Advanced ultrasound in reproductive medicine

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine

by

Dr Lewis Alan Nancarrow

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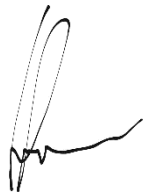
Declaration

I hereby declare that the work presented in this thesis is my own work and has not been submitted for any other degree.



Dr Lewis Nancarrow

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Reproductive medicine is a relatively young speciality in comparison to other fields of medicine. With its conception in the late 1970's there has been many advances, although there is still much to learn in this new area of medicine. Infertility affects one in seven heterosexual couples and can have huge social and financial impacts on a patient's life.

Ultrasound is a key component to the daily management of patients undergoing assisted reproductive treatments and the aims of this thesis are to highlight the importance of this vital tool and the additional benefits that can be gained from it when it is used to its full potential.

Initially, we created a questionnaire to assess embryo transfer technique in different units throughout the UK. This survey consisted of 38 questions, assessing various aspects of the embryo transfer technique. We had a good response rate of 47/79 (57%) and whilst reassuring practices were used in UK (i.e. ultrasound guided embryo transfers, soft transfer catheters), there was a large degree of discordance between different units. This variation in technique could explain the differences in live birth rate between 11-34% per embryo transfer and highlights the need for development of a standardised approach to embryo transfer, to ensure that evidence-based practice is followed in all units to optimise patient outcomes across the UK.

We performed a randomised controlled trial (RCT) comparing 4D ultrasound guided embryo transfers vs the clinical touch technique. We found a significant improvement in live birth rates in the 4D group vs the clinical touch technique group (41% vs 28% respectively, p=0.02). We also measured endometrial volume and thickness at the time of embryo transfer in the 4D group and found that it had no predictive value on pregnancy outcome, in keeping with other published literature.

In the final results chapter we performed an observational study assessing pregnancy site location and trophoblastic thickness and its impact on pregnancy outcomes. We recruited 300 patients at their initial early pregnancy scan following embryo transfer. We took a 3D image of the uterus and measured where the pregnancy was located in the cavity and also trophoblastic thickness. Miscarriage rates were higher in those pregnancies located in the lower half of the uterus and whose trophoblastic invasion was thinner.

To conclude, my work has highlighted the importance of using advanced ultrasound techniques in reproductive medicine. We recommend further studies comparing 4D ultrasound guidance vs 2D ultrasound guidance, as well as assessment of other ultrasound

features and biomarkers at early gestation scan to identify those at risk of complications in pregnancy so that they can be appropriately counselled and managed to limit both maternal and neonatal morbidity.

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List of abbreviations

2D: Two-dimensional

3D: Three-dimensional

4D: Four-dimensional

AFC: Antral follicle count

AGA: Appropriate for gestational age

AMH: Anti-mullerian hormone

ART: Assisted reproductive technologies

ASRM: American society of reproductive medicine

BFS: British fertility society

BMI: Body mass index

BPR: Biochemical pregnancy rate

COS: Controlled ovarian stimulation

CPR: Clinical pregnancy rate

CT: Computed tomography

CTT: Clinical touch technique

ELCS: Elective caesarean section

EMCS: Emergency caesarean section

EMT: Endometrial thickness

EMV: Endometrial volume

ERA: Endometrial receptivity array

ESC: Endometrial stromal cells

ESHRE: European society of human reproduction and embryology

ET: Embryo transfer

FHR: Fetal heart rate

FSH: Follicle stimulating hormone

GDM: Gestational diabetes mellitus

GnRH: Gonadotrophin releasing hormone

GnRHa: Gonadotrophin releasing hormone agonist

hCG: human chorionic gonadotrophin

HFEA: Human fertilisation and embryology authority

ICSI: Intra-cytoplasmic sperm injection

IUGR: Intrauterine growth restriction

IVF: In-vitro fertilisation

LBR: Live birth rate

LGA: Large for gestational age

LH: Luteinising hormone

LIF: Leukemia inhibitory factor

MIP: Maximal implantation point

MRI: Magnetic resonance imaging

MSD: Mean sac diameter

NHS: National health service

NICE: National institute for health and care excellence

OHSS: Ovarian hyperstimulation syndrome

OR: Odds ratio

PET: Pre-eclampsia

PGD: Preimplantation genetic diagnosis

PGT: Pre-implantation genetic testing

PGT-A: Pre-implantation genetic testing for aneuploidies

PGT-M: Pre-implantation genetic testing for monogenic disease

PUL: Pregnancy of unknown location

RCT: Randomised controlled trial

SGA: Small for gestational age

SPSS: Statistical package for the social sciences

TA: Transabdominal

TOP: Termination of pregnancy

TRV: Trophoblastic volume

TSH: Thyroid stimulating hormone

TT: Trophoblast thickness

TV: Transvaginal

UAPI: Uterine artery pulsatility index

UGET: Ultrasound guided embryo transfer

uNK: Uterine natural killer cells

WOI: Window of implantation

ZIFT: Zygote intrafallopian transfer

Chapter 1: Introduction

General introduction

Assisted reproduction is a dynamic field of medicine, which allows many couples with fertility problems to achieve parenthood. Over 8 million babies have been born as a consequence of assisted reproductive technologies (ART)[1] and in the future, as the population continues to delay childbearing until later in their reproductive life, the need for in-vitro fertilisation (IVF) will continue to grow[2]. Ultrasound is a key tool that is frequently used in everyday activities, in any IVF centre providing assisted reproductive services and this thesis focuses on examining some of the new roles that ultrasound may have in improving our current understanding and in predicting the outcomes of ART.

Infertility

Infertility is defined as a disease of the reproductive system with a failure to achieve a clinical pregnancy after 12 months or more of regular unprotected intercourse[3]. Infertility is a common occurrence in the UK with one in seven heterosexual couples affected [4], which can lead to distress, depression, as well as discrimination and ostracism with associated costs to individuals and society[5].

The estimated cost of ART in the UK is approximately £250 million per year (based on 0.13% of healthcare expenditure being used for ART) [6, 7]. The cost of a privately funded single IVF treatment cycle in the UK is estimated to be on average around £5000[8]. This, therefore accounts for roughly 18% of the average disposable income of a UK resident per year[6]. The cost per live birth reflects the relationship between the cost and the success of treatment and is often referred to as the cost-effectiveness ratio[6]. In the UK it is £32,696, which implies the significant cost to the health service and also to the patient if they are ineligible for publicly funded treatment[6].

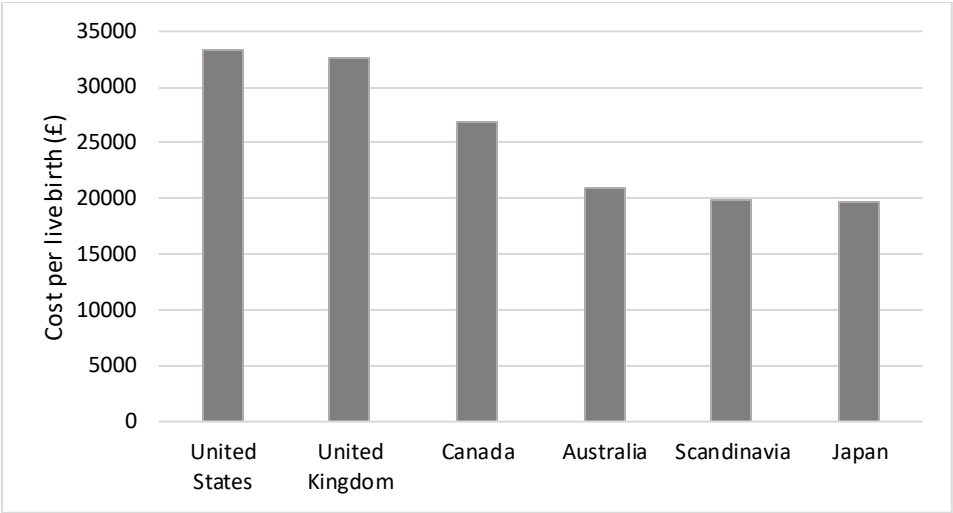


Figure 1. Cost effectiveness of IVF in developed countries[6]

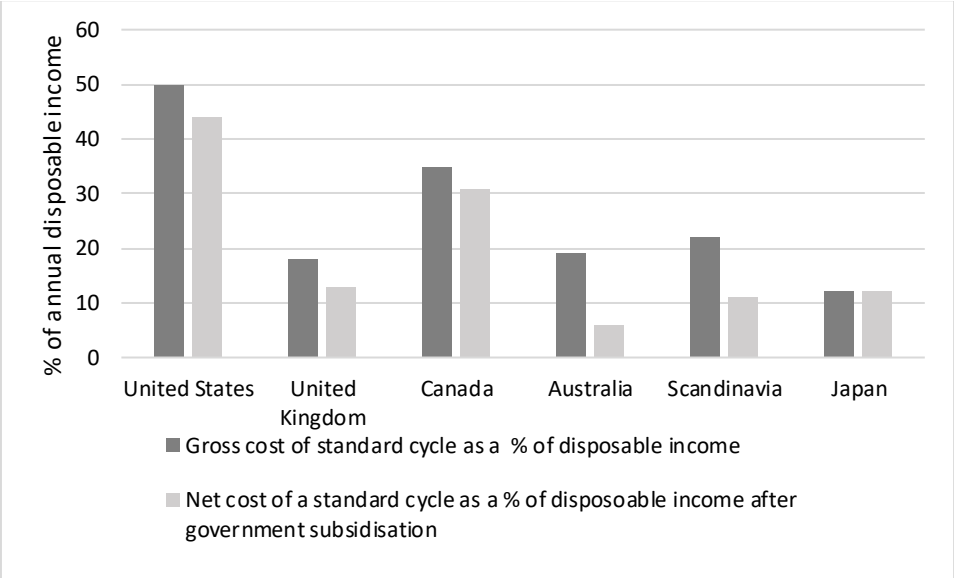


Figure 2. Average cost of a standard IVF cycle as a percentage of disposable income[6]

The known causes of infertility can be split into the individual causative factors relevant to either male or female. Infertility may also be the result of both the male and female partner having factors affecting their fertility and this is known as combined factor infertility. Whilst in other cases, if there is no evident cause of infertility identified, this is known as unexplained infertility. The prevalence of these causes is shown in Table 1.

Table 1. Causes of infertility [9]

Factors	Percentage (%)
Combined factors	40
Male Factors	26-30
Ovulatory dysfunction	21-25
Tubal factors	14-20
Other (e.g., cervical factors, peritoneal factors and uterine abnormalities)	10-13
Unexplained	25-28

Considering these particular conditions, treatment for infertility can be divided broadly into 3 particular types:

(1) Medical treatment, including methods such as ovulation induction;
(2) Surgical treatment, such as laparoscopic treatment for endometriosis or repair of tubal damage;
(3) ART such as IVF[4].

Fertility background

ART techniques aim to replicate the physiological processes involved in the human reproductive cycle[10-12] by following the below principles.

Human menstrual cycle and conception

The human menstrual cycle is typically 28 days long of which menstruation is the defining feature [13]. It is regulated by the cyclical and concert activity of the hypothalamo-pituitary-ovarian axis, working on the end effector organs, the ovary and the endometrium[14, 15]. There are two phases to the normal menstrual cycle, that can be defined by the changes in the ovary, the follicular phase, followed by the luteal phase; or by the endometrial changes, classified as the proliferative and secretory phases[13, 16-18].

The follicular phase

The follicular phase, begins on the first day of menses and ends at ovulation[13, 18]. Follicle stimulating hormone (FSH) starts to rise at the end of the previous menstrual cycle following a decline in steroid production from the corpus luteum. FSH, like all gonadotrophins, is a glycoprotein which is composed of an alpha and a beta subunit[19]. The alpha subunit in FSH is composed of the same 92 amino acids that are present in the alpha subunits of thyroid stimulating hormone (TSH), human chorionic gonadotrophin (hCG) and luteinising hormone (LH), whilst the beta subunits are unique to each gonadotrophin[20]. Secretion of FSH by the gonadotropic cells of the anterior pituitary, stimulates the recruitment and development of a cohort of preantral follicles within the ovaries into antral follicles[13]. FSH attaches to the membrane bound G-protein coupled FSH receptors in the granulosa cells on preantral follicles of the ovary, exerting the effect of activating the aromatase enzyme[13, 20, 21]. Aromatase enzyme converts androgens to oestrogen, leading to the subsequent release of oestrogen[13, 20]. As the follicular phase progresses, FSH stimulates growth of the antral follicle, leading to an increase in the number of granulosa cells and as a consequence an increase in oestrogen production. Development of a dominant follicle occurs during this time and is described in three stages: 1 – Recruitment, 2- Selection and 3-Dominance[13, 20, 22, 23]. Recruitment occurs from day 1-4 of the menstrual cycle and refers to the emergence of medium sized follicles between 2-5mm[13, 22]. Selection is where the largest of the recruited antral follicles continues to grow whilst the remaining follicles undergo atresia, which occurs around day 5-7[13, 22]. Dominance occurs by day 8 and is where the dominant follicle continues to increase in size, secreting higher levels of oestrogen and inhibiting the growth of the other subordinate follicles. This increase in oestrogen secretion, particularly estradiol-17β, engages in a negative feedback effect on the pituitary reducing the production of FSH[22]. The consequent decline in FSH limits subordinate follicular growth both by lack of direct stimulation and indirectly by creating a more androgenic microenvironment, leading to atresia of these subordinate follicles [13, 14, 22, 24]. It has been hypothesized that dominant follicles contain more granulosa cells and FSH receptors, therefore are more susceptible to FSH stimulation than the smaller subordinate antral follicles[22, 25].

The dominant follicle is responsible for 90% of the oestrogen production at this point, and rising oestrogen levels lead to the induction of the transmembrane LH receptors on the dominant follicle granulosa cells[22, 26]. This makes the granulosa cells more responsive to

LH stimulation and less dependent on the falling levels of FSH, which occurs due to negative feedback on the pituitary by the increased follicular oestrogen production.

Following selection, the dominant follicle increases in size with continued growth, until it reaches its pre-ovulatory stage, with a diameter of around 16-29mm[22, 27]. This is mediated by continued oestrogen release due to inhibin A secretion, along with increased aromatase activity due to increased granulosa cell numbers and secretion of insulin-like growth factor[22, 24, 28, 29].

The rise in oestrogen has a positive feedback effect on LH production [30]. It is unclear whether this is specific to the alpha or beta oestrogen receptor or both, but the rise in oestrogen stimulates gonadotrophin releasing hormone (GnRH) production from the hypothalamus, as well as directly stimulating release of LH from the anterior pituitary [30]. Oestradiol also acts by sensitizing the pituitary to GnRH by increasing GnRH receptors on the pituitary gonadotrophs[31] and potentially inhibiting GnRH metabolism[32]. Once oestrogen levels reach 200 pg/mL for more than 50 hours then LH begins to be secreted[13, 22, 30]. When oestradiol levels reach their peak, the positive feedback this has on the hypothalamus and pituitary gland results in the LH surge , which is the ovulatory signal for the dominant follicle and thus, results in subsequent ovulation[22].

Ovulation

The LH surge commences roughly 34-36 hours prior to ovulation, with the peak occurring between 12-24 hours prior to ovulation[13, 22]. The LH surge also stimulates the oocyte for final development and completion of meiosis 1 and expulsion of the first polar body preparing for fertilisation[33]. Luteinisation of the granulosa cells also occurs with the subsequent release of progesterone which is responsible for the midcycle FSH surge [13]. The combination of LH and progesterone leads to the production of prostaglandins and proteolytic enzymes that break down the collagen in the follicular wall, resulting in the release of the oocyte-cumulus complex[13, 33, 34]. The mid-cycle FSH surge is also thought to increase granulosa cell LH receptors whilst also freeing the oocyte from follicular attachments[13]. Oestradiol levels fall prior to the LH peak, potentially due to downregulation from LH or from inhibition from rising progesterone levels[13, 35].

The released oocyte is collected by the fimbrial end of the fallopian tube and is transported towards the uterus by ciliary motility and contractions of the tubal smooth muscle[36, 37].

Fertilisation takes place in the fallopian tube where the oocyte meets with the ascending sperm.

The luteal phase

The luteal phase occurs after the LH surge and usually last around 14 days. Following ovulation, the remaining granulosa cells still within the follicle become enlarged and vacuolated in appearance. This process is called luteinisation and results in the terminal differentiation of the mural granulosa and theca cells into luteal cells, forming the corpus luteum[38]. This differentiation from granulosa cell to luteal cell switches the primary function of the cell from a predominately secreting oestrogen to secreting progesterone. This occurs with the cessation of granulosa and theca cell proliferation by downregulating cyclin D2 and is followed by hypertrophy of these cells[38]. The LH surge causes a downregulation of genes specific to the granulosa cell, whilst upregulating those genes specific to luteal function[38]. Genes downregulated as a consequence of the LH surge include *Cyp19a1*[38, 39] and Inhibin A[40] both of which are key to the production of oestrogen[13]. Meanwhile, there is increased expression of the enzyme *Cyp11a1* and the cholesterol mobilizing protein *Star* both of which are required for the synthesis of progesterone[38, 41, 42]. The corpus luteum is a temporary endocrine organ which signals and prepares the oestrogen primed endometrium for the arrival and implantation of a fertilised ovum[13, 34]. Peak function of the corpus luteum occurs roughly between 8-9 days following ovulation, which correlates with the endometrial implantation window[13, 34]. This peak in function is shown with raised progesterone and oestradiol levels along with peak vascularity of the corpus luteum as seen on ultrasound scan[13, 33]. Both the luteinised theca and granulosa cells are involved in steroidogenesis; however, it is thought the larger granulosa cells are the greater contributors[13, 34]. Continued LH secretion maintains the corpus luteum in the interim period until implantation. If no pregnancy implants then no hCG is released and the corpus luteum will undergo luteolysis, forming the corpus albicans[13, 34]. This occurs roughly 9-11 days following ovulation and can be due to a number of luteolytic agents including oxytocin, vasopressin, prostaglandin F2α and matrix metalloproteinases [13, 43-45]. The declining function of the corpus luteum and reduced progesterone release results in constriction of the spiral arterioles supplying the endometrium[13, 14], leading to tissue ischaemia and subsequent sloughing of the functional unit, the luminal and functionalis layers of endometrium resulting in menses[13, 14].

However, if fertilisation does occur, the fertilised ovum is transported along the fallopian tube into the uterine cavity where it implants within the endometrium[36]. hCG starts to be produced from the syncytiotrophoblasts once successful embryo implantation has occurred and this in turn promotes the continued function of the corpus luteum and luteal support required to maintain the endometrium, and as a consequence supporting the early pregnancy[36].

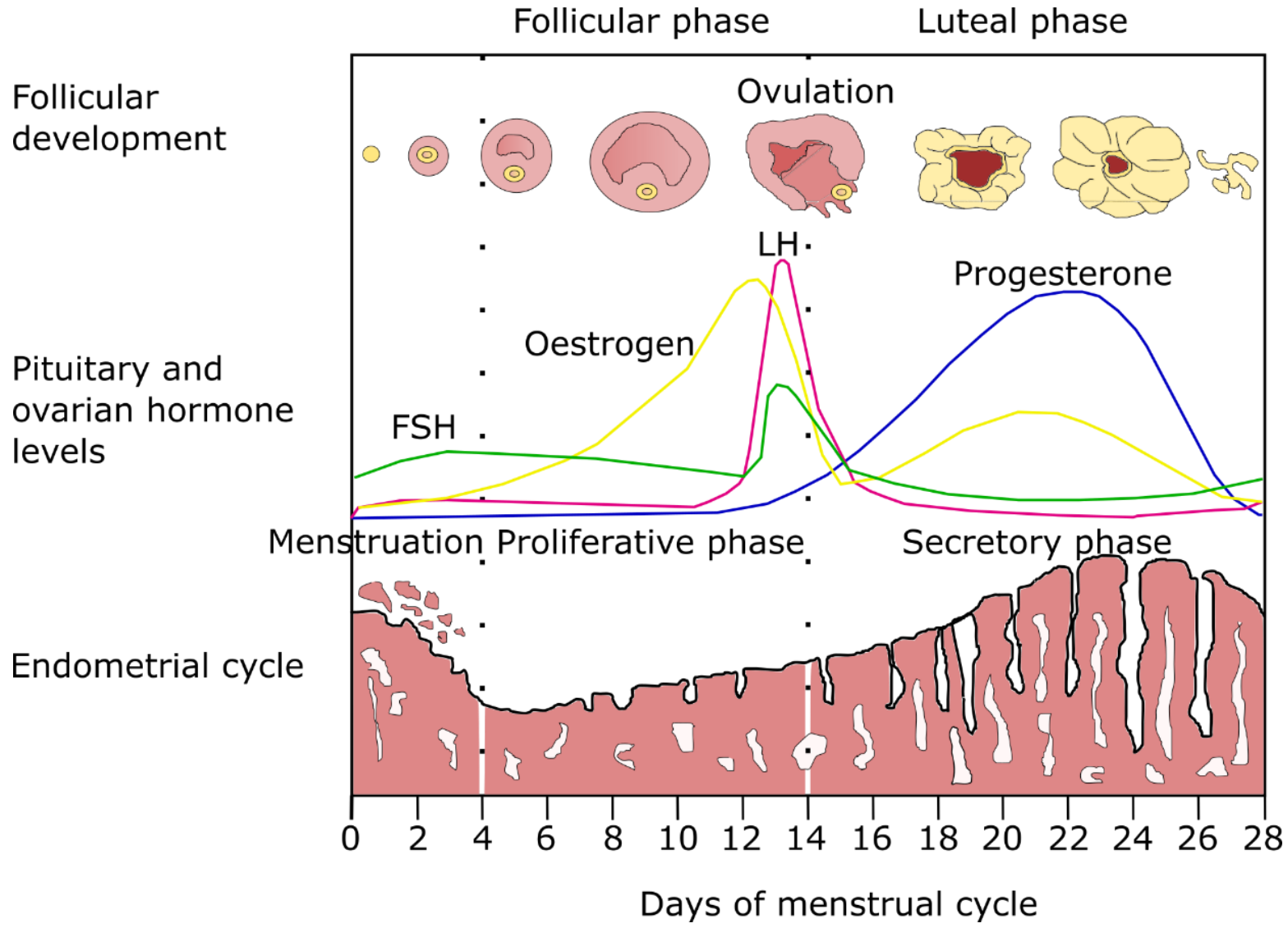


Figure 3. The menstrual cycle

Implantation

Preconditions for implantation

During the follicular and luteal phase of the ovarian cycle, the endometrium undergoes both structural and functional remodelling with both oestrogen and progesterone being key hormonal regulators facilitating these changes[46]. During the proliferative (follicular) phase there is a regeneration of the endometrium under the influence of rising oestrogen levels with proliferation of the epithelium, stroma and vascular endothelium[46]. The rising levels of oestrogen also helps with the expression of the progesterone receptor, enabling the endometrium to be able to respond to progesterone during the secretory (luteal) phase. Progesterone plays a key role in the maintenance of endometrial homeostasis via signalling of stimuli following attachment to the progesterone receptor. Progesterone also has a morphological impact on the endometrial stromal cells (ESC) converting them from fibroblast-like mesenchymal cells to secretory epithelioid like cells in the mid-secretory phase[47, 48]. This process is called decidualization and these newly formed decidualized ESC are essential for implantation, conferring immunotolerance to the foetal semi-allograft, controlling trophoblast invasion and also nourishing and protecting the peri-implantation conceptus[48, 49]. Decidualization occurs approximately 6 days after ovulation just prior to the window of implantation (WOI)[47]. This usually occurs between day 20-24 of a regular menstrual cycle[37, 48] indicating the optimal time for embryo implantation and is dependent on the functional communication between a blastocyst and the receptive endometrium[37].

During the WOI, the receptive endometrium expresses a number of different genes that encourage implantation of the fertilised ovum, now called a blastocyst. As the blastocyst enters the uterine cavity from the fallopian tube, it adheres to the apical surface of the epithelium and penetrates into the underlying sufficiently decidualised uterine stroma[37]. Implantation process, thus can be divided into 3 different stages: apposition; adhesion/attachment and invasion/penetration [37].

Apposition

Apposition is the first step of implantation when communication between the endometrium and blastocyst begins. As the blastocyst enters the uterine cavity, it expresses adhesion molecules such as L-selectin and it begins to roll around the endometrium[37]. These adhesion molecules ensure that the blastocyst can tether itself to

the uterine epithelium by interacting with the L-selectin oligosaccharide-based ligands, as well as trophinin and heparin-binding epidermal growth factor, that are present on protrusions of the epithelial cells, which are called pinopodes [37, 50]. As the blastocyst begins to adhere to these pinopodes, suitable alignment is required in relation to inner cell mass of the embryo to ensure proper apposition is achieved [37]. Also present is a pericellular matrix of glycoproteins and glycolipids from the luminal epithelium which also aid apposition [37]. Mucin-1 is one of these molecules and acts as an anti-adhesion molecule, by preventing apposition of the embryo in less favourable locations within the uterine cavity [37].

Adhesion/attachment

Removal of endometrial mucins along with others seem to be necessary for successful blastocyst adhesion to the luminal epithelium of the endometrium [37, 50]. During the adhesion phase the blastocyst induces cleavage of Mucin-1 at the implantation site to enable successful attachment [51]. A number of chemokines and cytokines are necessary for adhesion and they work by attracting the blastocyst to the area of implantation, with one of the most important proteins identified being Leukemia inhibitory factor (LIF) [37, 52]. It reaches maximal levels during the mid-secretory phase of the menstrual cycle and studies have shown that deficiencies in LIF are more common in those women who are infertile [53]. There have been a number of studies identifying the important role of LIF in implantation, with mice that were null for the LIF failing to implant embryos or appropriately decidualize the uterus [52, 54, 55]. Integrins are also reported to be necessary for adhesion to the pinopodes of the epithelium aiding implantation. Heterodimer αVβ3 is one such integrin and is expressed both by the trophoblast cells of the blastocyst as well as the luminal epithelial cells and it works by encouraging recognition of the blastocyst from the endometrium [37]. Abnormal expression of this integrin is associated with conditions associated with reproductive failures, e.g., recurrent miscarriage and infertility [56].

Invasion

In this phase, the trophoblast cells of the blastocyst, penetrate the endometrial luminal epithelium to gain entrance into the endometrial stroma with the aim of reaching the maternal vasculature [37]. Thin folds develop on the trophoblast cells called invadopodia and these grow between adjacent endometrial epithelial cells, degrading the basement membrane and allowing for trophoblastic invasion into the endometrial stroma [37]. As the

trophoblast cells invade and proliferate into the endometrial stroma, they begin to differentiate into the inner cytotrophoblast cells and the outer syncytiotrophoblast cells. The blastocyst continues to invade into the stroma and is usually completely embedded 8 days after ovulation[37]. Syncytialization occurs whereby the syncytiotrophoblasts invade the luminal epithelium creating fluid filled spaces that are separated by trabeculae [37]. The cytotrophoblast cells proliferate within the trabeculae, which are arranged radially from the blastocyst, creating the primary chorionic villus. This progresses onto the formation of secondary and tertiary villi, which is part of process of placentation [37].

Abnormalities in the above-described processes can have a significant impact on a couple's ability to become pregnant, making it necessary for intervention from appropriate specialists to help patients to conceive.

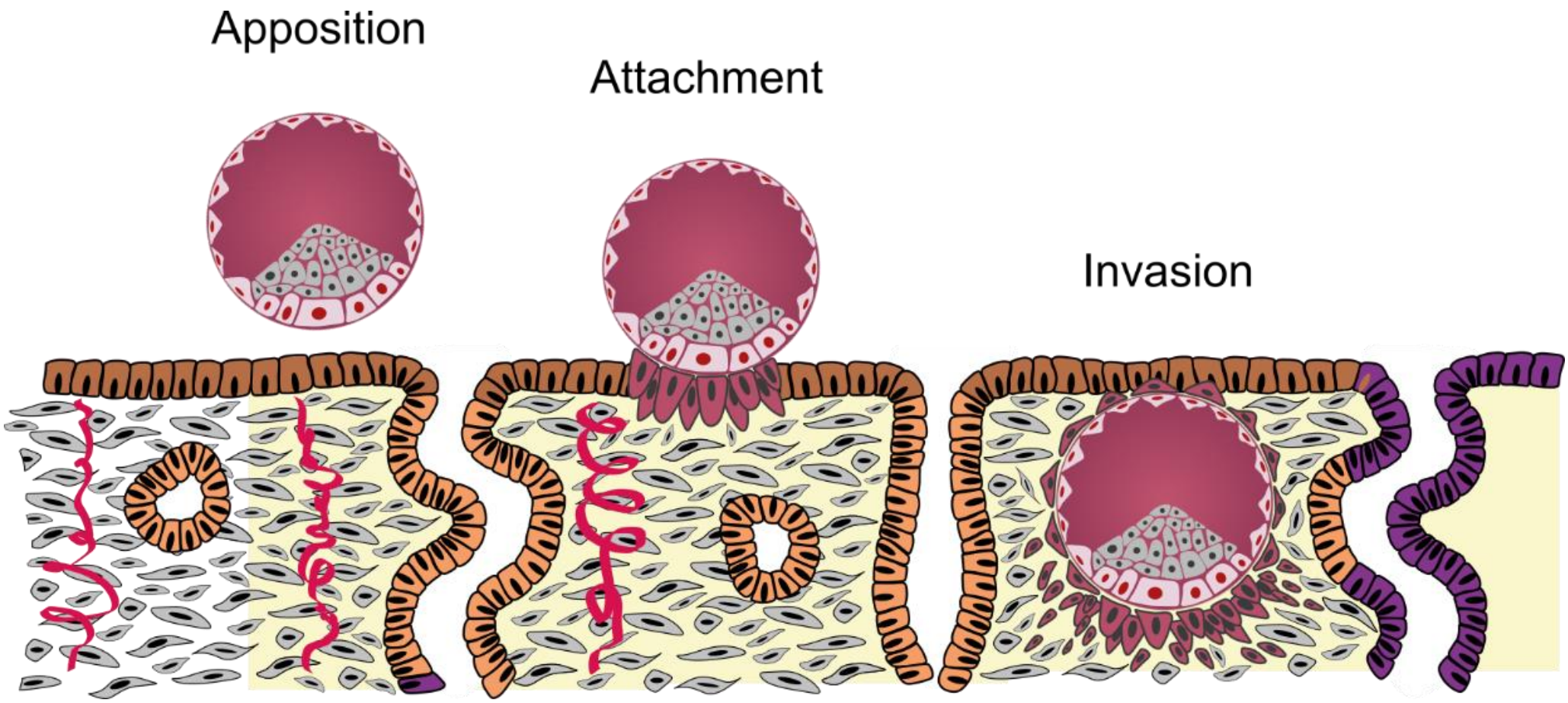
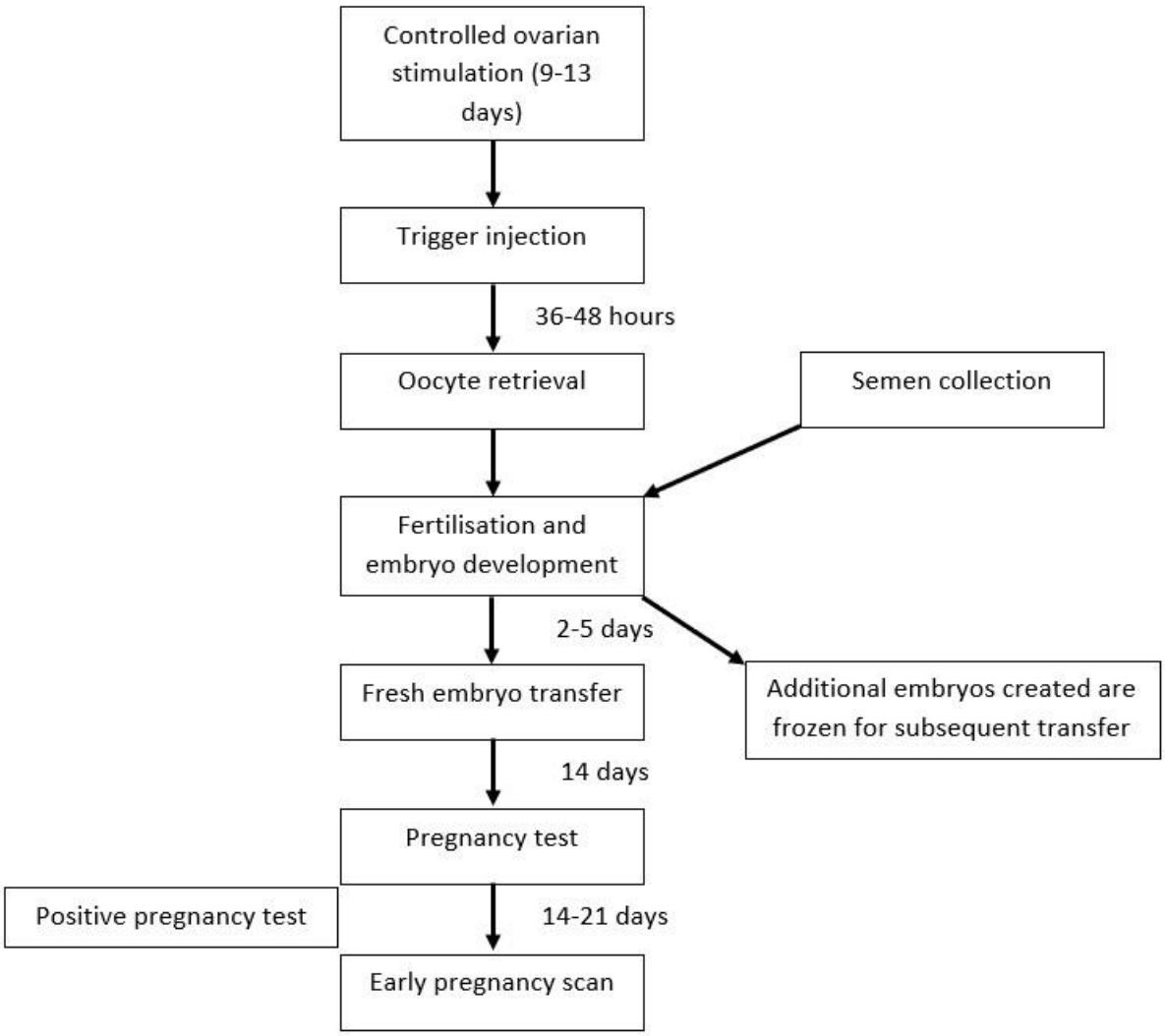


Figure 4. Implantation (Adapted from Tempest et al. 2021) [57]

The IVF process

There are distinct stages within the IVF process that involves attainment of gametes (egg and sperm), creation of an embryo, and replacement of that embryo in to a synchronized and receptive endometrium in hope of subsequent implantation and pregnancy [58]. Those distinct stages and their history and advances are described in more detail below.

