing data. Some hormonal profile data was missing at the beginning of the study. Table 4.15 summarises the Median and range of oestradiol and progesterone at the different timelines.

Table 4.15 Hormonal profile for fresh cycles

Hormonal profile	Median	Range	Total Number
Oestradiol at trigger	8887	720-23363	89
Progesterone trigger	1.0185	0.050-4.49	68
Oestradiol at egg collection	5176.76 (mean)	2816.64 (std)	93
Progesterone at egg collection	11.725	1.19-46.76	94
Oestradiol at embryo transfer	5234.5	158.90-44769	82
Progesterone at embryo transfer	60	5.59-60	82

Oestradiol to progesterone ratio was computed for both trigger and egg collection. This was then converted into categorical data by dividing into tertile groups, and chi squared tests were performed for primary (clinical pregnancy) and secondary outcomes (live birth).

There was no statistical difference for oestradiol to progesterone (E2:P) ratio at trigger or egg collection. Progesterone at embryo transfer levels were mostly 60 ng/ml (which

is higher than the upper limit for the analyser). To achieve an accurate result would entail a complex dilution process, which was both complex and time consuming for the lab technician. The high level is due to the exogenous progesterone as well as progesterone from the corpora lutea, so a decision was made at the beginning of the study to accept that progesterone is high if it is >60 ng/ml. Therefore E2:P ratio was not computed and no further analysis for progesterone at embryo transfer was performed.

4.4.5.1.1 Hormonal profile and endometrial pattern

One-way ANOVA was used to explore the relationship between endometrial pattern and the hormonal profile. Table 4.16 summarises the mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at trigger.

Table 4.16 The mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at trigger in fresh cycles.

		N	Mean	Std. Deviation
Oestradiol at Trigger	Triple	50	8749.12	4692.32
	Early Intermediate	28	10511.42	5959.66
	Late Intermediate	6	10229.83	2795.07
	Homogenous	1	13121.00	.
	Total	85	9485.60	5059.22
Progesterone at Trigger	Triple	40	0.88	0.546764
	Early Intermediate	22	1.08	0.63
	Late Intermediate	3	1.02	0.24
	Homogenous	1	4.49	.
	Total	66	1.01	0.71
Oestradiol: Progesterone ratio at trigger	Triple	39	13220.16	8841.96
	Early Intermediate	22	16298.83	20249.19
	Late Intermediate	3	10371.56	6444.09
	Homogenous	1	2922.27	.
	Total	65	13972.28	13674.03

One-way ANOVA was conducted to compare the relationship between endometrial pattern and progesterone levels at trigger point. Interestingly, the results showed that triple and early intermediate pattern are associated with low progesterone levels. In contrast, late intermediate and homogenous pattern are associated with higher progesterone levels. This is clearly demonstrated in figure 4.4 which shows the mean of progesterone levels for endometrial pattern at trigger. The result was statistically significant [$F(3, 62) = 13.2, p < 0.001$]. **Error! Reference source not found.** shows the mean of progesterone levels for endometrial pattern at Trigger.

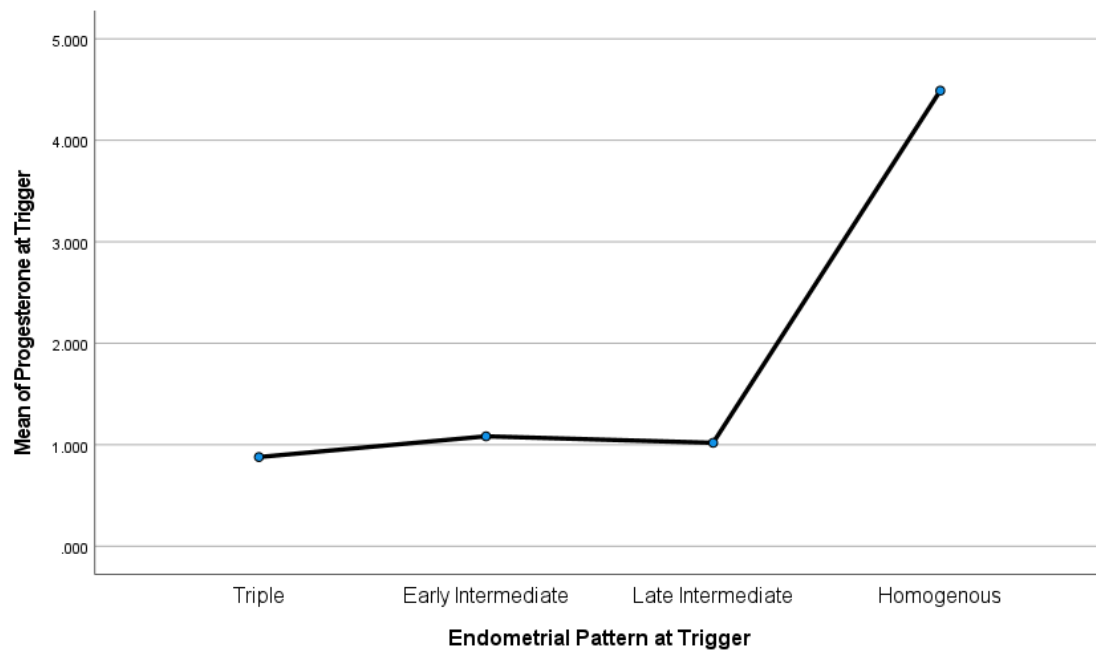


Figure 4.4 Mean of progesterone levels for endometrial pattern at Trigger

A one-way ANOVA was conducted to compare the relationship between endometrial pattern and oestradiol levels at trigger point. The results were not statistically significant [$F(3, 81) = 0.951$, $p = 0.420$]. Figure 4.5 shows the mean of oestradiol for endometrial pattern at Trigger.

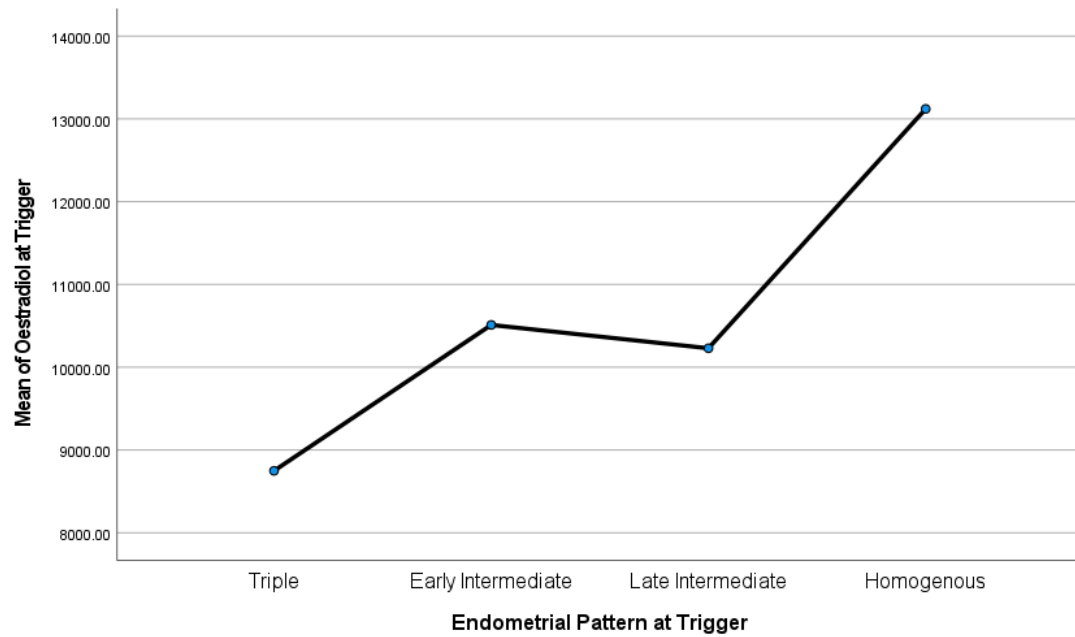


Figure 4.5 Mean of oestradiol levels for endometrial pattern at Trigger

One-way ANOVA was conducted to compare the relationship between endometrial pattern and the oestradiol: progesterone ratio at trigger point. Although, the results showed a trend of high oestradiol: progesterone ratio with triple and early intermediate pattern which sharply drops with late intermediate and homogenous pattern, the result was not statistically significant [$F(3, 61) = 0.527$, $p = 0.666$]. Figure 4.6 shows the mean of oestradiol: progesterone ratio for endometrial pattern at Trigger.

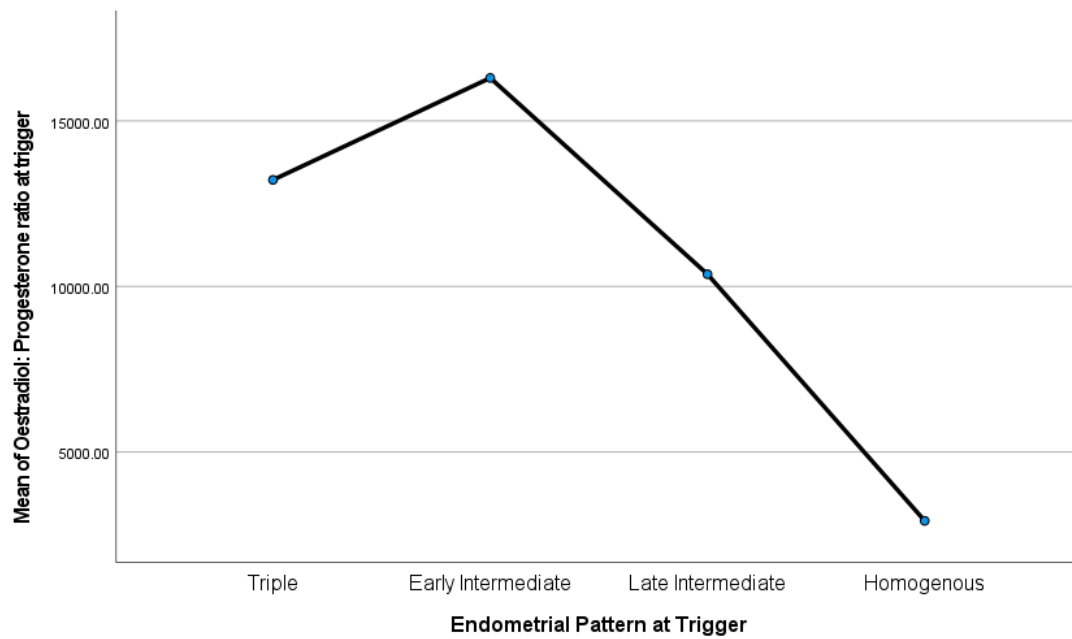


Figure 4.6 Mean of Oestradiol: progesterone ratio at trigger

The mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at egg collection are shown in Table 4.17 below.

Table 4.17 The mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at egg collection.

		N	Mean	Std. Deviation
Oestradiol at egg collection	Triple	13	4808.57	3598.94
	Early Intermediate	34	4854.77	2491.23
	Late Intermediate	33	5484.00	3107.29
	Homogenous	7	6082.43	2095.03
	Total	87	5185.32	2872.64
Progesterone at egg collection	Triple	14	10.46	8.60
	Early Intermediate	34	10.75	3.82
	Late Intermediate	33	14.38	8.35
	Homogenous	7	16.07	6.86
	Total	88	12.49	7.04
Oestradiol: Progesterone ratio at egg collection	Triple	13	523.99	280.23
	Early Intermediate	34	530.09	437.38
	Late Intermediate	33	451.88	263.15
	Homogenous	7	453.15	261.04
	Total	87	493.32	341.10

One-way ANOVA was conducted to compare the relationship between endometrial pattern and progesterone levels at egg collection. Similar to the results at trigger, triple and early intermediate pattern were associated with low progesterone level while late intermediate and homogenous pattern were associated with high progesterone levels. However , the results were not statistically significant [$F(3, 84) = 2.60, p = 0.057$]. Figure 4.7 shows the mean of progesterone for endometrial pattern at egg collection.

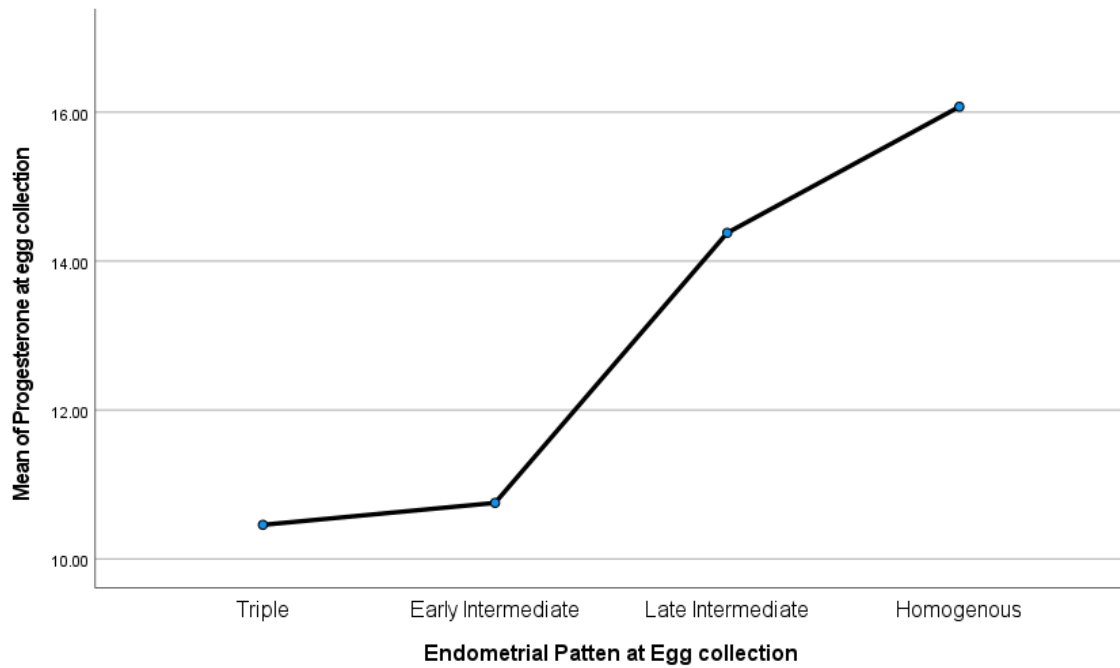


Figure 4.7 Mean of progesterone levels for endometrial pattern at egg collection

A one-way ANOVA was conducted to compare the relationship between endometrial pattern and oestradiol levels at egg collection. The results were not statistically significant [$F(3, 83) = 0.562$, $p = 0.641$]. Figure 4.8 shows the mean of oestradiol for endometrial pattern at egg collection.

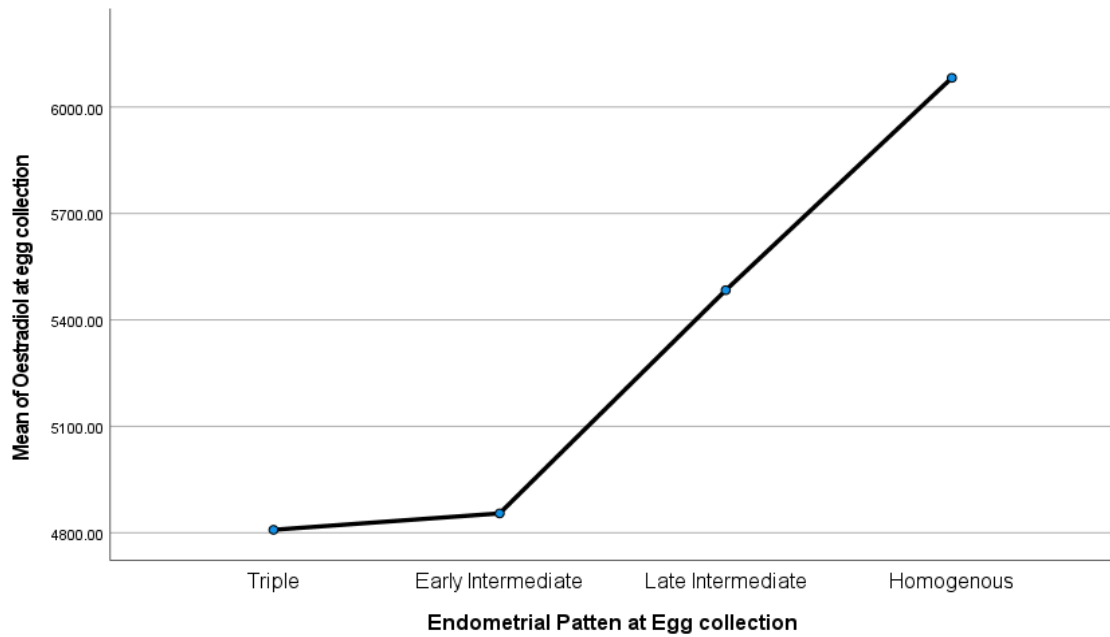


Figure 4.8 Mean of oestradiol levels for endometrial pattern at egg collection

One-way ANOVA was conducted to compare the relationship between endometrial pattern and the oestradiol: progesterone ratio at egg collection. The result was not statistically significant [$F(3, 61) = 0.353$, $p = 0.787$]. Figure 4.9 shows the mean of oestradiol: progesterone ratio for endometrial pattern at egg collection.

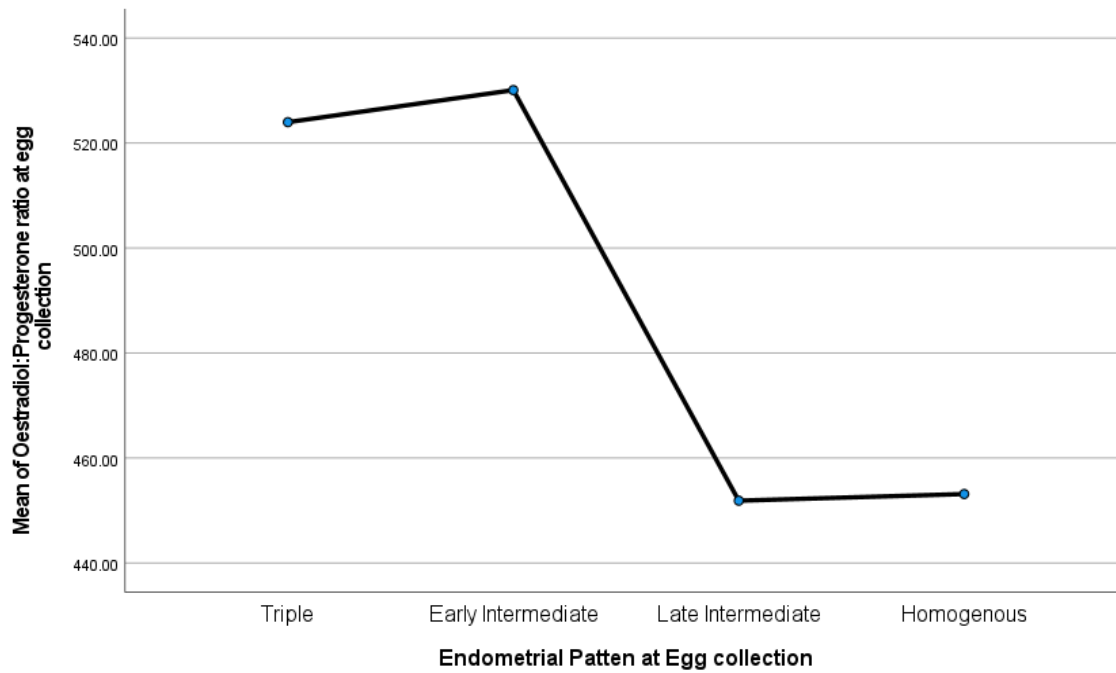


Figure 4.9 Mean of Oestradiol: progesterone ratio for endometrial pattern at egg collection

The oestradiol to progesterone ratio could not be computed at embryo transfer in fresh cycles as the progesterone level was >60ng/ml for the majority of the samples, which is more than the upper limit for the analyser. Table 4.18 below summarises the mean and standard deviation of oestradiol and progesterone for the endometrial pattern at embryo transfer.

Table 4.18 The mean and standard deviation of oestradiol for the endometrial pattern at fresh embryo transfer.

		N	Mean	Std. Deviation
Oestradiol at embryo transfer	Early Intermediate	2	3528.50	2863.08
	Late Intermediate	46	5625.33	3439.85
	Homogenous	29	8405.48	9906.85
	Total	77	6617.94	6733.47

One-way ANOVA was conducted to compare the relationship between endometrial pattern and oestradiol levels at embryo transfer. There was a trend of a linear increase of oestradiol levels from early intermediate pattern to homogenous pattern, however the results were not statistically significant [$F(2, 74) = 1.767$, $p = 0.178$]. Figure 4.10 shows the mean of oestradiol for endometrial pattern at embryo transfer.

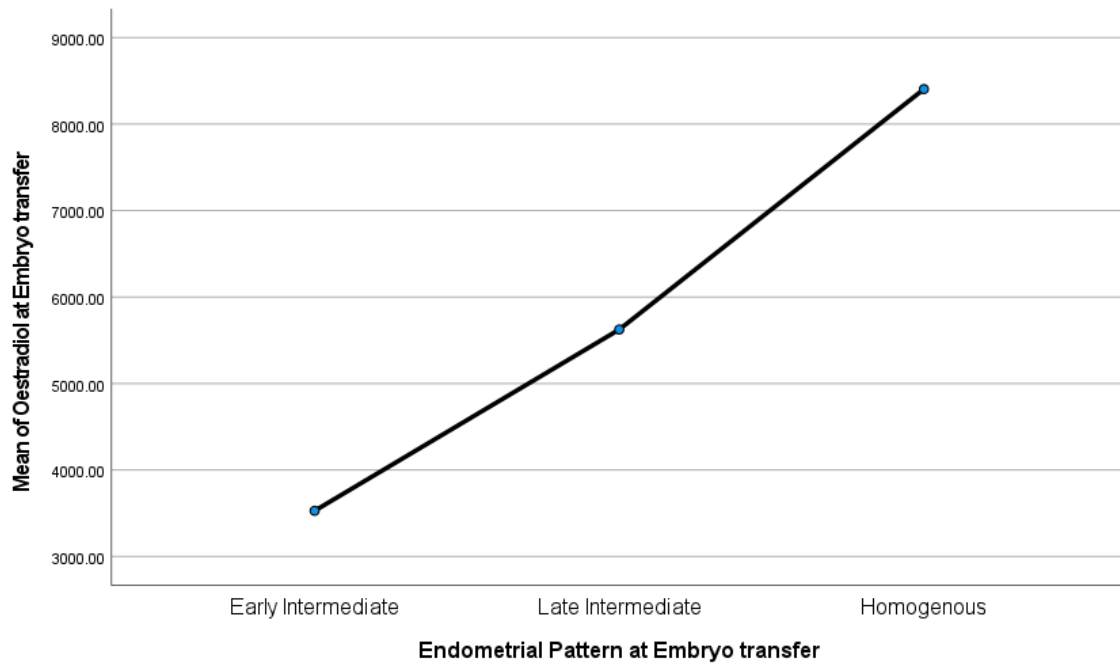


Figure 4.10 Mean of oestradiol level for endometrial pattern at embryo transfer

4.4.6 Frozen embryo transfer cycles

A total of 75 frozen embryo transfer cycles were included in this study.

- 72 cycles had endometrial preparation with hormone replacement therapy.
- One lady had natural cycle “unmedicated” for frozen embryo transfer.
- One lady had HRT for endometrial preparation but was a fresh embryo recipient and her transfer was coordinated with her donor.
- One lady received a short flare and standard dose HRT.

4.4.6.1 Primary outcomes for frozen embryo transfer cycles

Endometrial pattern was assessed at two time points, day 15 of the cycle (if the endometrium was deemed adequately prepared, then embryologists were informed to coordinate the thawing process and frozen embryo transfer). Initiation of progesterone was normally started on the same day or the next day and embryo transfer of a blastocyst occurred five days later. The second time point was embryo transfer.

The results were not statistically significant for endometrial pattern at frozen embryo transfer. Table 4.19 below summarises the odds ratio for clinical pregnancy related to the endometrial pattern in frozen embryo transfer cycles.

Table 4.19 Summary of the odds ratio for clinical pregnancy related to the endometrial pattern in frozen embryo transfer cycles

Endometrial Pattern at day 15					
Classification	Number	Clinical pregnancy (%)	OR	95% CI	*p-value
Triple	43	27 (63%)			0.244
Early Intermediate	19	11 (58%)	0.815	0.271-2.45	
Late Intermediate	5	1 (20%)	0.148	0.015 to 1.44	
Homogenous	1	1 (100%)	1.8	0.069 to 46.811	
Total	68				
Endometrial Pattern at Embryo transfer					
Classification	Number	Clinical Pregnancy (%)	OR	95% CI	*p-value
Triple	0	NA	NA	NA	
Early Intermediate	1	1 (100%)			0.863
Late Intermediate	60	34 (56.7%)	0.43	0.02 to 11.09	
Homogenous	14	9 (64.3%)	0.58	0.019 to 16.72	
Total	75				
<i>*p value based on Fisher Exact test</i> <i>Triple is the reference pattern at day 15, Early intermediate is the reference pattern at embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.6.2 Secondary outcomes for frozen embryo transfer cycles

4.4.6.2.1 Live birth outcomes for endometrial pattern in frozen embryo transfer cycles

The results were not statistically significant for endometrial pattern in frozen embryo transfer cycles. Table 4.20 below illustrates live birth outcomes for endometrial pattern at frozen embryo transfer.

Table 4.20 Summary of the odds ratio for live birth related to the endometrial pattern in frozen embryo transfer cycles

Endometrial Pattern at day 15					
Classification	Number	Live birth	OR	95% CI	*p-value
Triple	43	24 (56%)			0.340
Early Intermediate	19	9 (48%)	0.713	0.241-2.105	
Late Intermediate	5	1 (20%)	0.198	0.020-1.920	
Homogenous	1	1 (100%)	2.388	0.092 to 61.919	
Total	68				
Endometrial Pattern at Embryo transfer					
Classification	Number	Live birth	OR	95% CI	*p-value
Triple	0	NA	NA	NA	
Early Intermediate	1	1 (100%)			0.665
Late Intermediate	60	29 (48.3%)	0.31	0.012 to 7.969	
Homogenous	14	8 (57.1%)	0.44	0.015 to 12.544	
Total	75				
<i>*p value based on Fisher Exact test</i> <i>Triple is the reference pattern at day 15, Early intermediate is the reference pattern at embryo transfer.</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.6.2.2 Biochemical pregnancy outcomes for frozen embryo transfer cycles

The results were not statistically significant for endometrial pattern in frozen embryo transfer cycles. Table 4.21 below illustrates biochemical pregnancy outcomes for endometrial pattern at frozen embryo transfer.

Table 4.21 Biochemical pregnancy outcomes for endometrial pattern at day 15 and at embryo transfer in frozen embryo transfer cycles

Endometrial Pattern at day 15					
Classification	Number	Biochemical pregnancy	OR	95% CI	*p-value
Triple	43	29 (67%)			1.0
Early Intermediate	19	13 (68%)	1.046	0.328-3.332	
Late Intermediate	5	3 (60%)	0.724	0.108-4.839	
Homogenous	1	4 (100%)	1.474	0.057 to 38.482	
Total	68				
Endometrial Pattern at Embryo transfer					
Classification	Number	Biochemical pregnancy	OR	95% CI	*p-value
Triple	0	NA	NA	NA	
Early Intermediate	1	1 (100%)			1.0
Late Intermediate	60	40 (66.7%)	0.659	0.0257 to 16.89	
Homogenous	14	10 (71.4%)	0.78	0.026 to 22.98	
Total	75				
<i>*p value based on Fisher Exact test</i> <i>Triple is the reference pattern at day 15, Early intermediate is the reference pattern at embryo transfer</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.6.2.3 Implantation outcomes for frozen embryo transfer cycles

The results were not statistically significant for endometrial pattern in frozen embryo transfer cycles. Table 4.22 below illustrates the odds ratio for Implantation related to the endometrial pattern in frozen embryo transfer cycles.

Table 4.22 Summary of the odds ratio for Implantation in relation to the endometrial pattern in frozen embryo transfer cycles

Endometrial Pattern at day 15					
Classification	Number	Implantation	OR	95% CI	*p-value
Triple	43	27 (63%)			0.715
Early Intermediate	19	11 (58%)	0.815	0.271-2.450	
Late Intermediate	5	2 (40%)	0.395	0.060-2.623	
Homogenous	1	1 (100%)	1.8	0.069 to 46.811	
Total	68				
Endometrial Pattern at Embryo transfer					
Classification	Number	Implantation	OR	95% CI	*p-value
Triple	0	NA	NA	NA	
Early Intermediate	1	1 (100%)			0.862
Late Intermediate	60	35 (58.3%)	0.46	0.018 to 11.86	
Homogenous	14	9 (64.3%)	0.58	0.019 to 16.72	
Total	75				
<i>*p value based on Fisher Exact test</i> <i>Triple is the reference pattern at day 15, Early intermediate is the reference pattern at embryo transfer</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.6.3 Subgroup analysis

4.4.6.3.1 Single blastocyst transfer (top quality embryo) for frozen embryo transfer cycles

Subgroup analysis was performed for frozen cycles where a single top quality blastocyst was transferred. Grade A or B embryos were considered top quality (see appendix 3). This was performed for the primary outcome of clinical pregnancy and the secondary outcome of live birth at day 15 and embryo transfer.

4.4.6.3.2 Clinical pregnancy outcomes for top quality blastocyst in frozen embryo transfer cycles

Clinical pregnancy was calculated for women who had single embryo transfer with a top quality blastocyst, either grade A or B. Only 37/75 frozen cycles had a single embryo transfer with top quality embryo.

The results were not statistically significant. Table 4.23 below summarises the rate and odds ratio for clinical pregnancy outcomes related to the endometrial pattern in frozen embryo transfer cycles where a single top quality embryo was transferred.

Table 4.23 The rate and odds ratio for clinical pregnancy outcomes related to the endometrial pattern in frozen embryo transfer cycles (single top quality blastocyst).

Endometrial Pattern at day 15					
Classification	Number	Clinical Pregnancy	OR	95% CI	*p-value
Triple	24	16 (6%)			0.605
Early Intermediate	8	4 (50%)	0.50	0.098-2.540	
Late Intermediate	2	1 (50%)	0.50	0.028-9.076	
Homogenous	0	0	NA	NA	
Total	34				
Endometrial Pattern at Embryo transfer					
Classification	Number	Clinical Pregnancy	OR	95% CI	*p-value
Triple	0	0			
Early Intermediate	0	0			
Late Intermediate	31	19 (61.3%)			0.394
Homogenous	6	5 (83.3%)	3.158	0.328-30.426	
Total	37				
<i>*p value based on Fisher Exact test</i> <i>Triple is the reference pattern at day 15, Early intermediate is the reference pattern at embryo transfer</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.6.3.3 Live birth outcomes for top quality blastocyst in frozen embryo transfer cycles

The results were not statistically significant. Table 4.24 below summarises the rate and odds ratio for live birth outcomes related to the endometrial pattern in frozen embryo transfer cycles where a single top quality embryo was transferred.

Table 4.24 The rate and odds ratio for live birth outcomes related to the endometrial pattern in frozen embryo transfer cycles (single top quality blastocyst).

Endometrial Pattern at day 15					
Classification	Number	Live birth	OR	95% CI	*p-value
Triple	24	14 (58%)			0.849
Early Intermediate	8	4 (50%)	0.714	0.143-3.559	
Late Intermediate	2	1 (50%)	0.714	0.040-12.829	
Homogenous	0	0	NA	NA	
Total	34				
Endometrial Pattern at Embryo transfer					
Classification	Number	Live birth	OR	95% CI	*p-value
Triple	0	0	NA	NA	
Early Intermediate	0	0	NA	NA	
Late Intermediate	31	18 (58.1%)			0.532
Homogenous	6	4 (66.7%)	1.44	0.23 to 9.11	
Total	37				
<i>*p value based on Fisher Exact test</i> <i>Triple is the reference pattern at day 15, Early intermediate is the reference pattern at embryo transfer</i> <i>OR= Odd ratio, CI= Confidence interval, NA= Not applicable</i>					

4.4.6.4Secondary objectives

4.4.6.4.1 Change in endometrial pattern in frozen embryo transfer cycles

Sequential change in endometrial pattern during the different time lines was investigated using the McNamar test. Change in endometrial pattern was evaluated

using the McNamar test (Table 4.25). There were 68/75 cycles who had both a day 15 scan and an embryo transfer sequentially. No triple patterns were seen at embryo transfer. Unfortunately, a p value could not be generated.

Table 4.25 Change in endometrial pattern from day 15 to day of frozen embryo transfer

		Early Intermediate	Late Intermediate	Homogenous	Total
Endometrial pattern at day 15	Triple	1	37	5	43
	Early intermediate	0	16	3	19
	Late intermediate	0	4	1	5
	Homogenous	0	0	1	1
Total		1	57	10	68

4.4.6.4.2 Endometrial thickness

Endometrial thickness was measured at day 15 and frozen embryo transfer. The data was converted to categorical data by dividing into tertile groups, and chi squared tests were performed for primary (clinical pregnancy) and secondary outcomes (live birth).

There was one lady with known resistant thin endometrium who had endometrial thickness of 5.3 mm in day 15. This has increased to 5.6 mm (her optimal thickness) by the time of embryo transfer. She wanted to proceed with embryo transfer after counselling but did not get pregnant. All other women had endometrial thickness of more than 7 mm by the time of frozen embryo transfer. The results show that

increasing endometrial thickness is associated with increasing clinical pregnancy rates (Table 4.26) as well as increasing live birth rates, (see. Table 4.27). However, these results were not statistically significant.

Table 4.26 Clinical pregnancy outcomes for endometrial thickness in frozen cycles

Endometrial Thickness at day 15					
Endometrial thickness (mm)	Number	Clinical pregnancy (%)	OR	95% CI	p-value
5.3-8.3	28	18 (64%)			0.668
8.4-10.2	22	13 (59%)	0.802	0.254-2.531	
10.3-13.8	25	13 (52%)	0.602	0.200-1.811	
Total	75				
Endometrial Thickness at Embryo transfer					
Endometrial thickness (mm)	Number	Clinical pregnancy (%)	OR	95% CI	p-value
5.6-9.8	26	15 (58%)			0.915
9.9-11.4	24	15 (63%)	1.222	0.393-3803	
11.5-17	25	14 (56%)	0.933	0.308-2.828	
Total	75				
<i>OR= Odd ratio, CI= Confidence interval</i> 5.3-8.3 is the reference thickness group at day 15 and 10.3-13.8 is the reference thickness group at embryo transfer					

Table 4.27 Live birth outcomes for endometrial thickness in frozen cycles

Endometrial Thickness at day 15					
Endometrial thickness	Number	Live birth (%)	OR	95% CI	p-value
5.3-8.3	28	16 (57%)			0.648
8.4-10.2	22	11 (50%)	0.750	0.244-2.303	
10.3-13.8	25	11 (44%)	0.589	0.199-1.749	
Total	75				
Endometrial Thickness at Embryo transfer					
Endometrial thickness	Number	Live birth (%)	OR	95% CI	p-value
5.6-9.8	26	15 (58%)			0.708
9.9-11.4	24	11 (46%)	0.621	0.203-1.898	
11.5-17	25	12 (48%)	0.677	0.244-2.044	
Total	75				
<i>OR= Odd ratio, CI= Confidence interval</i> 5.3-8.3 is the reference thickness group at day 15 and 10.3-13.8 is the reference thickness group at embryo transfer					

4.4.6.5 Hormonal profile at frozen embryo transfer

Oestradiol and progesterone levels were measured at day 15 and embryo transfer. Oestradiol to progesterone ratio was computed for both day 15 and frozen embryo transfer. This was then converted into categorical data by dividing into tertile groups and chi squared tests were performed for primary (clinical pregnancy) and secondary outcomes (live birth). Table 4.28 illustrates the median and the range for oestradiol and progesterone.