What does IVF involve?



Controlled ovarian stimulation (COS)

Originally, as part of IVF, there was no ovarian stimulation, patients were monitored during their regular menstrual cycle by measuring LH levels in their urine to determine when ovulation was due to occur[59]. Oocyte retrieval was then done as soon as the LH rise was

detected, but this method was imprecise, with up to 30% of patients ovulating prematurely and losing the oocyte[58].

Controlled ovarian stimulation (COS) is a process with the aim of ensuring development of an optimal number of ovarian follicles leading to the collection of mature oocytes[58] and is the first step in IVF. If a small number of oocytes are collected, then chances of a live birth are reduced. In the study by Dhillon et al the live birth rate (LBR) for patients with 2 versus 20 eggs was 20% vs 80% respectively[60]. However, the induction of ovulation is a delicate balance, as an excessive follicular response is associated with higher risk of ovarian hyperstimulation syndrome (OHSS), which is a potentially serious, life-threatening condition for the woman[58]. OHSS is characterised by an increased capillary permeability which can cause a major fluid shift from the intra-vascular compartment into the extravascular areas, which includes the abdominal cavity, lungs and pericardium. This results in a hypovolaemic state which if not managed appropriately can lead to multiorgan failure, whilst also increasing the risk of venous thromboembolism[61]. Therefore, careful treatment planning to achieve the maximum number of mature oocytes without compromising the woman's health is paramount.

Since its introduction to ART, there has been a number of COS protocols developed including "long" gonadotrophin releasing hormone agonist cycles to (GnRHa), "Short" GnRH antagonist and also Co-flare cycles[58]. Monitoring of stimulation has also improved with ongoing advancement in ultrasound technology and hormonal monitoring[58]. The cycle type that is most commonly used is the short cycle, this has the benefit that it is, by definition, shorter in duration in comparison to the long cycle and also allows for the use of a GnRH-agonist trigger, which can significantly reduce the chance of OHSS, particularly with those patients deemed most at risk[58].

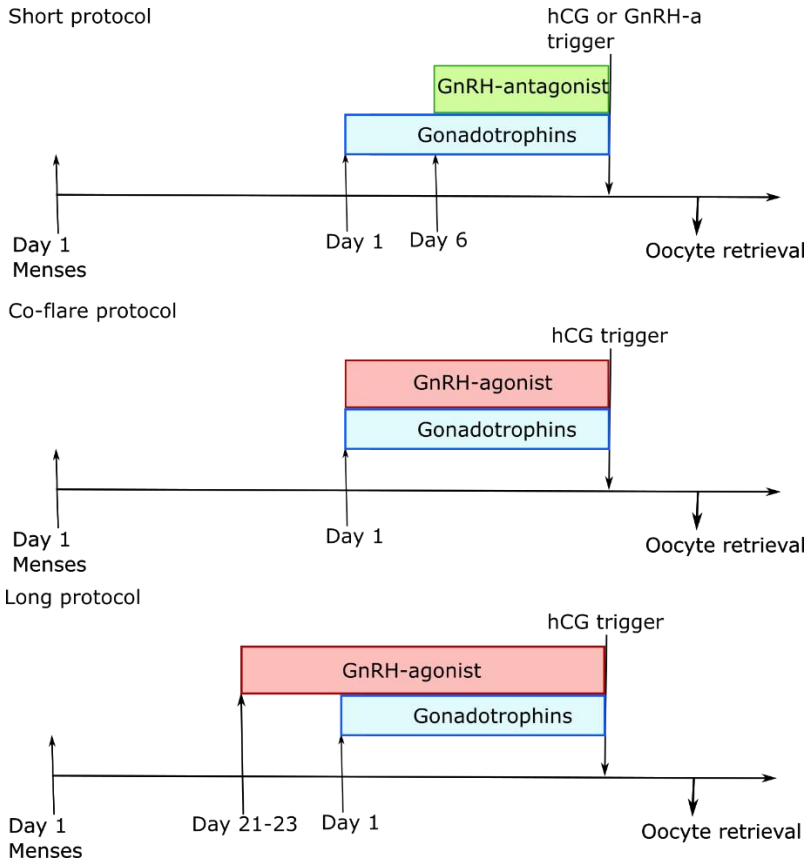


Figure 5. Short, long and Co-flare stimulation cycles

COS involves administering exogenous gonadotrophins to stimulate follicular growth within the ovaries until a certain level of maturity is attained [58]. The types of gonadotrophins may differ, depending on the drug(s) used, with the predominant ingredient of these drugs being FSH although they often contain smaller amounts of LH through the purification aspect[62]. The dose of gonadotrophin administered is routinely determined on factors such as a patients age, body mass index (BMI) and ovarian reserve testing (anti-mullerian hormone (AMH) level and antral follicle count (AFC))[63]. As previously highlighted, the aim is to retrieve the optimal number of oocytes whilst not overstimulating and causing the woman to develop OHSS[63]. Ultrasound imaging is the predominant method for monitoring the response of the ovaries to the stimulation medication [64]with most IVF units aiming for between 12 and 15 oocytes[65-68]. During the COS cycle, GnRH-agonists (e.g. Buserelin) or GnRH antagonists (e.g. Cetrotide) are used to prevent premature ovulation and the consequent loss of oocytes in to the pelvic cavity due to spontaneous ovulation[58]. Once the follicles have reached a target size of 17mm, it is assumed that appropriate oocyte maturity has been achieved[69, 70]. To induce ovulation, a trigger

injection of hCG or GnRH-agonist is administered to stimulate the final oocyte maturation prior to the oocyte retrieval.

Oocyte retrieval

Following the administration of the final trigger injection, oocytes are usually aspirated via a transvaginal (TV) route, usually 36 hours later[71] under ultrasound guidance[72].

Initially oocyte retrieval was performed laparoscopically, but this approach had its disadvantages. The laparoscopy, as well as the general anaesthetic are both procedures that are not without potential complications (e.g., Bowel injury and aspiration respectively), whilst also being more expensive and time consuming[58, 73].

With the development of ultrasound technology, transabdominal (TA) ultrasound guidance oocyte retrievals began in the 1981[74]. This negated the need for the more expensive laparoscopic method. However, one of the main disadvantages was the pain experienced by the patient[75]. Although local anaesthetic was used at the puncture site on the abdomen, patients still found it an uncomfortable procedure and often general anaesthesia was still required[75]. Studies showed no difference between number of oocytes retrieved between laparoscopic or TA ultrasound guided approaches[58, 75] however, the need for general anaesthetic in both resulted in a lack of appreciation and utilisation of the TA ultrasound approach[58].

This was followed on by transurethral and TV approaches to oocyte retrieval, but it was the TV approach which became the preferred route for aspiration. The advantage of using the TV ultrasound method was that it is better tolerated by the patient, it can be done under sedation or local anaesthetic[76] it allows for superior imaging of the ovary and complication rates are rare[58]. There was no need to puncture the abdominal wall or urinary bladder and the distance between puncture site and ovary transvaginally was much shorter [58].

TV ultrasound guided oocyte retrieval is now the gold standard approach and will be used in the majority of patients undergoing IVF treatment[58].

TA ultrasound and laparoscopic oocyte retrievals are still performed but are reserved for more difficult cases or where access to the ovary vaginally is poor[77, 78].

Fertilisation

In the beginning IVF was the only option for infertile couples. In IVF, fertilisation of the oocyte occurs in the lab when the semen is co-cultured together with the oocyte, with the spermatozoa fertilising the oocyte during this period of incubation[79]. However, if the semen sample is abnormal, the likelihood of fertilisation is reduced. A number of techniques were developed to assist with fertilisation in cases where semen samples were abnormal, such as zona drilling (creating a hole in the zona to allow easier access for the spermatozoa) and injection of spermatozoa into the perivitelline space (space between the zona pellucida and cytoplasm)[58], however the fertilisation levels were still low due to the inability of these methods to quickly block polyspermy (fertilisation of the oocyte by more than one spermatozoon, causing an abnormal number of chromosomes) [58]. This led to development of intracytoplasmic sperm injection (ICSI) in 1991, which allowed embryologists to physically inject a spermatozoa into the cytoplasm of the oocytes [80]. The first ICSI baby was born in 1992 and since then ICSI fertilisation rates, even for severe male factor infertility, have been comparable to conventional IVF[81].

ICSI is a more time consuming and costly process and when used in non-male factor infertility has significantly lower fertilisation and implantation rates when compared to IVF[82]. Therefore, it should be reserved only for the sub-group of patients who may benefit from the process.

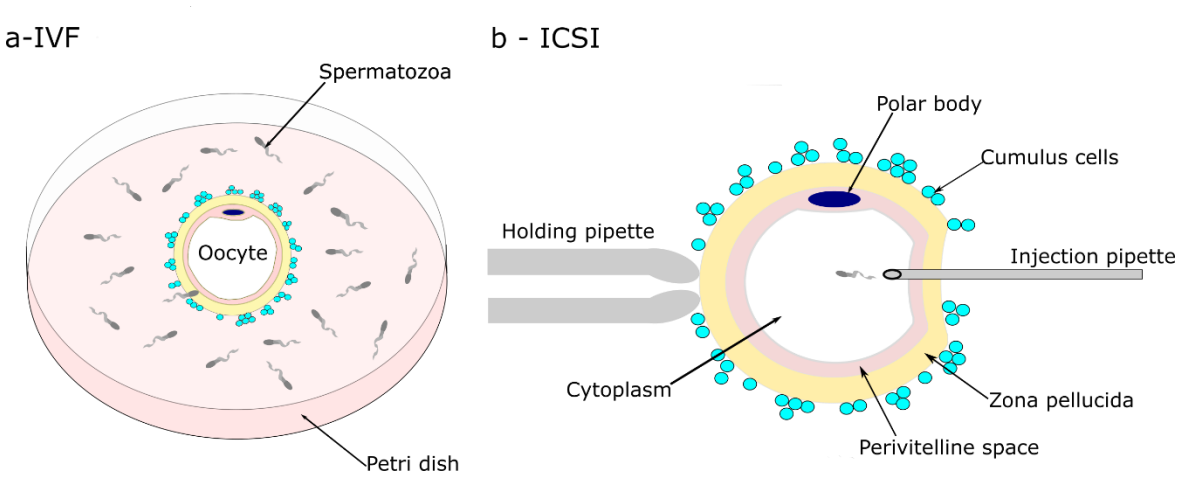


Figure 6. A- IVF fertilisation, B- ICSI fertilisation

After successful fertilisation, the resulting embryo(s) is assessed by an embryologist and will be graded based on its progress according to the well-established and expected

development pattern for early human embryos[83]. The fertilised oocyte, now called the zygote will undergo a number of mitotic cell divisions, with each cleavage of the cell resulting in the creation of another cell known as a blastomere [84].

At around day 3, around 8 blastomeres are expected to be present in the zygote, and at this stage it is called a morula[85]. The blastomeres in the morula will continue to divide to create the blastocyst, a cellular mass of around 100 cells that has a configuration of tightly compacted ball of cells. The histological organisation contains an inner cell mass, a ring of trophoectoderm cells on the periphery and a fluid filled centre known as the blastocoel[84]. Grading of the embryos is based on the Gardner and Schoolcraft method and evaluates the degree of blastocyst expansion, the consistency of the inner cell mass and the cohesiveness of the trophoectoderm[86].

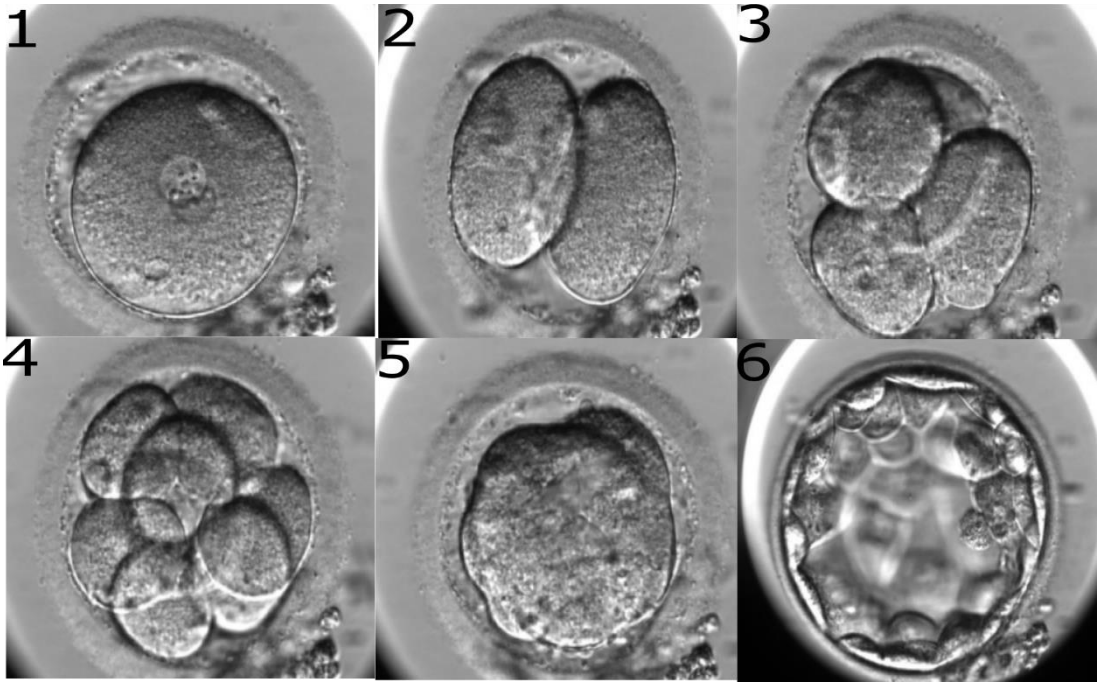


Figure 7. Embryo development. 1. Day 1 check – normal fertilisation, 2 –Day 2 – 2 cell embryo, 3- Day 2- 4 cell embryo, 4- Day 3- 8 cell embryo, 5- Day 4- Morula, 6- Day 5- Blastocyst

Timing of the ET depends on the embryos progress. Embryos can be transferred at the cleavage stage (day 2-3 days following fertilisation) or blastocyst stage (day 5-6 following fertilisation)[87]. In the past ETs had been performed at the cleavage stage[58]. This was mainly due to the suboptimal culture media that was used at the time that lacked the amino acids and vitamins that would allow the embryo to progress to the blastocyst stage

[58]. Since then, the quality of culture media has improved, allowing for continued progression of the embryo to the blastocyst stage [58]. Delaying ET until the blastocyst stage has numerous advantages including better embryo grading and selection, improved implantation rates and reduced time to pregnancy [58, 88, 89]. Cleavage stage ET are, however, still performed and can be considered in women with poor prognostic factors such as age >35 years, fewer available embryos for transfer and multiple previous failed IVF attempts [89]. This is due to their risk of losing viable embryos in the process of extended embryo culture [89].

Luteal support

Following oocyte retrieval, if fresh embryo replacement is anticipated, there is a need for luteal support [90, 91]. The current hypothesis relates to the luteal phase deficiency following IVF due to the supraphysiological levels of steroids secreted by a high number of corpora lutea during the early luteal phase [91]. This directly inhibits the LH release via negative feedback actions at the hypothalamic-pituitary axis, resulting in luteolysis [91, 92] and without additional luteal phase support, the pregnancy is likely to fail [93].

Luteal phase support can be provided in numerous different ways including oral progesterone tablets, Intramuscular progesterone injections, progesterone pessaries and hCG injections [90, 94]. There is no evidence of superiority in any of the above options, however vaginal progesterone appears to be better tolerated with less side effects than other methods of progesterone administration [94] and is consequently the most commonly used option in European countries [95]. hCG increases the risk of OHSS and is therefore not the routine first line option for luteal phase support [90, 94]. Recently the combination of progesterone and GnRHa have been used for luteal support, which has shown promising improvements in clinical pregnancy rate (CPR) and LBR. However, further high quality randomised controlled trials (RCT) are recommended before this method of support can be recommended in routine practice [90].

Embryo transfer

Once the uterine cavity has been primed with exogenous progesterone, the patient will then proceed with their fresh ET [8]. For this the patient is placed in lithotomy position and the vagina and cervix are cleaned with saline soaked cotton wool or gauze [96]. A catheter containing the embryo is then passed through the cervix and into the uterine cavity, where

the embryo is deposited[97]. This is done using a non-touch technique to avoid contamination of the catheter tip[98].

There are two predominant methods:

- Clinical touch technique (CTT), which is a 'blind' procedure where the embryo is deposited 6 cm from the external os or to the middle of the uterine cavity based on the practitioners' tactile sense[97].
- 2D ultrasound guided technique where the catheter tip is visualised on scan, aiming to deposit the embryo 1-2cm from the fundus of the uterine cavity[97].

Following the fresh ET (transfer of the embryo in the same COS cycle), patients continue with luteal support until positive pregnancy test (14-17 days after oocyte retrieval[99]), which is equivalent to being gestational age of four weeks. hCG secreted from the invading syncytiotrophoblasts will maintain the corpus luteal function until the luteal-placental shift occurs between 6-8 weeks gestation[100]. This is when the production of progesterone to maintain the pregnancy shifts from the corpus luteum to newly developing placenta[100]. Whilst many units continue luteal support beyond confirmation of pregnancy test, there is little evidence to support this practice[101].

Other IVF Advances

Laboratory advances

Laboratory techniques are continually advancing and improving the understanding and progression of embryo development[58]. Culture media has moved on from the initial media that was made to mimic the female reproductive tract[58], and have been optimised for the in-vitro growth of embryos[58]. Media previously made within IVF laboratories have now been replaced by commercially produced media, reducing manufacturing errors and batch to batch variability[58].

Embryo development has previously been monitored by removing the developing embryo from a warm jacketed incubator and placing it under a microscope. This can be done up to 5 times prior to transfer and this regular removal from the incubator can expose the embryo to undesirable changes in temperature, humidity and gas composition[102]. Since then, the monitoring has progressed, with the introduction of time-lapse imaging incubators. Time lapse imaging allows for microscopic monitoring of embryos in a controlled environment minimizing exposure and stress to the developing embryo. Time

lapse imaging incubators have built in microscopes that do not require removal of the embryo from the incubator for assessment. They can take an image of a developing embryo every 10 minutes and can potentially lead to improved morphological evaluation and identification of dynamic markers [103]. However, a Cochrane review of the current evidence concludes that there is no good quality evidence supporting the use of time-lapse imaging when compared with standard incubation monitoring[104].

In recent years, as a consequence of time-lapse imaging, artificial intelligence-based methods have been developed as an aid to embryo selection. There are multiple experimental systems that can assess embryo implantation potential, and the use of morphometric analysis by time-lapse imaging, mathematical and statistical tools, as well as computer-assisted scoring [105] are examples of some of them.

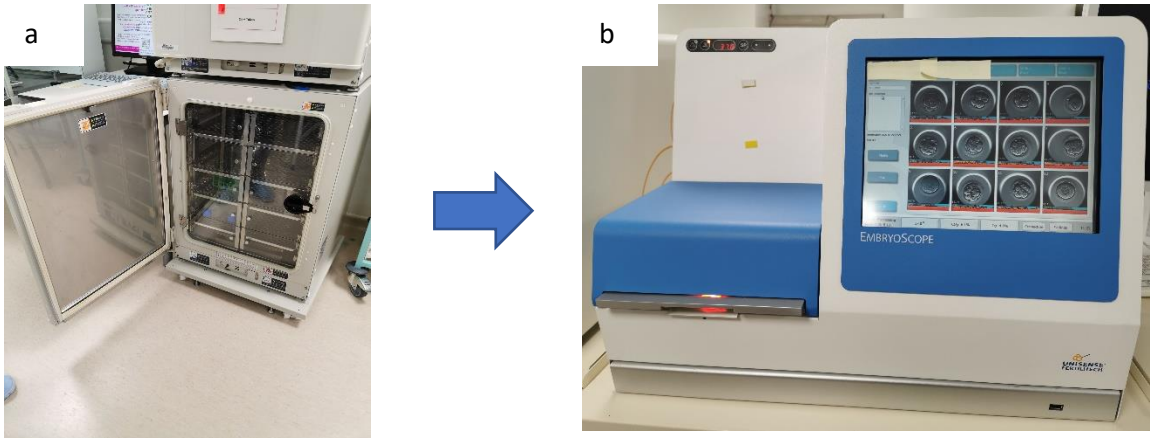


Figure 8- a. Warm Jacketed incubator and b. Time lapse incubator

Another advance in laboratory techniques is pre-implantation genetic testing (PGT). It was first developed in 1989 and allowed for the diagnosis of single gene defects in an embryo[106]. This progressed onto preimplantation genetic screening (now pre-implantation genetic testing for aneuploidies [PGT-A]) and preimplantation genetic diagnosis (PGD) (now preimplantation genetic testing for monogenic disease [PGT-M])[58]. PGT-A screens for any potential genetic disorder when one is not known (such as Down's syndrome), whilst PGD screens for a particular genetic condition that can be passed from parent to offspring (such as cystic fibrosis)[107]. This process involves taking a biopsy from the blastomere or trophoectoderm and amplifying the DNA to identify genetic abnormalities[58]. For those who are at risk of transmitting genetic disease PGT-M is of great benefit however PGT-A continues to be a topic for debate[58]. Those of increasing maternal age are more likely to have aneuploid embryos and these are known to have

poorer implantation rates and higher risk of miscarriage [108]. The theory behind PGT-A is that it improves chances of ongoing pregnancy as only euploid embryos are transferred, rather than aneuploid embryos which are known to be abnormal. However, this sort of genetic testing is not without risk, as biopsy of the embryo has the potential to cause damage to the developing embryo, although there is no evidence of any detrimental effect to the children that are born following this technique [109]. This procedure is also more costly and more time consuming and should be reserved for those patients who are of increasing maternal age, although it is not currently recommended as routine practice in either the UK or USA [110, 111]. Looking to the future, analysis of the culture media rather than an embryo biopsy may allow for a non-invasive approach to identifying genetic abnormalities [58].

Endometrial assessment and preparation

Endometrial histological assessment was introduced in the 1950s which helped provide an objective classification for endometrial dating [112]. Hysteroscopy was introduced in the 1980s which facilitated the diagnosis and management of intrauterine pathologies which could affect fertility such as polyps, fibroids and adhesions [58]. The use of ultrasound guided oocyte retrieval in 1986 prompted the further utilisation of ultrasound as a non-invasive approach to assess for endometrial morphology both before, during and after oocyte retrieval [58]. Ultrasound is the most commonly used tool employed to evaluate the endometrium, being able to assess endometrial morphology and volume, myometrial contractility and uterine perfusion [113]. However, it is unable to accurately predict molecular normalcy and implantation potential [58]. In the last twenty years there has been some advancement in implantation potential with the development of endometrial receptivity analysis. This assesses the transcriptomic signature of the human endometrium to determine when the WOI is and aims to improve synchronisation of ET with endometrial receptivity in the future [114-116].

Use of ultrasound in reproductive medicine

What is ultrasound

Ultrasound is used in nearly all branches of medicine and has a variety of both diagnostic and therapeutic uses[117]. Ultrasound works by emitting high frequency sound waves, above the level of human hearing, from ultrasound transducers that are placed either externally on the skin or internally[118]. Piezoelectric crystals are the most common element used in ultrasound transducers and work by emitting sound waves once an electric field is applied to them[119]. These crystals can also work in reverse by producing an electric field once sound waves come into contact with them. When the transducer emits a sound wave, this will be reflected back to the transducer once it comes into contact with boundaries between different tissue types (i.e., tissue, blood and bone)[119]. The electrical signals generated from these reflected beams are then processed and analysed to produce a 2D ultrasound image[120] (Figures 9 and 10).

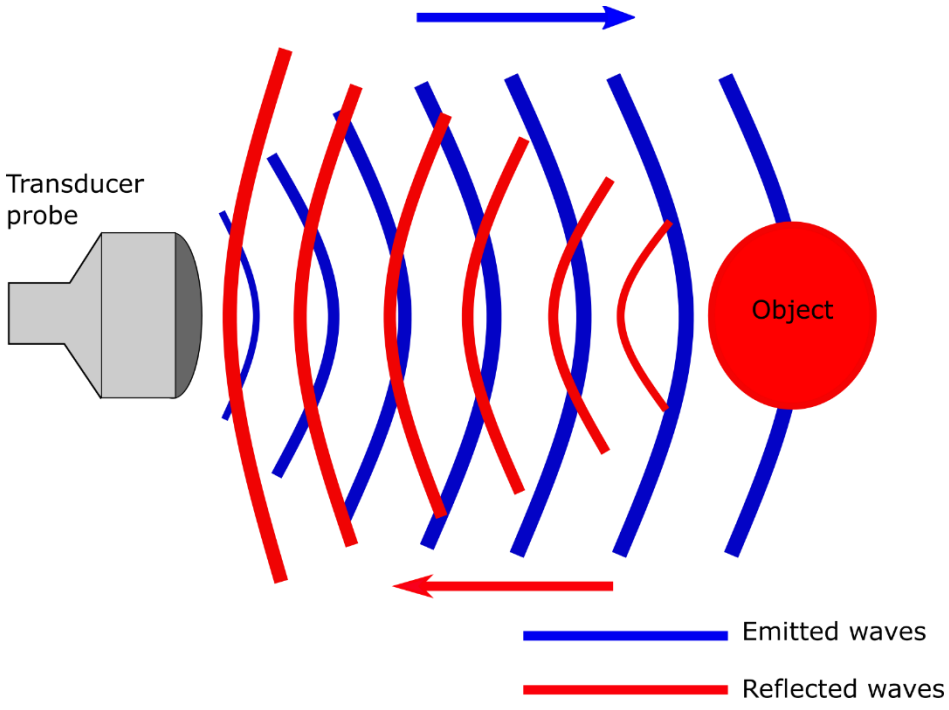


Figure 9. How ultrasound works

Figure 10. How TA ultrasound is performed

The benefit of ultrasound compared to other imaging modalities (e.g., magnetic resonance imaging (MRI) and computed tomography (CT) scanning) is that it is accessible, portable, cheaper and has a good safety profile [120].

Ultrasound in reproductive medicine

Ultrasound has been essential in nearly every aspect of reproductive medicine, since the first IVF success, back in 1978[121]. However, with advancing technology, the resolution of attained images has significantly enhanced its use in treatment from diagnosis of specific causes of infertility, monitoring of ovarian stimulation, facilitating oocyte retrieval and ET, whilst also being able to determine pregnancy location and viability[122].

Identification of pelvic pathologies

Prior to commencing fertility treatment, routine imaging of the pelvis is required to screen for pathologies known to affect conception and continuance of a pregnancy [122]. Ultrasound is a reliable test when determining pelvic pathologies, with a sensitivity of 88-100% and 86% when diagnosing ovarian cysts and hydrosalpinges respectively [122]. Identification of such pathologies can improve ART outcomes, for example, appropriate surgical management of a hydrosalpinx is of known benefit to ART success rates as the presence of a hydrosalpinx can reduce implantation rates by up to 50% [123].

Ultrasound is also able to identify the presence of uterine fibroids, polyps and congenital abnormalities, which may affect the success of IVF, although management of these pathologies remains controversial[122].

Assessment of ovarian reserve

Ovarian reserve is defined as the existent quantitative and qualitative supply of follicles in the ovaries that can potentially develop into mature follicles, which, in effect, determines a woman's reproductive potential[122]. It is important to assess ovarian reserve as it enables clinicians to identify both high and poor responders to COS, allowing for appropriate counselling and minimizing the risk of OHSS[124]. This can be assessed in a number of ways, using static measures (FSH, AMH), dynamic markers (following stimulation) or ultrasonographic markers (AFC, ovarian volume and ovarian blood flow)[124, 125].