Table 4.28 Hormonal profile at frozen embryo transfer

Hormonal profile	Median	Range	Total Number
Oestradiol at day 15	846.55	313-2911	54
Progesterone at day 15	0.087	0.05-0.724	53
Oestradiol at frozen embryo transfer	785.9	166.3-7034	67
Progesterone at frozen embryo transfer	13.36	5.59-60	67

The results were not statistically significant for Oestradiol to progesterone (E2:P) ratio on day 15.

At frozen embryo transfer, the odds of becoming pregnant with a high E2:P ratio (>80.27) is 7% lower when compared with a low E2:P ratio ≤ 44.25 . This decreases further for the middle group. The odds of becoming pregnant with E2:P ratio of (44.26 - 80.26) is 84% lower when compared with low E2:P ratio ≤ 44.25 . The results are statistically significant with p value of 0.006. Table 4.29 below summaries the clinical pregnancy outcomes for oestradiol: progesterone ratio at frozen embryo transfer.

Table 4.29 Clinical pregnancy outcomes for oestradiol: progesterone ratio at frozen embryo transfer

Test	Classification	Number	Clinical Pregnancy	OR	95% CI	p-value
E2:P ratio at frozen embryo transfer	≤ 44.25	23	16 (69.6%)			0.006
	44.26 - 80.26	22	6 (27.3%)	0.164	0.045 to 0.597	
	≥80.27	22	15 (68.2%)	0.938	0.265 to 3.313	
Total		67				
*p value based on Fisher Exact test ≤ 44.25 is the reference E2:P ratio						

The odds of having a live birth with a high E2:P ratio at frozen embryo transfer (>80.27) is 57% lower when compared with a low E2:P ratio ≤ 44.25 . This decreases further for the middle group. The odds of having a live birth with E2:P ratio of (44.26 - 80.26) is 87% lower when compared with low E2:P ratio ≤ 44.25 group. The results are statistically significant with a p value of 0.006. Table 4.30 below summarises the live birth outcomes for oestradiol: progesterone ratio at frozen embryo transfer.

Table 4.30 Live birth for oestradiol: progesterone ratio at frozen embryo transfer

Test	Classification	Number	Live birth	OR	95% CI	p-value
E2:P ratio at frozen embryo transfer	≤44.25	23	16 (69.6%)			0.006
	44.26 - 80.26	22	5 (22.7%)	0.129	0.034 to 0.489	
	≥80.27	22	11 (50%)	0.438	0.129 to 1.481	
Total		67				
<i>*p value based on Fisher Exact test</i> <i>≤ 44.25 is the reference E2:P ratio</i>						

4.4.6.5.1 Hormonal profile and endometrial pattern in frozen cycles

One-way ANOVA was used to explore the relationship between endometrial pattern and the hormonal profile. Table 4.31 summarises the mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at day 15.

Table 4.31 The mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at day 15.

		N	Mean	Std. Deviation
Oestradiol at day 15	Triple	33	820.25	448.97
	Early Intermediate	16	1109.45	613.68
	Late Intermediate	4	927.83	144.84
	Homogenous	1	898.30	.
	Total	54	915.35	496.49
Progesterone at day 15	Triple	32	0.14	0.14
	Early Intermediate	16	0.15	0.17
	Late Intermediate	4	0.12	0.05
	Homogenous	1	0.09	.
	Total	53	0.14	0.14
Oestradiol: Progesterone ratio at day 15	Triple	32	10465.16	9816.73
	Early Intermediate	16	11750.81	7301.94
	Late Intermediate	4	8859.90	4657.80
	Homogenous	1	9981.11	.
	Total	53	10722.99	8644.41

One-way ANOVA was conducted to compare the relationship between endometrial pattern and progesterone level at day 15. The result was not statistically significant

and the levels were all low <1 [$F(3, 49) = 0.094$, $p = 0.963$]. Figure 4.11 shows the mean of progesterone levels for endometrial pattern at day 15.

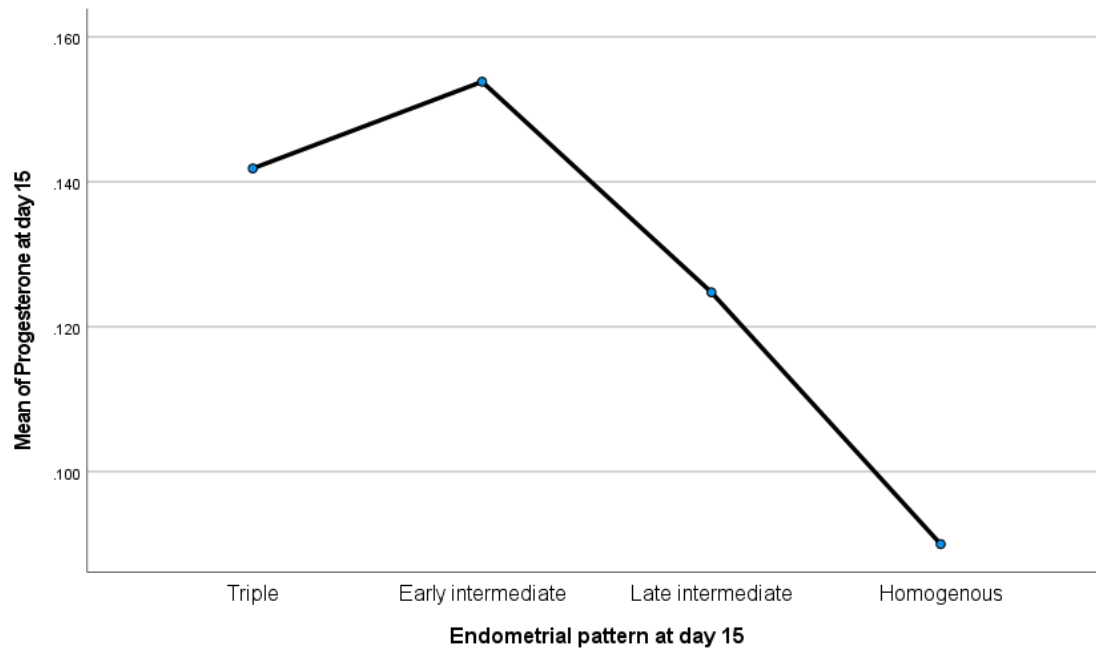


Figure 4.11 Mean of progesterone levels for endometrial pattern at day 15

A one-way ANOVA was conducted to compare the relationship between endometrial pattern and oestradiol levels at day 15. The results were not statistically significant [$F(3, 50) = 1.236$, $p = 0.306$]. Figure 4.12 shows the mean of oestradiol for endometrial pattern at day 15.

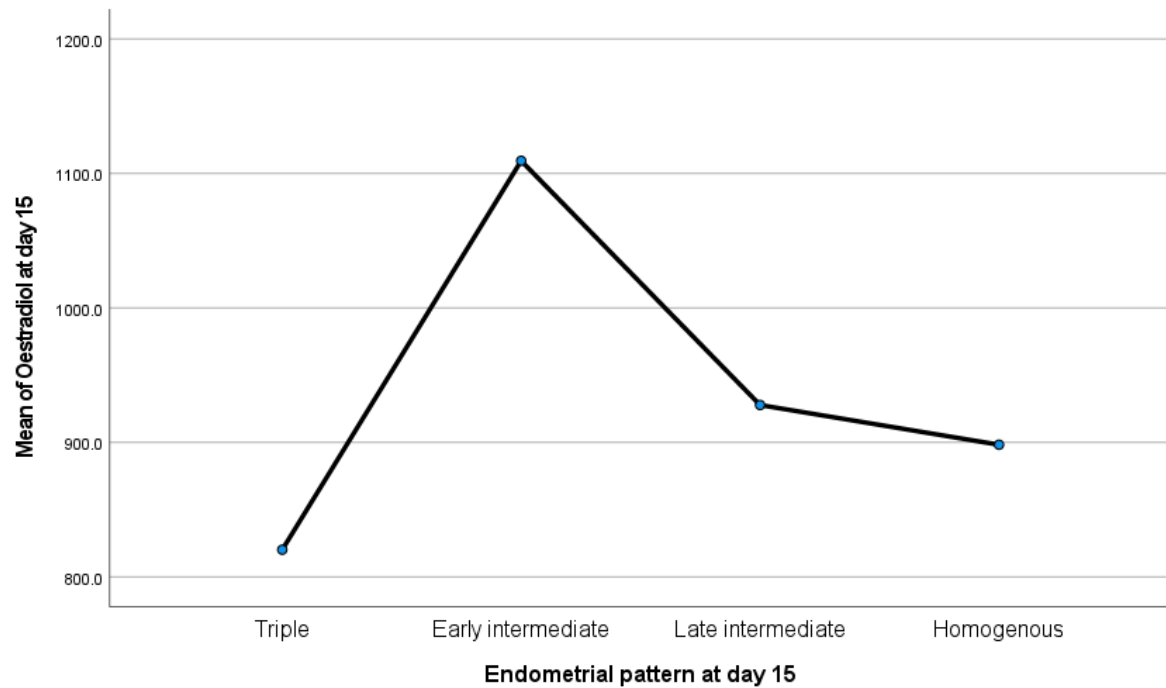


Figure 4.12 Mean of oestradiol levels for endometrial pattern at day 15

One-way ANOVA was conducted to compare the relationship between endometrial pattern and the oestradiol: progesterone ratio at day 15. The result was not statistically significant [$F(3, 49) = 0.142, p = 0.934$]. Figure 4.13 shows the mean of oestradiol: progesterone ratio for endometrial pattern at day 15.

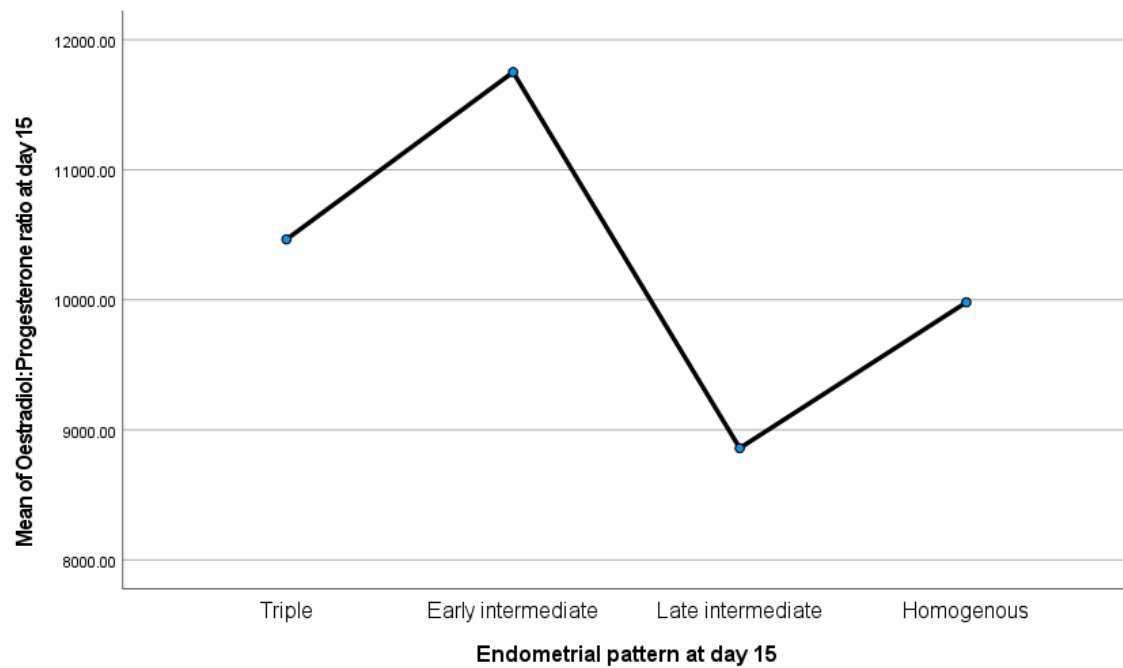


Figure 4.13 Mean of Oestradiol: progesterone ratio at day 15

There was no triple pattern at embryo transfer. The mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at frozen embryo transfer are shown in Table 4.32 below.

Table 4.32 The mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern at frozen embryo transfer.

		N	Mean	Std. Deviation
Oestradiol at embryo transfer	Early Intermediate	1	341.80	.
	Late Intermediate	55	853.03	358.60
	Homogenous	11	1875.08	1931.71
	Total	67	1013.20	906.98
Progesterone at embryo transfer	Early Intermediate	1	10.17	.
	Late Intermediate	55	15.43	8.89
	Homogenous	11	16.63	5.96
	Total	67	15.55	8.41
Oestradiol: Progesterone ratio at embryo transfer	Early Intermediate	1	33.61	.
	Late Intermediate	55	66.05	37.93
	Homogenous	11	154.28	186.80
	Total	67	80.05	87.05

However, one-way ANOVA was conducted to compare the relationship between endometrial pattern and progesterone levels at frozen embryo transfer. The results were not statistically significant [$F(2, 64) = 0.295$, $p = 0.745$]. Figure 4.14 shows the mean of progesterone for endometrial pattern at frozen embryo transfer.

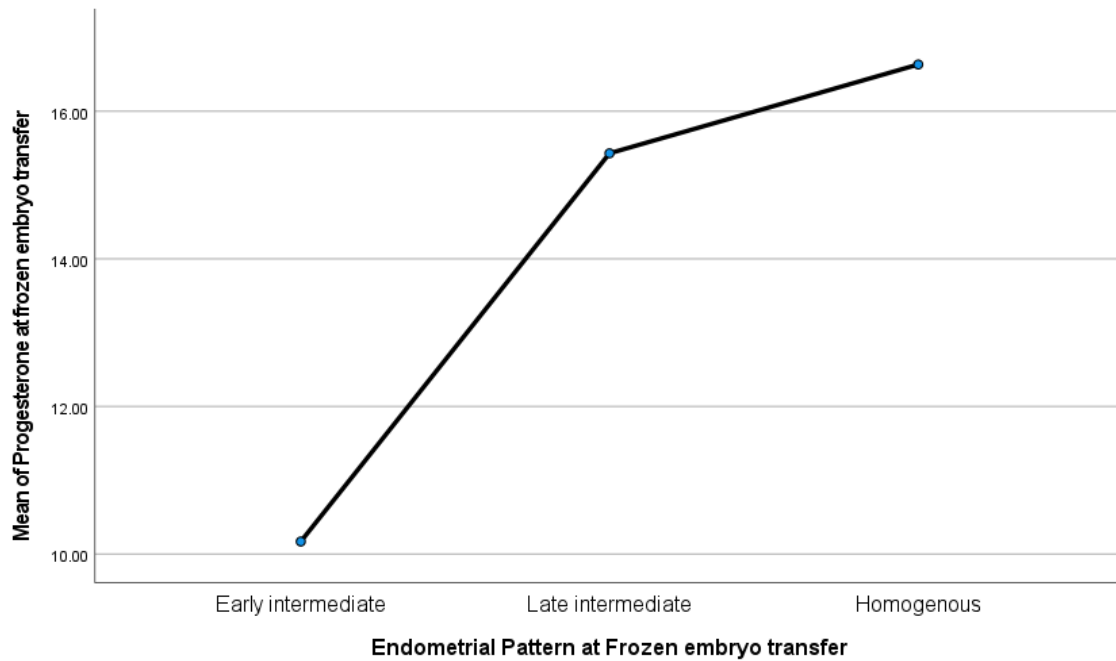


Figure 4.14 Mean of progesterone levels for endometrial pattern at frozen embryo transfer

One way ANOVA was not valid in exploring the relationship between endometrial pattern and oestradiol or oestradiol: progesterone ratio at frozen embryo transfer as the test of homogeneity (Levene test) was significant for both (p value <0.001).

4.5 Discussion

The results of the study are in support of the hypothesis that endometrial pattern can be used to predict the outcome of IVF/ICSI. Women with either a triple or early

intermediate pattern at egg collection are much more likely to have a live birth than women with a late intermediate or homogenous pattern. None of the women with homogenous pattern (8/108) at egg collection had a live birth. Women with a homogenous pattern at egg collection were 95% less likely to become pregnant when compared to women with a triple pattern (clinical pregnancy & live birth OR 0.05, 95% CI 0.0027 to 1.05). This could be due to accelerated endometrial development (Lessey 2000b) (Lessey 2011, 2000a; Lessey and Young 2019). This result is statistically significant with p value of < 0.05 and is in agreement with Sharara et al (Sharara, Lim, and McClamrock 1999) who found that patients who had a homogeneous, hyperechogenic pattern have a significantly lower implantation rate when compared to patients with a triple line pattern at egg collection (Heger, Sator, and Pietrowski 2012). Furthermore, they also noted that there is a change from triple pattern to homogenous pattern between trigger and egg collection in 12.6% of the cycles. (Sharara, Lim, and McClamrock 1999) (Heger, Sator, and Pietrowski 2012).

In this study, women with late intermediate pattern at egg collection were 35% less likely to become pregnant when compared to women with a triple pattern (clinical pregnancy OR 0.65, 95% CI 0.21 to 1.98) . This result is statistically significant with a p value of 0.009.

Although Bassil et al. (Bassil 2001) did not find any significant differences in endometrial thickness, width, length, growth and pattern in pregnant cycles compared to non-pregnant cycles, they did note a change in endometrial pattern from a multi-

layered (triple) to a homogeneous, hyperechogenic pattern between the day of egg collection and the day of embryo transfer in 22.2% of cycles. (Heger, Sator, and Pietrowski 2012).

In this study, the change in endometrial pattern from trigger to egg collection was statistically significant with a p value <0.001. 30 women had triple pattern at trigger which changed to early intermediate at egg collection, 13 women had their pattern change from triple at trigger to late intermediate at egg collection and 2 women had their pattern change from triple at trigger to a homogenous pattern at egg collection. However, it was not possible to assess the change in endometrial pattern between egg collection and embryo transfer due to small numbers.

Other studies which are in support of the hypothesis include Welker et al. (Welker et al. 1989), who found that a triple pattern on the day of egg collection positively influenced implantation. Gonen et al, (Gonen and Casper 1990) also showed that women with multi-layered patterns on the day before egg collection had significantly higher pregnancy rates (Heger, Sator, and Pietrowski 2012).

Jarvela et al (Järvelä et al. 2005) also found a significantly higher pregnancy rate in women with a triple-line pattern compared to those with a homogeneous one, both after FSH stimulation and on the day of egg collection (Heger, Sator, and Pietrowski 2012).

There was no triple pattern at embryo transfer. Only 2 women had an early intermediate pattern, and none of them had a live birth. Therefore, having a triple or early intermediate pattern at embryo transfer is unfavourable as it could be due to delayed endometrial development (Lessey 2000b, 2011, 2000a). The odds of having a live birth with a homogenous pattern at embryo transfer is almost 2 times higher than an early intermediate pattern (live birth OR 1.79, 95% CI 0.08 to 40.82). Similarly, women with a late intermediate pattern were almost 5 times more likely to become pregnant than women with an early intermediate at embryo transfer. This result is statistically significant with a p value of 0.031. However, the confidence interval is wide for both of these results (given that there were only 2 women with early intermediate pattern at embryo transfer).

Other studies that examined endometrial pattern at trigger time include Zhao et al, (Zhao et al. 2014) and Ahmadi et al (Ahmadi et al. 2017) who both showed that having a triple line at trigger point is favourable. The results from my study for endometrial pattern at trigger point did not show any statistical significance, as it was split between triple and early intermediate pattern. The number of clinical pregnancies although not statistically significant was 55% for triple pattern and 41% for the early intermediate. These two patterns (triple and early intermediate) are likely grouped together and both called “triple” in studies that use the three grade classification system.

Ideally, I wanted to assess single transfer embryo with top quality embryos, but only 76/116 fresh cycles had a single embryo transfer and of those, only 63 had top quality

embryo transferred. The decision of how many embryos to transfer is a clinical one. Therefore, subgroup analysis of single top quality embryos did not show any significant results due to the small numbers. Similarly, in frozen cycles only 37/75 had a single top quality embryo transferred and the results were not statistically significant.

For fresh cycles, clinical pregnancy and live birth outcomes seem to increase with increasing endometrial thickness, although the results are not statistically significant. This might be due to the fact that cycles with a thin endometrium were already excluded from the study, as per Nurture clinical protocol. The same applies to frozen cycles.

Endometrial Doppler did not seem to impact pregnancy outcomes. This could be due to small numbers.

Despite the limitation in the hormonal profile data in fresh cycles, the graphs of mean oestrogen, progesterone and endometrial pattern, show increasing levels in oestrogen and progesterone from triple, to early intermediate, to late intermediate, to homogenous, reflecting the stages of endometrial development. Oestrogen is essential for endometrial proliferation while progesterone is absolutely critical for implantation and pregnancy maintenance (Young 2013). Interestingly, the results comparing the relationship between endometrial pattern and progesterone levels at trigger showed that triple and early intermediate patterns are associated with low progesterone levels while late intermediate and homogenous patterns are associated with higher progesterone levels. These results are statistically significant with p value

<0.05. This is in keeping with our hypothesis suggesting that if there is a premature rise in progesterone at trigger/ovulation. This can cause “accelerated endometrial development” from proliferative endometrium to secretory endometrium, leading to asynchrony between the endometrium and the embryo, and resulting in implantation failure. The increasing hyperechogenic change on ultrasound illustrates the elevated progesterone levels as clearly demonstrated in figure 4.4 (late intermediate and homogenous pattern). It also illustrates the relationship between hormonal profile and our “novel pattern recognition” classification system, thus providing a possible explanation of how favourable and unfavourable endometrial patterns on ultrasound can help predict pregnancy outcomes.

In frozen cycles, endometrial patterns did not appear to have an impact on pregnancy outcomes, possibly due to small sample size.

In frozen cycles, the odds of becoming pregnant and having live birth was higher in women with low oestrogen to progesterone ratio (≤ 44.25) than women with high (>80.27) or medium ($44.26 - 80.26$) oestrogen to progesterone ratio. The results are statistically significant with p value of 0.006. These results suggest having a low oestrogen: progesterone ratio is favourable in achieving clinical pregnancy and live birth, emphasising what is already known about the critical role of progesterone in implantation and pregnancy maintenance. It could also suggest that the ratio and interaction between these two hormones is probably more important than the

individual hormone level by itself. (Lessey, Yeh, et al. 1996; Lessey 2000a; Young 2013; Lessey and Young 2019)

4.6 COVID 19 impact, limitations, technical difficulties and handling of missing data

The biggest challenge encountered in this study was the attrition rate due to the exclusion criteria. I knew beforehand that many women would not progress to embryo transfer after having their data collected at trigger and egg collection. The main reasons for this were a decision to “freeze the embryos” due to a risk of ovarian hyperstimulation syndrome, and failure of fertilisation or blast formation. Similarly, a smaller number of women in frozen cycles would not have embryo transfer due to a thin endometrium. I therefore needed to over recruit with regards to the initial numbers of women to achieve the target of 200 eligible cycles. I was well on target to achieve this, however this was disrupted due to COVID 19. Despite all of this, 230 cycle scans’ (540 scans) were performed and of those 191 cycles were eligible to be included (467 scans were analysed).

One of the limitations of the study pertained to missing data. Data was missing due to a number of reasons – either due to clinical commitments resulting in lack of clinician availability to perform a scan (this was random), or occasionally related to patient factors such as patients arriving late for a scan or unclear images. The trigger scan was largely performed by nurses and a sonographer. To minimise the missing data, although endometrial patterns were analysed as cine loop, where there was a clear

“still image” of triple pattern recorded at trigger, this was accepted and recorded as triple pattern for the study as this will still be accurate. However, the same cannot be said for other endometrial patterns as the “still image” will not accurately reflect the pattern and will depend on when the image was frozen.

As the embryo transfer scan is the only additional scan in this study, I had to do most of the scans myself. However, when I was not available due to other clinical commitments, the scans were often not performed. To tackle this problem, it was suggested that the women would be asked to attend their scans just prior to the actual embryo transfer in theatre, so that the scan could be performed by the operating clinician or myself if available. The issue with this approach was that the study scan needed to be performed on a relatively empty bladder, while the embryo transfer needed to be performed with a full bladder. This approach was therefore abandoned after a few weeks of trialling, as some of the scans were unclear due to full bladder. I therefore, reverted to the original method where women were asked to attend half an hour earlier than their scheduled time with an empty bladder for the study scan to occur. They were then encouraged to drink while waiting for their embryo transfer.

Despite all of the technical difficulties, every attempt was made to minimise the amount of missing data for the primary test (endometrial pattern) and the endometrial pattern was only recorded when a clear image can be seen. 110/116 endometrial patterns were recorded at trigger, 108/116 at egg collection and 106/118 at embryo transfer.

For frozen cycles 68/75 patterns were recorded at day 15 and 75/75 patterns were recorded at embryo transfer.

4.7 Conclusion

In conclusion, a novel classification system of endometrial patterns has been developed and tested. Women with triple and early intermediate patterns at egg collection were more likely to become pregnant than women with a homogenous pattern. Women with late intermediate and homogenous patterns at embryo transfer were more likely to become pregnant when compared to women with early intermediate pattern. None of the women with a homogenous pattern at trigger point had a live birth. There were no women with a triple pattern at embryo transfer. Currently many IVF centres will cancel cycles and recommend “embryo freezing” depending on endometrial thickness. Endometrial pattern can provide more useful information, and the results from this study demonstrate that endometrial pattern can be utilised as an additional tool to improve IVF/ICSI success rate. Women with unfavourable endometrial patterns (for example homogenous pattern at trigger/egg collection) can be counselled to freeze their embryos depending on the other clinical parameters. Studies with a larger sample size and randomised controlled trials are needed to validate these results.

Chapter 5 The biopsy study exploring the relationship between endometrial pattern on ultrasound, endometrial histology, and microarray

5.1 Background

Endometrial pattern on ultrasound can reflect the cyclical changes in the endometrium (Miyara et al. 1996; Forrest et al. 1988; Bakos, Lundkvist, and Bergh 1993; Yoshimitsu et al. 1987). Noyes (Noyes, Hertig, and Rock 1950) criteria has been the gold standard in dating the endometrium for over 50 years. Despite its limitations, and in the absence of a gold diagnostic test in dating the endometrium, it still remains as one of the most efficient ways of dating the endometrium.

5.2 Study aims and objectives.

The aim of this study is to look at the endometrial pattern in relation to endometrial receptivity, and to assess if there is any correlation between the endometrial pattern on ultrasound scan, histology, and genetic markers of receptivity.

5.3 Methods

5.3.1 Ethical Approval

Ethical approval was obtained from Faculty of Medicine & Health Sciences Research Ethics Committee, Nottingham University Hospitals. Reference Number 87-1808.

5.3.2 Study setting

The study took place at Nurture, Nottingham, UK.

5.3.3 Participants recruitment and eligibility criteria

Women who were electing to have endometrial scratch as part of their IVF were invited to take part in the study. The inclusion criteria included women who were over 18 years old undergoing IVF or ICSI, using own eggs or donated eggs, and able to sign an informed consent. Women with a known pathology that affect the endometrial cavity (polyp, submucosal fibroid), or have septate uterus or more complex congenital uterine anomaly have been excluded.

5.3.4 Visit schedule

The study involved a single visit which took place on the planned day of the endometrial scratch. Women were asked to abstain from sexual intercourse or to use barrier methods of contraception in that cycle. The endometrial scratch normally take place on day 19-24, around the mid luteal phase.

Women were given a urine testing kit to check for ovulation (an LH or “ovulation” kit (Clearblue, Geneva, Switzerland). This involved testing for luteinising hormone (LH)

around day 10-14 of the menstrual cycle. The day when the test was positive was defined as LH surge day (LH+0).

On the day of the endometrial scratch, a urinary pregnancy test was performed to exclude pregnancy. The day of the scratch was recorded in relation to LH surge. For example, LH+3 indicates that the endometrial scratch was performed 3 days after the LH surge. For women having endometrial scratch prior to LH surge, LH levels were retested until they were positive (LH surge), and the day was calculated retrospectively, for example LH-2. For women who were recruited after their ovulation date had passed, the ovulation day was assumed to be day 14 in a regular 28 day cycle.

Prior to the biopsy, a transvaginal ultrasound scan to assess for endometrial pattern and thickness was performed. 10 ml blood samples for oestradiol and progesterone were taken and analysed inhouse at Nurture laboratory by lab technicians, according to standard protocols.

I recruited the women, scanned them on the day of endometrial scratch, recorded their endometrial pattern, and took their blood for oestradiol and progesterone levels at Nurture fertility. I performed the majority of the endometrial scratches but occasionally assistance was required with difficult scratches. I performed the RNA extraction at the Queens Medical Centre (QMC). I sent the purified RNA to the team at Sutton Bonnington who performed microarray analysis. The translational team at the histology department in QMC prepared the slides for histological assessment. Dr

Soomro (Consultant histopathologist at Nottingham City Hospital) assessed the endometrial samples and assigned the histological classification according to Noyes criteria.

5.3.5 Endometrial scratch steps

The Pipelle biopsy was done in the pre-IVF-treatment cycle. The participant was positioned in the Lloyd Davis position, and the cervix was visualised using a speculum inserted into the vagina. The cervix was cleaned with sterile water. The endometrial biopsy was performed using Pipelle de Cornier endometrial biopsy sampler (Laboratories CCD, France), or Wallace endometrial sampler (Cooper Surgical, USA). The sampler was passed through the cervix into the uterine cavity. These steps were repeated (up to 4 times) until an adequate sample were obtained. The sample was then divided into 2 pots. One pot contained formalin to be sent to the Queens' Medical Centre (QMC) histology department, where the translational research team prepared the samples for histological assessment and natural killer cells analysis (NK cells). The second sample was snap frozen in liquid nitrogen and stored at -80° C at QMC and analysed for microarray.

5.3.6 Ultrasound scanning and Image acquisition.

In addition to the biopsy, ultrasound scanning was used to record the endometrial patterns. The endometrial pattern was recorded according to the “novel pattern recognition” classification system. See appendix 3

- Pattern 1: Triple
- Pattern 2: Early intermediate
- Pattern 3: Late intermediate
- Pattern 4: Homogenous

Images were obtained in the sagittal view by using TVUS using Voluson E8 or E10 with a 5-9MHz Transducer (GE Healthcare, Chicago, IL, USA). We optimised each image for each participant individually, to obtain the best view/image of the endometrial patterns. We avoided using a pre-set as it affected the quality of the images due to the variation between participants.

5.3.6.1 Two-dimensional (2D) ultrasound settings

The power was set to 100%. Focus was set to 5 cm deep but that was adjusted when needed, depending on the patient. Tissue harmonics started as high, and were adjusted to mid, low or off, depending on best image obtained. Gain was adjusted for the best image possible with maximal differentiation between the layers. Finally, dynamic contrast was adjusted to obtain the best image.

5.3.7 Protocol for Histological preparation and assessment

The translational research team at the histology department of QMC prepared the samples for histological assessment. The samples arrived in 10% neutral buffered formalin, and were allowed to fix overnight. The sample and cassette were labelled with the identification number and the sample was placed into the cassette and processed overnight on Thermo Excelsior tissue processor (Thermo Fisher Scientific, USA), using different reagents in the following order: formalin, followed by the Industrial methylated spirit (IMS), then xylene, and lastly, wax. The tissue was then imbedded in paraffin wax and the block was placed on ice to cool to allow sectioning. Two sections at 4µm were taken for Haematoxylin and Eosin (H&E), and CD56 (immunocytochemistry).

The sections were put under water then in haematoxylin solution for 20 minutes, then under tap water for 30 seconds. The sections were then differentiated in acid alcohol for a few seconds until the background was clear, and then under tap water for 30 seconds. After that, they were placed in borax solution for 30 seconds then back to tap water for another 30 seconds. They were then placed in Eosin solution for 10 seconds, then finally they were washed in tap water, dehydrated, cleared and mounted. The final result was that the nuclei should stain blue, and the cytoplasm as pink/red. The chromatin should be distinct where present, and there should be clear separation between the different components. The sections were then sent to the histopathologist for assessment.

The endometrial samples were assessed by Dr Soomro (Consultant histopathologist at Nottingham City Hospital) who was blinded to the day of the cycle. The samples were classified according to the Noyes criteria (Noyes, Hertig, and Rock 1950) as following:

- Early proliferative
- Mid proliferative
- Late proliferative
- Early secretory
- Mid secretory
- Late secretory

5.3.8 Protocol for purification of RNA from endometrial sample

The endometrial samples were placed in 2 ml microcentrifuge tubes containing 1 stainless steel beads (3-7 mm mean diameter). The tubes were kept in dry ice. Once RNA was ready to be purified, the tubes were placed at room temperature (15-25° C) and the lysis buffer was added to each tube (20 ml of RTL buffer and ethanol 200mcg). The tubes were then placed in the TissueLyser adapter Set 2x24 (QIAGEN, USA). The TissueLyser was then operated for 2 minutes at 20-30 Hz. The adapter set was disassembled and the tubes were rearranged so that the tubes nearest to the TissueLyser were now outer, to ensure uniform disruption and homogenisation of the

tissue. After that, the TissueLyser was reassembled again and operated for another 2 minutes at 20-30 Hz. It was then proceeded with RNA purification. At the end of this process, an amount of 600 µl of lysate was obtained. 200 µl of the lysate was added to 150 µl of the lysis buffer. This was pipetted directly into the QIAshredder spin column (QIAGEN, USA) and placed in a 2 ml collection tube and centrifuged for 2 minutes at full speed. 1 volume of 70% ethanol was added to the homogenised lysate and mixed well by pipetting. 700µl of the sample, including any precipitate that may have formed, was added to an RNeasy spin, placed in a 2 ml collection tube and centrifuged for 15 seconds at $> 8000 \times g$. 700µL of the buffer RW1 was then added to the RNeasy spin column and centrifuged for 15 seconds at $> 8000 \times g$ to wash the spin column membrane.

Following that, 500µl buffer RPE was added to the RNeasy spin column and centrifuged for 15 seconds at $> 8000 \times g$ to wash the spin column membrane. The RNeasy spin column was then placed in a new 1.5ml collection tube. 35µl RNase free water was then added directly to the spin column membrane and centrifuged for 1 minute $> 8000 \times g$ to elute the RNA.