Ovarian volume remains an area of controversy regarding its ability to determine ovarian reserve. A number of studies have shown reduction in oocytes collected and pregnancy rates in those with reduced ovarian volumes (<3cm^3)[126, 127], whereas other studies failed to show a significant difference between ovarian volumes of normal and poor responders[128, 129]. A systematic review by Broekmans et al concluded that ovarian volume has little clinical application in the prediction of poor pregnancy response[122, 130].

Antral follicle count (AFC) is based on the number of follicles in an ovary that measure between 2-9mm in size and is known to be a reliable determinant of ovarian reserve[122]. There is a strong positive correlation between AFC and AMH, and when they are combined have the potential to better evaluate ovarian reserve[131].

Ovarian blood flow as a predictive marker for ovarian response has been assessed by a few studies[132-136]. A multiple regression analysis reviewing a number of predictive variables, including AFC and ovarian stromal index (measure of intensity of blood flow), found that the best predictor of ovarian response was AFC followed by the ovarian stromal index[134]. Ovarian blood flow is rarely used clinically and is essentially a research tool[122].

Monitoring during COS

Initial monitoring of ovarian stimulation was performed by repeated hormone analysis on either serum (Estradiol, progesterone and LH) or urine (LH) samples[137]. This was an unreliable way on its own to determine follicular response and more traumatic to the

patient when it came to repeated blood samples being taken[69]. Monitoring of follicular size with ultrasound allowed a less invasive mode of monitoring whilst giving more consistent results on how the ovaries responded to stimulation. A meta-analysis in 2014 showed no additional benefit of adding hormonal monitoring to follicular tracking with ultrasound[138]. It's now routine practice worldwide to monitor ovarian stimulation with 2D transvaginal (TV) ultrasound, with 3D ultrasound techniques also being applied [122, 137].

TV ultrasound guided oocyte retrieval

The initial technique for oocyte collection has also changed since those early days of IVF. The first oocytes retrievals were performed laparoscopically, with follicles that appeared of a significant size drained [59]. Laparoscopy is an invasive surgical procedure and carries a higher risk of complication, such as bowel or vascular injury, in comparison to TV aspiration (1-12.5/1000 vs 4/1000 respectively)[139, 140]and is now rarely done in modern IVF. Laparoscopy is therefore reserved for cases where TV access to ovaries is not possible[73, 78].

Current practice now involves TV ultrasound scanning under sedation[141]. A needle is passed along the top of the ultrasound probe and is inserted through the top of the vagina into the ovarian follicles to aspirate the fluid and the oocytes contained within[141]. This approach with ultrasound guidance causes minimal trauma to the patient whilst also maintaining constant visualisation of pelvis and needle location, reducing the chances of complication and risk[73]. The added benefit in comparison to the laparoscopic technique is that dominant follicles situated within the ovary that wouldn't be visualised laparoscopically, are seen on ultrasound therefore wouldn't be missed during the procedure[142].

Additionally, during the oocyte retrieval, morphological abnormalities of the endometrium can be identified. If the endometrial thickness is too thin or if there is fluid within the endometrial cavity, this can have a negative effect on pregnancy outcome [143-145]. Ultrasound imaging allows for identification of a suboptimal endometrium and can guide management options to help improve embryo implantation rates.

With advances in ultrasonography however there can be many more uses for this vital piece of equipment and with the right expertise, information gained from ultrasound can be used to further enhance our knowledge of physiological processes involved in IVF, whilst

subsequently improving our management and success rates within this field of medicine[122, 137].

The use of Ultrasound in Embryo transfer

History of the embryo transfer

The steps and processes used for ET have changed little since the conception of IVF[59]. The first ET used a soft catheter with the embryo preloaded into the catheter, which was passed through the external cervical os into the uterine cavity, with gentle expulsion of the embryo once in position[59](Figure 11). However, since then, there has been no further progression to this particular aspect of ART[58].

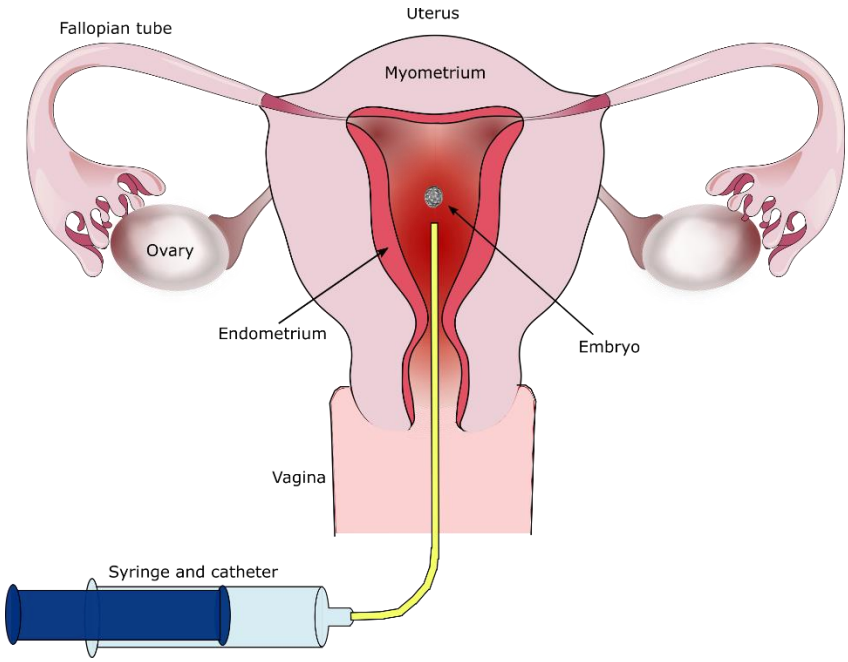


Figure 11. Embryo transfer

Why is embryo transfer so important?

It is estimated that up to 30% of all cycle failures may be due to poor practice relevant to the embryo transfer technique[146] and the pregnancy rates differ depending on the clinician performing the transfer[147].

ET is intended to be as atraumatic as possible whilst depositing the embryo at the optimal implantation site[58]. Possible trauma at the time of ET may disrupt the endometrium,

cause bleeding from the cervix or the endometrial cavity or cause pain at the time of transfer[148, 149].

Traumatic transfers are more likely to occur as a consequence of a 'difficult' transfer however the definition of a difficult transfer varies and is subjective due to individual views of different practitioners[149]. Definitions of a difficult transfer can include one or more of the following aspects of the ET:

- Degree of discomfort felt by the patient at the time of ET
- Presence of blood on catheter tip
- Need for change in the equipment used for the average ET (stylet, tenaculum, outer sheath)
- Duration of ET and if multiple attempts were required.
- The need for cervical dilatation
- Anatomical position of the uterus (significant anteversion/anteflexion or retroversion/retroflexion)
- Presence of cervical stenosis

Difficult or traumatic ET are known to be associated with increased uterine contractions, and their increased frequency negatively impacts embryo implantation rates [129]. Fanchi n et al showed that those women with < 3 uterine contractions (visualised by ultrasound) per minute at the time of ET had a CPR of 53% in comparison to those who had >5 contractions per minute only had a CPR of 14%[150].

The frequency of contractions is increased, when using a tenaculum to manipulate the cervico-uterine junction [151]. A tenaculum is a toothed pair of grasping forceps that is used in this context to straighten the uterocervical junction, principally in those patients with acute anteversion or retroversion of the uterus. The precise mechanism of how the tenaculum causes an increase in uterine contractions is unknown, but one theory is that mast cells stimulated by the tenaculum can cause the release of inflammatory reaction mediators, which can stimulate endometrial and myometrial contractions[152]. ET deemed easy or intermediate in difficulty had a 1.7-fold increase in pregnancy rates in comparison to difficult transfers in a retrospective analysis of 4807 IVF/ICSI cycles[153].

Anticipation of difficult transfers may help to strategize and reduce the difficulty of the subsequent transfers and consequently improve pregnancy rates [154]. For example, those

at risk of cervical stenosis (i.e., those with previous cone biopsies[155]) may benefit from a mock ET [149, 156]. This is when a mock ET is performed and if the catheter is unable to be passed through the cervix, then cervical dilatation/canalization[157] can be performed prior to the actual ET to allow for a less traumatic passage of the catheter[156, 157]. In extreme cases where a vaginal route is not an option (I.e., congenital cervical atresia[158]) then ET can be performed by transferring the embryo through the myometrium into the endometrial cavity (transmyometrial ET) or by laparoscopically depositing the embryo into the fallopian tube (zygote intrafallopian transfer [ZIFT]). Both these options are more traumatic, whilst increasing surgical risks and unit workload [146], and should be reserved for those cases where catheterisation of the cervix is not feasible.

Catheter types

Soft and hard catheters are both used for ET. Soft catheters are more likely to follow the contour of the uterine cavity, less likely to have mucous plugging and less likely to cause trauma or endometrial disruption[159]. Hard catheters can be easier to pass however are more likely to cause trauma. A Cochrane review of the two types of catheter, found soft catheters to have significantly better pregnancy rates in comparison to the hard catheters (odds ratio 1.34 in favour of soft catheter) and routine practice now recommends ET to be performed with a soft catheter where possible [160].

Ultrasound guided embryo transfers

The use of ultrasound in ET was first discussed by Strickler et al in 1985 [161]. They hypothesised that under ultrasound guidance, visualisation of the catheter tip allows for accurate deposition of the embryo and may correlate to improved pregnancy outcomes[161]. Having guidance at the time of transfer would hope to minimize trauma and as discussed previously this could significantly improve IVF outcomes, with the ability to navigate difficult anatomy using the 'blind' CTT being more unpredictable[162] (Figure 11).

The exact deposition point for optimal ET has yet to be defined[163], however recent reviews have suggested there is fair evidence that ET catheters should be positioned in the upper or middle area of the uterine cavity [147, 164]. There are a number of studies that recommend ET at least 1cm away from the fundus [165-170], although, there is limited evidence for deposition >2cm from the fundus [147, 164]. Those deposited <1cm from the fundus showed reduced implantation rates in comparison to those deposited >1cm from

the fundus [171], yet there is currently no recommendation on exactly where to deposit an embryo[163]. Figure 12 shows what can be seen when performing an ET under ultrasound guidance.

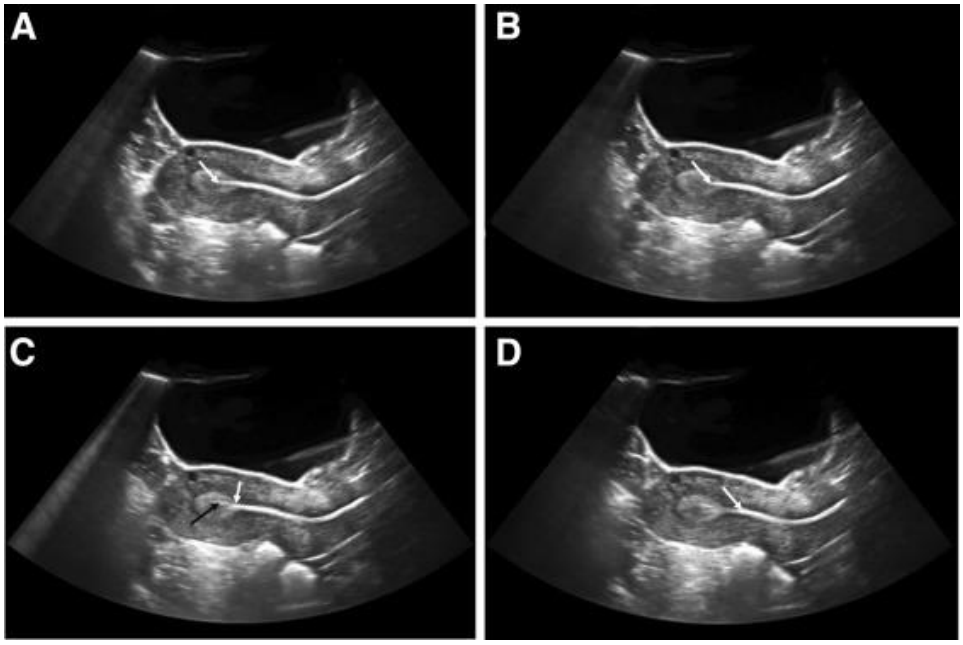


Figure 12. Ultrasound guided ET (UGET).[154] (**A**) The soft catheter is passed through the internal os into the uterus. (**B**) The position of the catheter tip of the catheter is adjusted to ensure proper positioning from the fundus. (**C**) The embryo is expelled into the uterine cavity at the precise location from the fundus. **D**) The catheter is withdrawn whilst maintaining pressure on the syringe plunger. *White arrow* indicates catheter tip; *black arrow* indicates catheter contents after transfer.[154]

The disadvantages of using ultrasound guidance for ET is the need for additional members of staff, a longer duration to perform the transfer and the need for a full bladder [172]. Although, filling the bladder is also a requirement in some units for the CTT, as it is thought that this straightens the uterocervical junction allowing for an easier transfer. Another point that has been suggested is that with ultrasound guidance it is sometimes necessary to move the catheter to identify the tip placement, this can cause disruption to the endometrium, opposing the initial benefit thought to be achieved by using ultrasound guidance[173].

Meta-analyses have been performed to compare the two methods of CTT versus 2D UGET. The first Cochrane review by Brown et al [97] compared 21 RCT studies that reviewed a total

number of 6218 women. The result from this review showed that UGET resulted in a higher CPR (OR 1.31) and LBR (OR 1.47) in comparison to the CTT.

Similar results were found in the meta-analysis by Cozzolino et al [174] which reviewed 14 RCTs including a total of 5503 women. Ultrasound guided transfers in this review were also found to have both higher CPR (OR 1.41) and LBR (OR 1.49) in comparison to CTT.

Despite the evidence showing CPR and LBR with the use of ultrasound guidance, it is not routine practice in all reproductive medicine centres and continues to be an ongoing dispute between different clinics and clinicians[175]. This is likely due to one of the RCTs that was in the both the meta-analysis showing no difference between the clinical touch method vs the UGET [176]. This study by Drakeley et al reviewed 2294 women and found no difference between the two transfer techniques[176]. It accounted for over a third of all the participants in the above meta-analyses and when taking into account the additional costs, staff and time required for the ultrasound guided technique, it is likely a reason why this particular technique is not routine practice in all units[175, 176]. However, based on the findings of the multiple meta-analyses, according to the guidance from National institute for health and care excellence (NICE)[4], ultrasound guidance is currently the recommended technique for ET..

Transabdominal and transvaginal scan guidance

Traditionally ultrasound guidance has been performed TA rather than TV, with the majority of studies comparing UGET and the CTT using the TA method[97, 174]. The use of TV ultrasound guidance was first documented in 1991[162] although it is not routinely used as the method of ultrasound guidance for ET. Due to its superior image quality, TV ultrasound is the imaging modality of choice when assessing the female genital tract, introducing the potential for improved outcomes when using this modality for ET[177].

There are a number of disadvantages to the TA approach to ultrasound guidance during ET; Firstly, an additional appropriately trained practitioner may be required to perform the scan; Secondly, a full bladder is preferred to obtain a good image of the uterus (bladder distension can cause the patient discomfort and also delay transfers if the bladder is not sufficiently distended[178]); Thirdly, in obese women or those with a retroverted uteri, TA scanning may not provide the quality of image required to ensure optimal placement of the embryo[179].

TV ultrasound guidance has some advantages over TA ultrasound in that; only one practitioner is needed; the bladder does not need to be filled prior to transfer with a concomitant reduction in pain, anxiety and discomfort having been previously noted[178, 180] and there is improved visualisation of the uterus and ET location[174].

When TV ultrasound has been compared to TA ultrasound within a meta-analysis, including 3 RCTSs, there was equal efficacy in CPR (OR 1.05) and ongoing and LBR (OR 1.19) were found[174, 178, 180, 181]. However, limitations were noted in these RCTS. Porat et al results were affected by the small numbers involved, Bodri et al was only aiming to determine equivalence of the two techniques not superiority and Karavani et al focused their power analysis on reduction of patient discomfort[174].

When two observational studies were analysed, they found improved CPR with TV UGET with one comparing TV ultrasound to CTT (implantation rates 15.2 vs 7% respectively, $p<0.01$)[182] and the other compared TV vs TA ultrasound guidance (pregnancy rate per ET 38%, n = 800 vs 30%, n = 3910 respectively; P=0.0004) [177].

With the large retrospective review by Larue showing improvement with TV ultrasound guidance, a definitive conclusion following the meta-analysis was not achieved[174]. All studies did show improved patient comfort with the TV approach, meaning improved patient satisfaction[174].

Table 2. Advantages and disadvantages of ET techniques

ET technique	Advantages	Disadvantages
CTT	• One practitioner required • Least amount of training • Less costly • Shortest duration	• Exact deposition point unknown • Patient unable to visualise procedure
TA ultrasound guidance	• Known embryo deposition point • Patient can visualise the procedure	• Second operator required • Full bladder required • Additional cost of training and equipment

TV ultrasound guidance	• Better resolution of ultrasound image • Known embryo deposition point • Improved patient comfort • Single operator • Patient can visualise the procedure	• Additional training required • Additional cost of training and equipment

3D and 4D ultrasound guided embryo transfers

2D ultrasound allows for improved determination of catheter in comparison to CTT. But controversy still exists between ideal placement of embryos in the uterine cavity, with studies only highlighting generalized locations with 2D measurements, not being able to take into consideration variances in uterine anatomy between different patients[183]. 2D imaging only allows for a single slice of the uterus to be seen at a specific time whilst 3D imaging allows for multiple views from different perspectives enabling a complete view of the uterine cavity. This ability to see the entire cavity may enable more accurate embryo deposition points in both normal and abnormal uterine cavities, such as unicornuate or bicornuate uteri[183].

The first use of 3D UGET was in 2000[184]when 3D TA guidance was utilised and it was possible to see the exact position of the catheter tip during ET. It was hypothesized that by determining the exact position of the catheter tip, the optimal transfer site could be identified and this could improve embryo implantation rates[184].

In a cohort of 699 patients, it was shown that for every millimetre away from the fundus the chance of clinical pregnancy increased by 11%[185]. This is based on depositing the embryo at the fundus (0mm), 1-5mm from the fundus and >5mm from the fundus and is based on a multivariate logistic regression model[185]. This is not in keeping with current recommendations of depositing the embryo more than 1cm from the fundus, however it does highlight that having an accurate measurement of catheter tip placement should be of the upmost importance when performing the ET.

The ability of 3D imaging to correctly identify catheter tip placement was shown in the study by Fang et al[186]. Just before embryo deposition 2D and 3D images were taken of the uterine cavity with the catheter 1-1.5cm from the uterine fundus based on the 2d measurement. The subsequent review of these measurements then allocated patients into groups looking at the difference between the 2D and 3D distances from the fundus, group 1, <3mm; group 2, 3-5mm; group 3, 6-9mm and group 4, >10mm. Group 4 showed a significant decrease in clinical CPR in comparison to the other 3 groups and showed that if there is a significant difference between the 2D and 3D embryo deposition points then this can have a significant impact on CPR[186]. This study also showed that nearly 43% of patients had a difference of >3mm between the 2D vs 3D scan[186], which as stated previously by Pope et al can have significant impact on ET success rates[185].

3D imaging creates a full picture of the uterine cavity and can allow the clinician to determine exactly where the catheter tip is, ensuring that embryo deposition occurs in the optimal area. 3D ultrasound also allows for identification of uterine anomalies such as arcuate, septate or bicornuate cavities and can allow for adjustment of embryo deposition accordingly[186].

The maximal implantation point (MIP) has been shown to be the effective deposition point for an embryo[187]. This has been hypothesized as an area where natural pregnancies would likely implant, as it follows the trajectory that an embryo would take when entering the uterine cavity from the tubal ostia [183]. It also has the added benefit of being where the endometrium is thickest and the vascular flow is maximal[183]. An observational study, using 3D/4D found that deposition at the MIP showed an increase of 10% in their pregnancy rates when compared to their previous 2D guided practice[187]. This is the only study assessing 4D ultrasound guidance and with no other evidence available to support its use, uptake of this particular technique is not widely applied. The uptake of this particular technique is also less likely with the need for additional training to perform 4D ultrasonography, as well as needing more advanced and costly ultrasound machines [188-190].

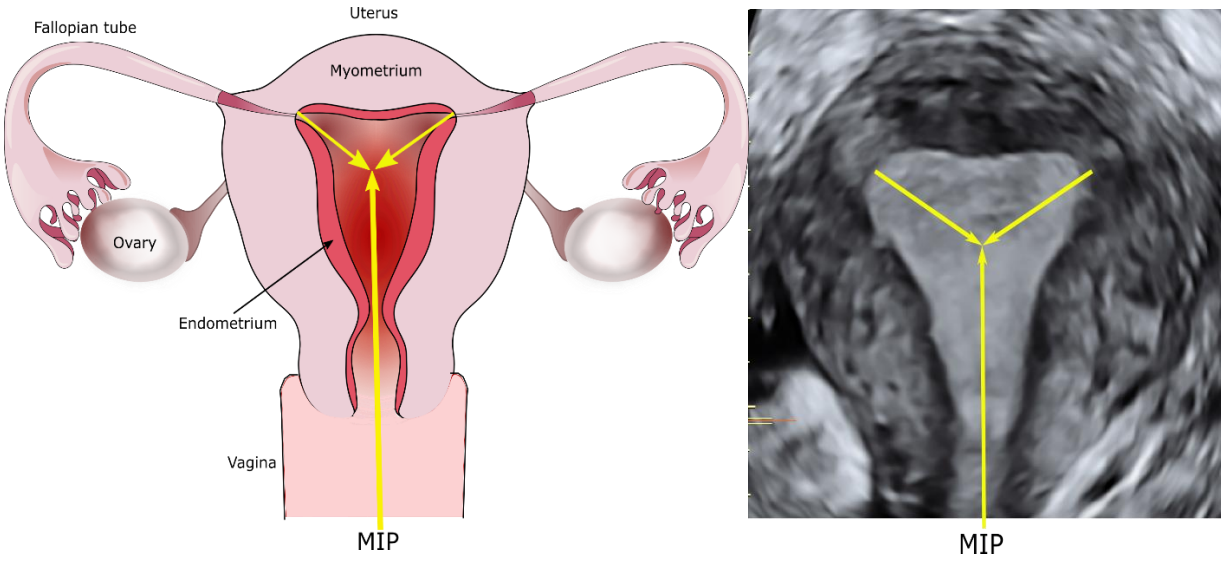


Figure 13. MIP as highlighted by Gergely et al [183]

To date there has only been one RCT comparing 3D ultrasound guided transfers with 2D guidance. This study by Saravelos et al showed no difference between 2D and 3D guided ET [191]. Although the 3D approach is a more modern and advanced technique, the study by Saravelos et al did not advise to use it as an approach to improving clinical outcomes [191]. The limitations of this study were that it used an unselected population and was underpowered to assess differences in subsets of women [191]. As this is the only RCT comparing 3D vs 2D ET, further studies are required to confirm or refute the findings shown here.

3D ultrasound guidance does have its own disadvantages. Being similar to 2D ultrasound guided transfers, it needs an additional practitioner to perform the scan (unless it is done TV), it requires more advanced scan machines and probes and also requires practitioners to be appropriately trained in 3D ultrasonography; all of which have cost implications. 3D imaging doesn't allow for a 'live' image, with recurrent sweeps required to determine correct placement of the catheter [191]. This prolongation in ET time may have an impact on the ET success rate [192] and duration of transfer was not included in the study by Saravelos et al [191].

The use of 4D ultrasound guided transfers has only been reported in one manuscript which shows improvement in their pregnancy rates when compared to their 2D guided success rates prior to adoption of the 3D/4D technique in their prospective observational study of 5073 patients [187]. Implementing 4D guided ET allows for a live image of the uterine

cavity, without increasing the duration of the ET that would likely occur when performing repeated 3D sweeps.

Measurements at the time of embryo transfer

Endometrial Thickness

Endometrial thickness (EMT) is one of the most common measurements used to determine endometrial receptivity[193, 194]. Endometrial receptivity is the ability of the endometrium to create the optimum environment for embryo development and implantation[193]. EMT can be measured easily and quickly with a 2D ultrasound probe and requires minimal training for the person performing the scan [195].

Having a thin endometrium is thought to have a negative impact on embryo implantation following ET, but the definition of a thin endometrium varies between studies [196]. It has been shown that pregnancies can be achieved with an EMT of >4mm [197] although there is still debate as to what the cut off for transfers should be as different studies have had different definitions of a thin EMT, (<6mm <12mm)[196]. A number of meta-analyses have looked at the impact of a thin EMT on CPR with varying results. All have stated that having a thicker EMT improves CPR[193, 196, 198], however, the cut offs for the minimum EMT have been documented at 6mm[193] or 7mm[196]. The measurement of EMT also varied between the meta-analyses with some measuring the EMT on the day of hCG trigger [196, 198]or with others measuring it on the day of ET[193]. With thin EMT only counting for a small percentage of the IVF patients this also makes it more difficult to draw conclusions on success rates when they only make up a small proportion of the IVF population (0.7-1.5% <7mm, 2.5-9.1% <8mm)[199].

There have been a number of studies that have looked into whether the endometrium can be too thick. Some of these found a decrease in pregnancy rates with an EMT >14mml [117, 200, 201] although there are other case series showing pregnancies in women with an EMT>20mm[202, 203].

EMT is the most commonly investigated marker for endometrial receptivity yet numerous studies and meta-analyses have failed to show any difference in mean EMT between pregnant and non-pregnant groups[193]. The mean difference between pregnant and non-pregnant EMTs was -0.5-1.16mm[193] or 1mm[198], depending on the review, with one questioning whether this result was clinically meaningful [198]. With the inter-observer variation when scanning the EMT thought to be 1.5mm [204], this does question that

although a thicker mean EMT is statistically significant in terms of pregnancy rates this may not actually be clinically significant[193]. It was determined that EMT has no predictive capacity for occurrence of pregnancy but it is thought to be a factor that can assess the probability of conceiving after IVF (EMT ≤ 7 mm: OR 0.42 (95% CI 0.27–0.67] *P* = 0.0003)[196].

Endometrial volume

The use of endometrial volume (EMV) as a potential marker for endometrial receptivity has been studied for over 20 years[205]. EMV can be accurately measured using 3D ultrasound and has shown to have a high degree of reproducibility[206] however, 2D ultrasound lacks this reproducibility between practitioners and also has a higher mean error rate [207].

Similarly, to endometrial thickness there is conflicting results with regards to EMV and its ability to detect endometrial receptivity[193]. The EMV for good outcomes following ET vary with some stating a EMV >2ml a prerequisite for good endometrial receptivity [206], whilst others notice a significantly lower pregnancy rate when the EMV <2.5ml [208]. More recent studies have also showed a positive correlation between EMV and pregnancy rates[209, 210].

However, other studies noted no difference between non-pregnant groups and pregnant groups when measuring EMV [211, 212]. One study did notice that then EMV for the pregnant group had reduced in size on the day of egg collection in comparison to the EMV taken on the day prior to hCG administration, but there was no change in the EMV for the non-conception group[211]. As a single measurement EMV was deemed poor at predicting pregnancy outcomes[211, 212]. However, when used in conjunction with other criteria, it may be able to determine whether to transfer or cryopreserve an embryo [212].

A subsequent review in 2012[213] found that EMV was ineffective at predicting pregnancy and highlight that its likely more of an interaction of the blastocyst and endometrium which are more important, yet it was still quoted that an EMV of <2-2.5ml may significantly reduce pregnancy rates.

The timing of EMV measurements differed between the studies with some performing EMV on day of hCG trigger or oocyte retrieval and with the other studies measuring the EMV on the day of ET [206-214].

As ultrasound technology continues to improve there may be further advancements in its ability to determine endometrial receptivity. Although it may not be the sole measurement

in the determination of endometrial receptivity, when used in combination with other investigations, such as endometrial biopsies, subendometrial doppler flow and uterine contractility, this may enhance the clinicians' ability to identify when to perform a transfer or cryopreserve an embryo[213] (Figure 14).

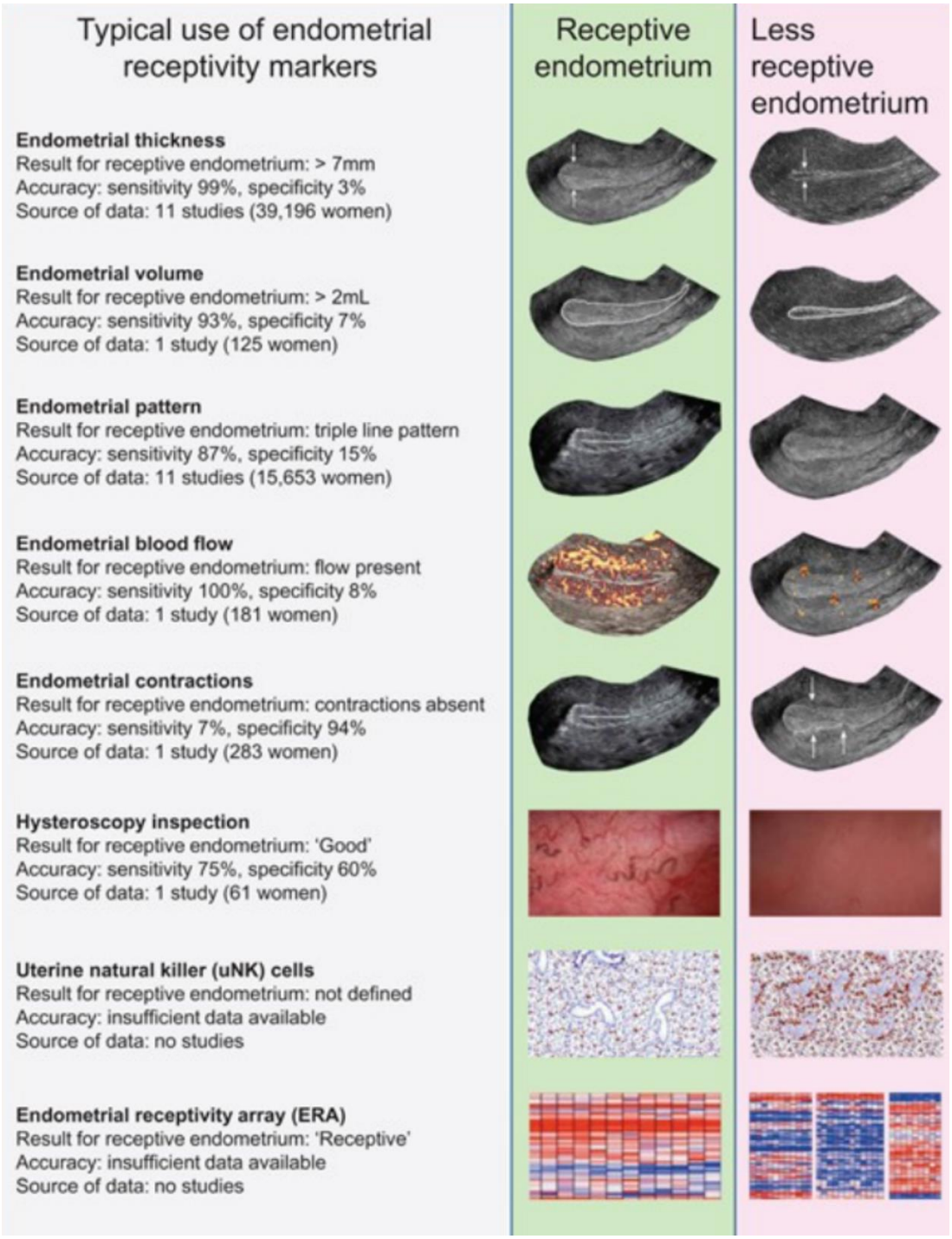


Figure 14. Summary of the prognostic accuracy of different endometrial receptivity markers for clinical pregnancy Craciunas et al[193]

Implantation site of pregnancy

The implantation site can be determined using ultrasound imaging[215-217]. Prior to the implementation of ultrasound, the only way to determine implantation site was opportunistically at the time of hysterectomy (which were performed for various therapeutic reasons)[218-220]. The implantation site varies between women, in both natural and IVF conceptions but implantation tends to occur more frequently in the fundal region (80-90% natural vs 66% IVF)[169, 215, 217]. In IVF, more pregnancies occur in the middle and lower region of the uterine cavity (10-20% Natural vs 34% IVF)[169, 215, 217]. In natural pregnancies those that implanted lower within the uterine cavity were more likely to miscarry but this was not seen in those pregnancies conceived following IVF [215-217].