5.3.9 Preparing samples for microarray (Protocol used in the lab)

The tissue sample was first excised and removed from storage. They were cut and weighed and placed in 2 ml microcentrifuge tubes containing 1 stainless steel bead (3-7 mm mean diameter). The sample was kept on dry ice until it was ready for usage. It was then placed in a collection tube. The tissue was disrupted in buffer RLT, and

lysate homogenised. To prepare this, 10microlitres of the B-ME was added per 1ml of buffer RLT. This was done for 10 samples at a time (8000 microlitres of RLT buffer was added to the falcon tube, and then 80 microlitres of BME was added into the hood). Following that, 700 microlitres of pre prepared buffer RLT (with added BME) was added to the sample in the collection tube. The tissue was immediately disrupted and homogenised using the TissueLyser until it was uniformly homogenous. The TissueLyser was set at 25Hz for 2 minutes. It was then placed in ice for one minute, and then lysed again for 2minutes. 400microlitres of the lysate was pipetted directly into a QIAshredder spin column in a 2ml collection tube and centrifuged for 2 minutes at full speed. The top and sides of these tubes were labelled. 1 volume (700 microlitres) of 70% ethanol was then added to the cleared lysate and mixed immediately by pipetting. Up to 700 microlitres of the sample including any precipitate that may have formed was transferred to an RNeasy spin column. The lid was gently closed and the sample was centrifuged for 23 seconds at 8500xg. The flow through was discarded, and the process was repeated with the left-over volume. 350microlitres of buffer RW1 was then added and spun for 23 seconds at 8500xg. 80 microlitres of DNase solution was added to each tube (10microlitres of DNase I and 70 microlitres of RDD buffer). The samples were then left at room temperature for 15 minutes. 350 microlitre of buffer RWI was then added, and the samples spun for 23 seconds at 8500xg. 500 microlitre of Buffer RPE (with added ethanol) was added to the RNeasy spin column. The lid was closed gently, and the sample centrifuged for 20 seconds at 8500xg to wash the spin column membrane. The flow through was then discarded. Using the same collection tube, 500 microlitre Buffer RPE was added to the RNeasy

spin column again. The lid was gently closed and centrifuged for 2 minutes at 8500 xg to wash the spin column. After centrifugation, the RNeasy spin column was gently removed from the collection tube so that the column did not contact the flow through, to prevent carryover of ethanol. The RNeasy spin column was placed in a new 2 ml collection tube, and the old collection tube with the flow-through was discarded. The lid was gently closed and centrifuged at full speed for 1 minute. This step was performed to eliminate any possible carryover of buffer RPE, or residual flow-through that may remain on the outside of the RNeasy spin column. The RNeasy spin column was then placed in a new 1.5 ml collection tube. 30 microlitre of RNase free water was then directly added to the spin column membrane. The lid was gently closed and centrifuged for 1 minute at 8500xg to elute the RNA.

The samples were then shipped to Sutton Bonington campus at the University of Nottingham in dry Ice for microarray analysis.

5.3.10 Microarray protocol

Microarray was performed by the School of Biosciences laboratory, at the Sutton Bonington campus, the University of Nottingham. For microarray experiments, total RNA was first reverse transcribed into single-stranded cDNA with T7 promoter sequence at the 5' end and then converted to double-stranded cDNA, which acted as a template for in vitro transcription. Next, antisense RNA was synthesised and amplified by in vitro transcription of the second-stranded cDNA template using T7 RNA polymerase. Enzymes, salts and inorganic phosphates as well as unincorporated

nucleotides were removed to prepare the cRNA for 2nd cycle single-stranded cDNA synthesis. Single-stranded cDNA was then fragmented, labelled and hybridized onto the Clariom S arrays (#902926, Affymetrix, High Wycombe, Bucks, UK).

Whole-genome transcriptome analysis was conducted by hybridizing X biological samples of total RNA per treatment to the Clariom S arrays. All steps of sense cDNA synthesis, fragmentation and hybridization were performed according to the manufacturer's protocol (GeneChip® 3000 System, Affymetrix).

Gene expression profile data was generated as CEL files and subjected to analysis by the Partek Genomics Suite 6.6 software. Quality Control (QC) metrics were checked by examining surface defects, hybridization, labelling, and a ratio of the 3' probe set to the 5' probe set (3'/5' ratio) to provide the quality of the microarray data. The values were log2 transformed and quantile normalisation using the Robust Multi-array Average (RMA). The list of genes of interest were comprised of genes upregulated or downregulated by at least 1.5-fold, with adjusted p-value + FDR <0.05.

5.3.11 Statistics and data analysis

Statistical analysis of the results was performed using the Statistical package for the Social Sciences (SPSS, Chicago, IL, USA) software. The distribution of the data was checked for normality using a normal probability plot. The statistical significance of the data was analysed using the relevant statistical techniques.

To assess the relationship between the hormonal profile and the endometrial pattern, the Levene test was performed to test for homogeneity in the first instance, and if not significant (p value >0.05), one way ANOVA test was then used to assess the relationship between hormonal profile and endometrial pattern.

To assess the relationship between the histology and the hormonal profile, the Levene test was performed to test for homogeneity in the first instance, and if not significant (p value >0.05), one way ANOVA test was then used to assess the relationship between the hormonal profile and the endometrial histology.

The principal component analysis (PCA) was used to explore the relationship between the endometrial pattern, microarray and the histology. The PCA plot was generated by Partek Genomics Suite 6.6 software. PCA is an exploratory multivariate statistical technique and is one of the oldest and most widely used to analyse large amounts of the dataset, such as microarray datasets (Cadima and Jorge 2016; Raychaudhuri, Stuart, and Altman 2000). It displays a multidimensional dataset in three dimensions, which allows visualizing the samples and their distances from one another in a colourful scatter plot, and allows clear visualization of the gene's expression pattern (Elhaik 2022). The PCA utilises a comprehensive mathematical approach to identify the data with the maximum amount of variance (this hereafter defined as the first principal component (PC1) by drawing a line across the data). The second largest variance would be a line that cuts through the first one by 90° , hereafter is defined as the second principal component (PC2). In other words, PC1 would represent the most

variation in the data and PC2 would represent the second most variation in the data, PC3 representing the third most variation in the data, etc. Respectively, PC1 and PC2 represent the x- and y-axis on the PCA graph (Vyas and Kumaranayake 2006).

5.3.12 Missed data

Patients who had no or insufficient endometrial samples obtained were excluded from the study. Patients where the endometrial pattern was unclear were excluded from the study.

5.4 Results

5.4.1 Participants

30 women were recruited. One woman had 3 cycles. 2 women were excluded from the start as one woman had didelphys uterus and another patient cancelled her appointment. There were 2 women where the endometrial scratch was performed, however no samples were obtained. There were 3 women where the endometrial pattern was unclear due to axial uterus. 24 women had full data. The flow chart in Figure 5.1 shows the recruitment steps.

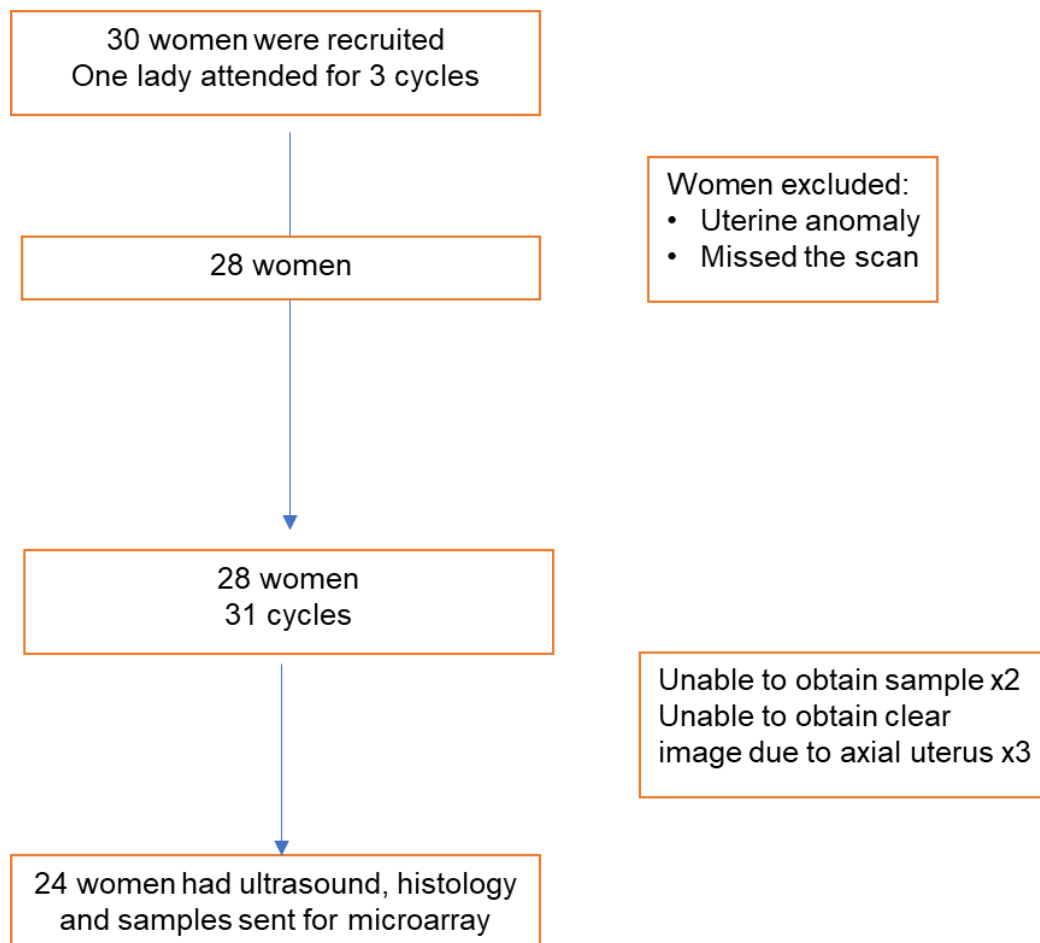


Figure 5.1 Flow chart of recruitment

5.4.2 Participants characteristics

The age of the patients ranged from 25-42 years ($M \pm SD = 35 \pm 4.9$). Only 7 women were captured prior to their ovulation and given LH kit. Other characteristics are described in Table 5.1 which illustrates participants demographics.

Table 5.1 Participants demographics in the biopsy study

	M±SD or Median (Interquartile range) or Number (%)
Age	35±4.9
BMI (kg/m ²)	24±3.6
Duration of subfertility (months)	36 (24)
Parity	0
Length of cycle (days)	30±7.0
Cycle day	22.1±2.9
Average ovulation day	13±2.9
Endometrial thickness	11.4±3.0
NK positive	9 (50%)
No of previous miscarriages	0
No of previous ectopic	0
No of previous terminations	0
Oestradiol (pg/ml)	486.3±213.6
Progesterone (ng/ml)	10.6±6.9
<i>N=24</i> <i>M= Mean, SD= Standard deviation, BMI= Body Mass Index. NK: Natural killer cells (only reported in 18 women)</i>	

Only 7 women had LH test. 6 of them had regular cycles and their ovulation day was day 13 (x5) and day 12 x1. Interestingly one lady had irregular cycles and her ovulation day was day 22 and the biopsy was conducted on day 25 and it showed proliferative endometrium.

5.4.3 Causes of subfertility

Unexplained subfertility was the main cause, followed by male factors. Table 5.2 illustrates the different causes of subfertility.

Table 5.2 Causes of subfertility in the biopsy study

Cause of subfertility	Number (%)
Unexplained	9 (43%)
Male factors	6 (29%)
Ovulatory	3 (14%)
Tubal factors	1 (13%)
Endometriosis	2 (10%)

5.4.4 Results of endometrial pattern

As the endometrial sample is taken around the mid luteal phase, there were no triple patterns. Table 5.3 summaries the number and percentages of endometrial pattern.

Table 5.3 The number and percentages of endometrial patterns

Endometrial pattern	Number (%)
Triple	0
Early intermediate	4 (17%)
Late intermediate	18 (75%)
Homogenous	2 (8%)

5.4.5 Results of histology

There were 27 samples that had histological diagnosis according to Noyes criteria, however as endometrial pattern was unclear in 3 women, only 24 samples were included for analysis (Noyes, Hertig, and Rock 1950).Table 5.3 summaries the number and percentages of endometrial histology.

Table 5.4 The number and percentages of endometrial histology

Endometrial histology	Number (%)
Proliferative	5 (21%)
Early secretory	6 (25%)
Mid secretory	11 (46%)
Late secretory	2 (8%)

5.4.6 Results of PCA

5.4.6.1 PCA plot of endometrial pattern and histology

The PCA plot demonstrates a positive correlation between the endometrial pattern on ultrasound and histology. Early intermediate pattern is likely to represent proliferative to early secretory changes of the endometrium, and is clearly separated from the homogenous pattern and the histological changes of mid and late secretory phase. There is a clear overlap between late intermediate and homogenous, and mid to late secretory endometrium. Figure 5.2 shows PCA plot exploring the relationship between ultrasound endometrial pattern and endometrial histology.

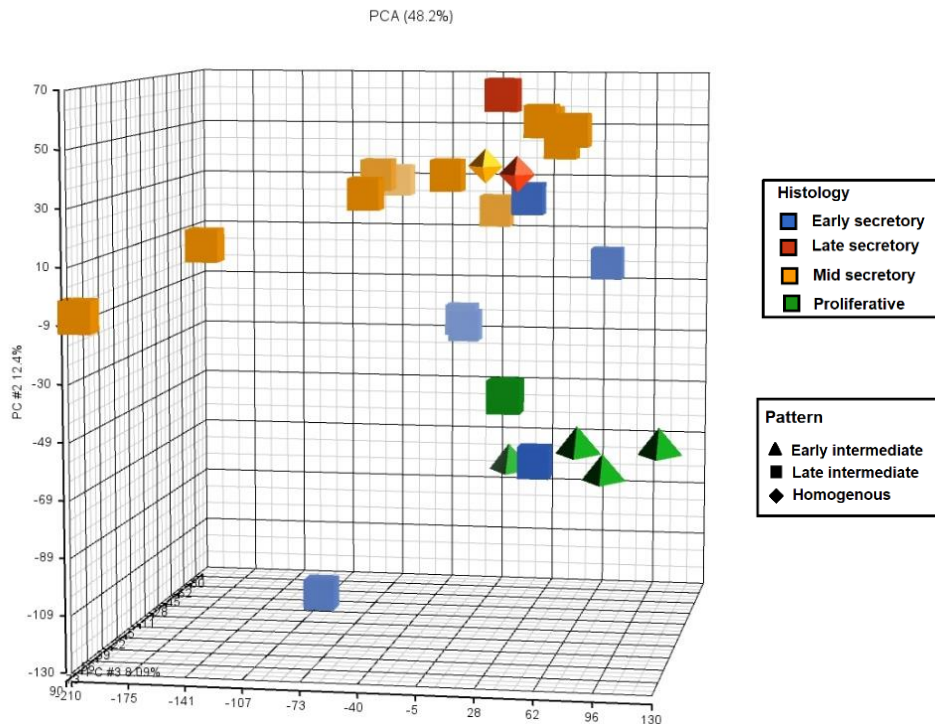


Figure 5.2 PCA 3-dimensional scatter plot exploring the relationship between ultrasound and histological endometrial pattern. Endometrial pattern on ultrasound is illustrated by geometric shapes while histology is illustrated by the different colours (blue, red, orange and green). The PCA plot shows that early intermediate pattern on ultrasound is closely related to proliferative endometrium histology (green pyramids). These are clustered in the lower aspect of the PCA plot. This is clearly separate and distinct from the homogenous pattern/ late secretory histology which is clustered in the upper aspect of the PCA plot. There is some overlap between late intermediate and homogenous patterns and mid to late secretory histology.

5.4.6.2 PCA plots of endometrial pattern

As seen in Figure 5.3, gene expression in late intermediate pattern is scattered across the graph and overlapped with the early intermediate pattern and homogenous pattern. However, early intermediate pattern and homogenous pattern did not overlap.

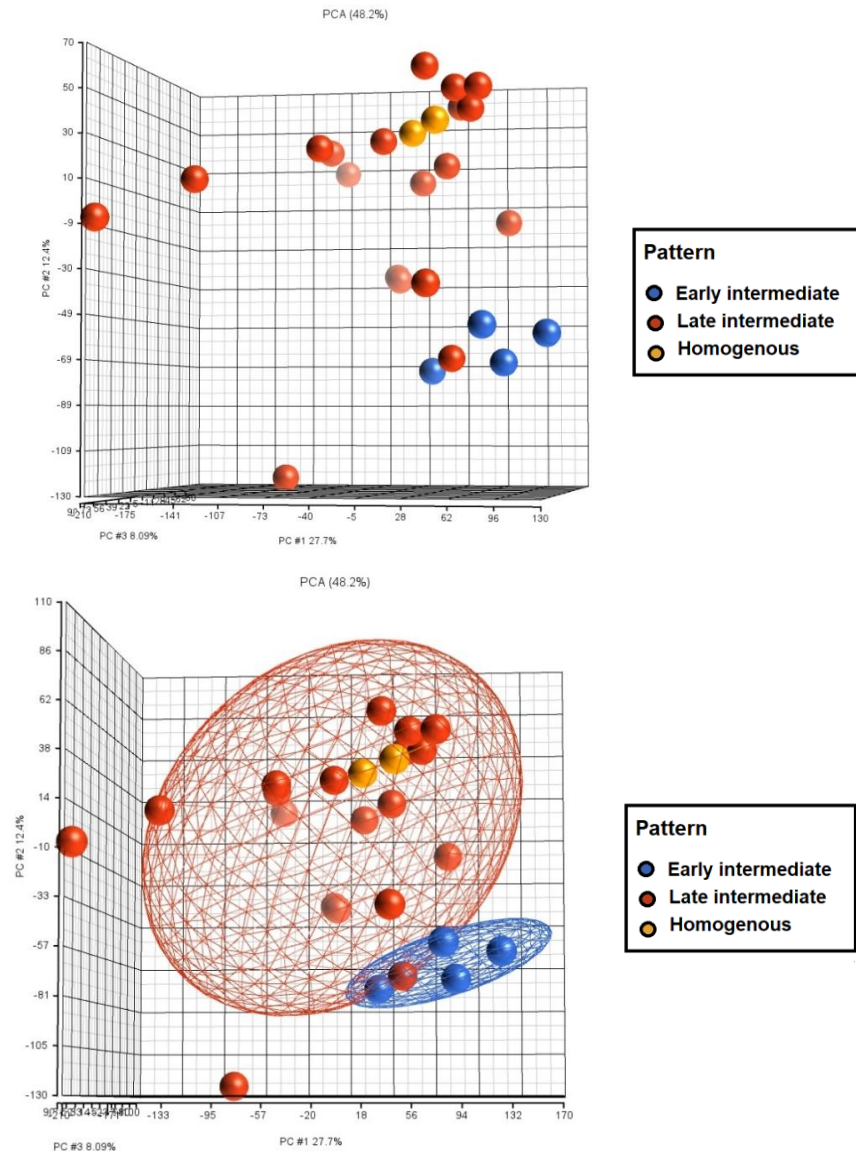


Figure 5.3 PCA graph of gene expression patterns in three endometrial patterns

The graph shows a higher representation of late intermediate pattern (red colour) than other patterns. It also shows an overlap between late intermediate pattern and homogenous pattern (orange colour). Early intermediate pattern (blue colour) although overlapped with late intermediate pattern, is clearly distinguished from homogenous pattern.