As previously stated, when depositing an embryo, studies have suggested aiming for 1-2cm from the uterine fundus[97]. In 2000 a study looked at depositing embryos in the mid-fundal region of the endometrial cavity and rescanning these women in early pregnancy to evaluate the implantation site and compare this to the embryo deposition point[216]. Of the 22 pregnancies, 81% of them implanted in the mid-fundal region where they were originally deposited, with the remainder migrating to elsewhere within the uterine cavity, 2 of the pregnancies being tubal ectopics[216]. Migration of the pregnancies was thought to be due to wavelike movements of the endometrium, but with low numbers it is difficult to determine the true relationship between embryo deposition and implantation[216].

A study in 2006 looked at implantation sites of pregnancies following embryo deposition in the mid-cavity[169]. Under 2D guided abdominal ultrasound they measured the endometrial cavity length from the fundus to the internal os and then deposited the embryo at the midpoint[169]. This was based on a number of previous studies identifying the middle of the cavity to be the best place for deposition[185, 221, 222]. The study by Cavagna et al noted that the number of pregnancies implanting into the middle of the uterine cavity following ET was higher in comparison to those patients who conceived naturally (29.8% vs 9 -15% respectively), however the majority of implantations still occurred in the upper portion of the endometrial cavity (66%)[169]. These results promote the idea of embryo migration following ET and lead the question with regards to potential influence of endometrial contractility on embryo implantation sites[169].

There has been only one other study looking into embryo site implantation and its correlation following embryo deposition in 2015[223]. This study reviewed the position of

the air bubble in the uterine cavity at 1 minute and 60 minutes following ET and then scanned the patients 3 weeks after to determine gestation sac location and its correlation with air bubble position[223]. It found that 40.8% of gestation sacs correlated to their air bubble position at 1 min, which increased slightly to 50.7% when compared to air bubble position at 60 minutes[223]. This poor level of agreement challenges the initial assumption that embryo implantation occurs at the site of deposition[216], however, the paucity of studies does not supply sufficient evidence to be able to confirm or deny a correlation between embryo deposition point and pregnancy implantation site.

Another issue with this most recent study is that it observed the migration of the air bubble following ET as the site of embryo-deposition. The air bubble can be seen on scan is a surrogate marker of the embryos position as the embryo itself cannot be seen on ultrasound[223]. Following the air bubble migration after transfer may not necessarily reflect the embryo migration/location. The migration of the air bubble may also be due to buoyancy of the air bubble, rather than uterine contractions, which would be the more likely cause of an embryo to migrate[223].

Two of the three studies discussed above, used day 2-3 embryos for transfer with the remaining study not stating the age of the embryo[169, 216, 223]. However, it is around day 5-6 when blastocysts tend to hatch out of the zona pellucida, with a number of studies showing that implantation rates are better with embryos at this stage [224-226]. This raises questions about whether there would be more of a correlation between ET deposition site and implantation site when day 5 blastocysts are transferred.

Trophoblastic invasion at early gestation scan

Trophoblast cells make up the outer layer of cells of the blastocyst and have important roles in the development of the placenta and endocrine support of the early pregnancy[227]. Trophoblast invasion plays a key role in implantation and the successful continuation of a pregnancy[228]. Trophoblastic invasion and migration occur following adhesion of the embryo to the endometrial epithelium[46]. Trophoblasts differentiate into cytotrophoblast and syncytiotrophoblasts and invade and migrate into the maternal decidua[46]. The aim of invasion is to reconstruct the maternal arteries, converting them from muscular vessels to sinusoidal sacs lined with endovascular trophoblast [46], ensuring sufficient blood flow to a developing fetus to encourage placental efficiency and fetal

viability[46]. Abnormal trophoblastic invasion has been linked with a number of pregnancy related conditions including recurrent spontaneous abortion, pre-eclampsia (hypertension and proteinuria in pregnancy), miscarriage and intrauterine growth restriction [229-231].

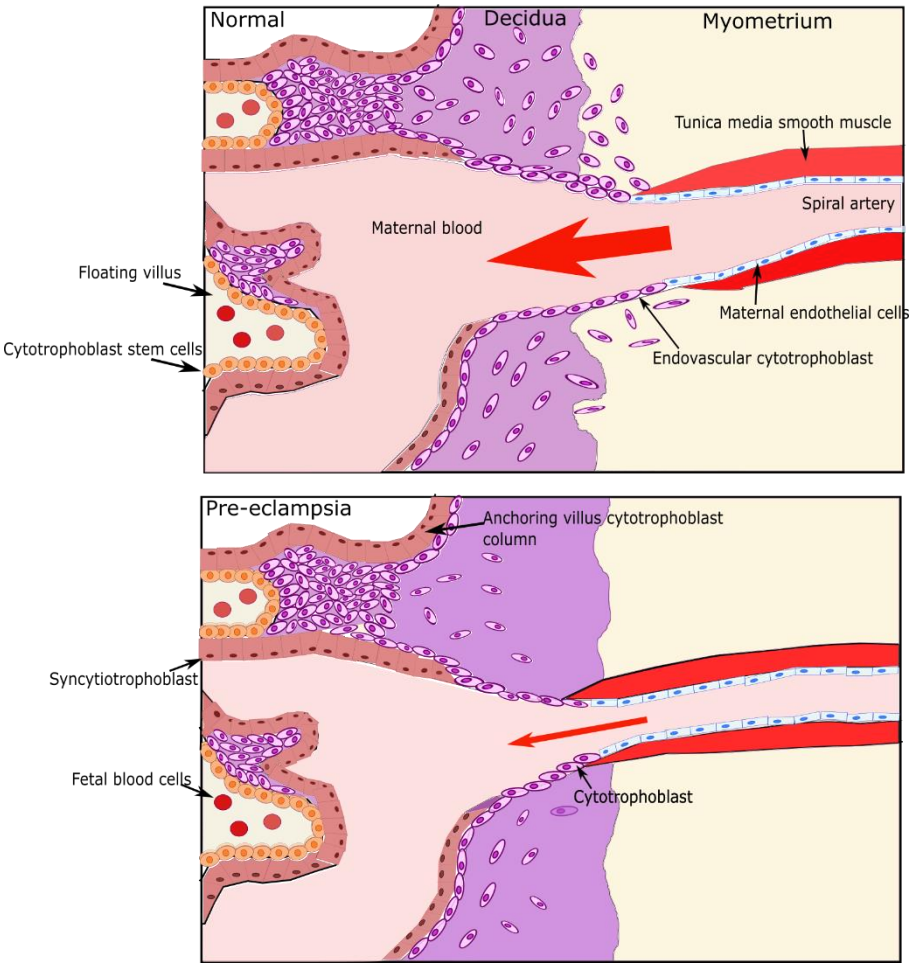


Figure 15. Normal and abnormal (pre-eclampsia) trophoblast invasion

Two studies have previously reviewed the relationship between trophoblast thickness (TT) and early pregnancy loss [232, 233]. The first study showed that if there was a difference >3 between gestational age in weeks and TT then there was an increased risk of miscarriage ($P < 0.001$) [232]. For example, if the gestational age was 8 weeks, then there was an increased risk of miscarriage if the TT was 4mm in comparison to 8mm. This study looked at 'normal' pregnancies and did not mention whether it included IVF pregnancies. It reported that all patients had regular periods however there is no mention to show exactly when their last menstrual period (LMP) was or how they calculated a patient's gestation.

The most recent study assessed multiple ultrasound features and their association with subsequent miscarriage[233]. They found no association with TT and subsequent miscarriage[233]. However, they did find that a combination of fetal heart rate, mean uterine arterial pulsatility index and trophoblast volume can be used in a predictive model for early gestation miscarriage[233].

Both studies only reviewed early pregnancy losses and did not follow the patients to review the incidence of obstetric complication such as intrauterine growth restriction (IUGR), pre-eclampsia and stillbirth.

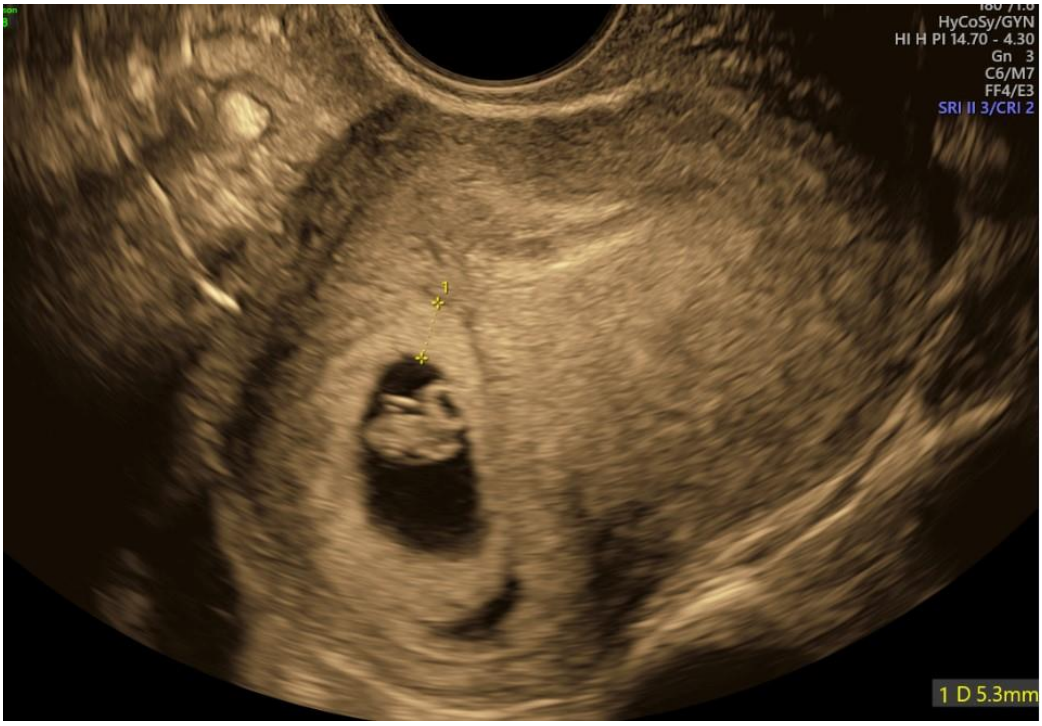


Figure 16. 7-week gestation scan showing TT

Summary

This chapter shows how much ART practices have progressed since its inception in the 1970's. During this time there have been numerous advances in this field with developments in ovarian stimulation and monitoring, as well as laboratory processes and procedures[58]. Ultrasound is a key tool in the management of modern ART yet it is rarely used to its full potential. This chapter highlights the inconsistencies in the literature and the need for further investigation into the use of advanced ultrasound techniques in reproductive medicine.

Aim of thesis

Firstly, we wanted to determine the current embryo transfer techniques in use in the fertility units in the UK to obtain contemporaneous information on the use of ultrasound during embryo transfers. Secondly, we aimed to determine if 4D ultrasound guidance can be of benefit to embryo transfer success rates in comparison with the current technique used at the Hewitt fertility centre, which is the clinical touch technique. Finally, we want to determine if pregnancy site location and trophoblast invasion using ultrasound images have an impact on predicting pregnancy outcomes in ART pregnancies. The findings presented in this thesis are of interest to practicing clinicians in reproductive medicine to improve patient care, as well as being relevant to researchers to focus on further studies to confirm the clinical utility of 4D Ultrasound in embryo transfers and in predicting pregnancy outcomes and also to fill the gaps in knowledge identified. Therefore, the data produced in this thesis may benefit patients, clinician and researchers in the speciality of reproductive medicine.

Chapter 2: National survey on embryo transfer technique

Introduction

Transferring a good quality embryo in to an appropriately prepared uterine cavity is an integral part of the in IVF process and a fundamental step in conception[147]. Reproductive medicine as a speciality and the IVF process in particular, have seen significant changes over the past 40 years, with many developments in both clinical practice and laboratory procedures[58]. However, during this time, there has been little change in the ET technique originally developed by Steptoe et al[59, 175].

The best ET technique would deliver the embryo to the optimum location within the uterine cavity, in the least traumatic way without disturbing the primed uterine environment[175]. The first described ET technique introduced and delivered a preloaded embryo with a soft catheter, into the uterine cavity via the cervical canal [59]. The intra-uterine position of the catheter tip for embryo deposition was either determined by measuring 6cm from the external cervical os or by measuring the cavity length with a dummy transfer prior to the actual ET[97]. The first ultrasound guided ET was reported in 1985[161], and 30 years later, a Cochrane review concluded that ultrasound guidance should be the recommended and preferred method for ET[97]. Despite this Cochrane guidance, a lack of universal implementation exists, demonstrated by two recent surveys, showing wide variation in ET techniques[175, 234]. The reason for this is thought to be multifactorial, with most of the published data on efficacy of ET techniques being conflicting, inconclusive or affected by confounding variables dependent on either the practitioner or the technique[174-176, 235-237]. This is an important issue in IVF research, for example, studies using different embryo deposition points of 1, 1.5 or 2cm from the fundus, and measuring the outcome of clinical pregnancy are confounded by the embryo deposition site[97, 176, 238-240]. Use of patient relaxant, direction of the removal of the ET catheter and duration of bedrest following transfer are some of the other possible discordances between studies[175]. Such differences could also impact outcomes between trials[185], resulting in misinterpretation of the available evidence. The lack of consensus that exists at the present time, may also be due to the apparent absence of a robust, specific guideline highlighting the practice of ET technique. Such guidelines from professional organisations such as the British Fertility Society (BFS), European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive

Medicine (ASRM) may facilitate standardisation of best evidence-based practice, which is a fundamental first step towards improving clinical outcomes in IVF.

The last UK survey on ET was conducted nearly 2 decades ago and their main recommendation was the need for a standardised national protocol to be implemented for ET[241]. Since then, new evidence on subtle differences in the ET technique had suggested that they may affect the success of IVF[166, 241, 242]. Examples for these include two separate Cochrane reviews recommending the use of ultrasound guidance, as well as the use of soft catheters for ETs[97, 243]. However, a universally available, standardised, national guideline or protocol for practitioners in IVF units in the UK is yet to be produced. Our aim, therefore, was to evaluate and gain insight in to the current clinical practice regarding ET, in the UK. This data will aim to provide the basis for future attempts to harmonise the practice in the UK with the formulation of a standardised protocol and will allow to place the data presented in the subsequent chapters in the context of national practice in the UK.

Materials and Methods

The Survey

Initial literature review and clinical opinion was obtained from local practitioners at the Hewitt Fertility Centre, Liverpool, which is one of the larger NHS IVF units in the UK with approximately 1800 fresh IVF/ICSI cycles being performed per annum. The initial survey questions, reviewing current ET techniques and practice pertinent to individual practitioners, were formulated in August 2018. The initial survey, including 33 questions, was modified after being peer reviewed by 5 other fertility specialists from around the country, resulting in the final 38 question survey (Appendix).

The survey questions were informed by current evidence relating to different aspects of the ET technique. The questions in the final survey included demographic information on the unit (type of practice, number of embryo transfers per year, location) and important outcome measurements (including biochemical pregnancy rate (BPR) CPR and LBR). We also included those relevant to the ET technique (such as the type of catheter used, the use of ultrasound guidance, how practitioners clean the cervix) and those relevant to the practitioners involved during the ET (which professionals were involved and their experience).

Physician and ET preparation

Our survey included questions that cover many steps involved in the preparation of the patient prior to ET. These include currently recommended practices as well as practises that prevail despite not presently being recommended, for example, the use of patient relaxant[147, 244]; the use of ultrasound scanning gel as lubrication for the speculum [245, 246]. The recommended practice we surveyed included the use of sterile gloves for transfer whilst avoiding direct contact with the tip of the catheter [247]. The use of saline or sterile water for cleansing the cervix and for the lubrication of the speculum that is advised, with the expectation to reduce microbial burden and for improving patient comfort[96]. Some practitioners may also use culture media for the same purpose, but the supporting evidence for its use is lacking [248-251] and we sought to collect data on that.

The benefit of removal of cervical mucous prior to ET is questionable with conflicting evidence, where some studies recommending removal[147, 164, 252, 253],whilst others, including a meta-analysis have failed to show any significant benefit [254, 255]. Similarly, there is inconsistent practice among practitioners regarding the flushing of the cervical canal with contradictory evidence for benefit [254-258]. Ultrasound guidance is recommended for ET, either performed via a transabdominal or transvaginal route [97, 147, 164, 174, 187]. The main benefits of this is to identify if fluid is present in the endometrial cavity, since in that case, ET is to be avoided [145]. There is however, insufficient evidence to support aspiration of endometrial fluid[235, 259-261].

A mock or practice embryo transfer, is performed prior to ET and assesses uterine position, ease of transfer and helps inform clinicians regarding the most appropriate type of catheter to use[262]. The timing of mock transfers varies between clinicians and practices and can be done either prior to treatment cycles, or in the treatment cycle, at oocyte retrieval or immediately prior to transfer. However, the timing of mock transfer has not been shown to impact pregnancy rates[262].

Practices such as use of warmed speculum, cleaning of the cervix with cotton wool or gauze swabs or the designation of the practitioner who perform the ultrasound scan (clinician, radiologists, nurse practitioner etc) have been probed for their proposed benefit in improving patient comfort, reducing risk and in improving conception rates, yet without robust supporting evidence.

ET procedure

The questions contained within our survey comprised of practices which may or may not have supporting evidence. For example, the limited evidence on the 3 main techniques of ET does not demonstrate any significant difference between the methods in improving pregnancy rates[263, 264].

There are three main techniques of ET:

1- Trial with transfer;
   Trial with transfer involves performing a mock ET with a trial catheter, then removing it and replacing it with a second catheter loaded with an embryo and depositing it in the desired place

2- Afterload technique
   The afterload technique involves placing a ET catheter 1cm past the internal os and then removing the inner sheath. Then an embryo is preloaded into another inner catheter and is passed through the original outer sheath to the desired point in the uterine cavity.

   and ;

3- Direct technique.
   The direct technique involves inserting a loaded catheter directly into the desired place within the uterine cavity.

There have been only 2 studies comparing the direct and afterload technique of ET, without significant evidence for superiority one showing the afterload technique to improve clinical pregnancy rates(although significance was not reached) [264] and the other showing no difference between the two[263].
Similarly, soft catheters[160, 243], and embryo deposition points in the upper/middle portion of the uterine cavity between 1-2cm from the uterine fundus[147, 164] are recommended practice whilst the use of a tenaculum is not recommended[265, 266]. Slow and steady pressure for expulsion of the embryo is preferred to rapid expulsion however studies have failed to show statistical significance[147, 164, 235].

Post procedure

The evidence base for subsequent re-transfer of retained embryos not altering the clinical pregnancy rates is fair [147, 267-272], yet no studies have compared retransfer with the original catheter or a new one.
Similarly, studies support the immediate withdrawal of the catheter following embryo deposition[147, 164, 273, 274], with a few recommending rotation of the catheter on removal[275, 276]. The presence of blood on the catheter has an uncertain significance[147] and the immediate mobilisation following transfer is recommended [277-280] since prolonged bedrest was deemed to be detrimental[281].

The final electronic survey was emailed through SurveyHero (www.surveyhero.com) to all clinical leads in the 79 Human Fertilisation and embryology authority (HFEA) registered units that were performing ETs in the UK, in December 2018. SurveyHero is an online anonymous survey tool, and no patient identifiable data was collected. Electronic reminders were sent out in the interim 6-month period when they were requested to respond, and where there was no response from clinical leads, other consultants within the same unit were contacted requesting a response to the survey. To remove duplication or inaccuracy of responses from a particular unit, the name of the organisation was included. If multiple responses were received from the same unit, the first response from that unit (after confirming concordance with duplicate responses) was used in the analysis.

Ethical consideration

The survey did not involve human or animal research and did not collect any personal or patient identifying data, thus, a formal Ethical Review Body approval was not required. The electronic Survey was available as an open-access questionnaire to the invited IVF practitioners in the UK, who voluntarily answered the study questions. Data collected for this study was anonymous, with no patient or person identifiable information.

Statistical Analysis

This survey was not designed as a comparative study or powered to detect differences, thus in line with our research aims of the current national practice in the UK, we report summary statistics of the data obtained from the survey. Where possible, the Statistical package for the Social Sciences (SPSS) for Windows (Version 26; IBM Corporation, USA) was used to analyse categorical data using the $\chi 2$ test or the students paired t-test for continuous data.

Results

Sixty-one out of the 79 clinics responded, and following exclusions the final number of responses analysed were 47(Figure 16.).

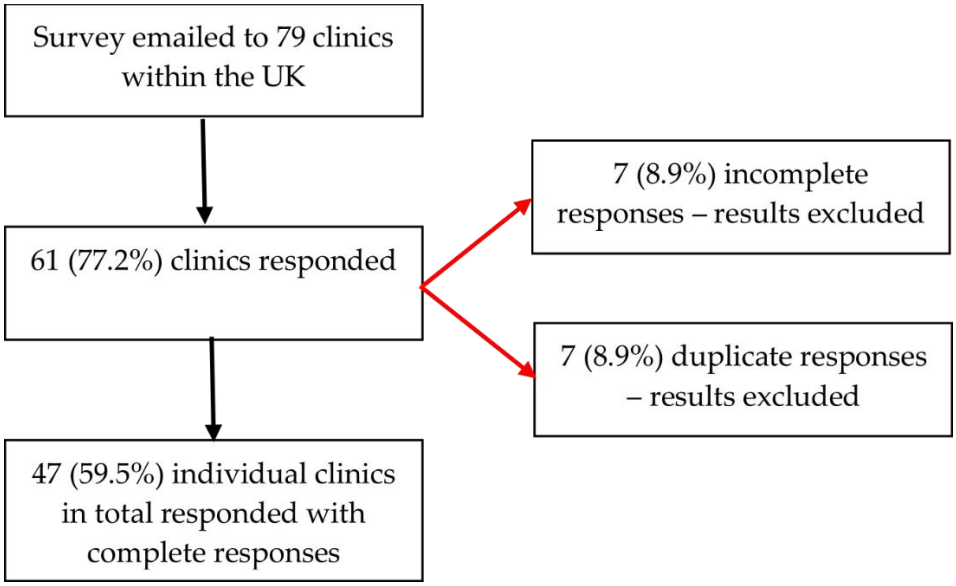


Figure 16. Flowchart of survey respondents

Demographics of the units

Of the 47 responses, 2 (4%) were from clinics accepting only NHS patients; 36 (77%) clinics accepted both NHS and privately funded patients and the remaining 9 (19%) clinics treating only privately funded patients (Table 1).

Most clinics (27/45; 60%) based their success rates on the CPR. Clinics estimated their success rates for CPR and LBR with 23 (49%) clinics estimating their CPR to be between 40-50% per ET, and 28 (60%) of the clinics estimating their LBR to be between 30-40% per ET) (Table 3).

Table 3. Unit demographics

Types of IVF practice n (%)	
NHS	2 (4)
NHS and Private	36 (77)
Private	9 (19)

Basis of ET success n (%)	
Positive pregnancy test	13 (28)
Clinical pregnancy rate	27 (57)
Live birth rate	5 (11)
No response	2 (4)
Persons performing the ET n (%)	
Consultant only	18 (38)
Consultant and nurse	14 (30)
Consultant, registrar and nurse	7 (15)
Consultant and registrar	6 (13)
Nurse only	2 (4)
Estimated clinical pregnancy rates per embryo transfer n (%)	
20-30	3 (6)
30-40	18 (38)
40-50	23 (49)
50-60	1 (2)
60-70	0 (0)
>70	1 (2)
No response	1 (2)
Estimated Live birth rate per embryo transfer n (%)	
20-30	13 (28)
30-40	28 (60)

40-50	3 (6)
50-60	0 (0)
60-70	1 (2)
No response	2 (4)

The human fertilisation and embryology authority (HFEA) separately publishes data on success rates measured as the LBR per embryo transfer[282]. Although the majority of units report their LBR per ET to be between 30-40% (28/47 60% of units) according to the HFEA data from 2017[282], most units actually had a LBR per ET between 20-30% (31/47 66%) (Figure 17).

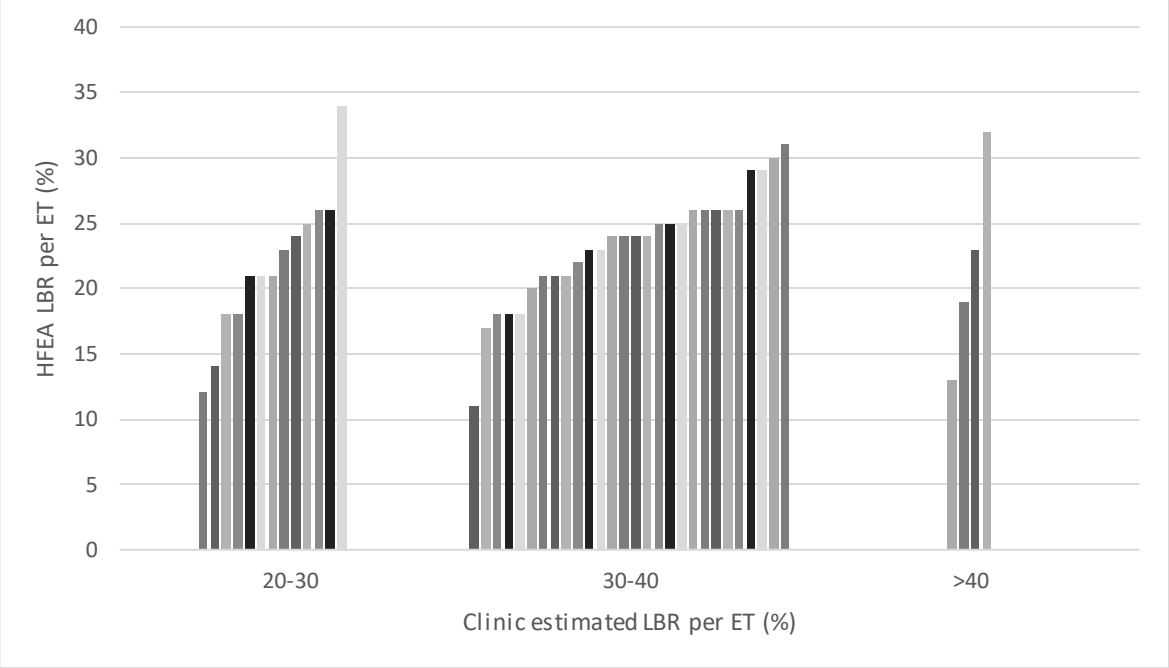


Figure 17. Clinic estimated LBR vs HFEA LBR per ET

Embryo transfers

Seven clinics (15%) allowed individuals to utilise their preferred ET technique and no zygote intrafallopian transfers were performed by any of the clinics (Table 4).

Table 4. Number of transfers performed by units

Presence of standardised technique within the unit n (%)	
Standard technique	40 (85)
Technique based on individual preference	7 (15)
Number of ETs performed in the unit per year n (%)	
<500	7 (15)
500-1000	20 (43)
1000-1500	10 (21)
1500-2000	2 (4)
>2000	8 (17)
Number of transmyometrial transfers per year n (%)	
10	1 (2)
5	2 (4)
3	1 (2)
2	7 (15)
1	6 (13)
0	30 (64)

When the published HFEA clinic success rates were considered, those clinics performing more transfers appear to have better LBR than those performing less ET's (Table 5).

Table 5. Number of ETs relating to average HFEA LBR

Number of ETs	**Number of clinics**	**Average HFEA LBR (%)**
<500	7	20.1
500-1000	20	22.8
1000-1500	10	22.2
1500-2000	2	28.5
>2000	8	24.3

ET preparation

All units use sterile gloves (100%) and most do not use sedation for ET (94%) (Table 6). Forty-three (91%) of the clinics cleaned the cervix prior to ET and 33 (72%) removed cervical mucous with a cotton wool swab. Most units (78%) would abandon the ET if there was fluid within the endometrial cavity on ultrasound. Thirty-nine (83%) of the clinics performed ultrasound guided ET with nursing staff performing the majority of the ultrasound scanning (92%).

Table 6. Patient and practitioner preparation prior to ET.

Patient relaxant n (%)	
None	44 (94)
Voltarol	1 (2)
Sedation when required	1 (2)
Sedation	1 (2)
Sterility of Procedure n (%)	
Sterile gloves after handwashing	27 (57)
Aseptic technique	18 (38)

Scrubbed and gowned	2 (4)
Warmed speculum n (%)	
Yes	11 (23)
No	36 (77)
Lubrication on speculum n (%)	
None	10 (21)
Culture media	1 (2)
Normal Saline	23 (49)
Sterile water	11 (23)
Ultrasound gel	2 (4)
What is used to clean the cervix n (%)	
Normal Saline	34 (72)
Media from lab	7 (15)
Not cleaned	4 (9)
Sterile water	2 (4)
Instrumentation used to clean the cervix n (%)	
Cotton wool	23 (50)
Gauze sponge on forceps	19 (41)
Cotton wool and Gauze	2 (4)
Pipette	1 (2)
N/A	1 (2)
Removal of endocervical mucous n (%)	

Cotton wool	29 (63)
Aspirate	4 (9)
Cotton wool and flush	4 (9)
Flush	2 (4)
Not removed	7 (15)
Embryo transfer technique n (%)	
2D ultrasound guidance	38 (81)
3D ultrasound guidance	1 (2)
Clinical touch technique	7 (15)
Dummy ET and measurement of cavity length	1 (2)
Person performing the ultrasound scan n (%)	
HCA	8 (17)
Embryologist	1 (2)
Nurse	36 (77)
Doctor	4 (9)
Ultrasound technician	1 (2)
Approach to fluid within the endometrial cavity n (%)	
Abandon the transfer	35 (74)
Aspirate the fluid and continue with transfer	7 (15)
Continue with the transfer	3 (6)
No response	2 (4)

Use of a routine mock transfer n (%)	
For specific indication	27 (57)
Not routinely done	10 (21)
Immediately before transfer	4 (9)
At oocyte retrieval	2 (4)
Before cycle begins	4 (9)

ET technique

The most common ET technique was the afterload technique (53%), with 100% of respondents using soft catheters (Table 7). Clinics generally used (72%) a stylet for less than 25% of their transfers and the routine use of tenaculum was uncommon. Most (91%) reported deposition of the embryo in the upper or middle portion of the uterine cavity, although, exact deposition points from the uterine fundus varied from 0.5cm to over 2cm. Embryo retention following transfer was <5% in all clinics with 31 respondents (66%) re-transferring the embryo in a new catheter when this occurred.

Table 7. ET technique

Embryo transfer technique (n%)	
Afterload technique	24 (53)
Trial with transfer technique	12 (27)
Direct technique	9 (20)
ET catheter preference n (%)	
Wallace	29 (62)
Cook	22 (47)
Kitazato	6 (13)
Surepro	2 (4)

Labotect	1 (2)
Use of stylet n (%)	
All the time	1 (2)
>50% of transfers	6 (13)
25-50% of transfers	5 (11)
<25% of transfers	34 (72)
Never	1 (2)
Use of a tenaculum n (%)	
Never	9 (19)
Several times in career	18 (38)
<10% of transfers	18 (38)
<30% of transfers	2 (4)
Approximate location of catheter tip in uterine cavity n (%)	
Upper third	18 (38)
Middle third	25 (53)
Lower third	4 (9)
Approximate distance embryo is deposited (cm) from uterine fundus n (%)	
0.5	1 (2)
1	10 (21)
1.5	12 (26)
2	5 (11)

>2	4 (9)
Don't measure	15 (32)
Who depresses the plunger once the catheter is in place n (%)	
Clinician	34 (72)
Embryologist	13 (28)
Speed and process of embryo deposit n (%)	
As slowly as possible	7 (15)
Slow pace with steady pressure	29 (62)
Moderately fast with steady pressure	11 (23)
As quick as possible	1 (2)
Approach to retained embryos n (%)	
Re-transfer in same catheter	19 (40)
Re-transfer in new catheter	31 (66)
Frequency of retained embryos n (%)	
<1% of ET	35 (74)
1-5%	12 (26)
Presence of blood or mucous on catheter tip n(%)	
<5%	22 (47)
5-10%	18 (38)

10-20%	5 (11)
20-30	2 (4)
Duration catheter left inside cavity following embryo deposition n (%)	
Immediately removed	6 (13)
5-10 seconds	18 (38)
10-20 seconds	17 (36)
30 seconds	5 (11)
1 minute	3 (6)
Direction catheter removed n (%)	
Straight	21 (45)
Rotate as removed	25 (53)
Both	1 (2)
Patient remaining supine after transfer n (%)	
Get up immediately	32 (68)
5-10 minutes	15 (32)

Clinics were asked to rank how they would deal with a difficult transfer and what steps they would take (Figure 18). When faced with a difficult transfer, the majority responded claiming to use a stylet and use cervical dilators was the most infrequent response.

Rank	Choice	Distribution	Score	Times Ranked
1	Use a stylet		242	45
2	Change to another catheter		201	40
3	Use of tenaculum		147	43
4	Keep trying		128	33
5	Call for help		126	38
6	Freeze embryo and transfer on another day		77	42
7	Use of cervical dilators		75	32
		Lowest Highest		

Figure 18. If there is difficulty in ET, what would be your preferred options in order 1-7

When the respondents were asked what they thought to be the most important aspect was with regards to ET, the majority of responses suggested guidance with ultrasound and good consistent technique (Figure 19). Interestingly, there were 3 responses stating that a slow steady transfer improves chances of success whilst 3 other responses urged speedier transfers.

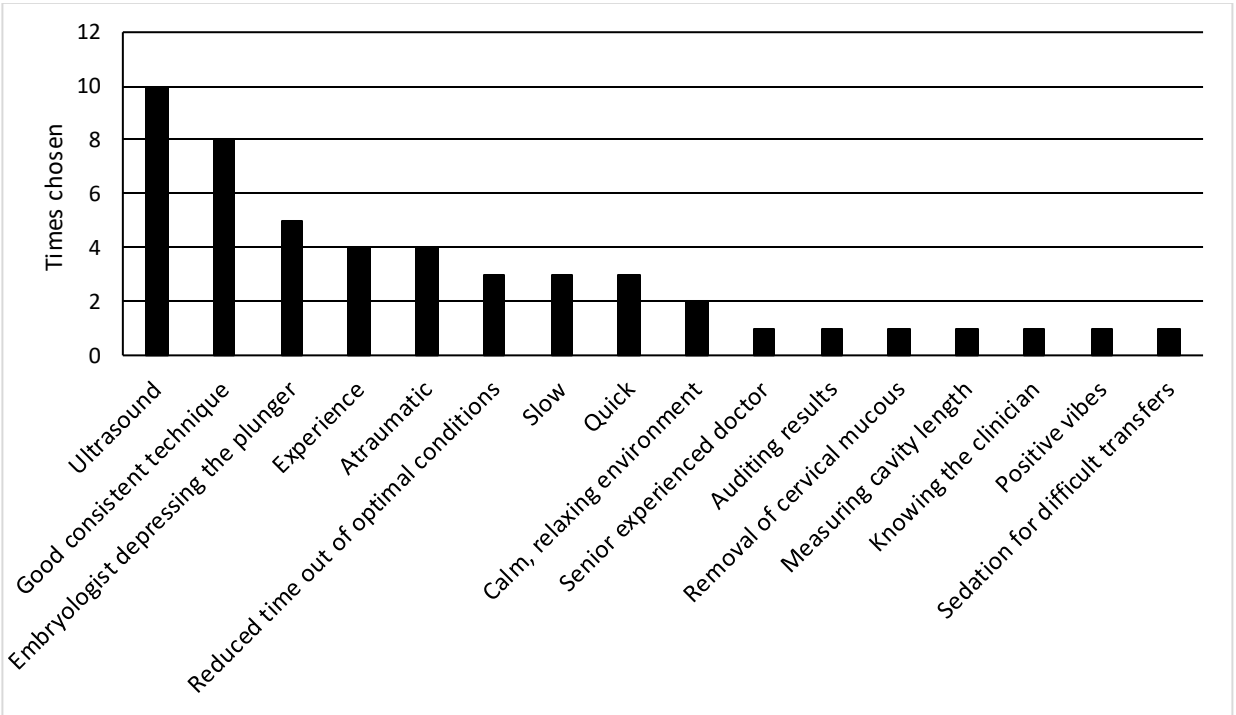


Figure 19. Most important aspects of embryo transfer.

The average LBR per ET in the UK is 21% [283]. The mean and median LBR from the clinics that responded, thus included in our survey was 23%. We therefore split the respondent clinics into low LBR (<23%) and high LBR (≥23%) groups. The only differences in technique between the two groups was how the clinics approached fluid within the endometrial cavity; those in the low LBR were siginificantly more likely to aspirate the fluid or continue with transfer in comparison to the high LBR group (p= 0.007). The high LBR group were more likely to use the CTT (6 vs 2) however this was not statistically significant.

When comparing the LBR published by HFEA for units, very similar results were observed between those units that use ultrasound guidance and those which used clinical touch technique (CTT). For the CTT, the LBR was 22.8% (SD+/-3.06) compared to 22.4% (SD+/-5.4) for the ultrasound guided group (p=0.873).

Duplicate results

There were 5 clinics where multiple responses were received. Four clinics had two respondents to the survey with one clinic sending 4 responses from different clinicians. Interestingly none of the responses were the same. The four clinics with two respondents all reported having a standardized technique for embryo transfer. This was not reflected in the duplicate answers as the number of matching repsonses were 28/38 (74%), 27/38 (71%), 23/38 (61%) and 22/38 (58%).
The clinic where four responses were received, Three clinicians reported a technique based on individual preference with the remaining one clinician reporting a standardized technique. Only 13/38 (34%) responses were matching but this could be due to the first 3 practitioners performing transfers based on their own preference.

Similarily to the ASRM survey, all aspects of the embryo transfer were analysed for concordance and discordance among the respondents[175]. Areas where there was a degree of concordance was assumed when 70% of the units gave the same response. This level of concordance was achieved in 10 of the responses. This included (1) clinician depressing the plunger at ET, (2) abandoning the transfer if fluid was present in the endometrial cavity, (3) having the nurse perform the ultrasound scan, (4) ultrasound guidance for ET, (5) unwarmed speculum, (6) no routine analgesic cover or patient relaxant, (7) presence of a standardized technique for ET, (8) use of stylet, (9) Use of sterile gloves and (10) use of soft catheters.

A Level of discordance was noted when <60% of clinics gave a similar response to a particular aspect of the embryo transfer. This occurred in 11 of the questions. These were (1) basis of ET success, (2) sterility of procedure, (3) lubrication on speculum, (4) instrumentation used to clean cervix, (5) use of a routine mock transfer, (6) ET technique, (7) use of tenaculum, (8) approximate location of catheter tip in uterine cavity, (9) approximate distance uterine fundus embryo deposited, (10) duration of cather left inside the cavity following embryo deposition and (11) direction of catheter removed.

Discussion

This contemporary national survey updates the 16-year-old previous survey on ET technique in the UK and highlights the existing wide variation in practice with no standardised approach to the procedure prevailing in the UK. It therefore emphasizes the urgent need for a standardized national protocol to ensure best outcomes for women undergoing IVF in the UK[241].

Over the years there have been many changes in ET techniques in general, with new evidence demonstrating the benefit of particular practices, such as the use of ultrasound guidance[97], soft catheters[160, 243, 284] and avoiding prolonged bed rest following transfer[279] to improve outcome. Reassuringly, the majority of units that responded, appear to acknowledge the new evidence in their practice (83% ultrasound guidance, 100% soft catheters and 68% immediate mobilisation). Interestingly, we unexpectedly found no significant difference in LBR between clinics regardless of the use of ultrasound guidance.

Positioning of the embryo catheter in the upper or middle third of the cavity was the practice in 91% of the units, in line with the systematic reviews[147, 164]. However, this apparently excellent practice should be considered with caution since some survey responders appear to have different interpretations of the terms upper, middle and lower third of the cavity (Figure 20). They determined the upper third of the cavity as 0.5-2cm, middle third 1->2cm and the lower third as 1.5->2cm from the fundus. Among those respondents who measure the distance from the fundus, 85% will place the catheter 1-2cm from the fundus of the uterus. Frequency of depositing the embryo at the upper third of the cavity increased to 97% if we include those who transfer at >2cm, thus, in keeping with the recommendations from the Cochrane reviews[147, 164, 285]. This draws attention to the need for clarity in a future guideline/study protocol, where embryo deposition is described.

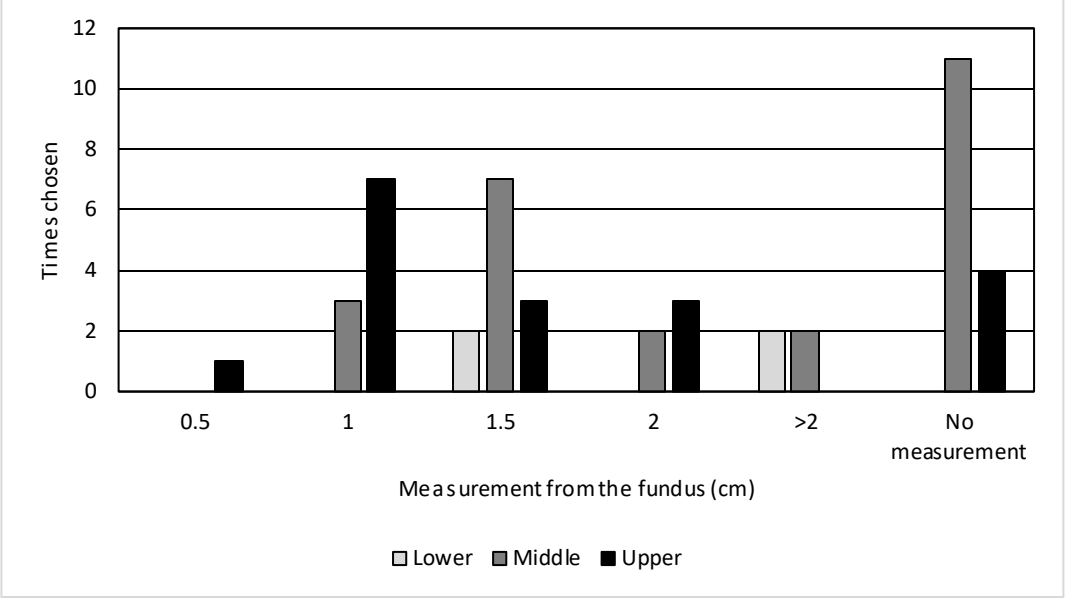


Figure 20. Units interpretation on distance from fundus to location in uterine cavity.

There have been two recent systematic reviews of the literature investigating the particulars of the embryo transfer technique and the impact it has on success rates [147, 164]. The role of anaesthetic or analgesics during the embryo transfer is not recommended and this concurs with current practice in the UK with only one unit reporting using regular sedation for embryo transfers. Particularly with the additional risk anaesthesia adds to the procedure, it is best to avoid unless in specific cases where the procedure would not be tolerated when the patient is awake.

The review found that no specific glove type is recommended for embryo transfer [147] and therefore whether scrubbed, aseptic technique or sterile gloves are used, there is no concern with reference to current UK practice based on our survey results.

The responses to this survey indicate that the majority of units only perform mock transfers for particular individuals. However there is insufficient evidence at present to identify when is the optimal time [164].
The use of soft catheters has been supported by both reviews, showing an increase in pregnancy rates when compared to embryo transfers with hard catheters [147, 164]. This is reflected in the opinions of practitioners in the UK with all clinics using soft catheters.

Both reviews supported the use of ultrasound guidance for embryo transfer, showing good evidence that its use improves IVF outcomes [97, 147, 164]. Survey results showed that the

majority of units adopted this approach, however with 15% not using ultrasound guidance it does show there is room for improvement.

There is fair evidence supporting the removal of cervical mucous prior to transfer [147, 252, 253] reflecting the results of this survey with 85% of units following this evidence.

Despite the available evidence supporting immediate withdrawal of the catheter following embryo expulsion[147, 164, 273, 274] only 6 units (13%) adhered to this, with the remaining units allowing a delay prior to removal. There was no significant difference in pregnancy rates between the groups regardless of this practice [273, 274], however this practice may unnecessarily prolong the uncomfortable procedure for the patient without conferring any benefit.

All units report embryo retention rates at <5% in keeping with previously quoted incidence rates[235]. Maintaining a low retention rate would help reduce patient anxiety and reduce time that the embryo is outside of the incubator/optimal conditions, with prolonged transfer times known to have a detrimental effect on pregnancy rates[286, 287] , although the re-transfer of retained embryos has not shown to be detrimental[267, 269-272].

Conversely, there are areas with room for improvement. Alarmingly, 21% of respondents claimed that they would either aspirate (15%) or proceeded with transfer (6%) when there was fluid identified within the endometrial cavity, despite available advice to the contrary[145, 235]. The frequent use of a tenaculum in some units is another such concern. The use of a tenaculum is not only painful, but can also have a negative impact on embryo implantation rates due to increased uterine contractions due to stimulating oxytocin release[152, 265, 266]. Therefore, it should only be used in difficult ETs, yet surprisingly, it was the third most popular option to be used for difficult transfers.

One other interesting feature identified in our survey was that the majority of respondents estimated their LBR to be between 30-40%. However the 2017 HFEA data reported most of the clinics having a LBR between 20-30%[283]. Although it is possible that this is due to the HFEA data being 2 years older than when the clinics responded to our survey, this may also be relevant to the personal perception versus actual figures, and further highlights the important impact such discrepancies may have when patients are counselled by the clinicians in these units. Relevant to this, CPR was the preferred marker of success for the responders, since presumably it is an easily and relatively rapidly attained marker of

success, with the majority of clinics performing the initial scan themselves to confirm a pregnancy thereby acquiring this data. Subsequently, patients may be lost to follow up and accurate LBR data is more difficult to collate [288]. Importantly, LBR is a mandatory outcome to be reported in the UK and possibly the most relevant data to the patients. However, publicising the CPR, which is naturally higher than the LBR, may be more attractive to patients [289].

Whilst there are a number of questions where concordance is observed in this survey there are more responses that differ than are similar. This lack of standardisation amongst units can be one of the reasons why LBR between clinics range from 11-34% [282]. If standardisation of ET were to occur, it could potentially highlight other imperfect areas within the entire IVF process, in addition to ET, that may also have an impact on the LBR.

Standardization could also reduce research bias, which has previously been noted by Gambadauro et al [290]. When reviewing published trials in IVF there is very little information about the methods and execution involved in the ET and this could potentially be a source of performance bias as there is currently no core outcome set [290, 291]. Our findings are in agreement with a previous survey conducted by the ASRM [175], which also highlighted the need for standardization. As a consequence of their survey the ASRM have been able to produce a protocol for ET suitable for the North American practice [147, 171, 175]. We also anticipate this survey would also facilitate the launch of a similar national/European protocol following discussion with representative bodies such as the BFS and/or ESHRE.

The main limitation of this survey was that we did not achieve full coverage of all UK IVF units. The response rate was reasonably high (59%), but we accept that this survey is not necessarily representative of universal practice within the UK. The data obtained is qualitative and should be interpreted as such, but it is meant to highlight the variations in current practice within the UK and to prompt conversations on how to standardisation could be achieved in ET technique.

The strengths of this survey are that it is the first of its kind in UK, comprehensively and systematically dissecting out the practice of the ET procedure. It has emphasized the concordance, discordance and areas of improvement required in certain practices involved in the ET process, identifying the areas in need of a standardized approach.

ET techniques have been shown to have a significant impact on pregnancy rates [166, 292, 293] and this variation between practices could have an influence (along with other factors of the IVF process) on a units success rates. In a field of medicine where every percent counts, slight changes could result in significant improvement in success rates and patient satisfaction. We therefore have a responsibility to ensure that all patients receive best evidence-based care and this survey brings to light that this may not be the case at least in some areas of the ET process in the UK.

Recommendations

The previously mentioned reviews have made recommendations based on the literature they have reviewed. This can be seen in Table. 8.

Table 8. Recommendations for ET

Recommendation	ASRM guideline[147]	Saravelos et al [164]
Removal of cervical mucous	Grade B evidence	Grade B evidence
Use soft ET catheters	Grade A evidence	Grade A evidence
Abdominal ultrasound guidance	Grade A evidence	Grade A evidence
Embryo transfer to central or upper cavity	Grade B evidence	Grade B evidence
Immediate catheter withdrawal	Grade B evidence	Grade B evidence
Immediate ambulation	Grade A evidence	Grade A evidence
Immediate re-transfer of retained embryo	Grade B evidence	Grade B evidence

Based on the other findings of this survey, current common practice in the UK is as follows:

1. No routine use of anaesthesia or analgesia
2. Use sterile gloves
3. No use of warmed speculum
4. Use sterile water or normal saline for speculum lubrication
5. Clean the cervix with normal saline or laboratory media
6. Use cotton wool or guaze to clean the cervix and remove mucous
7. Abandon transfer if fluid within the endometrial cavity
8. Perform mock transfer for specific indication
9. Afterload technique
10. Use a stylet when required or anticipated difficulty
11. Avoid the use of tenaculum/vulsellum
12. Slow and steady pressure of plunger
13. Remove the catheter either straight or rotational

Conclusion

This is the first survey, which sheds light on contemporary practice and attitudes among different units regarding ET in the UK. It highlights the urgent need for standardisation in ET, a process that is vital for IVF success rates. Such standardization of practice will facilitate practitioner training, research and ultimately IVF success rates. The lack of evidence for best practice that prevails in many areas of the ET procedure will need to be overcome with a consensus expert meeting and review of all literature. This survey has further demonstrated the need for studies to identify the best ET technique and the optimum location within the uterine cavity for embryo implantation. Such information will be essential to improve ET success rates. We therefore believe that areas of discordance identified in our survey, where there is insufficient evidence for the most favourable method, will guide future research to fill the gaps in our current knowledge. Furthermore, the information from this survey highlights the clinical importance of studies undertaken during my MD studentship, presented in the subsequent chapters in this thesis.

Chapter 3: 4D Ultrasound Guided Embryo Transfer vs. Clinical Touch Technique: a randomised controlled trial

Introduction

Most aspects of IVF have developed and changed dramatically since their introduction [58] and this includes ovarian stimulation, oocyte recovery and in-vitro techniques of fertilisation. In contrast, the technique of ET remains largely unaltered [59, 175]. A recent review of European IVF practice found the CPR per ET to be 34.8% [294], suggesting two thirds of ETs were unsuccessful in achieving a pregnancy. This may be due to causes relevant to suboptimal embryos (e.g., chromosomal abnormalities [295]), or defective endometrium (e.g., unreceptive or deficient endometrium[295]), but this could also be related to an inadequate ET technique [265, 295].

The original approach to ET was the CTT, performed by transferring the embryos at a fixed distance from the external cervical os (approximately 6cm [97]) or undertaking a 'dummy' procedure (utilising a uterine sound) to assess the length of the uterine cavity and determine the distance required for the actual ET [237]. The CTT is essentially a "blind" procedure with the aim of depositing the embryo within 10mm of the uterine fundus [237]. Due to the blind nature of the CTT, the optimal site of embryo deposition is not visually determined and there is increased likelihood of the catheter indenting or embedding into the endometrium [237, 296]. Inadvertent endometrial trauma or contact with the fundus may induce high frequency uterine contractions leading to migration of the embryo to suboptimal areas within the uterine cavity or even complete expulsion from the cavity[150]. These events would clearly have a significant negative impact on CPR and LBR [297, 298].

The use of ultrasound at the time of ET was first proposed in 1985, with the anticipation that a more accurate, less traumatic positioning of the catheter tip near the uterine fundus would improve CPR[161], allowing individualised placement of the embryo [299], and facilitating the navigation of the uterocervical junction[97]. UGET has the added benefit of allowing the patient to visualise the procedure, which improves her experience and reduces anxiety levels [181, 300, 301].

A 2010 Cochrane review comparing two dimensional (2D) UGETs with the CTT(n=3622) concluded detecting no difference in LBR (OR 1.14(95%CI 0.93 to 1.39)), but demonstrated

a significant increase in CPR (OR 1.38 (99%CI 1.16 to 1.64)) with UGETs [237]. In 2016, a follow up Cochrane review (n=5859) further demonstrated an increased chance of a LBR/ongoing pregnancy with 2D UGETs, compared with CTT (OR 1.47, 95% CI 1.30 to 1.65; 13 trials; n = 5859 women; I^2 = 74%; low-quality evidence)[97]. Four trials were added to the previous 2010 review, with the largest study (Drakeley et al 2008, n=2295) showing no difference between UGET and CTT[97, 176]. The overall evidence included was deemed to be of low quality due to methodological flaws; e.g. only 2 studies used computer-generated randomisation, lack of detail regarding allocation concealment and randomisation method and high heterogeneity between studies with different embryo deposition points, day of transfer and number of embryos transferred [97, 285, 302, 303]. UGET is recommended by the National Institute of Clinical Excellence (NICE) in the UK and appears to be the preferred method globally, since it is used in 77% of ETs worldwide[234]. However, a significant proportion of units still employ CTT[304], the low quality of evidence for clear benefit found in the 2016 Cochrane review is likely to be the reason for this[97].

Traditionally, UGETs have been performed using TA 2D ultrasound scans[180] with a full bladder. A TA approach is not as invasive as a TV scan and bladder distension may straighten the utero-cervical angle, thus making it easier to pass the ET catheter[265]. Disadvantages associated with TA UGET when compared to the TV approach include, the requirement of an additional practitioner, a full bladder (which can cause discomfort during ET)[305], poorer resolution[174] and suboptimal image quality in some women (e.g. obese or those with a retroverted uterus) [179]. 2D ultrasound lacks the ability to visualise the entire uterine cavity and a designated fixed reference point using a 2D image may not be sufficient for precise embryo positioning, particularly in those with uterine malformations, such as a bicornuate uterus[186]. Three-dimensional (3D) and four-dimensional (4D) imaging of the uterus, which is presently available, allows visualisation of finer detail of the cavity with greater clarity, enabling spatial awareness in terms of dimensions and volume[183, 186]. It is assumed that more accurate placement of the transfer catheter deposits the embryo(s) at the optimal site (the maximal implantation point), thus, resulting in an improvement in pregnancy outcomes[184, 186].

A feasibility study showed that TA 3D ultrasound could confirm correct placement of a trial catheter prior to ET, but the subsequently ETs were not under ultrasound guidance [184]. A further feasibility study reported the ability of 3D and 4D ultrasound to ensure correct catheter placement via the TA route with a resulting increase in pregnancy rate of 10% with 3D/4D UGETs, when compared with 2D scans[306]. This study did not use a specific

distance from the fundus but declared that the embryo was released at the MIP point[183], which was determined by following the trajectory of the fallopian tubes into the middle of the cavity and this could be tailored to an individual's anatomical differences[183].

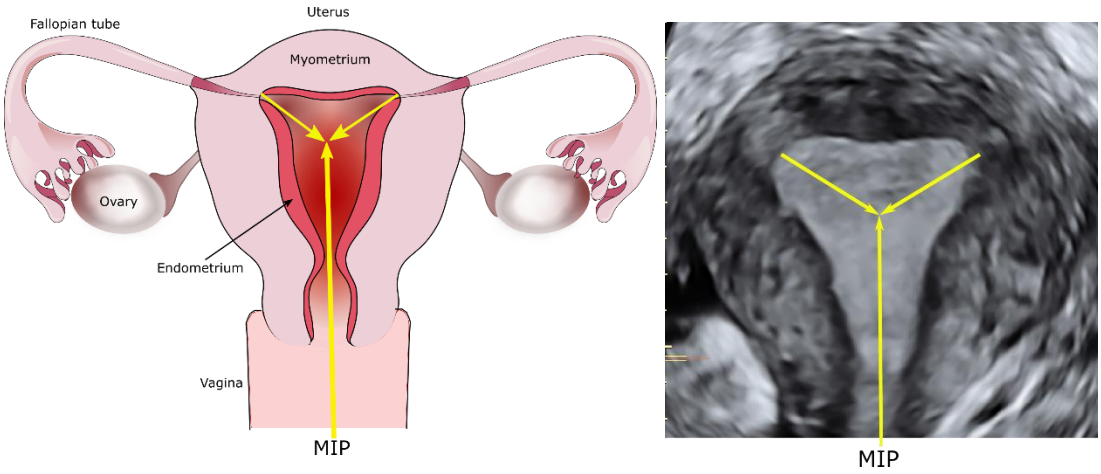


Figure 21. Maximal implantation point[183]

4D ultrasound allows for a real-time 3D view of the uterine cavity, which negates the delays that repetitive 3D sweeps would require, whilst still obtaining the improved spatial awareness of the uterine cavity[190]. This provides an accurate view of the MIP, with embryo deposition at this point potentially leading to improved outcomes.
Kitazato (CE 0086 International (Single Use) Kitazato Medical Co. Tokyo) catheters are soft catheters with a 30-degree curve at the distal end, which has made it possible to perform a TV 4D ultrasound scan at the same time as the ET. This catheter facilitates an easy and atraumatic entrance into the uterine cavity and due to the inbuilt 30-degree curve, has the added benefit of staying in position whilst removing the speculum and inserting the transvaginal ultrasound probe.

The primary aim of this project was to determine if 4D UGETs resulted in higher LBR when compared with the CTT.

Methods

Study design

This was a prospective randomised controlled (un-blinded) parallel trial (RCT) comparing two techniques for ET (4D UGET vs CTT) conducted in a single, NHS fertility centre in the UK. The Hewitt Fertility centre is one of the largest reproductive medicine units in the UK, performing around 1800 IVF cycles per annum. The study received ethical approval from

the Liverpool central research ethics committee on the 9th December 2016 (REC ref: 16/NW/0588) and was registered to ISRCTN registry on the 6th of February 2018 (IRAS 202857, IRSCTN 79955797).

Sample size

In 2015 our unit trialled the 4D UGET technique on an unselected population of 50 patients. The CPR for this group was 40% versus the standard unit CPR of 25% (CTT ET performed).

Based on this feasibility trial, the sample size was calculated. Assuming an expected CPR in the intervention group of 40% and the control group of 25%, to achieve an 80% power to detect this expected difference, (with a significance level of 5%), 149 subjects per group would be required. With an estimated withdraw/non-evaluable subject rate of 5%, we aimed to recruit a total of 157 subjects per group, leading to a total required sample size of 314 subjects. Recruitment commenced in July 2018 and finished in December 2019, patients were followed up until they achieved a live birth, if they conceived from the index ET.

Study population

All consecutive women attending the unit on the day of their ET, were assessed for eligibility according to the inclusion and exclusion criteria detailed in table 9. These inclusion and exclusion criteria were imposed to reduce the number of variables and heterogeneity that could affect pregnancy outcomes. When eligible, women who provided informed written consent were recruited in to the trial.

Table 9. Eligibility criteria

Inclusion criteria	**Exclusion criteria**
Undergoing fresh or frozen single blastocyst ET	Known or suspected hydrosalpinx
Able to provide written informed consent	Fluid within the endometrial cavity
	Gross distortion of endometrium (e.g., fibroids, bicornuate uteri etc.)
	Previous myomectomy

	Previous randomisation
	Significant health issues, e.g., HIV, Hepatitis C, Hepatitis B, previous trachelectomy
	≥2 embryos transferred

These exclusion criteria were agreed upon to limit the number of variables that could affect pregnancy outcomes, ensuring the most homogenous data. The presence of hydrosalpinx, fluid in the cavity or distortion of the endometrial cavity are known to negatively impact implantation rates therefore were excluded [123, 145, 261, 307, 308]. Similarly, previous myomectomies increase the risk of intrauterine adhesions, known to inhibit embryo implantation [309]; Those with significant health issues are more likely to have failed implantation or early pregnancy loss, such as those with inflammatory bowel disease, abnormal thyroid or prolactin hormone levels or autoimmune issues and were therefore not included in this study[310]. Transferring more than one embryo is known to increase pregnancy rates but also multiple pregnancy rates and their associated complications [311], therefore, to standardise outcomes only single ET s were included in this study.

Randomisation

The study numbers were generated at the beginning of the trial using a computer generated online block randomisation tool using a one to one ratio (https://www.sealedenvelope.com/simple-randomiser/v1/lists)[312]. The random numbers were centrally and consecutively distributed in sealed opaque envelopes to the patients recruited.

Control Group

Women in the control group underwent ET according to the accepted standard practice in the unit during the study period, the CTT. The soft, Wallace Classic ET Catheter (18 or

23cm) with centimetre graduations (Smiths Medical International Ltd, UK, CE marked) was the first line catheter utilised and stylets were only used according to clinical requirements (Figure 22).