5.4.6.3 PCA plot of endometrial histology

PCA showed clustering of the endometrium tissue samples with some overlapping between late secretory and mid secretory histology, and between early secretory and proliferative histology. There was a clear distinction between the mid secretory/late secretory and the proliferative histology (4). Figure 5.4 shows PCA graph of gene expression patterns in four different histology patterns

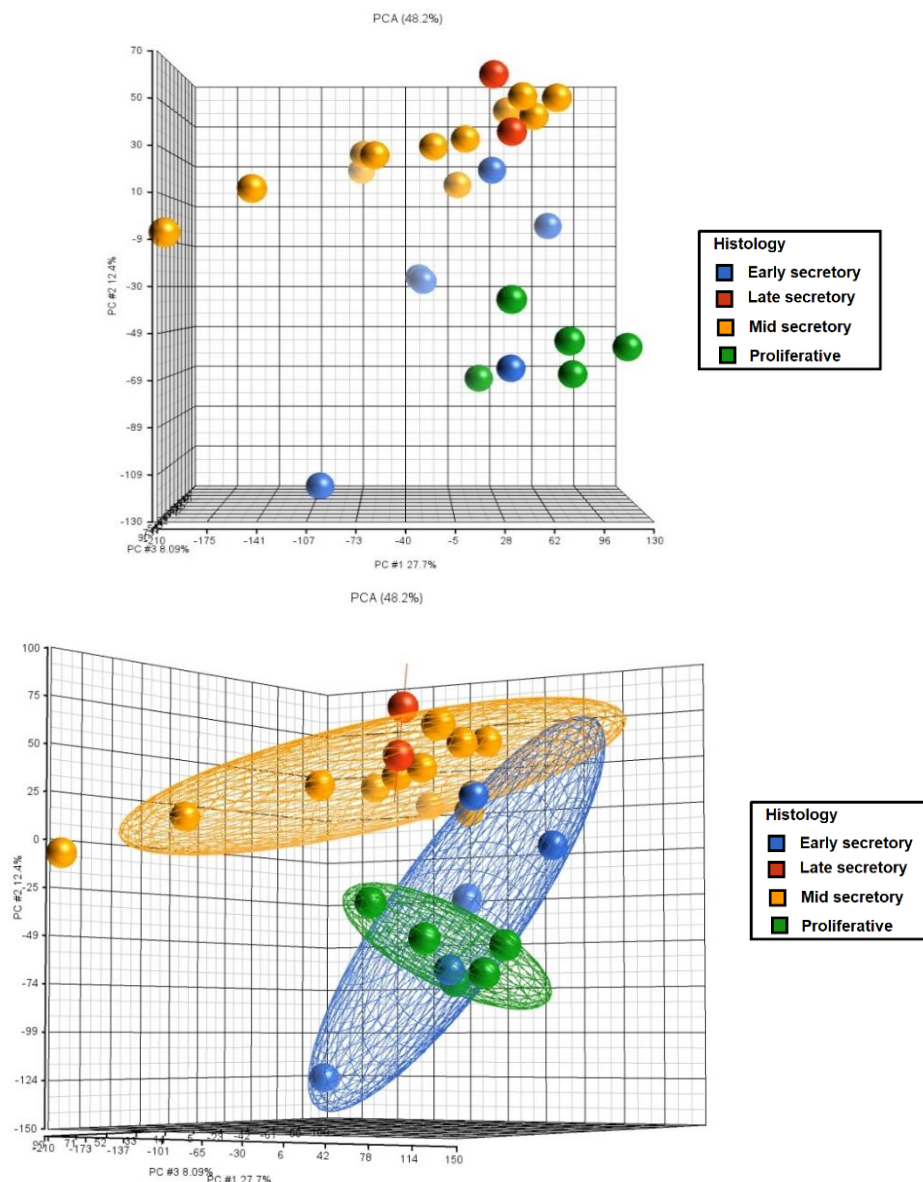


Figure 5.4 PCA graph of gene expression patterns in four different histology patterns

The proliferative histology (green colour) has a distinctive cluster from the late (red colour) and mid secretory (orange colour). The early secretory histology (blue colour) overlapped with all clusters.

5.4.6.1 Endometrial pattern and the hormonal profile

One-way ANOVA was used to explore the relationship between the endometrial pattern and the hormonal profile. Table 5.5 summarises the mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial pattern.

Table 5.5 The mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio on the day of endometrial scratch

		N	Mean	Std. Deviation
Oestradiol	Early Intermediate	4	286.82	70.88
	Late Intermediate	18	503.46	226.47
	Homogenous	2	577.90	157.82
	Total	24	473.55	217.62
Progesterone	Early Intermediate	4	5.30	3.90
	Late Intermediate	18	11.59	5.55
	Homogenous	2	22.57	2.70
	Total	24	11.46	6.51
Oestradiol: Progesterone ratio	Early Intermediate	4	154.91	224.79
	Late Intermediate	18	272.94	972.96
	Homogenous	2	25.37	3.95
	Total	24	232.64	844.01

One-way ANOVA was conducted to explore the relationship between the endometrial pattern and progesterone levels on the day of the biopsy. Early intermediate pattern was associated with low progesterone levels while late intermediate and homogenous pattern were associated with higher progesterone levels. The results were statistically

significant [$F(2, 21) = 7.245, p = 0.004$]. Figure 5.5 shows the mean of progesterone for the endometrial pattern on the day of endometrial scratch

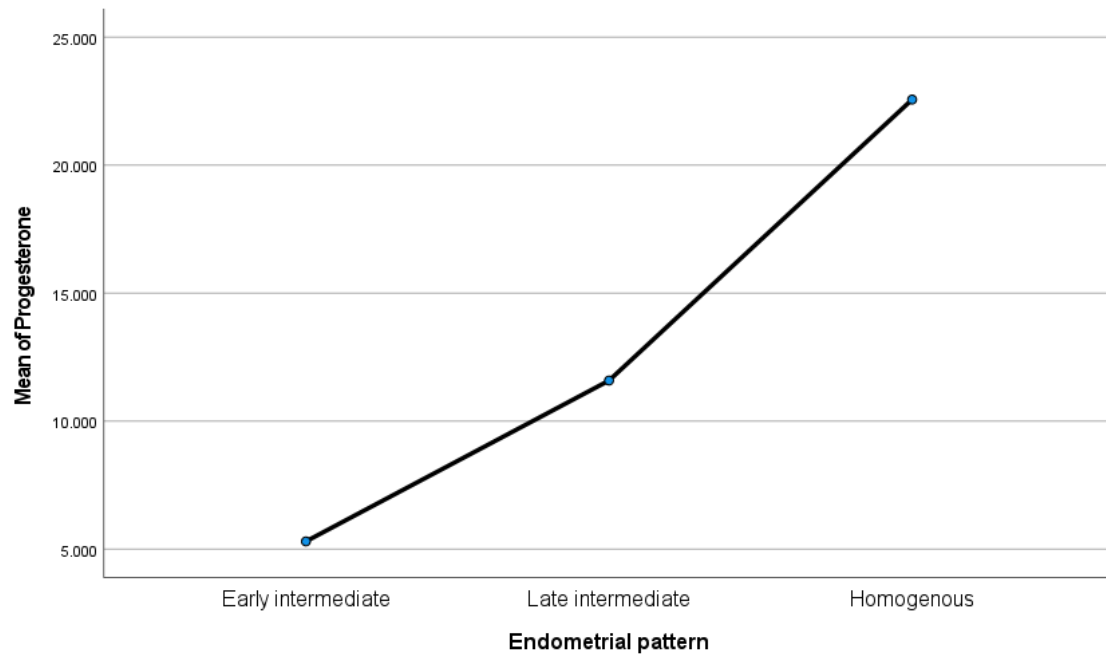


Figure 5.5 The mean of progesterone for endometrial pattern on the day of endometrial scratch

A one-way ANOVA was conducted to explore the relationship between the endometrial pattern and oestradiol level on the day of the biopsy. The results were not statistically significant [$F(2, 21) = 2.042, p = 0.155$]. Figure 5.6 shows the mean of oestradiol for the endometrial pattern.

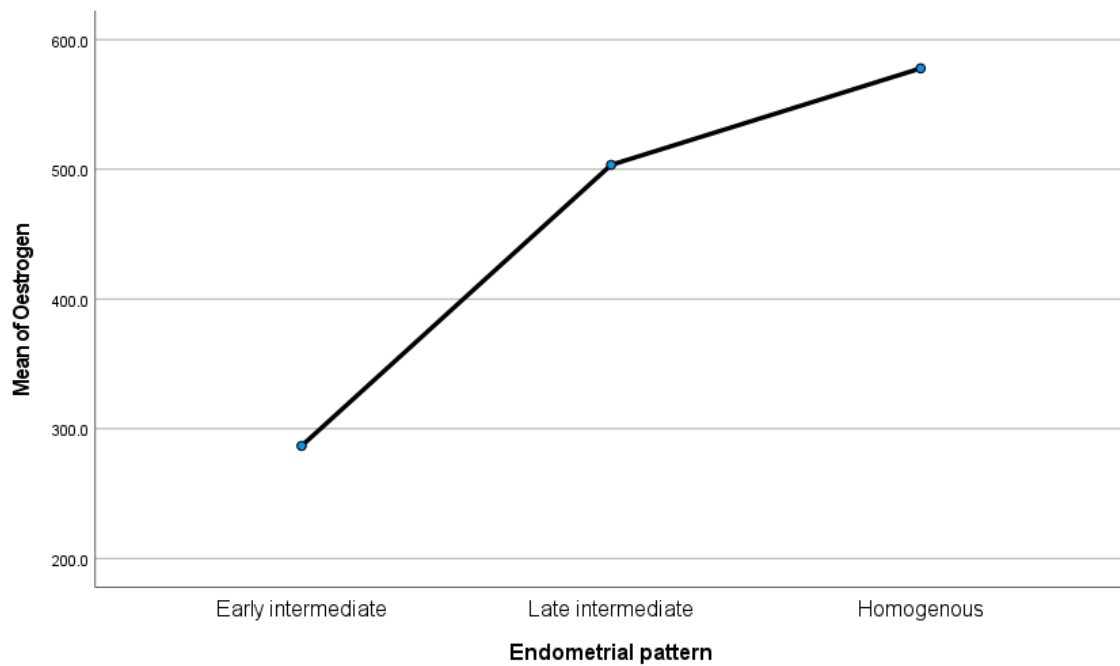


Figure 5.6 The mean of oestradiol for endometrial pattern on the day of endometrial scratch

A one-way ANOVA was conducted to explore the relationship between the endometrial pattern and oestradiol: progesterone ratio on the day of the biopsy. The results were not statistically significant [$F(2, 21) = 0.090$, $p = 0.914$]. Figure 5.7 shows the mean of oestradiol: progesterone ratio for the endometrial pattern.

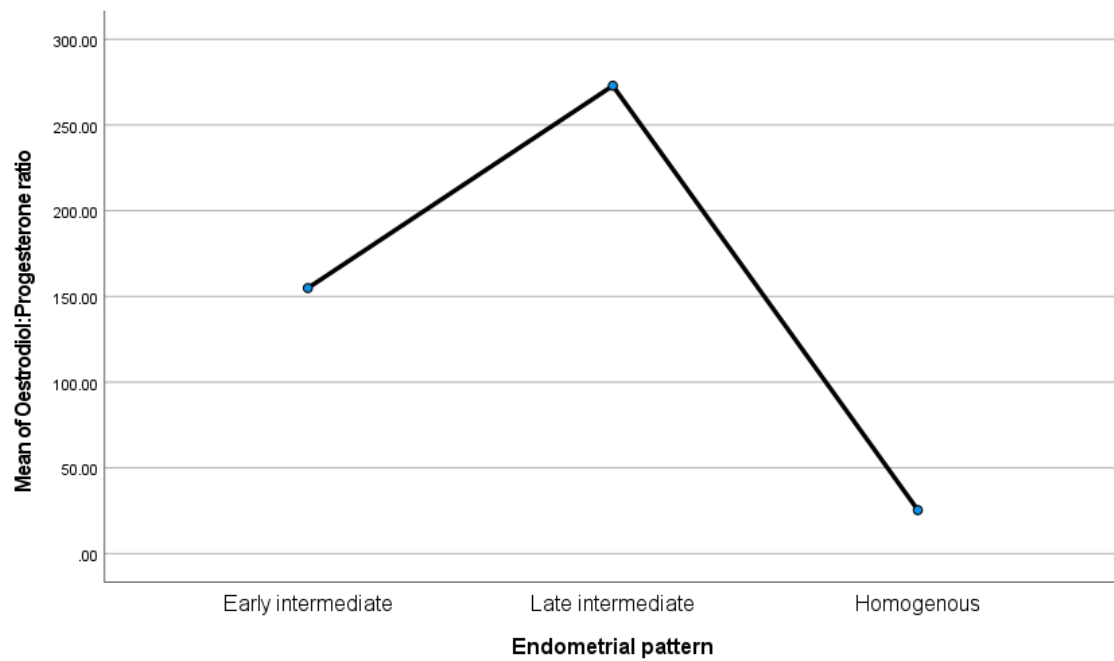


Figure 5.7 The mean of oestradiol: progesterone ratio for the endometrial pattern

5.4.6.1 Endometrial histology and the hormonal profile

One-way ANOVA was used to explore the relationship between endometrial histology and the hormonal profile. Table 5.6 summarises the mean and standard deviation of oestradiol, progesterone and oestradiol: progesterone ratio for the endometrial histology.

Table 5.6 The mean and standard deviation for oestradiol, progesterone and the oestradiol: progesterone ratio

		N	Mean	Std. Deviation
Oestradiol	Proliferative	7	496.63	299.85
	Early secretory	6	503.67	230.79
	Mid secretory	11	448.69	165.50
	Late secretory	2	604.75	119.85
	Total	26	486.29	213.56
Progesterone	Proliferative	7	3.15	3.85
	Early secretory	6	13.78	7.48
	Mid secretory	11	12.27	4.05
	Late secretory	2	17.84	9.38
	Total	26	10.60	6.94
Oestradiol: Progesterone ratio	Proliferative	7	2278.41	3676.27
	Early secretory	6	49.69	32.90
	Mid secretory	11	38.22	14.68
	Late secretory	2	37.28	12.88
	Total	26	643.92	2065.80

One-way ANOVA was conducted to explore the relationship between the endometrial histology and progesterone levels on the day of the biopsy. The results show that there are low progesterone levels in proliferative endometrium which increase in the secretory phase reaching a maximum in late secretory phase. The results were statistically significant [$F(3, 22) = 6.911$, $p = 0.002$]. Figure 5.8 shows the mean of progesterone levels for the endometrial histology.

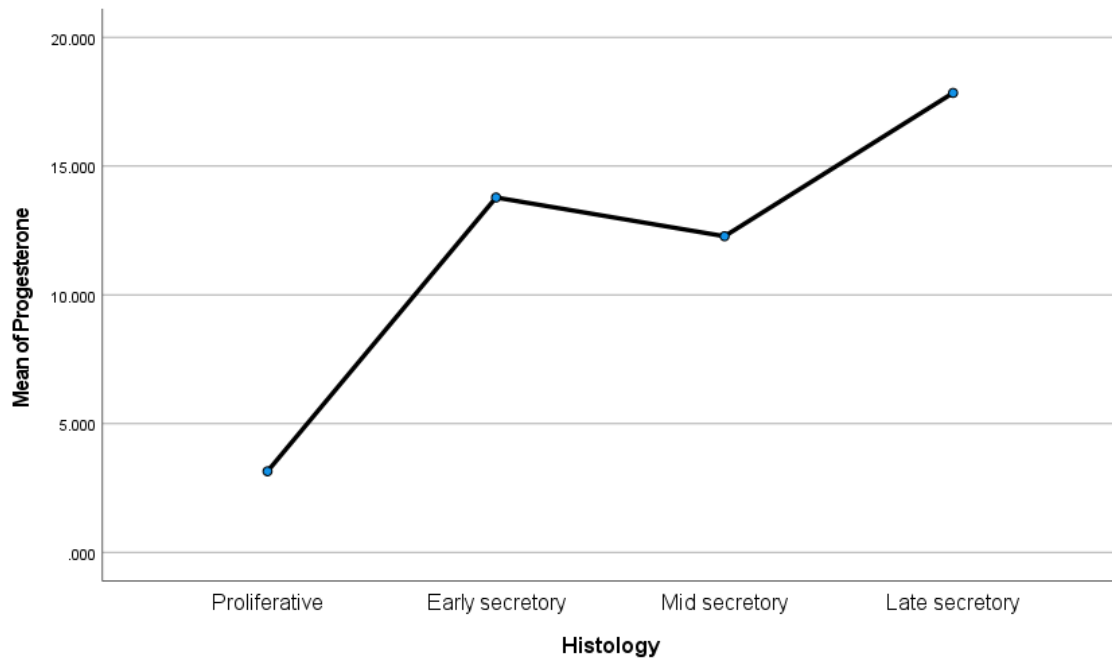


Figure 5.8 The mean of progesterone levels for the endometrial histology

One-way ANOVA was conducted to explore the relationship between the endometrial histology and oestradiol levels on the day of the biopsy. The results were not statistically significant [$F(3, 22) = 0.310$, $p = 0.818$]. Figure 5.9 shows the mean of oestradiol for the endometrial histology.

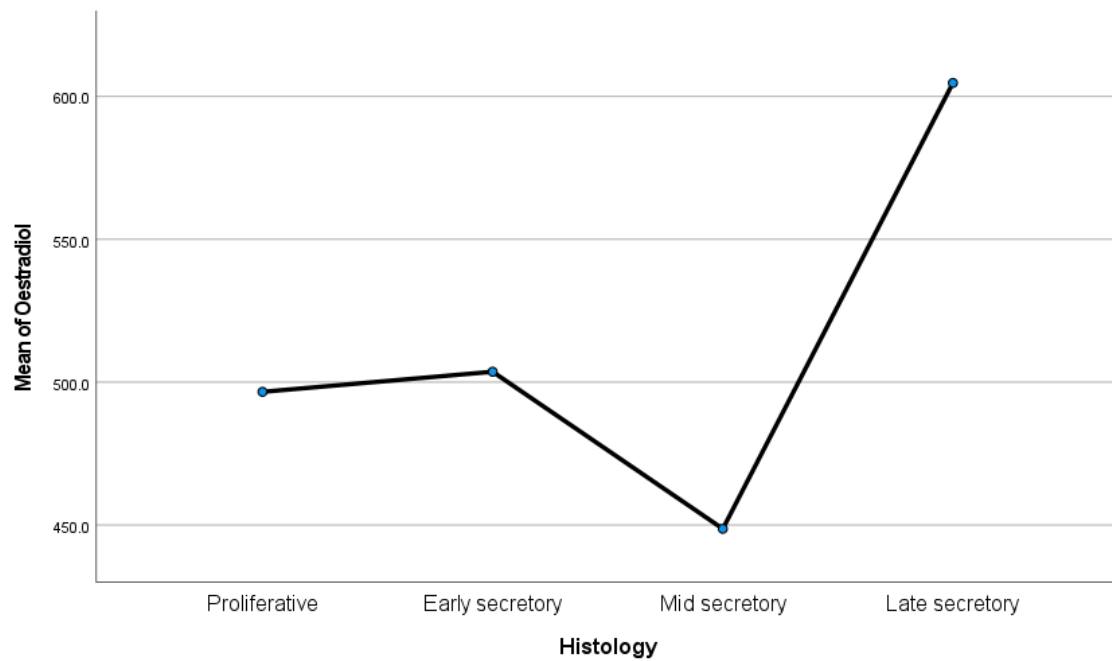


Figure 5.9 The mean of oestradiol levels for the endometrial histology

One way ANOVA was not valid in exploring the relationship between endometrial histology and oestradiol: progesterone ratio as the test of homogeneity (Levene test) was statistically significant (p value <0.001).

5.5 Discussion

The PCA found a distinctive distribution that separate the mid/late histology from the proliferative histology. This may represent a different expression pattern amongst this

histology. There was also a clear distinction between early intermediate pattern on ultrasound and the homogenous pattern.

The most exciting finding of this study was the positive correlation between the “new endometrial pattern recognition” classification system and endometrial histology on PCA plots according to its transcriptomic signature, providing a “biological basis” for our novel pattern recognition classification system.

Moreover, the hormonal data showed a linear correlation between the expression of oestrogen and progesterone, and the endometrial pattern on ultrasound. The correlation between progesterone and the endometrial pattern was statistically significant, signifying the important role that progesterone plays in regulating endometrial receptivity, and that endometrial pattern does reflect the cyclical and hormonal changes of the endometrium. There is greater correlation between the hormonal profile and endometrial pattern than between the hormonal profile and histology, suggesting that ultrasound is a better indicator of these changes (Grunfeld et al. 1991).

One of the limitations of the study resulted from the endometrial pattern disparity in the samples. Ideally, there should be equal numbers of samples in each category of the endometrial pattern and histology diagnosis, to allow accurate comparison. However, as the endometrial scratch is routinely undertaken around the mid luteal phase, and women who were electing to undertake this invasive procedure as part of their treatment were opportunistically recruited, it was expected that no women would

exhibit triple pattern. This posed a limitation in allowing accurate comparison between pattern and histological diagnosis.

A further limitation occurred when some women elected to undertake endometrial scratch late in the cycle. These women were recruited too late in the cycle for the window of ovulation to be captured, and their figures further contributed to the limitation of the data , as it created difficulties in accurately dating the cycle.

Another limitation of the study was in its use of the Noyes criteria to date the samples. This is because the Noyes criteria has its own limitations and inconsistencies, as it has been shown to date cycles with an error margin of a few days(Lessey and Young 2019). In the absence of a gold diagnostic test however, this limitation had to be accepted.

A further limitation of the study was that it is not possible to perform these tests in a treatment cycle due to the adverse effects on implantation. These tests were therefore conducted in natural cycles, which had a different endometrial response to a controlled ovulation cycle.

5.6 Conclusion

The PCA plot showed a clear correlation between the endometrial pattern and histology. Early intermediate pattern is closely related to the proliferative phase, and is distinct from the homogeneous pattern. In the mid to late secretory endometrium,

there was an overlap between the early intermediate and late intermediate pattern, and between the late intermediate and homogeneous pattern. The endometrial pattern and histology are closely related which is expressed in the PCA plot according to their transcriptomic signature. Further testing is required to further elucidate the “genomic” factors regulating this relationship.

Chapter 6 General discussion and conclusion

6.1 Summary of findings

It is generally acknowledged that prediction of the assisted reproduction depends on many factors. The endometrial patterns as detected by ultrasound is one of these factors and therefore has been studied (as reviewed in chapter 2). The research of the predictive ability of endometrial patterns to predict the success rates of the IVF/ICSI have yielded conflicting results.

A systematic review and meta-analysis have been conducted in order to identify the reasons for the conflicting results. The evidence regarding the ability of the endometrial pattern in predicting the treatment outcomes was studied. The results of this review showed that women with triple pattern at trigger are more likely to become pregnant when compared to women with a homogenous pattern (OR 1.89 95% CI:1.39, 2.58). Similarly, women with a homogenous pattern at fresh embryo transfer were more likely to become pregnant compared to women with an intermediate pattern (OR 0.53 95% CI 0.31, 0.92). This indicates that not only the endometrial patterns but also the timing of detecting these patterns is important determinant of the success of the treatment.

Although a reliable method in conducting this review was followed, its findings do need to be interpreted with caution because of its limitations. First, the quality of evidence collected from some reviewed studies could be considered low to

moderated. Second, the substantial heterogeneity of the studies. Third, the different classification systems were used in the reviewed studies which made it challenging to accurately assess the predictive ability of endometrial patterns. Each classification system might have a degree of inter-observer and intra-observer variability that limit their usefulness in predicting the outcomes. Therefore, I performed further search to develop a novel “endometrial pattern recognition” classification system based on the census statements produced by The IETA and reviewing the literature. Next, I conducted three studies to assess the efficacy of this classification system.

The reliability for inter-observer and intra-observer variation of the new classification system was assessed. I collected anonymised random cine loops of endometrial patterns while scanning on different machines with different quality, to reflect real day to day scanning. I attempted to collect as many as possible to reflect different endometrial patterns. During this study, I noted that it was difficult to accurately assess the endometrial pattern on a still image, and that a sweep of the endometrium was required to be able to fully assess the endometrium, and to reflect normal practice/routine scanning.

The interobserver agreement using Cohen Kappa was checked and were able to attain 0.90 which is almost perfect agreement between two observers (myself and my supervisor) looking at 60 cine loops. Once we agreed on the cine loops, it was time to test it with other observers. To have a benchmark, we decided to test the current classification system (three grade) and that produced 0.61 which is substantial

agreement between operators, however as mentioned before it was a high result due to the fact that the triple pattern was overclassified.

The new classification system was introduced and taught to the same operators and after a good period of time asked them to retest the new classification system. This produced similar results, with moderate to substantial agreement. Looking at the results in further detail however, showed some discrepancies between the operators. Most operators were able to achieve between 80-90% accuracy, suggesting that better results could be achieved with training. The strength of this study is the use of cine loops to reflect dynamic scanning with large number of cine loops as well which allowed to test for inter-observer as well as intra-observer variation. However, more training is needed and this needs to be re- tested again. I would focus on image quality, longer cine loops less and less videos and focus on inter-rater variability.

Under the direction of my supervisors, I designed the main study protocols to assess whether this “novel pattern recognition” endometrial classification system can impact or predict successful outcome. The study was pre-registered on trials.gov with specific inclusion and exclusion criteria, and pre-defined objectives and outcomes to address some of the pitfalls of other studies. We proposed to look at 200 cycles between fresh and frozen cycles. We managed to recruit over 180 women of which 155 women were eligible, who underwent more than 230 cycles (of which 191 were eligible to be included). In the design of the study, care was taken to collect as much information as possible but with the least inconvenience to the patients. The study was therefore

designed with no extra visits to the patient, as it was felt they were already required to attend numerous appointments as part of their IVF treatment. For fresh cycles, we aimed to collect data at 3 time points (around the trigger point, egg collection and embryo transfer). For women undergoing frozen embryo transfer we collected data at 2 time points, around day 15 of the cycle (when they normally visit, but coordinated with the embryologist so that their embryo transfer and progesterone were initiated) and the day of embryo transfer. The results of this study showed that women with a homogenous pattern at egg collection were 95% less likely to become pregnant when compared to women with a triple pattern (clinical pregnancy and live birth OR 0.05, 95% CI 0.0027, 1.05). It also showed that women with late intermediate pattern at egg collection were 35% less likely to become pregnant when compared to women with a triple pattern (clinical pregnancy OR 0.65, 95% CI 0.21, 1.98). These results are statistically significant with a p value of 0.05. Endometrial pattern was recorded blind to the outcome. The main limitation was the power of the study as larger sample is needed. There were also some missing data. Different operators scanned on different machines which can contribute to the limitation. However, patterns were recorded as cine loops and single operator classified the patterns to reduce bias.

Finally, the last study was an opportunity to explore the relationship between histology, endometrial pattern using our “novel pattern recognition classification system” on ultrasound, and Microarray in women electing to have endometrial scratch. The results of this study showed there is a positive correlation between endometrial pattern and histology on PCA plots. The main limitation is the need to use Noyes criteria which

has its own limitation. Some women booked late in the cycle after ovulation which limiting the ability to date their cycles. There was disparity in the sample size with some groups more than others (mid secretory endometrium was overrepresented in the samples given the timing of the samples which can contribute to the limitation of the results).

In conclusion, these studies support that endometrial pattern can help predict implantation. However, it should be used as an additional tool to help counsel women regarding embryo transfer when unfavourable patterns are detected.

6.1 Future directions

In this thesis, a new endometrial classification system was developed, its reliability, its prediction abilities, and its association with histological changes in the endometrium were tested. However, further research is still required to reassess this classification system.

First, there is a need for a well powered randomised controlled trial to test the predictive ability of endometrial patterns on ultrasound to predict implantation. The ideal population, women having single top quality blastocyst to reduce the impact of the embryo factor.

Second, the reliability of this system ought to be re-tested within other centres and with other professionals. Retraining professional personnel in identifying the pattern

recognition classification system. It could be more effective to used less cine loops. It can be compared to other classification systems that I did not use in my study which can prove its efficacy.

For the endometrial biopsy study, the main limitation resulted from the small samples and the disparity of the different groups. To remedy this, larger studies with more balanced groups of endometrial pattern/histology are required. To further explore the “window of implantation” phenomenon, a study retesting microarray against the endometrial receptivity array, may be of future interest. Finally, following on from the positive relationship between progesterone and endometrial pattern, a potential study exploring the relationship between progesterone and microarray may provide further insight into the role of progesterone in endometrial receptivity.

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Appendices

Appendix 1: Search criteria

Pubmed Medline	((Fertility[Mesh] AND "Infertility, Female"[Mesh]) OR ("Embryo Implantation, Delayed"[Mesh]) OR (Intracytoplasmic injection\$ [All Fields]) OR (("reproductive techniques, assisted"[MeSH Terms]) OR (Assisted reproductive technologies[Text Word])))AND("ultrasonography"[MeSH Terms] OR "ultrasounds"[MeSH Terms] OR ultrasound[Text Word]) AND (Endometrium [Mesh Terms])
Ovid (Medline) AND PsycINFO	1.(IVF or in vitro fertili\$ation or in-vitro fertili\$ation or in vitro fertili\$ation) 2.((intracytoplasmic or intracytoplasmic) adj5 sperm inject\$) 3.ICSI 4. Assis* reproduct* Techn* 5. 1 or 2 or 3 or 4 6. (ultrasound or ultrasonograph\$ or ultrasound or ultra sonograph\$) 7. exp Ultrasound/ 8. exp sonogr*/ 9. 5 or 6 or 7 10. 4 and 8 11. exp Endometr*/
Cochrane Gynaecology and Fertility Group Specialised Register	1. exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/ 2. (in Vitro adj2 fertili\$) 3. (ivf or icsi) 4. (intracytoplas\$ adj2 sperm). 5. (embryo transfer\$ or ET) 6. invitro fertili\$ 7. Reproductive Techniques, Assisted 8. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 9. (ultrasound or ultrasonograph\$ or ultra-sound or ultra-sonograph\$) 10. exp Ultrasonography/ 11. 9 OR 10 12. Endometrial Pattern 13. 8 AND 11 AND 12
CINHAL	1. ICSI 2. TX intracytoplasmic sperm injection* 3. TX IVF 4. (MM "Fertilization in Vitro") 5. TX vitro fertilization 6. TX vitro fertilisation 7. TX embryo* N3 transfer* 8. Assisted reproduction 9. S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8

	10. (MH "Ultrasonography") OR "ultrasound" 11. TX Sonography 12. S10 OR S11 13. TX Endometrial pattern 14. S9 AND S12 AND S13
Embase	1 exp fertilization in vitro/ 2 exp intracytoplasmic sperm injection/ 3 exp embryo transfer/ 4 (in?Vitro adj2 fertili\$) 5 (intracytoplas\$ adj2 sperm)
Web of Science	TOPIC: ((Intracytoplasmic sperm injection) OR (In vitro fertilisation)) AND TOPIC: (Ultrasound) AND TOPIC: (Endometrial pattern)
CENTRAL	IVF in Title Abstract Keyword OR ICSI in Title Abstract Keyword AND "ultrasound" in Title Abstract Keyword AND Endometrial pattern in Title Abstract Keyword - (Word variations have been searched)
ClinicalTrials.gov	(IVF OR ICSI) AND (Ultrasound)
ISRCTN	IVF or ICSI within Condition: Infertility. Interventions: Ultrasound
Uk clinical trials gateway	Infertility female

Appendix 2: Table of included studies

Study	Study design	IVF, ICSI, both	Number of patients	Pattern Classification system	Timeline	Outcomes	Conclusion
Bassil (Bassil 2001)	Prospective	IVF, fresh cycles	145 patients, 170 cycles	Triple & homogenous	Egg collection & embryo transfer	Clinical pregnancy although not clearly defined	No effect
Bergh et al. (Bergh, Hillensjö, and Nilsson 1992)	Prospective	Fresh IVF	100 patients For embryo transfer only 70 were analysed	Multi-layered, Isoechogenic and homogenous	Trigger & egg collection	Clinical pregnancy although not clearly defined	More patients with triple pattern conceived than intermediate pattern
Coulam et al. (Coulam et al. 1994)	Prospective	Fresh IVF only included	107 women fresh cycles only included	Multi-layered and non multi-layered	Trigger	Clinical pregnancy clearly defined	Positive effect in donor cycle (not included here) noted with triple pattern
Check et al. (Check et al. 1993)	Observational	IVF only	273 fresh cycles	Hypoechogenic with well defined outer walls (triple), iso-echogenic, homogenous	Trigger	Clinical pregnancy not clearly defined	No pregnancies with homogenous pattern
Check et al. (Check et al. 2013)	Retrospective	IVF, fresh & frozen cycles	2759 fresh & 2244 frozen	Triple & Isoechogenic, they did not include homogenous	Trigger and coordination	Clinical pregnancy and live birth	No difference between triple & Isoechogenic.

Chen et al. (Chen et al. 2010)	Retrospective	Both Fresh cycles	2896 fresh cycles	Triple and no triple line	Trigger	Clinical pregnancy	Combined analysis of thickness and pattern good predictor of outcome at trigger (triple and increased thickness)
Dechaud et al. (Dechaud et al. 2008)	Prospective	Both fresh	124 patients Included 112	Homogenous and triple	Trigger	Pregnancy rate although not clearly defined	Triple line with thickness and doppler at trigger predicts good outcome
Dickey et al. (Dickey et al. 1992)	Prospective	Fresh IVF only included	75 cycles	Type A homogenous, B intermediate, C multi-layered. Only included type B and C	Trigger	Clinical pregnancy	For GIFT/TET (not included here) there was higher proportion of fecundity and continuing or delivered pregnancies for type C than for type B endometrium.
Eichler et al. (Eichler et al. 1993)	Prospective	Fresh	148 women and 148 cycles	Triple, intermediate and homogenous	Trigger	Clinical pregnancy	No effect
Fanchin et al. (Fanchin et al. 2000b)	Prospective	Fresh IVF	221 women 228 cycles	Computerised assessment of extent of hyperechogenic transformation of the endometrium over the endometrial thickness <30% (classified as Triple Line) 31%– 40% (classified as Triple Line) 41%–50%(classified as Intermediate)	Trigger	Clinical pregnancy	The more advanced is the hyperechogenic transformation of the endometrium at the time of hCG administration, the lower are the pregnancy rates and implantation rates in IVF-ET.