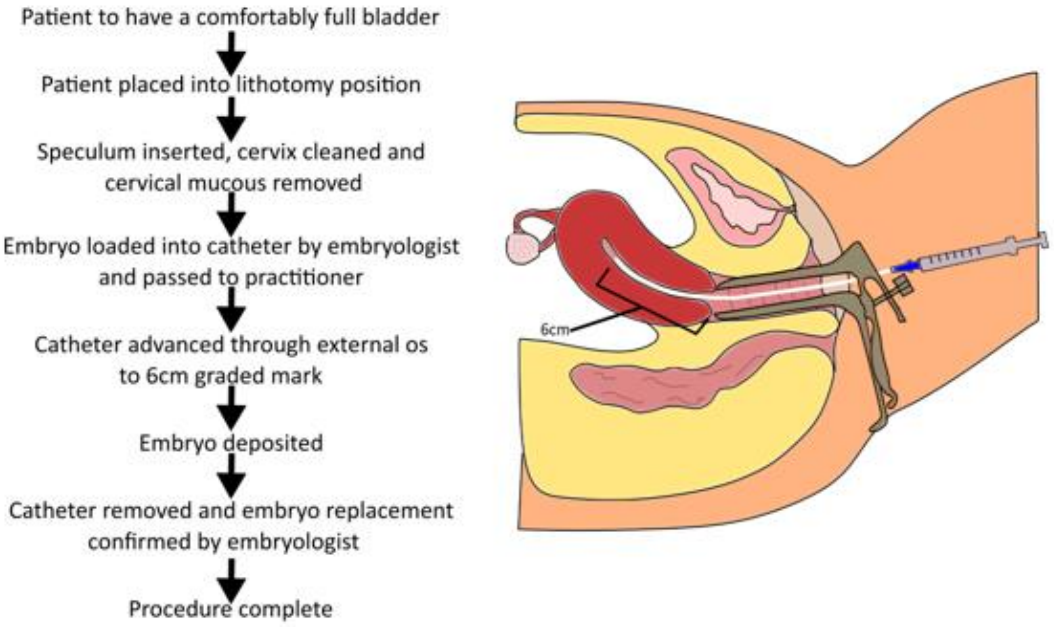


Figure 22. Flow chart depicting the steps taken during the CTT in the control group.

Intervention group

The Kitazato ET Catheter Inner 3Fr. 40cm Guide 30° / 20cm. Ref 223340 (CE 0086 International (Single Use) Kitazato Medical Co. Tokyo) has been designed to allow TV replacement of the embryo under TV ultrasound guidance. Ultrasound scans were performed using a General Electric Volusen E8 ultrasound machine with a 3D/4D RIC5-9-D transvaginal probe (GE Medical Systems Kretztechnik GmbH & Co, Austria) (Figure 23).

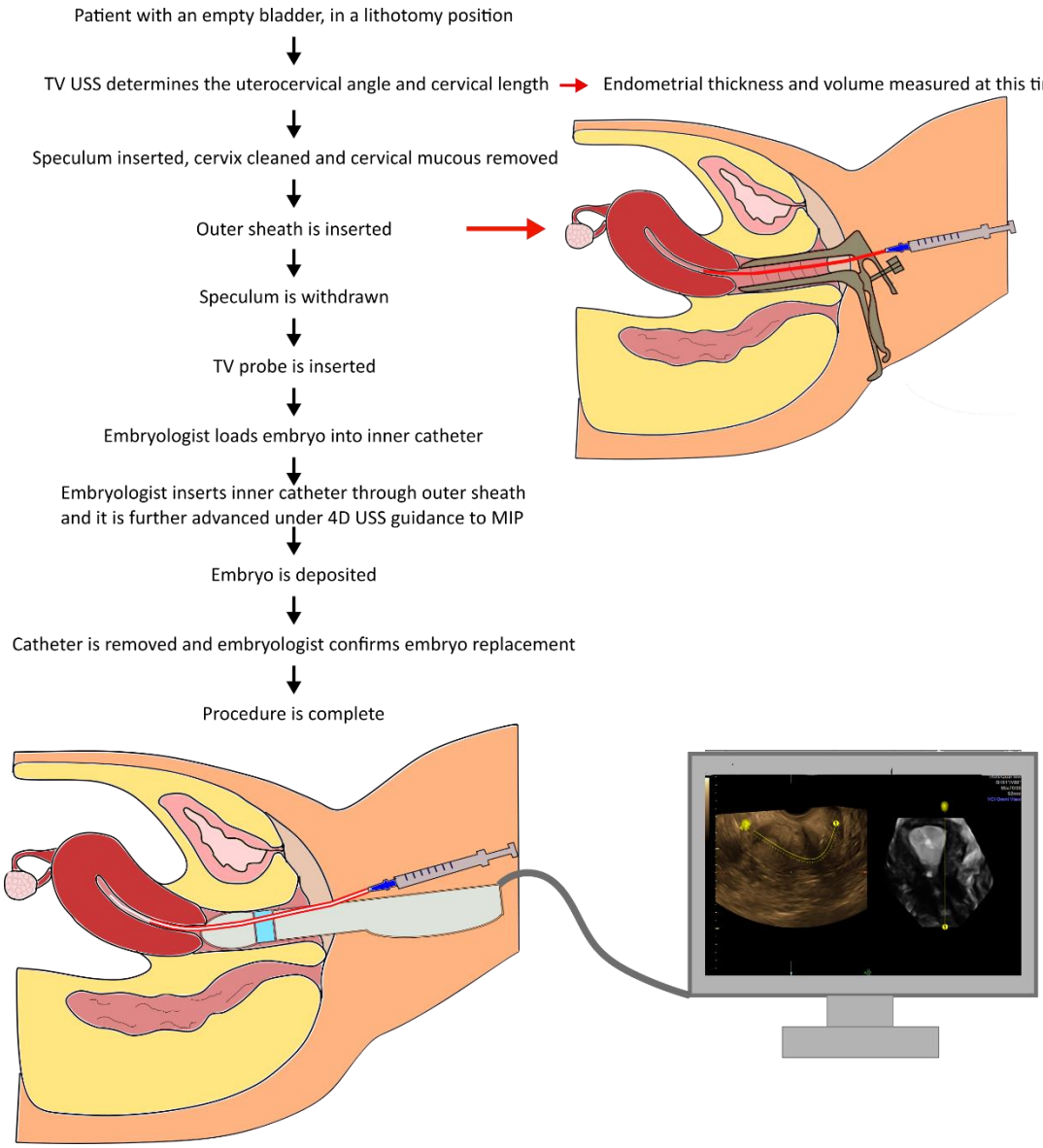


Figure 23. Flow chart depicting the steps taken during the ET process using the Kitazato catheter in the intervention group.

Outcomes

Clinical

The primary outcome measure of this study was the LBR. The secondary outcomes included BPR, CPR, miscarriage rate, ectopic pregnancy rate, multiple pregnancies. Duration of the procedure was also recorded from the initial patient preparation until embryo deposition. Those in the intervention group also had endometrial thickness and volume measured at the time of the transfer.

Patient satisfaction

All participants completed a questionnaire after their ET, where they graded comfort and satisfaction associated with the procedure on a numerical scale from 1-5, where 1 corresponded to being extremely uncomfortable or unsatisfied and 5 linked to being very comfortable and satisfied. The questionnaire also contained a free text comments section for participating respondents to document their judgments and views on the procedure.

Clinician satisfaction

Clinicians performing the ET also ranked the ease of the above two procedures from 1 to 5, with 1 corresponding to being uncomplicated and straightforward and 5 being very difficult.

Statistical analysis

All data was entered and analysed in the Statistical package for the Social Sciences (SPSS) for Windows (Version 26; IBM Corporation, USA). Continuous data was analysed using the students *T*-test, whilst categorical data was analysed using the χ^2 test. One-way ANOVA test was used to compare the means of more than two groups. Significance was set at two-sided p-value of < 0.05.

Results

A total of 320 patients were recruited over 17 months and 25 were subsequently excluded as presented in the flowchart in Figure 24.

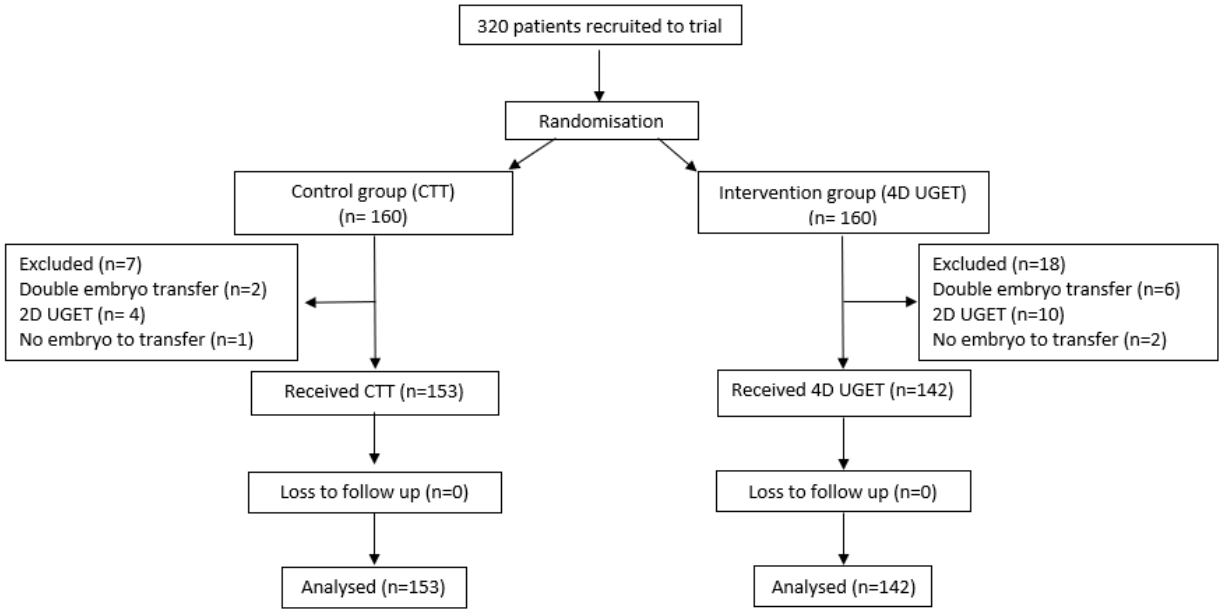


Figure 24. Flow diagram of recruitment and exclusions

Baseline characteristics

The baseline characteristics for study population are summarized in Table 10.

Table 10. Baseline characteristics

	CTT (n=153)	4D UGET (n=142)	P-value
Age (years) [mean ±SD]	34.04 [4.14]	33.12 [3.86]	0.05
BMI (kg/m^2) [mean ±SD]	25.43 [5.40]	24.85 [3.58]	0.29
Duration of infertility (years) [mean ±SD]	3.68 [2.29]	3.66 [2.02]	0.94
Type of infertility Primary [n, %] Secondary [n, %]	 75 (49) 78 (51)	 73 (51) 69 (49)	 0.68

AMH [mean ±SD]	25.31 [23.35]	25.73 [24.37]	0.88
Number of previous IVF/ICSI attempts [mean ±SD]	1.31 [0.69]	1.32 [0.77]	0.84
Number of previous ETs [mean ±SD]	1.30 [1.76]	1.18 [1.48]	0.51
Type of stimulation cycle Agonist [n, %] Antagonist [n, %] Embryo recipient [n, %]	 12 (8) 132 (86) 9 (6)	 8 (6) 128 (90) 6 (4)	 0.59
Oocytes retrieved	14.8	13.7	0.27
Type of ET Fresh [n, %] Frozen [n, %]	 63 (41) 90 (59)	 48 (34) 94 (66)	 0.19
Embryo quality: Good [n, %] Average [n, %] Poor [n, %]	 93 (61) 42 (27) 18 (12)	 90 (63) 37 (26) 15 (11)	 0.89

The causes of infertility were many and varied (Figure 25).

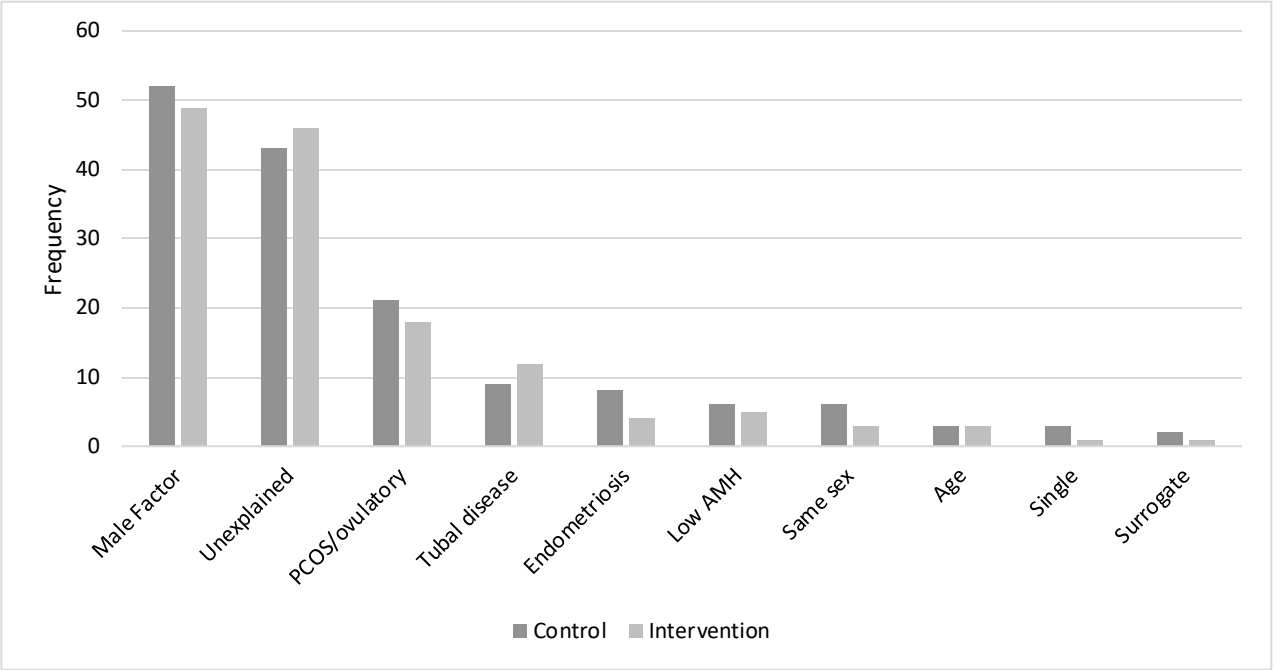


Figure 25. Causes of infertility

All of the 4D UGET were performed by one practitioner with the majority (95%) of the CTT also being performed by the same practitioner. There were 3 other practitioners who performed 7 of the CTT ETs.

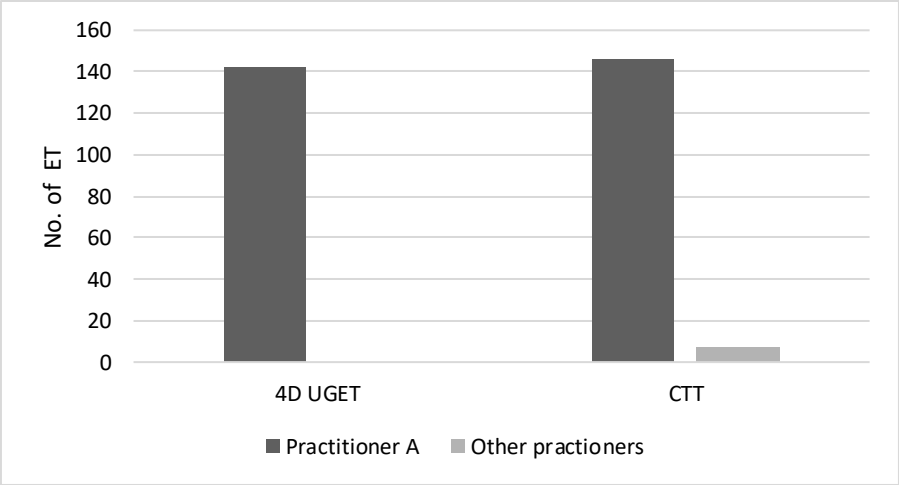


Figure 26. Practitioners involved in ET

Outcome analysis

Clinical outcomes:

There was a statistically significant improvement in the LBR for the 4D UGET when compared with the CTT group (41% vs 28% respectively, p= 0.02). This was also replicated in the BPR and CPR (Table 2, Figure 27). In the control group, there was one cervical ectopic pregnancy. In the intervention UGET group, there was one tubal ectopic pregnancy, which required salpingectomy, two pregnancies of unknown location (PUL), managed conservatively, and one termination of pregnancy (TOP) due to fetal abnormality.

The time taken to perform 4D UGET was significantly longer than the CTT ETs, and this is related to the additional steps required with the procedure (Table 11.).

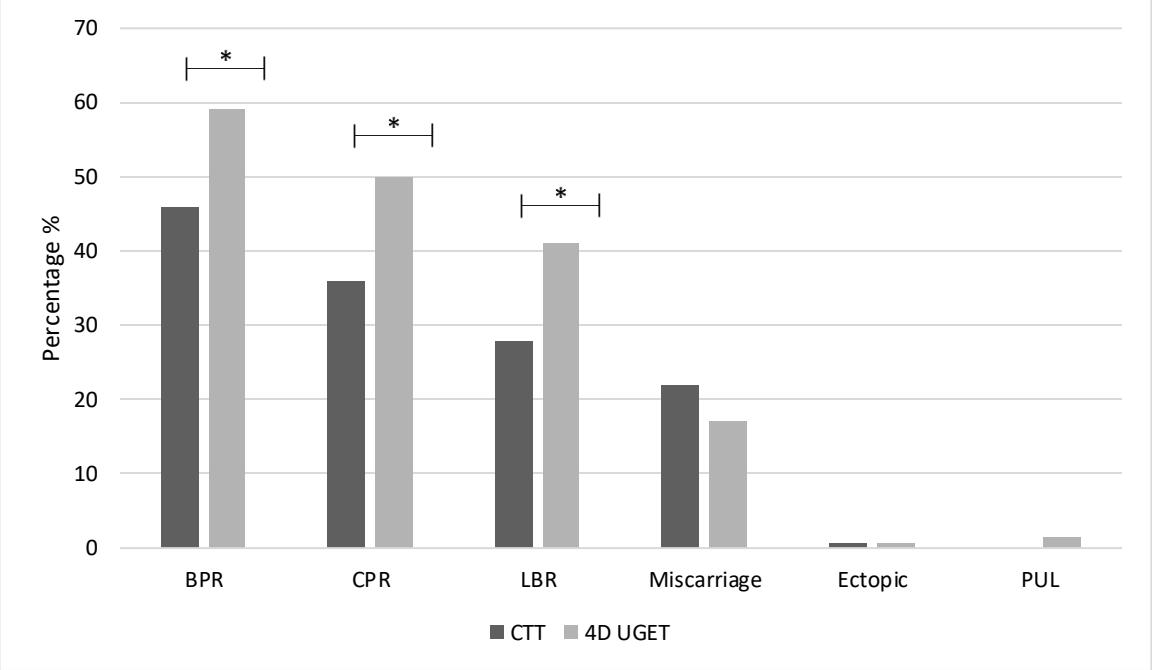


Figure 27. Primary and secondary outcome measures. (* = p <0.05)

Table 11. Clinical outcome measures

	CTT (n=153)	4D UGET (n=142)	P-value	Odds ratio (95% Confidence interval)
Biochemical pregnancy rate [n, %]	70 (46)	84 (59)	0.02	1.71 (1.08-2.72)
Clinical pregnancy rate [n, %]	55 (36)	71 (50)	0.02	1.78 (1.12-2.84)
Live birth rate [n, %]	43 (28)	58 (41)	0.02	1.77 (1.09-2.87)
Miscarriage [n, %]	12 (22)	12 (17)	0.49	0.73 (0.30-1.79)
Ectopic pregnancy [n, %]	1 (0.6)	1 (0.7)	0.96	1.08 (0.07-17.3)
PUL [n, %]	0 (0)	2 (1.4)	0.14	1.01 (0.99-1.03)
TOP [n, %]	0 (0)	1 (0.7)	0.30	1.01 (0.99-1.02)
Duration of procedure (minutes) [mean ±SD]	10.28 [2.18]	15.77 [2.62]	<0.01	NA

Ease of procedure: The ease of performing the procedure between groups did not show any statistical significance, however, 14 transfers were converted to 2D ultrasound guidance (4/157 (3%) in the control group and 10/152 (7%) in the 4D UGET intervention group). Those that were converted to 2D ultrasound in the control group were due to the difficultly experienced navigating the uterocervical angle during CTT. In the intervention group, conversion was due to problematic visualisation of the uterine cavity using 4D ultrasonography due to the uterus being axial in position or thin endometrium. Conversion to 2D US allowed for better visualisation of the catheter tip at the time of transfer.

Table 12. Ease of the procedure

	CTT (n=153)	4D UGET (n=142)	P-value
Ease of procedure [n, %]			
1	130 (85)	113 (80)	
2	9 (6)	10 (7)	0.66
3	12 (8)	16 (11)	
4	2 (1)	3 (2)	
5	0	0	

Patient satisfaction: The women did not report experiencing a significant difference in patient discomfort (p=0.17) or satisfaction (p=0.08) between the two procedures (Table 13). However, those in the 4D UGET arm of the trial were significantly more likely to write a comment following the procedure (p<0.001) and their comments were more likely to be positive (p<0.001). Sixty one percent (87/142) participants in the 4D UGET group commented, most of these comments (81) were complimentary, while only 7% (11/153) commented in the CTT group with only 4 positive comments. Thirty-six women stated their preference was UGET in comparison with CTT in their comments (Table 14).

Table 13. Patient satisfaction

	CTT (n=153)	4D UGET (n=142)	P-value
Patient comfort	4.44 (±SD 0.77)	4.31 (±SD 0.80)	0.17
Patient satisfaction	4.93 (±SD 0.39)	4.99 (±SD 0.12)	0.08

Table 14. Comments regarding ET

	Positive	Neutral	Negative	Total	*P* value
Control arm	4	4	3	11	<0.001
Intervention arm	81	6	0	87	

Endometrial assessment: The intervention group had endometrial thickness (EMT) and endometrial volume (EMV) measured at the time of ET (n=141, one patient unfortunately did not have saved images). No difference was observed in EMT or EMV between those who had a live birth and those who did not (p= 0.19 and p=0.84 respectively). Women who underwent a fresh ET had a significantly thicker EMT (p<0.001) and higher EMV (p<0.001) without an impact on the pregnancy rates.

Table 15. Mean endometrial thickness and volume

		Number	Mean (mm)	Standard error	P= value
Endometrial thickness	Live birth	57	8.97 (±SD 1.66)	0.25	0.19
	No live birth	84	9.44 (±SD 2.27)	0.22	
	Miscarriage	11	9.45 (±SD 2.33)	0.67	0.38
	Fresh	48	10.25 (±SD 2.32)	0.33	<0.001
	Frozen	93	8.74 (±SD 1.70)	0.18	
		Number	Mean (cm^3)	Standard error	P= value
Endometrial volume	Live birth	57	5.04 (±SD 2.39)	0.32	0.85

	No live birth	84	4.95 (±SD 2.27)	0.25	
	Miscarriage	11	4.52 (±SD 1.78)	0.51	0.60
	Fresh	48	6.20 (±SD 2.54)	0.37	<0.001
	Frozen	93	4.36 (±SD 1.92)	0.20	

Women with an EMT between 7-12mm had a statistically significant increased BPR (p=0.04) when compared with those ≥12 or ≤7mm, however such difference was not observed for CPR (p=0.22) and LBR (p=0.23) (Table 16). When the 7-14mm range was considered, no difference was observed in BPR, CPR or LBRs outside of that limit (≤7mm or ≥14mm).

Table 16. Endometrial thickness / volume and pregnancy outcomes

		Biochemical pregnancy (n)		Clinical pregnancy (n)		Live birth (n)		Miscarriage (n)	
Endometrial thickness (mm)	≤7 + ≥12	8/21	P=0.04	8/21	P=0.22	6/21	P=0.23	2/8	P=0.30
	7-12	75/120		63/120		51/120		9/75	
Endometrial volume(cm^3)	≤2.5	10/18	P=0.76	10/18	P=0.64	8/18	P=0.71	2/10	P=0.50
	≥2.5	73/123		61/123		49/123		9/73	

Discussion

Use of 4D ultrasound guidance for ET is associated with a statistically significant increased CPR when compared with CTT for ET. The 4D UGET method also led to a statistically significant improvement in BPR and LBR in comparison with CTT. To the best of our knowledge, this is the first RCT examining the clinical efficacy of 4D UGET and the 13% increase in pregnancy rate and LBR we observed, is in concordance with the previous observational study [24], that reported a 10% increase in pregnancy rates when 4D UGET was implemented into routine practice [306].

Patients included in our 4D UGET group achieved a LBR of 41%, almost double that of the current national LBR per single ET of 22-23%[283]. Our recent survey on ET practice in the UK suggested that the majority (85%) of clinics in the UK are using 2D ultrasound guidance with only a small proportion still using the CTT[304]. Therefore, we postulate the observed increase in pregnancy and LBR of the 4D UGET group in our study to be a result of optimal deposition of the embryo at the MIP in comparison to the blind, imperfect embryo deposition with the CTT.

Previous studies using 2D ultrasound proposed the best embryo deposition points to range from 0.5-2cm from the fundus of the uterine cavity, which is further endorsed by recent reviews recommending embryo deposition in the upper/middle third of the uterine cavity (between 1-2cm from the fundus)[147, 164]. However, the length of the uterine cavity is dependent on the patient, phase of the menstrual cycle and stimulation protocol[313-315], therefore, fixing a set distance from the fundus for embryo deposition is unlikely to be the optimum location for all patients. For example, 2cm from the fundus may be either in the upper third or in the lower third of the cavity, depending on the patient, this could have a significant impact on pregnancy outcomes[316]. Embryo deposition at the MIP allows for a patient-specific personalised approach, tracing the natural course of an embryo in to the uterine cavity from the fallopian tube.

We accepted an EMT of 7-12mm as optimal when scanning the participants in the 4D UGET group based on previous studies[117, 196]. We noted a significant improvement in BPR when the endometrium was between 7-12mm but this was not reflected in the CPR or LBR. Interestingly, those who had a fresh ET were more likely to have an increased EMT and EMV, likely due to the supraphysiological hormone state that occurs following ovarian stimulation[194], However, these findings had no impact on the LBR between the fresh and frozen subgroups. EMV had no impact on pregnancy outcomes, even those women with an EMV ≤2.5cm^3 still achieved a 38% LBR, this is in discrepancy to previous

recommendations[208, 213]. Similar to our study, these previous studies have performed TV EMV measurements using similar techniques on the day of ET[206, 208, 213, 317, 318]. In our study one experienced operator performed all the ultrasound measurements ensuring a consistent approach and technique. Previous studies have found high interobserver and intraobserver reliability and reproducibility when using 3D ultrasound EMV measurements[207, 213] supporting the role of 3D ultrasound for accurately measuring endometrial volume. Whilst some studies have determined what the optimal EMV should be, subsequent reviews and meta-analysis still find that EMV is a poor predictor of pregnancy outcome, in keeping with our findings.

Although the available evidence for 4D UGET is lacking, there have been a number of previous studies highlighting the benefit of obtaining 3D views of the endometrial cavity during ET[186, 319]. 3D UGET have been shown to give exact positioning of the catheter in the majority of cases, without increasing the duration of the ET procedure [184]. Further studies have also shown a discrepancy between embryo deposition point when comparing 2D and 3D images at the time of ET [186, 319], and a disparity between 2D and 3D measurements had a negative impact on subsequent pregnancy rates [186]. The only published RCT comparing 3D UGET with 2D UGET showed no difference between CPR between the two techniques[191]. That study however, did not collect data on the duration of procedure between groups and the need to adjust the catheter tip following repeated acquisition of the 3D image. Furthermore, the 3D images in that study were not 'live', thus requiring repeated 3D sweeps of the uterus, subsequent interpretation of the scan and potential adjustment of the catheter tip. This would increase the duration of the ET, which could lead to additional stresses to the embryo and subsequent deleterious effect on its successful implantation[192, 287]. Further concern with this approach would be the additional trauma from repeated catheter adjustment[235]. Furthermore, the study lacked power to assess difference in subgroups of women [191], thus, the benefit of 3D UGET over 2D UGET is yet to be fully elucidated.

The largest report of 4D UGET to date, albeit being a retrospective, observational study, found an increase in pregnancy rate of 10% when 3D/4D ultrasound guidance was implemented, demonstrating superiority of the method. However, the authors did not comment on any other changes to their practices that took place during the 5 years of data collection, which could have also attributed to the improved pregnancy rate [187, 306].

There are many postulated benefits of 4D UGET, including improved patient's experience and comfort as they can have an empty bladder during the procedure and they can visualise the transfer. Contrary to the expectation, there was no difference between the two methods for patient comfort or satisfaction scores. However, there were significantly more positive responses in relation to the 4D UGET approach with patients documenting the benefits of comfort and satisfaction due to reduced duration of speculum insertion, being able to have an empty bladder and the reassurance of being able to see the transfer on the ultrasound screen, in the free text comments section of our questionnaire. This may suggest that our questionnaire scores did not capture these subtle improvements in patient perception/assessment associated with the 4D UGET. Since our questionnaire was administered at the time of the ET, the observed superior clinical outcome associated with 4D UGET had no bearing on the patient satisfaction. Whilst our questionnaire scores failed to provide evidence for an improved comfort/satisfaction for the patients at the time of ET, 4D UGET group had the better clinical outcomes and it would be interesting to assess the patient experience at a different time point, in a future study, once the patient knew the outcome of the ET.

We found no difference between the groups when the clinicians assessed the ease of the procedure. The 4D UGET technique does require more skill to perform, however once the technique was learnt, applying it to clinical practice was often without complication. This observation needs to be considered with caution, since in this study; the same well-trained individual performed all 4D UGET procedures. For generalisability of this observation, further studies including multi-operators assessing the procedural ease of these techniques are required.

Interestingly, more 4D UGET procedures were converted to 2D UGET than in the CCT group. However, this was predominately due to suboptimal views of the uterine cavity but not because of difficulties in the procedure. The suboptimal views were commonly associated with an axial uterus (which led to more distortion of the image) or a thin endometrium obscuring clear outline of the uterine cavity. Manipulation of the uterus at the time of ET will have a negative impact on implantation, thus avoided in those with an axial uterus [150, 152]. ET can be cancelled in those with a thin endometrium, and the embryo can be frozen for transfer in a more favourable future cycle, although this may be associated with the potential risk of failed thawing or re-thawing of the embryo[320]. In our study, when conversion to 2D was required in the 4D UGET group, the procedure was effortless and

caused minimal delay or discomfort to the patient. Reassuringly, the observed higher conversions to 2D UGET in the 4D UGET group did not affect the superior BPR, CPR and LBR rates.

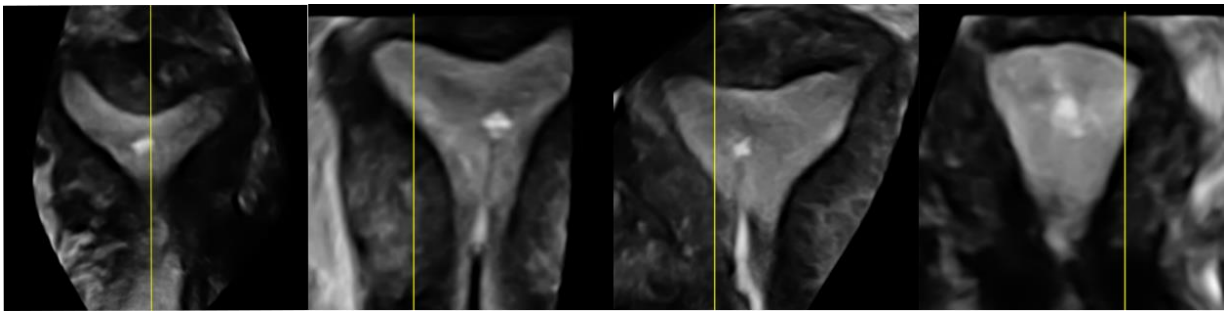


Figure 28. Variances in uterine cavity shape. Echobright areas within the uterine cavity show embryo deposition in trial patients.