				51%–60% (classified as Intermediate) 61%–70% (classified as Homogeneous) >70% (classified as Homogeneous)			
Gingold et al. (Gingold et al. 2015)	Retrospective	Fresh and frozen IVF	277 women & 356 cycles 176 fresh cycles & 180 frozen cycles in 152 women	Late proliferative (triple), early secretory (intermediate) & homogenous	Fresh trigger and embryo transfer. Frozen embryo transfer	Clinical pregnancy	Endometrial thickness does not affect clinical pregnancy rate but homogenous pattern is associated with a reduced implantation rate
Jarvela et al. (Järvelä et al. 2005)	Prospective	Fresh IVF	35 women and cycles	Triple and homogenous	Trigger and egg collection	Clinical pregnancy (gestation sac, FH not mentioned)	The presence of triple line pattern in the endometrium after FSH stimulation was strongly associated with the outcome of the IVF cycle.
Khalifa et al. (Khalifa et al. 1992)	Prospective	Fresh IVF	74 women	Homogenous and Mixed texture (triple)	Trigger and embryo transfer	Clinical pregnancy clearly defined	Endometrial pattern at trigger helpful in predicting outcome. However, the endometrial pattern on the day of embryo transfer does not appear to provide prognostic information.
Merce et al. (Mercé et al. 2008)	Prospective	Fresh Both	80 women 77 cycles	Triple and non triple	Trigger	Clinical pregnancy or histological	No effect

						products of conception	
Maugey-lauloma et al (Maugey-Laulom et al. 2002)	Prospective	Fresh Both	144 women and cycles	Multi-layered and homogenous	Embryo transfer	Ongoing pregnancy	Pregnancy was higher with triple pattern but it was not significant
Noyes et al. (Noyes et al. 1995)	Prospective	Fresh IVF	516 cycles	Solid (homogenous), ring (multi-layered), intermediate	Trigger	Clinical pregnancy clearly defined	Solid endometrium, a less favourable environment for embryo implantation.
Oliveira et al. (Oliveira et al. 1993)	Prospective	Fresh IVF	121 women, 139 cycles	Multiple layer and homogenous	Trigger	Clinical pregnancy clearly defined	No effect
Oliveira et al. (Oliveira et al. 1997)	Not stated	Fresh IVF	150 cycles	Multi-layered and homogenous	Trigger	Clinical pregnancy not clearly defined	Endometrial ultrasound in terms of pattern and thickness is of no prognostic value in IVF cycles on the day of administration of HCG. However, a minimum thickness has to be achieved for pregnancy to occur (7.0 mm). The presence of multi-layered endometrium appears more likely to favour pregnancy.
Oluborode et al. (Oluborode et al. 2020)	Retrospective	Both fresh and frozen	778 fresh and 256 frozen	Trilinear, semi-trilinear and unilinear	Trigger and frozen embryo transfer	Clinical pregnancy and live birth	No effect

Pandurgangi et al. (Pandurangi et al. 2021)	Prospective	Frozen	348 women	Trilaminar, isoechoic and hyperechoic	Day of progesterone initiation	Clinical pregnancy and live birth	No effect.
Puerto et al. (Puerto et al. 2003)	Comparative, observational	Fresh both	240 women	Homogenous, intermediate and trilaminar	Embryo transfer	Clinical pregnancy defined as intrauterine gestational sac (no mention of FH)	No effect
Sharara et al. (Sharara, Lim, and McClamrock 1999)	Prospective	Fresh IVF	96 women (included 86) 116 cycles (included 103)	Triple and homogenous	Trigger and egg retrieval	Clinical pregnancy	The endometrial pattern on the day of oocyte retrieval is more predictive of implantation success. A triple-line pattern may indeed be the sonographic parameter that mostly reflects endometrial receptivity, while a homogeneous pattern is a bad prognostic factor, regardless of endometrial thickness.
Sher et al. (Sher, Dodge, et al. 1993b)	Observational	Frozen	1332	Grade I Homogenous and grade II Halo pattern	trigger	Clinical pregnancy clearly defined	Combined effect of endometrial pattern and thickness, thick triple pattern associated with good outcome
Ugwu et al (Ugwu et al. 2022)	Prospective	Fresh	295	Triple, isoechoic and homogenous	trigger	Clinical pregnancy clearly defined	Utilised a prediction model incorporating age, endometrial pattern, thickness, BMI, ovarian reserve which showed positive correlation

Yang et al. (Yang et al. 2018)	Retrospective	Frozen Both	1512	Triple and no triple	Day of progesterone initiation)	Clinical pregnancy and live birth (clearly defined)	Endometrial thickness >8mm and triple line pattern had significantly higher clinical pregnancies but no difference in live birth and spontaneous abortion rates
Yuan et al. (Yuan et al. 2016)	Retrospective	Both fresh	10787	Triple, intermediate, homogenous	Trigger	Clinical pregnancy and live birth	Endometrial thickness is significant and independent predictor of pregnancy. No significant difference between patterns
Zackova et al. (Zácková et al. 2009)	Prospective	Both frozen	30	Triple and homogenous	Frozen embryo transfer day	Clinical pregnancy	Triple line endometrium on day of embryo transfer predicts good outcome
Zhang et al. (Zhang et al. 2016)	Prospective	Fresh both	529 women attrition to 435 women and cycles	Multi-layered and non multi-layered	Trigger	Clinical pregnancy	No effect.
Zhang et al. (Zhang et al. 2018a)	Retrospective	Frozen	1512 cycles	Triple, intermediate and homogenous	Day of progesterone initiation	Clinical pregnancy clearly defined	Showed no significant association between endometrial pattern and IVF outcome.
Zhao et al. (Zhao, Zhang, and Li 2012)	Retrospective	Fresh IVF	1933 women	Triple, intermediate and homogenous	Trigger	Clinical pregnancy clearly defined	Endometrial thickness and pattern independently affect pregnant outcomes. Combined endometrial thickness and pattern could not predict the outcome of IVF-ET when endometrial thickness was < 7 mm or >14 mm, while a triple-line pattern with a moderate endometrial thickness

							appeared to be associated with a good clinical outcome.
FH: Foetal Heart, GIFT: Gamete Intrafallopian Transfer, ZIFT: Zygote Intra-Fallopian Transfer,							

Appendix 3: “Pattern recognition” classification system of the endometrium ©

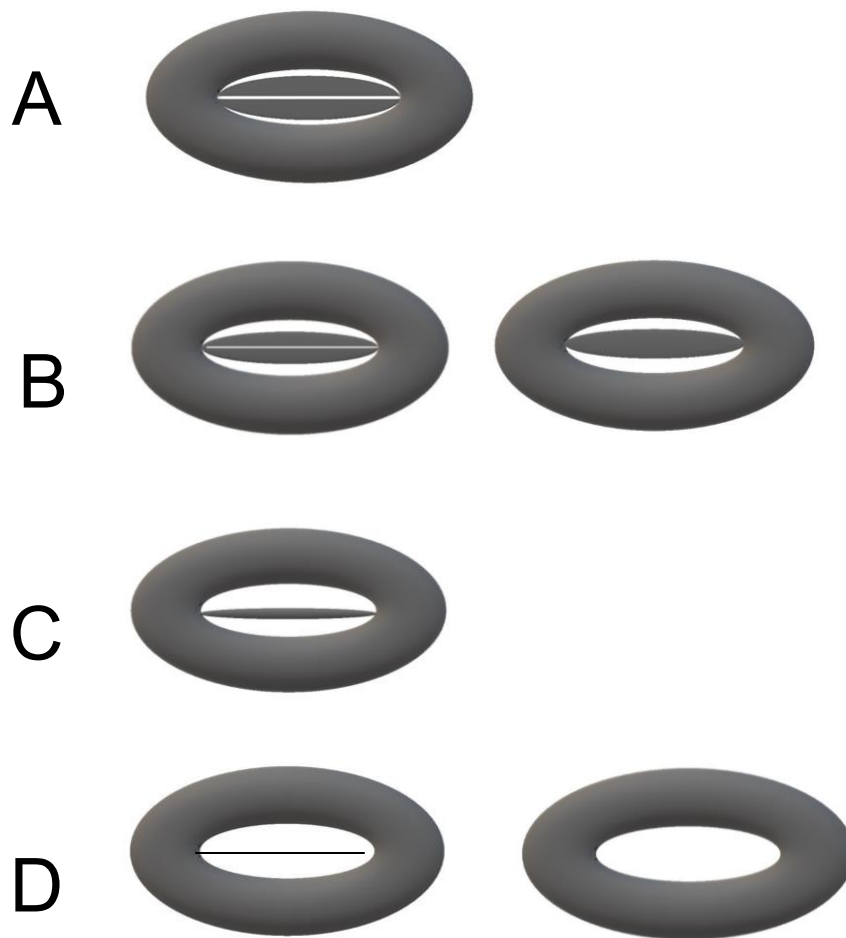
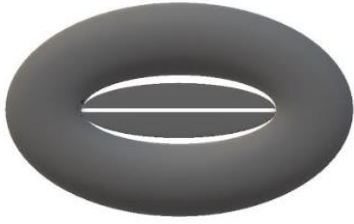
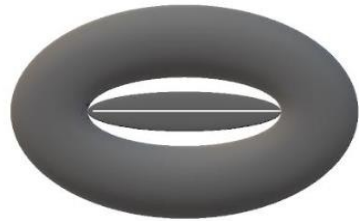


Figure xx. Endometrial “Pattern Recognition” classification system ©.

The figure shows the Endometrial pattern classification system used in this study. **A. Pattern 1: Triple:** 3 lines, hypoechogenic endometrium where the hyperechogenic area (white line on outside) is $< 1/3$ of the endometrial thickness. **B. Pattern 2: Early intermediate:** Hyperechogenic area (white lines on outside) is $\geq 1/3$ of the thickness and $< 2/3$ of the endometrial thickness. **C. Pattern 3: Late intermediate:** Hyperechogenic area (white line on outside) is $> 2/3$ of the endometrial thickness) **D. Pattern 4: Homogenous:** Hyperechogenic (completely white with or without a midline shadow).



Type 1: Triple or Trilaminar



**Type 2:
Intermediate or Triple/
intermediate**



**Type 3:
Intermediate**



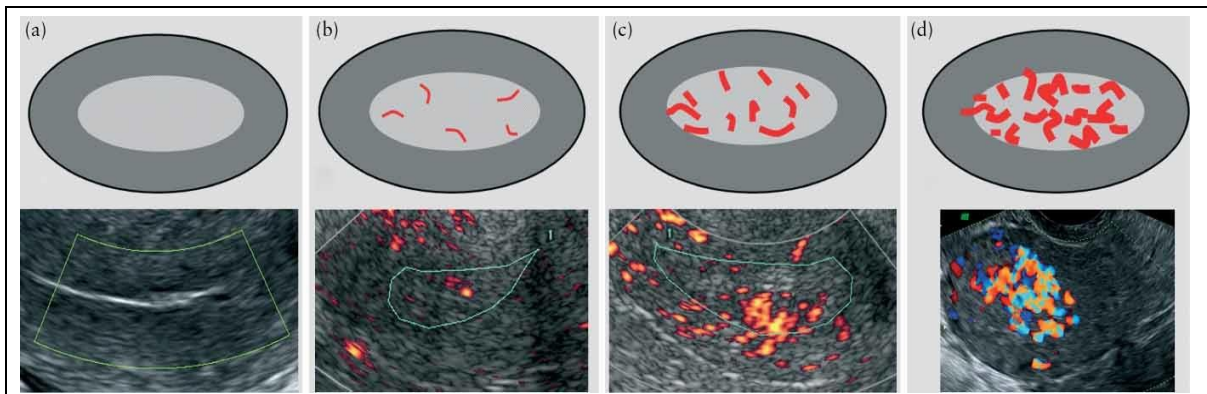
**Type 4:
Intermediate to
homogenous**



**Type 5:
Homogenous**

Initial stages in the development of the endometrial “pattern recognition”
classification system

Appendix 4: Colour doppler using IOTA setting and scoring system (Leone et al. 2010)



Colour Doppler assessment of the endometrium:

A colour score of 1 is given to indicate no colour, i.e. no flow (a);

A score of 2 indicates minimal colour, i.e. minimal flow (b);

A score of 3 indicates moderate colour, i.e. moderate flow (c);

And a score of 4 indicates abundant colour, i.e. abundant flow (d).

Appendix 5: The Nurture simplified blastocyst grading system.

Grade	Morphology
A	Expanded blastocyst with thinned ZP & cavity >50% of the embryos volume, with a clear ICM and cohesive TE. No excluded or degenerate cells
B	Full Blastocyst but not yet expanded with a clear ICM and cohesive TE. No degenerate or excluded cells
C	Blastocyst with a small ICM and/or irregular TE cells. Small area of degeneration or a single excluded cell may be present or a single large vacuole in the ICM
D	Blastocyst with no ICM and/or >1 excluded cell and/or a significant area of degeneration and/or multiple vacuoles in the ICM
Cavitating	Any sign of cavitation but ICM and TE not yet visible
Compacting	Any sign of compaction
Cleavage	Cells clearly visible and no sign of compaction

Appendix 6: Updated Blastocyst classification system (The fertility partnership)

Grade	Morphological Features
A	Expanded blastocyst with thinned ZP & cavity >50% of the embryos volume, with a clear ICM. TE consists of many cells and is cohesive.
B	Full Blastocyst but not yet expanded with a clear ICM. TE consists of many cells and is cohesive.
C	Blastocyst with a small ICM and/or TE consisting of fewer cells and is loosely cohesive.
D	Blastocyst with no ICM and/or TE consists of very few cells and is non-cohesive.
E	Any sign of cavitation but ICM and TE not yet visible
F	Any sign of compaction
Cleavage	Separate cells clearly visible and no sign of compaction

Appendix 7: Presentations

Poster presentations

The relationship between endometrial pattern on ultrasound, histology and microarray in endometrial biopsies at the at the British fertility conference in Edinburgh, UK in January 2024.

The prognostic accuracy of endometrial patterns on ultrasound to predict implantation at the at the British fertility conference in Edinburgh, UK in January 2024.

Endometrial ultrasound pattern to predict pregnancy in women undergoing embryo transfer: a systematic review and meta-analysis at the 34th World Congress on Ultrasound in Obstetrics and Gynaecology in Seoul, Korea in October 2023

The prognostic accuracy of endometrial patterns on ultrasound to predict implantation at the 34th World Congress on Ultrasound in Obstetrics and Gynaecology in Seoul, Korea in October 2023

The relationship between endometrial pattern on ultrasound, histology and microarray in endometrial biopsies at the 34th World Congress on Ultrasound in Obstetrics and Gynaecology in Seoul, Korea in October 2023

The endometrium on ultrasound. April 2021. IVF Worldwide Online congress via GE

Oral presentations

Oral poster presentation at the British fertility conference in Edinburgh, UK in January 2024. Endometrial ultrasound pattern to predict pregnancy in women undergoing embryo transfer: a systematic review and meta-analysis.

Oral poster presentation at the Royal college of obstetricians and gynaecologists world congress in June 2021 (online). Study on the inter-rater reliability of endometrial patterns on ultrasound.

Oral presentation at the Sue Watson presentation event: (Ultrasound Determinants of Endometrial receptivity) on 24/3/21, University of Nottingham, UK

Abstract accepted for oral presentation RCOG world congress 2020 (cancelled due to COVID 19. Study on the inter-rater reliability of endometrial patterns on ultrasound.

Sandpit research presentation, Study on the inter-rater reliability of endometrial patterns on ultrasound at the University of Nottingham

Appendix 8: Published and registered Protocols

Hiba Elhassan, Nick Raine-Fenning, Jim Thornton, Walid Maalouf. Endometrial pattern on ultrasound to predict successful implantation in women undergoing embryo transfer as part of assisted reproduction. PROSPERO 2021 CRD42021219015 Available

from: https://www.crd.york.ac.uk/prospERO/display_record.php?ID=CRD42021219015

Hiba Elhassan, Jim Thornton, Walid Maalouf, Nick Raine-Fenning,. The accuracy of endometrial ultrasound to predict implantation. Clinical trials NCT03860636

From: [Study Details | The Accuracy of Endometrial Ultrasound to Predict Implantation | ClinicalTrials.gov](#)

Published abstracts

Elhassan, H., Malouf, W., Thornton, J. G., Soomro, I., Castellanos, M., & Raine-Fenning, N. EP32.06: The relationship between endometrial pattern on ultrasound, histology and microarray in endometrial biopsies. Ultrasound in Obstetrics & Gynecology, 62, 304. <https://doi.org/10.1002/uog.27222>

Elhassan, H., Thornton, J. G., Malouf, W., & Raine-Fenning, N. EP32.15: The prognostic accuracy of endometrial patterns on ultrasound to predict implantation. Ultrasound in Obstetrics & Gynecology, 62, 306-307. <https://doi.org/10.1002/uog.27230>

Elhassan, H., Thornton, J.G., Malouf, W., Baig, H., Park, E., Alarbed, A., Odubamowo, K., Elhassan, H. and Raine-Fenning, N. (2023), EP32.12: Endometrial ultrasound pattern to predict pregnancy in women undergoing embryo transfer: a systematic review and meta-analysis. Ultrasound Obstet Gynecol, 62: 305-306. <https://doi.org/10.1002/uog.27227>