Strengths and Limitations

Our data from a large RCT including an unselected population of 320 women recruited and randomised on the day of their ET has provided robust evidence supporting the use of 4D UGET over CCT technique [176, 191, 236].

A curved catheter was required for 4D UGET, as the curve helped to stabilise the catheter upon removal of the speculum and subsequent insertion of the TV ultrasound probe thus, different types of soft catheters were utilised in our control and study groups. This may pose an inherent bias in our data; however, previous studies have confirmed non-inferiority between different soft catheters in altering birth rates [160, 321-324].

A small (1-2 seconds) time lag between the 4D ultrasound images was identified during the trial and had to be accounted for when performing the transfer. This issue was rectified with slow and steady catheter insertion, and good communication between the practitioner and the embryologist. Future advances in ultrasound technology is expected to further refine this minor issue.

4D ultrasonography is an advanced technique and is not a skill available in every assisted reproductive technology (ART) unit. Additional training is required to develop the technique for performing this type of ultrasonography and as it is currently untested against 2D UGET, therefore we are cautious to recommend routine use over 2D UGET practice, considering the additional cost and resource implications.

All of the 4D UGET and 146/153 (95%) of the CTT were performed by one experienced operator and although this reduced operator bias of the technique, we are not able to determine the effect of different practitioners of varying degrees of experience on the outcomes, thus the generalisability of our data cannot be confirmed. We documented the duration of the procedure from the start until deposition of the embryo, however, the length of time from when the embryo leaves culture until it is deposited in the uterine cavity maybe of more value as a surrogate of stress to the embryo.

Conclusion

Our study has demonstrated that 4D UGET significantly improves LBR in comparison to CTT. Future studies are warranted to assess the potential advantage of 4D UGET with 2D UGET to ensure the best ET methodology is utilised, thus, the highest CPRs are achieved. Implementation of 4D UGET in routine practice would require the development of training programs suitable for upskilling ART practitioners with the view to improving both ET outcomes and diagnosis and management of other relevant pathologies in reproductive medicine. Enactment of these advanced skills into routine care could be incorporated into a national guideline to ensure provision of best possible care for the patient throughout the UK.

Chapter 4: Optimal implantation site and trophoblastic thickness at early gestation scan – An observational study.

Introduction

Embryo implantation is the final step of the IVF process, which can be improved by a number of different factors: ET techniques [96, 166, 235], optimising the embryo deposition by ultrasound guidance during ET [97, 174], catheter type [160, 243] and determining the embryo deposition point [223, 285].

Although vital for a successful conception, the embryo implantation process is still not fully understood. It involves a complex interaction between a synchronized embryo and the endometrium [37]. The available evidence is somewhat contradictory, for example, existing evidence suggest that optimising the placement of the ET catheter within the uterine cavity improves pregnancy outcome [170], but there is lack of direct evidence to suggest that embryo deposition point is related to final implantation site [216, 223].

Ultrasound imaging is a key diagnostic tool used in the management of various gynaecological and obstetric conditions [325]. Standard practice in this area of medicine relies predominately on 2D ultrasound imaging [325], with TV ultrasound showing higher sensitivity and specificity at identifying pelvic pathologies in comparison to transabdominal (TA) ultrasound [184, 190, 326]. Furthermore, significant advances are also made in detailing pelvic anatomy and early pregnancy with the use of TV 3D/4D ultrasound scanning methods, which have superior image quality and detail [184, 190, 326].

Endometrial blood flow is the highest in the uterine fundus than in other areas of the uterus [327]. This led to the assumption that the fundal area would be the optimal site of implantation for an embryo [215, 327]. This assumption has been further supported by a number of studies assessing natural conceptions between 4-6 weeks gestation, reporting the majority of ongoing pregnancies to be located within the upper portion of the endometrial cavity, whilst a higher rate of miscarriage had been reported when the implantation site is in the lower half of the endometrial cavity [215, 217, 327]. In contrast, the only study looking at the implantation site in IVF pregnancies found a higher proportion of pregnancies implanted in the middle of the cavity (29.8% IVF vs 9.4% natural conception) and there was no difference in miscarriage rates despite the spatial location of the pregnancy within the uterine cavity [169].

Trophoblast invasion is a key part of the implantation process, and the extent of invasion determines the quality of anchorage and depth of the placenta[228]. Poor invasion increases the risk of miscarriage and other obstetric complications, such as pre-eclampsia and intrauterine growth restriction[228]. One previous study, reported that if the trophoblastic thickness (TT) was ≥3 mm less than gestation age (i.e. 4mm TT at 7 weeks gestation) that was associated with an increased risk of miscarriage, implying a very early placental insufficiency as the potential cause of pregnancy failure [232]. However, a subsequent study showed conflicting data demonstrating that miscarriage rates were not impacted by TT [233]. Both the studies assessed natural conceptions and did not include pregnancies related to ART. Typically, ART uses exogenous hormones, and the above studies could not account for this potential influence of exogenous hormones on the endometrium and subsequent trophoblastic invasion.

The aim of the study detailed in this chapter therefore, was to determine whether pregnancy site location and TT in IVF pregnancies with a single embryo transfer had an impact on early and late pregnancy outcomes. Outcomes we considered included miscarriage rates, live birth rates, and we also assessed their relationship with other late obstetric outcomes related to the placental function such as gestation at delivery, birth weight and obstetric complications such as small for gestation age (growth <10th centile[328]) (SGA) and pre-eclampsia.

Methods

This prospective observational study was approved by the East Midlands – Leicester Central Research Ethics Committee (REC 16/EM/0392). Women attending the Liverpool Hewitt fertility centre were included if they had a single embryo transfer and subsequent live viable intrauterine pregnancy confirmed on scan. There may be unknown mechanisms relevant to more than one embryo on the trophoblastic invasion and determination of pregnancy location is more difficult in multiple gestations. Therefore, only single embryo transfers were recruited. Uterine cavity abnormalities were also excluded due to known effect on implantation and association with higher miscarriage rate [295, 329] (Table 17).

Table 17. Inclusion and exclusion criteria

Inclusion criteria	**Exclusion criteria**
Single ET	≥2 embryos transferred
Single viable intrauterine pregnancy	Multiple gestations
Able to provide informed consent	Uterine cavity abnormalities (e.g. Submucosal fibroids, septate uteri)
	Intrauterine insemination (IUI) pregnancies
	Unable or unwilling to provide consent to the study

The Hewitt Fertility centre, is one of the largest reproductive medicine units in the UK, performing around 1800 IVF cycles per annum.

Women were approached and given verbal and written information (patient information sheet) (Appendix) after their early gestational scan (performed between 6 to 8 weeks of pregnancy). Once they agreed to participate in the study, consent was confirmed and written consent was obtained (Appendix). The ultrasound scan for the study was a 3D automated volume acquisition sweep and the images were stored for measurements to be calculated at a later time. All measurements were performed by a single experienced operator. All ultrasound scans were performed using a General Electric Volusen E8 ultrasound machine with a 3D/4D RIC5-9-D transvaginal probe (GE Medical Systems Kretztechnik GmbH & Co, Austria).

Implantation site was determined by measuring the smallest distance from the gestation sac to the anterior and posterior aspect of the uterus, the uterine fundus, the lateral edges of the uterus and the internal cervical os. If the distances were equal then the gestation sac was determined to be in the middle of that plane (i.e. 1.5cm from fundus and the internal os would be in the middle of the uterine cavity in the sagittal plane). The optimal resolution was ensured with utilisation of the high frequency transducer (5-9MHz), and TT was measured in the anterior-posterior diameter in the anterior aspect of the uterus.

The demographic variables collected included, woman's age, BMI, type of infertility, type of embryo transfer (fresh or frozen), the use of luteal support and embryo quality.

Patients who had a fresh ET had 11 days of luteal support as a standard practice in our unit, and those who had a stimulated frozen ET continued luteal support up until 12 weeks of

pregnancy. The luteal support was provided with 400mg progesterone pessaries or suppositories, which were taken twice daily. Those who had a natural frozen ET did not take any additional medications.

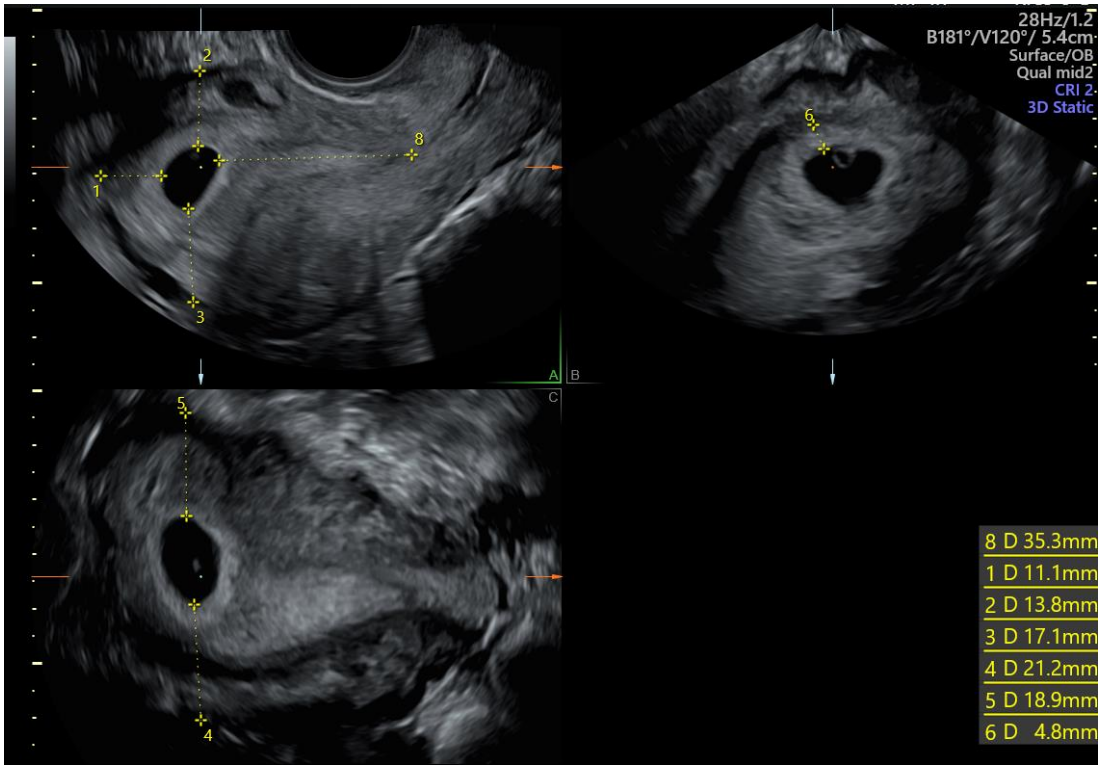


Figure 29. Measurements acquired. 1- Distance from fundus. 2- Distance from anterior uterine wall. 3- Distance from posterior uterine wall. 4-Distance from right uterine wall. 5-Distance from left uterine wall. 6- Trophoblastic thickness. 8- Distance from internal cervical os.

The primary outcomes we considered were live birth rates and miscarriage in relation to pregnancy location and TT. Still births and termination of pregnancies were also recorded, however their numbers were too small and therefore not included in the statistical analysis. Other secondary outcomes included placental location, birth weight and obstetric complications (pre-eclampsia (PET), small for gestational age (SGA), placenta praevia, gestational diabetes (GDM) and stillbirth).

Using the population-based growth chart, birth weights were classed as either SGA, appropriate for gestational age (AGA) or large for gestational age (LGA, growth >90th centile[330]).

Outcome data was obtained via local electronic hospital records or telephone call to recruited consented patients. Data was uploaded onto excel (Microsoft Excel 2019) prior to analysis.

Statistical analysis

Data was migrated from excel to Statistical Package for the Social Sciences (SPSS Version 26; IBM Corporation, USA) for analysis. Continuous data was analysed using students *T*-test whilst categorical data was analysed using χ^2 test. When means of more than two groups were compared one-way ANOVA test was used. Significance was achieved when the two-sided p-value was < 0.05.

Results

Three hundred women were recruited over a 14-month period (Aug 2018 to Oct 2019) from a single, NHS fertility centre in the UK. Of the total 300 women recruited, 277 (92.3%) achieved a live birth, 20 (6.7%) had a miscarriage, 2 (0.7%) had stillbirths and 1 (0.3%) had a termination of pregnancy (TOP) for trisomy 21.

Base line characteristics

The group of women who had a miscarriage were significantly older than the women who had a live birth but no further differences were noted in other baseline characteristics (Table 18).

Table 18. Baseline characteristics between live birth and miscarriages

	Live Birth (n=277)	Miscarriage (n=20)	P value
Mean Age (years)	33.5 (±SD 3.89)	35.9 (±SD 3.35)	**0.008**
Mean BMI (kg/m^2)	24.6 (±SD 3.41)	25.3 (±SD 2.54)	0.38
Type of infertility (n)			
Primary	120 (43%)	10 (50%)	0.77
Secondary	157 (57%)	10 (50%)	
Type of ET (n)			
Fresh	101 (37%)	4 (20%)	
Natural Frozen ET	98 (35%)	8 (40%)	0.30
Stimulated Frozen ET	78 (28%)	8 (40%)	

Embryo quality (n) Good Average Poor	(n=270*) 190 (70%) 64 (24%) 16 (6%)	 16 (80%) 4 (20%) 0	 0.84
BMI – body mass index, SD – standard deviation			

*7 of the embryos were transferred on day 3 therefore they were unable to graded according to the Gardner and Schoolcraft grading system.

Pregnancy site location

Having a fresh or a frozen ET did not impact on pregnancy site location as per Table 19. The provision or the type of luteal support also had no effect on where the pregnancy was implanted in the uterine cavity.

Table 19. Fresh or frozen ET and pregnancy site location

Pregnancy location	Fresh or Frozen ET		
	Fresh (n=113)	Frozen (n=187)	P value
Upper Middle Lower	105 (93%) 0 (0%) 8 (7%)	172 (92%) 1 (0.5%) 14 (7.5%)	0.73
Right Middle Left	61 (54%) 1 (1%) 51 (45%)	97 (52%) 6 (3%) 84 (45%)	0.43
Anterior Middle Posterior	47 (41%) 3 (3%) 63 (56%)	94 (50%) 4 (2%) 89 (48%)	0.34

Five women had 12 weeks of luteal support rather than routine 11 days due to clinical indications (I.e. recurrent miscarriage) for prolonged luteal support following their ET.

Table 20. Pregnancy location and luteal support

Pregnancy site location	Luteal support			P value
	11 days n= 108	12 weeks n=86	None n= 108	
Upper Middle Lower	98 (92.5%) 0 8 (7.5%)	79 (92%) 0 7 (8%)	100 (93%) 1 (1%) 7 (6%)	0.74
Right Middle Left	58 (55%) 1 (1%) 47 (44%)	48 (56%) 1 (1%) 37 (43%)	52 (48%) 5 (5%) 51 (47%)	0.32
Anterior Middle Posterior	45 (42%) 3 (3%) 58 (55%)	46 (54%) 2 (2%) 38 (44%)	50 (46%) 2 (2%) 56 (52%)	0.64

Women who miscarried were more likely to have a pregnancy located in the lower portion of the uterus compared with women who went on to have a live birth (35% vs 5.4%, p<0.01).

Table 21. Pregnancy location

Pregnancy location (n)	Live Birth (n=277)	Miscarriage (n=20)	P value
Upper Middle Lower	261 (94%) 1 (0.4%) 15 (5.4%)	13 (65%) 0 7 (35%)	**<0.01**
Right Middle Left	148 (53%) 7 (2.5%) 122 (44%)	7 (35%) 0 13 (65%)	0.40
Anterior	128 (46%)	12 (60%)	0.84

Middle Posterior	7 (2.5%) 142 (51%)	0 8 (40%)	

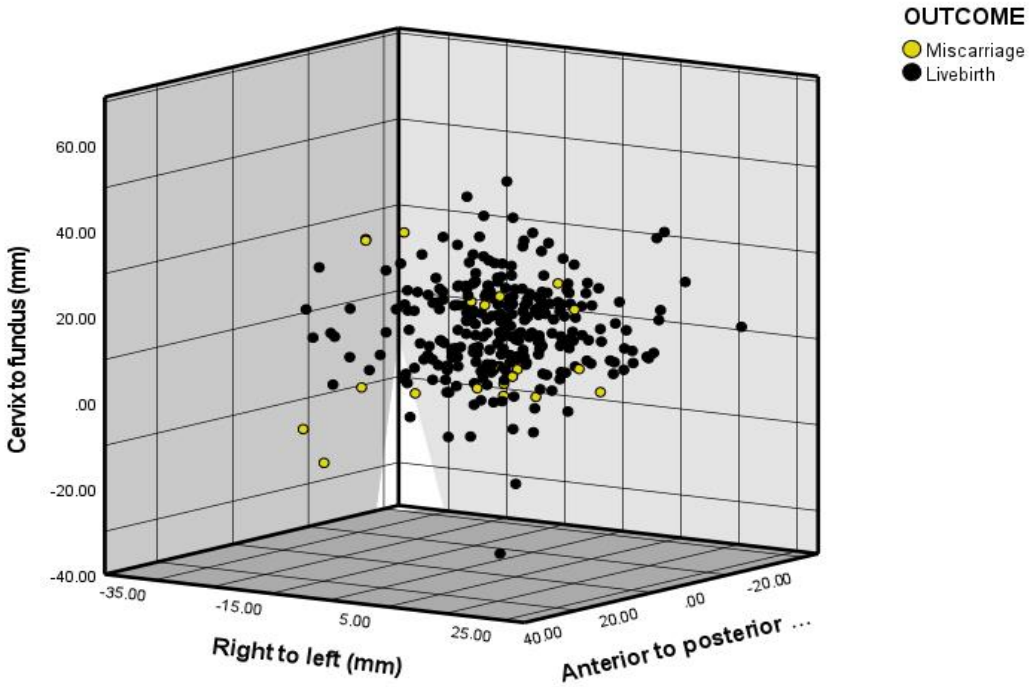


Figure 30. Location of the pregnancy in the uterine cavity

There was no significant relationship between pregnancy site location on birthweight, and gestational age at delivery. Pregnancies located anteriorly/posteriorly or upper/lower in the uterine cavity did not have a significant difference in gestational age at the time of delivery (p=0.94 and p=0.68 respectively). Interestingly however, pregnancies located in the middle of the uterine cavity rather than either the left or right side were more likely to be delivered at a lower gestational age (in Table 22, p<0.01) **and** were more likely to have late complications (p=0.04).

Table 22. Pregnancy site location and gestational age

	Number	Mean Gestational age (weeks)	P Value
Left	122	39.0	<0.01
Middle	7	36.8	

Right	148	39.0	

It should be noted that this was specific only to the transverse plane but a similar effect was not observed in the sagittal (upper/lower) or coronal (anterior/posterior) planes. When considering all complications, pregnancy location had no significant effect on cumulative complication rates. There was also no correlation between pregnancy location and placental site location (p=0.27).

Table 23. Pregnancy location and obstetric complications

		No complications (n=210)	Obstetric complications (n=67)	P Value
Anterior-posterior	Anterior Middle Posterior	97 (46%) 5 (2%) 108 (51%)	31 (46%) 2 (3%) 34 (50%)	0.96
Upper-Lower	Upper Middle Lower	198 (94%) 1 (0.5%) 11 (5%)	63 (94%) 0 4 (6%)	0.83
Left-right	Left Middle Right	99 (47%) 3 (1%) 108 (51%)	23 (34%) 4 (6%) 40 (60%)	**0.04**

In a further subgroup analysis of the impact of pregnancy location and complication rate along the transverse plane there was no difference in age or BMI. Considering specific complications, the only significant association was the pregnancy location in in the middle of the uterine cavity, in those who developed GDM (Table. 24).

Table 24. Subgroup analysis of pregnancies located on the left, middle or right with late complications

	Left (n=23)	Middle (n=4)	Right (n=40)	P Value
Mean age (years)	34.0 (±SD 4.01)	33.5 (±SD 3.11)	33.4 (±SD 4.00)	0.80
Mean BMI (kg/m^2)	25.75 (±SD 3.79)	24.91 (±SD 5.10)	25.13 (±SD 2.87)	0.76
Complications				
GDM	7	4	14	**0.03**
PET	3	2	8	0.22
SGA	3	0	4	0.73
Placenta praevia	4	0	2	0.21
Placenta accreta	0	0	2	0.50
LGA	5	1	8	0.96

Trophoblastic thickness

There was no difference in TT between those who had a fresh or frozen ET. Similarly, a difference was not detected in TT between those who had or hadn't received luteal support.

Table 25. Trophoblastic thickness

	Number	Mean trophoblastic thickness (mm)	P value
Type of transfer Fresh ET	N=300 113 (38%)	7.07 (±SD 2.17)	0.92

Frozen ET	187 (62%)	7.04 (±SD 2.13)	
11 days luteal support 12 weeks luteal support No luteal support	N=300 106(35%) 86 (28%) 108 (37%)	7.11 (±SD 2.21) 6.78 (±SD 1.99) 7.21 (±SD 2.19)	0.36
Embryo quality Good Average Poor	N=293 208 (71%) 69 (24%) 16 (5%)	7.17 (±SD 2.18) 6.75 (±SD 2.13) 6.49 (±SD 1.61)	0.22

TT was significantly more in those who went onto have a live birth and those who miscarried (7.2mm±SD 2.1 vs 5.5mm±SD 2.0 respectively, p<0.001) (Figure 31).

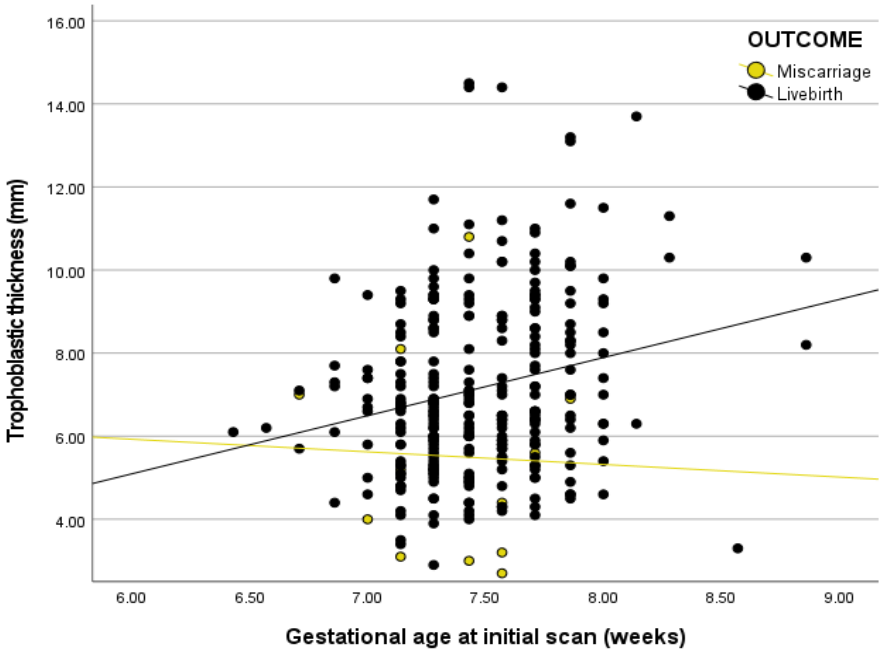


Figure 31. Trophoblastic thickness and gestational age

A weak negative correlation between TT was noted with gestational age at delivery, with a Pearson correlation coefficient of -0.43. No significant correlation was detected between TT and SGA, AGA and LGA groups (Pearson correlation coefficient $r^2$0.29, -0.08 and -0.04 respectively).

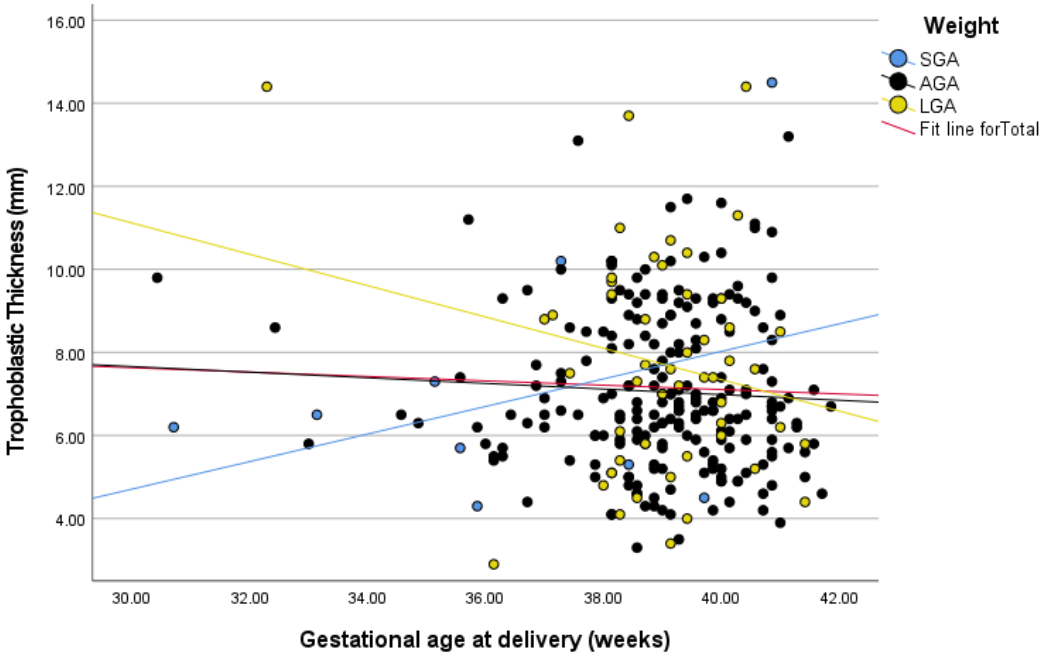


Figure 32. Trophoblastic thickness and gestational age in SGA, AGA and LGA infants.

There was no significant association between TT and birth weight., using one-way ANOVA test. There was no difference between SGA and AGA (p=0.75) or SGA and LGA (p=0.41) pregnancies, However, a significant difference in TT was noted between AGA group compared with LGA (p=0.01). Out of the 14 pregnancies that were diagnosed as LGA pre-natally, only 9 (64%) were above the 90th centile, and the remaining 5 (36%) were between the 75-90th centile.

Table 26. Birthweight and mean trophoblastic thickness

Birthweight	Number	Mean TT (mm)	Standard deviation	P value
SGA	18	7.13	2.84	0.05
AGA	190	6.97	1.88	
LGA	69	7.69	2.43	

For all live births, there was a weakly positive correlation between TT and birthweight (Pearson correlation coefficient 0.141). TT did not correlate with SGA, AGA and LGA groups (Pearson correlation coefficient 0.26, 0.08 and 0.08 respectively).

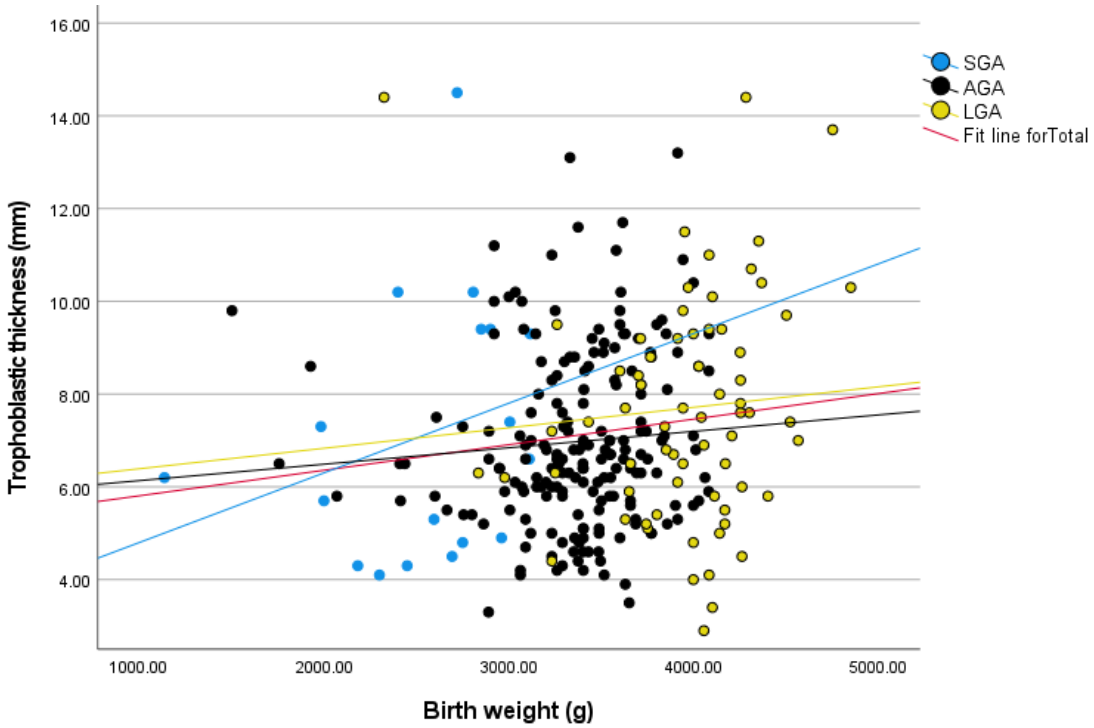


Figure 33. Trophoblastic thickness and birth weight in SGA, AGA and LGA infants.

Of the 277 live births, 67 (24%) had obstetric complications such as placenta praevia, placenta accreta, PET, GDM, SGA and LGA. TT differences did not identify those with or without obstetrics complications. Although an apparently thinner TT was observed in PET, GDM, placenta accreta and SGA, this difference did not reach statistical significance. LGA babies however had a significantly thicker TT when compared with those who weren't (p=0.001).

Table 27. Trophoblastic thickness and obstetric complications

	Number (277)	Mean trophoblastic thickness (mm)	Complication vs no complication P value
Obstetric complication -No -Yes	 210 (76%) 67 (24%)	 7.13 (±SD 2.09) 7.26 (±SD 2.19)	 0.68
GDM	25 (9%)	6.85 (±SD 2.04)	0.44
PET	13 (4%)	6.61 (±SD 1.61)	0.33
SGA	7 (3%)	6.41 (±SD 2.14)	0.34
Placenta praevia	6 (2%)	7.82 (±SD 3.13)	0.45
Placenta accreta	2 (1%)	6.05 (±SD 1.91)	0.46
LGA	14 (5%)	8.93 (±SD 1.73)	**0.001**

When all pregnancies with obstetric complications related to placental dysfunction (placenta praevia, placenta accreta, PET and SGA) were compiled together, there was no difference in TT compared with those pregnancies that had no obstetric complications (TT 6.77 ±SD 2.11 vs 7.13 ±SD 2.09 respectively, p=0.4).

Fresh vs frozen embryo transfer

We assessed if the type of embryo transfer and luteal support on the observed outcomes to consider their confounding attributes to the results. There was no significant difference between fresh and frozen embryo transfers on pregnancy outcome (live birth vs miscarriage, p=0.23). Babies born following frozen ET transfer had a significant increase in birthweight when compared with the fresh ET group (3526g vs 3353g p=0.009). There was no difference between those babies who had natural frozen ET vs stimulated frozen ET (3566g vs 3455g respectively, p=0.15). Babies were apparently born at a more advanced gestation following frozen ET than fresh embryo transfers but the difference was not significant (274 vs 270 days respectively, p=0.056).

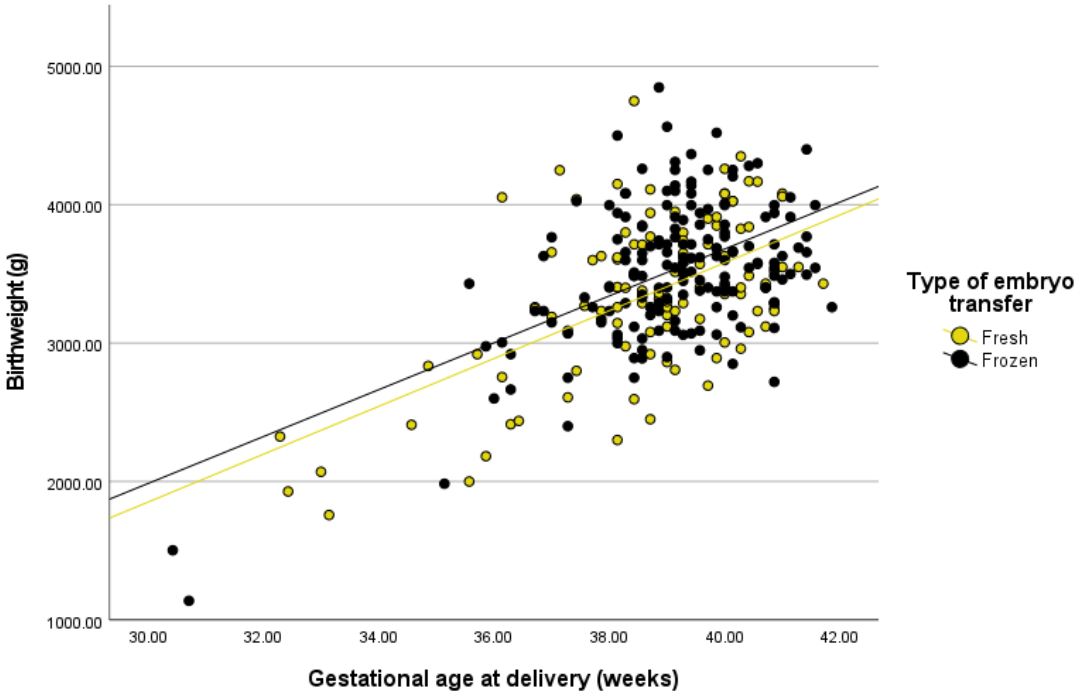


Figure 34. Birthweight in relation to gestation in fresh and frozen ETs.

There was no difference in obstetric complication rates between either the fresh or the frozen group (p=0.59).

Luteal support

There was no difference in type of luteal support provided to patients on live birth or miscarriage rates (p=0.3). There was a significant difference between birthweight and gestational age at delivery when comparing the different luteal support methods (ANOVA, p=0.023 and p=0.02 respectively). When comparing between the 3 different groups the only statistically significant difference was the babies who had 11 days of luteal support were significantly lighter and had shorter gestation at birth than those who had no luteal support (3357g +/- SD 570 vs 3566g +/-SD 386, p=0.003; 38.7 weeks +/-1.9 weeks vs 39.3+/-SD 1.2 weeks, P=0.02).

Table 28. Duration of luteal support, birthweight and gestational age at delivery

Duration of Luteal support	Birthweight (g)	Standard deviation	P value	Gestation (weeks)	Standard deviation	P value
11 days	3357	570		38.7	1.9	
12 weeks	3455	632	0.023	38.8	2.0	0.02

None	3566	386		39.3	1.1	

Type of luteal support also increased the risk of obstetric complications. Women who had 12 weeks of luteal support were more likely to have obstetric complications than those who weren't exposed to luteal support or those who had only 11 days of luteal support(p=0.006). However, when the individual complications were considered separately, no statistically significant differences were found.

Table 29. Obstetric complications and luteal support

Obstetric complication	11 days luteal support (n=101)	12 weeks luteal support (n=78)	No luteal support (n=98)	P Value
Yes	21 (21%)	29 (37%)	17 (17%)	0.006
No	80 (79%)	49 (63%)	81 (83%)	
Specific Complications				
GDM	5	11	9	0.11
PET	3	6	4	0.31
SGA	4	3	0	0.14
Placenta praevia	4	2	0	0.15
Placenta accreta	0	2	0	0.08
LGA	1	6	7	0.06

In total vaginal delivery was the most common mode of delivery at 36.1%, with emergency caesarean section (EMCS) (25.3%) and elective caesarean section (ELCS) (16.6%) the next

most common with instrumentals accounting for the remaining 22% (Figure 35).

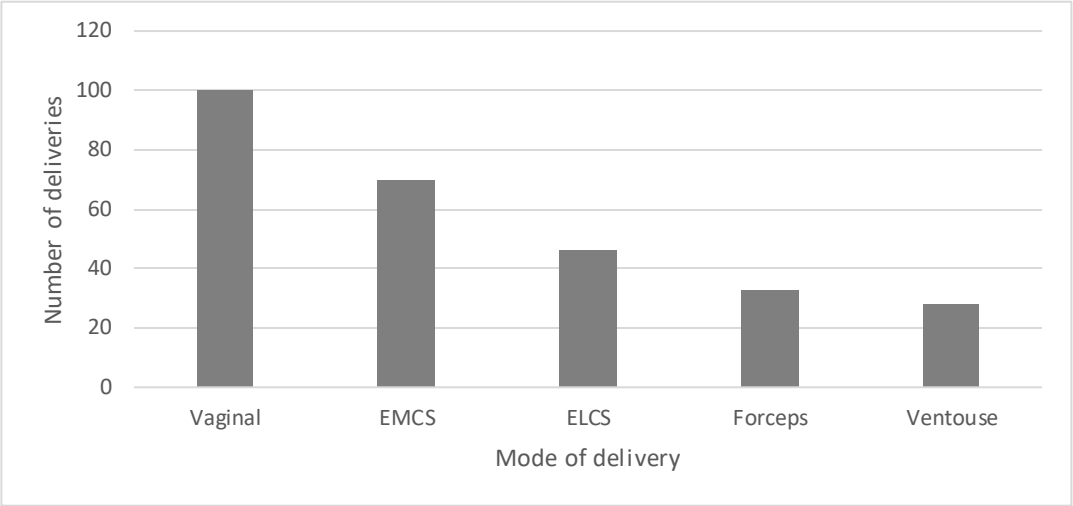


Figure 35. Mode of delivery

Discussion

In agreement with previous studies assessing natural conceptions our study examining IVF pregnancies has demonstrated an increase in miscarriage rates when pregnancies are located in the lower portion of the uterine cavity [215, 217]. Our findings are also in agreement with a previous study, using 2D ultrasound, reporting that a thinner TT is more likely to lead to miscarriage in IVF pregnancies [232].

We demonstrate that information on pregnancy location in an early scan gives useful information on the outcome of a singleton IVF pregnancy in the first trimester. This is important in counselling women as well as making appropriate management plans. The difference in outcome can be related to the relatively poor endometrial perfusion in the lower portion of the uterus when compared to the fundus [215, 327, 331], thus, a pregnancy establishing in the lower part of the cavity may be affected by the poorly perfused endometrial environment being less able to provide for the advancing requirements of an ongoing pregnancy. A previous study using 3D ultrasonography, examining IVF pregnancies however, reported that pregnancy location within the uterine cavity had no relevance to the miscarriage rate [169]. We assume the reasons for this discrepancy to be the smaller number of patients (n=63) included in the previous study, and their inclusion of multiple pregnancies (15 twins and 1 triplet) [169].

Considering the intracavity location of the pregnancy as a predictive marker of late pregnancy/obstetrics complications, when a pregnancy was located in the middle of the cavity, there was a significantly higher incidence of total occurrence of all obstetric complications we examined when compared with those pregnancies located more to the right or left side of the cavity. However, this significant difference was lost, when the complications were assessed individually, except for those who developed gestational diabetes. Similarly, those pregnancies in the middle of the uterus were more likely to be delivered earlier than those on the right or left. Location specific myometrial peristalsis and thus, intrauterine flow of fluid has been suggested and that can affect the placentation and placental function[332, 333]. Although we appreciate that this observation needs validation in further studies, future studies are warranted to explore if force pressure due to spatial differences in uterine peristalsis may incur sheer stress on placentation, contributing to GDM and preterm birth, particularly in the middle portion of the cavity. However, we are aware that the number of patients in the middle of the transverse plane were small (n=7) and whilst statistically significant may actually be a chance occurrence.

In 2000, the first study that associated TT with pregnancy outcome, proposed that a miscarriage can be predicted with a $\geq$ 3mm difference between TT and gestational age in weeks on 2D ultrasound (I.e. TT=3mm at 7 weeks gestation)[232]. Our data, in agreement also demonstrated that those who had a thinner TT were more likely to miscarry. Further more recent studies from the same authors from a private obstetric ultrasound practice in Australia included over 1000 natural conceptions and did not detect an association between TT and early pregnancy loss or maternal hypertensive disease[233, 334]. In their 2019 study, authors examined early pregnancy loss, and reported that trophoblastic volume (TRV), mean uterine artery pulsatility index (UAPI), fetal heart rate (FHR) and mean sac diameter (MSD) were all to be significantly lower in patients who miscarried. However, they did not find an association with TT and miscarriage [233]. The follow up period of this study was only until the end of the first trimester, thus any later miscarriages beyond first trimester would have not been reported. Furthermore, these studies only included natural conceptions with a different physiological path to conception and implantation than IVF or ICSI pregnancies, thus the data may not be relevant to our patient cohort [335, 336]. In the 2020study from the same group that examined the predictive value of the same early pregnancy ultrasound parameters on maternal hypertensive disease, suggested that TRV was the only marker that was significantly lower in hypertensive pregnancies. In concordance with our findings, they did not detect TT to have a significant relevance[334].

Unfortunately, that study only included maternal hypertensive disorders and did not collect data on any other obstetric complications such as SGA, GDM or other placental pathologies. All three previous studies only included natural conceptions thus, the data may not be relevant to IVF pregnancies. However, comparison of specific differences in early pregnancy ultrasonic parameters with pregnancy complications between a naturally conceived pregnancies and IVF pregnancies may unravel new research avenues to identify distinctive molecular pathways active in each to formulate specific therapeutic strategies.

Our study demonstrated a significant increase in TT in LGA babies compared to AGA babies. Increased trophoblastic invasion could lead to increased substrate and nutrient transfer to a developing fetus leading to fetal overgrowth[337]. The small number of pregnancies with an SGA baby meant that a significant difference was not identified when SGA group was compared with LGA group. A future study containing a larger cohort of patients would be required to clarify these findings. Interestingly, except for placenta praevia, women with all other late obstetrics complications studied, such as GDM, PET, SGA and placenta accreta had apparently thinner TT when compared with the control group without complications. A thin trophoblastic layer may represent suboptimal placentation from a pathophysiological perspective, and most of these complications have a relevance to suboptimal trophoblastic invasion, e.g. PET and SGA [230, 231, 338]. In contrast, placenta previa cases we include may have been related to a mere abnormal location without abnormal placentation, thus TT was not affected.

As secondary outcomes we also examined the differences in pregnancy outcomes relevant to fresh and frozen ET and as well as the impact of luteal support. In concordance with other studies[339] our data demonstrated that babies born from a frozen ET were more likely to have a higher birthweight and greater gestational age at delivery than those who were conceived with a fresh ET. Our data is in contrast to the previous meta-analysis, which reported that a frozen ET was associated with hypertensive disorders and LGA infants, and that a fresh ET was more likely to be associated with SGA[339]. The smaller sample size and differences specific to our local patient population may be the reason for these observed differences. Those who had no luteal support (natural frozen ET) and 12 weeks luteal support (stimulated frozen ET in the majority) in our study were more likely to have heavier babies at more advanced gestation in keeping with the previous publications[339, 340]. Interestingly, those patients who had prolonged luteal support (12 weeks) were more likely to have obstetric complications, although these complications were too heterogenous thus not sufficient numbers were included to do a subgroup analysis. Previously, in a larger

study, hypertensive disorders and placental pathologies have been identified as more prevalent in those who had stimulated frozen embryo transfers [341-343]. Most of the late pregnancy conditions such as pre-eclampsia, occur in only 3-5% of pregnancies [344] and thus, unfortunately our study was not sufficiently powered to detect the association of ET type with these relatively rate obstetric complications. In our unit, the routine practice is to offer natural ET (without luteal support) to women who ovulate. Those with anovulatory cycles would be offered a stimulated ET (12 weeks luteal support). Since anovulatory cycles are frequently observed in women with pre-existing medical conditions such as polycystic ovarian syndrome [345], this subgroup of patients are inevitably more at risk of obstetric complications due to their pre-existing medical conditions, not necessarily because of their prolonged luteal support. .

The NHS maternity statistics for 2019/20 showed vaginal deliveries in the UK to account for 57% of all deliveries, 31% caesarean section rates and 12% instrumental deliveries (12%) [346]. Interestingly, our cohort of patients were more likely to have a caesarean section (42%) in comparison to other modes of delivery, which is in keeping with previous data on modes of delivery in IVF pregnancies [347].

The strengths of this study are; that it is the first of its kind to consider association of pregnancy location and trophoblastic thickness measured by 3D ultrasound scanning with both early and late pregnancy complications in IVF pregnancies. Observer bias had been reduced by one experienced practitioner using the same ultrasound machine recording all measurements of pregnancy location and TT [348]. 3D imaging has previously been shown to improve inter-observer reproducibility in comparison to 2D imaging. It also allows for postprocessing reviews of the images, which would not be obtainable with 2D imaging [190, 349].

Limitations

Despite finding statistically significant differences for the primary outcome (miscarriage rates), this study was not sufficiently powered to detect significant associations of ultrasound features with the other secondary outcomes (PET, SGA etc.). 3D ultrasound is not routinely used in early pregnancy scanning since it requires more advanced and costly ultrasound machines and training. This potentially limits the translatability of our research into routine clinical practice.

Determining the pregnancy site location was based on scans between 6 -8 weeks in gestation. Whilst pregnancy location was determined using this method, in future it may be more beneficial to consider an earlier scan in the pregnancy to determine the true implantation site. Previous studies have conflicting results regarding embryo deposition point and pregnancy location[169, 216, 223]. The majority of the ETs in our study were done without ultrasound guidance, using the clinical touch technique, therefore we were unable to clarify if embryo deposition point correlated with pregnancy location.

We did not collect data on ethnicities of the patients in the study, therefore we were unable to use a customised growth chart when determining birth centiles. We therefore used the population growth chart developed by the World Health Organisation to determine whether babies born were SGA, AGA or LGA[350]. There is currently no conclusive evidence of superiority between customized or population-based growth charts[351, 352], therefore, we assume this to be of a lesser limitation of our data.

Finally, we did not include a control group in our study. IVF patients are a higher-risk group, therefore in future it may be beneficial to compare IVF pregnancies with natural conceptions.

Future studies

Future studies to confirm our results should include a larger cohort of patients, including other ultrasound markers such as trophoblast volume, mean gestational sac diameter, fetal heart rate and mean uterine artery pulsatility index. An appropriate control group of naturally conceived pregnancies and the use of other biochemical markers, as well as facilitating studies into trophoblast invasion could lead to the development of more effective therapeutic strategies, promoting optimal trophoblast invasion to minimise the burden and impact of early and late pregnancy complications in IVF pregnancies.

Conclusion

This study is the first of its kind to show a thinner TT and pregnancy location in the lower half of the uterus are more likely to result in miscarriage in IVF pregnancies. It has also suggested that TT and luteal support has a potential impact on pregnancy complication rates and further larger studies are required to determine this link. These findings along with other ultrasound markers such as trophoblastic volume, mean uterine artery pulsatility index and fetal heart rate can be used to develop an algorithm, to better counsel and manage those at risk of early pregnancy loss and obstetric complications.

Identification of those at risk may also prompt increased monitoring during pregnancy, such as attendance at high-risk antenatal clinics and extra growth scans, with the aim of reducing both maternal and neonatal morbidity. The findings of this study therefore highlights the important contribution that advance ultrasound can make in an IVF pregnancy, beyond embryo-implantation, in predicting the outcome of ongoing pregnancy.

Chapter 5: Discussion

The overall aim of the studies included in this thesis was to determine whether advanced ultrasonography can be used to improve or predict outcomes in reproductive medicine. Ultrasound has been an essential tool in assisted reproductive practice since its initial introduction in 1978[353], however, despite the continued advances in ultrasound technology[69, 137, 190] application into clinical practice has not followed swiftly[354]. This could be due to a number of reasons. For example, the new advanced ultrasonography techniques require sufficient supportive evidence on efficacy and safety [354], and they necessitate additional training, quality assurance, financial and resource allocation before implementation in to routine clinical use [190, 354].

Use of advanced ultrasonography in reproductive medicine covers a vast area, with improved techniques assessing different aspects of the ART treatment cycle, starting from improvement in identification of pelvic pathologies, monitoring the stimulation cycle, ET and detecting markers for adverse pregnancy outcomes in the first trimester [122, 137, 190, 233, 354].

The particular areas my studies explored were the use of advanced ultrasound for ET and its use in early pregnancy to determine the impact of pregnancy site location and trophoblastic thickness (TT) on pregnancy outcome. It is envisaged that the work described in this thesis will contribute to the current understanding of the use of ultrasonography in ART and will provide future avenues for research.

Chapter 2 – National survey on embryo transfer technique

The survey conducted and presented in this chapter provided a sound basis for the subsequent study by providing insight into the current ET practice in the UK. This work was of upmost important as the last similar survey assessing ET practice nationally was over 20 years ago[241]. Considering the changes that have taken place over the last two decades in ART practice, there was an obvious need for a contemporaneous survey on the national practice and preferences in ET techniques. All reproductive centres in the UK were invited to complete our survey that included 38 questions detailing the various aspects of ET. A satisfactory response rate was achieved, which allowed generalisable conclusions to be drawn about national practice.

Of the 29 questions relating to ET technique, only 10 (34%) questions had responses that showed a degree of concordance (>70% with the same response). This implies that there is a large variation in most aspects of the ET technique between different units. Only two questions resulted in the same answer from every clinician who completed the questionnaire and these confirmed that all clinicians used sterile gloves and soft catheters for ET. The lack of standardisation in ET techniques identified from this survey could explain the wide range of LBR between the clinics (ranged from 11-34%).

This survey also highlighted that there are number of practices which are prevailing in the UK, despite sufficient available evidence to confirm their negative impact on ART outcomes. This included the routine use of tenaculum at the time of ET and not abandoning ET when fluid is seen in the endometrial cavity.

The use of ultrasound guidance for ET still appears to be a contentious issue and although the majority of units use ultrasound guidance, 8 (17%) units did not. Interestingly, the HFEA data did not demonstrate a significant difference between the LBR for those units that used ultrasound versus those that did not for ET.

Whilst the majority of clinics deposited the embryo in the upper/middle portion of the uterus, clinician's interpretation of this point varied between 0.5cm to > 2cm from the fundus of the uterus. This raises the question – what distance from the fundus defines the upper/middle portion of the uterine cavity?

Surprisingly, clinicians were not fully aware of the HFEA recorded ET success rate of their unit. The majority of the responders estimated their LBR per ET being between 30-40%, but in reality, the majority of the clinics had a success rate between 20-30%. This could be due to the HFEA data predating the survey by 2 years, but it is also possibly related to the differences between personal perception and actual recorded HFEA data. This inaccuracy may affect patient counselling in the respective units.

In summary, this survey has helped to highlight inconsistencies and potential areas that could be improved with regards to ET. Based on our findings and current evidence [147, 164, 171], we have subsequently developed a stepwise approach to performing an ET [304].

ET techniques have been shown to have a significant impact on pregnancy rates [166, 292, 293] and this variation in practice identified by the survey could have an influence (along with other factors of the in-vitro fertilisation (IVF) process) on a unit's success rate. In a

field of medicine where every percent matters, slight changes could result in significant improvement in patient outcomes and satisfaction.

Implementing a standardised approach to the ET technique would eliminate areas of poor practice currently employed by some centres and thus, ultimately lead to improved patient outcomes. Future prospective clinical studies will need to be undertaken to ensure best practise but until such data is available, guidance can be provided through consensus meetings involving experts from all stakeholder groups, which can be further informed by comprehensive and systematic reviews of the literature.

Chapter 3 – Four-dimensional ultrasound guided embryo transfer randomised controlled trial

UGET has been a subject of debate since it was first used back in 1984[161]. Since then, there have been numerous studies and systematic reviews to assess the benefit of ultrasound guidance at the time of ET[97, 174, 237]. Systematic reviews found an increase in the CPR between 5-10%, when ultrasound guidance was used in comparison to the CTT[97, 174]. However, the overall quality of the evidence using the GRADE methodology was low and studies only included TA ultrasound guidance[97]. The largest study to date comparing UGET vs CTT was done at our unit, the Hewitt Fertility Centre. The study by Drakeley et al, recruited over 2000 patients which accounted for over a third of the population included in the systematic reviews and found no difference between the two methods of ET[176]. Therefore, the CTT has remained as the standard technique for ET at the Hewitt fertility centre and some other clinics in the UK.

Whilst there have been further studies into the use of advanced ultrasonography at the time of ET, there has been no previous RCTs using 4D UGET. We therefore performed the first RCT assessing TV 4D UGET versus our current standard practice, the CTT.

320 patients were recruited to the trial and we detected a significant increase in the CPR and the LBR in those who had a 4D UGET vs those who had the CTT . No differences in any of the baseline characteristics, ET difficultly or patient satisfaction between these two groups were noted.

Currently in the UK, average LBR per ET is 24%[355] and the results of our trial showed that the use of 4D ultrasound can potentially increase that success by over 70%. Interestingly, ETs using the CTT also showed a higher LBR than the UK national average. Considering 83% (as per chapter 2) of the units are using TA ultrasound, it would be of interest to demarcate

how much of an impact TV 4D ultrasound guidance has over TA 2D ultrasound guidance on ET success rates. The same person performed all of the 4D UGET in our trial, which may have positively influenced the outcomes and doesn't allow effect of multiple practitioners on the final success rates to be considered. Future studies should address these limitations and assess their impact on ET success rates.

Embryo deposition point for the 4D group was based on the MIP, which was initially devised by Gergely et al [183]. This allowed tailoring the ET to the patient specific uterine anatomy. Such personalised approach to ET is preferable to a previously proposed arbitrary measurement [285]. For example, 2cm from the fundus can result in embryos being deposited in either the upper, middle or even lower portion of the uterus depending on the individual uterine anatomy, which can have a negative impact on implantation [164, 175].

When conducting this trial, I found TV scanning offered a number of advantages over TA scanning. It did not require a full bladder, that can often cause patient discomfort; it allowed for clearer images and did not require an extra practitioner to be present. Patients generally found the procedure comfortable. Whilst previous studies comparing TV to TA scanning did not identify a difference in pregnancy rates, they did report improved patient comfort in the TV group[178].

The findings from this study, demonstrated that 4D UGET significantly improves the clinical outcomes, compelling us to change our current practice of CTT for the benefit of our patients. The challenges to such change in practice are that not all of our practitioners are trained to perform 4D ultrasound. This will invoke additional training and associated costs to the unit. 2D ultrasound is a comparatively easier and less time-consuming technique to learn than 4D scanning, therefore an RCT comparing 4D vs 2D TV UGET will help to make a financially and clinically sound decision on change of practice.

The Cochrane review in 2016 noted an odds ratio of 1.31 for the CPR of those who had 2D UGET vs CTT. Our trial showed an odds ratio of 1.78 for CPR with 4D UGET, markedly more than the performance of 2D UGET as detailed in the previous Cochrane review. However further RCTs would be required to determine the true difference between 2D and 4D UGET. Whilst this may not be a clinically significant improvement, patients may consider this information to make a choice regarding their method of ET, given the option.

Chapter 4- Optimal implantation site observational study and trophoblastic thickness

There has been conflicting evidence regarding the relevance of pregnancy implantation site on pregnancy outcomes[169, 215, 217, 327]. Studies assessing natural conception found that successful pregnancies were more likely to be located in the upper half of the uterine cavity and those in the lower half were more likely to miscarry [215, 217]. This observation was not replicated in those who had undergone fertility treatment [169]. A higher proportion of gestation sacs were found in the middle of the cavity in women undergoing ART and no obvious increase in miscarriage rate were reported dependent on pregnancy site[169].

Similarly, very few studies exist examining TT on early gestation scan and its impact on pregnancy outcome. Two studies on natural conceptions, one in 2000 and another in 2019, have reported contradictory results on whether TT had an impact on early pregnancy loss[232, 233]. This knowledge required further clarification, particularly in women undergoing ART, thus, we recruited 300 patients to our trial with the primary outcome to determine if the location of pregnancy site and TT had an impact on the miscarriage rate. This prospective observational study included women following a single ET, with subsequent live single intrauterine pregnancy. A 3D image of the uterus was taken at the early gestation scan and these images were stored on the ultrasound machine for measurements to be calculated at a later date. The patients were followed up until live birth and further information was collected on secondary outcomes including obstetric complications, birthweight and gestational age.

Miscarriages were more common in pregnancies located in the lower half of the uterus and in those with a thinner TT. Pregnancies located in the middle of the uterine cavity rather than the left or right side were noted to be associated with gestational diabetes but this is unlikely to have a clinical significance. Those diagnosed as large for gestational age at late gestation were more likely to have an increased TT. An apparently decreased TT was observed with pregnancy complications such as GDM, PET, SGA and placenta accreta, when compared with those who did not have any complication, however this observation did not reach statistical significance.

Our ultrasound findings of pregnancy location and miscarriage were in keeping with the previous studies in natural conceptions[215, 217]. Our results also supported the previous evidence that decreased TT was associated with an increased risk of miscarriage [232].

While we were recruiting, a further study, which assessed numerous ultrasound markers in natural conceptions and their impact on pregnancy outcome was published [233]. The authors included TT as one of the outcomes but also examined other parameters such as trophoblastic volume, uterine artery pulsatility index, fetal heart rate and mean sac diameter. Their study did not find a significant difference in TT on early pregnancy outcomes but correlations were found with the other ultrasound markers [233]. This study only focused on the first trimester, thus, potentially missing later miscarriages and late pregnancy outcomes.

Despite finding statistically significant differences for the primary outcome (miscarriage rates), our study was not sufficiently powered to detect associations with late obstetric outcomes as described above. However, we did identify some significant differences. Adequately powered further future studies will confirm the validity of our results.

The use of these ultrasound findings could help to identify those patients at risk and therefore facilitate appropriate counselling, risk stratification and improved management pathways. Identifying non-invasive biomarkers and facilitating studies of trophoblast invasion could lead to the development of more effective therapeutic strategies targeting later complications of pregnancy.

Further studies are required to see if there is an association between embryo deposition point and subsequent implantation site. This could lead to the development of novel methods to ensure embryo deposition at the optimal point of the cavity, an avenue for further investigation.

Overall conclusion

Ultrasound is an essential tool in the day-to-day management of patients undergoing fertility treatment, although it is rarely used to its full potential. Attempts should be made to utilise these advanced techniques to the maximum benefit of the patients and their management.

The body of work included in this thesis has highlighted the significant impact advanced ultrasonography has on predicting and managing a woman undergoing fertility treatment. It has informed us on ways to improve embryo implantation by more precise embryo deposition with 4D UGET, and how a standard early scan could predict outcomes in the

index pregnancy. Additionally, this work has identified future avenues of research to pursue.

Introduction of advanced ultrasonography techniques may incur additional training and costs to ART units, however, since it can significantly contribute to the delivery of a healthy baby with ART, the additional resource allocation is justified.

Appendices

Ethical approvals

NHS
Health Research Authority

North West - Liverpool Central Research Ethics Committee
3rd Floor
Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 020 71048008

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

09 December 2016

Mr Richard Thomas Russell
Consultant Gynaecologist & Subspecialist in Reproductive Medicine & Surgery
Liverpool Women' NHS Foundation Trust
The Hewitt Fertility Centre
Crown Street
Liverpool
L8 7SS

Dear Mr Russell

Study title:	**3D / 4D Ultrasound Guided Embryo Transfer vs. Clinical Touch Technique: a randomised controlled trial**
REC reference:	**16/NW/0588**
IRAS project ID:	**202857**

Thank you for your letter of 02 December 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Mrs Carol Ebenezer, nrescommittee.northwest-liverpoolcentral@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation

as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see

"Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet completed any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
IRAS Application Form [IRAS_Form_11072016]		11 July 2016
Non-validated questionnaire	1.0	01 July 2016
Participant consent form	1.0	16 March 2016
Participant information sheet (PIS)	1.0	16 March 2016
Participant information sheet (PIS)	1.1	30 November 2016
Referee's report or other scientific critique report	1	27 January 2016
Research protocol or project proposal	3.0	21 March 2016
Response to Request for Further Information		02 December 2016
Summary CV for Chief Investigator (CI)		16 May 2014

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

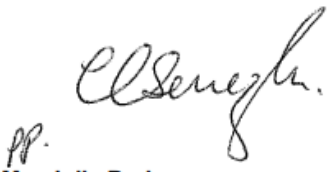
HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/NW/0588	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely



Mrs Julie Brake
Chair

Email:nrescommittee.northwest-liverpoolcentral@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Mrs Louise Hardman, Liverpool Women's NHS Foundation Trust*

NHS
Health Research Authority

East Midlands - Leicester Central Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

20 September 2017

Mr Richard Thomas Russell
Consultant Gynaecologist and Subspecialist in Reproductive Medicine
Liverpool Women's NHS Foundation Trust
Crown Street
Liverpool
L87SS

Dear Mr Russell

Study title:	**Determining the Optimal Embryo Implantation Site**
REC reference:	**16/EM/0392**
IRAS project ID:	**204885**

Thank you for your letter of 19 September 2017 responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the sub-committee.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation

as revised.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA Approval (England)/ NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" above).

Approved documents

The documents reviewed and approved by the Committee are:

Document	*Version*	*Date*
Covering letter on headed paper [Cover Letter]		18 September 2017
IRAS Application Form [IRAS_Form_22082016]		22 August 2016
IRAS Application Form XML file [IRAS_Form_22082016]		22 August 2016
IRAS Checklist XML [Checklist_22082016]		22 August 2016
Participant consent form	1.0	22 August 2016
Participant information sheet (PIS)	1.0	22 August 2016
Participant information sheet (PIS)	1.0	18 June 2017
Research protocol or project proposal	1.0	22 August 2016
Summary CV for Chief Investigator (CI)		

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance

We are pleased to welcome researchers and R & D staff at our RES Committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

16/EM/0392	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project.

Yours sincerely

pp. S. O'Neil

Mr Murthy Nyasavajjala
Chair

Email: nrescommittee.eastmidlands-leicestercentral@nhs.net

Copy to: *Miss Louise Hardman, Liverpool Women's NHS Foundation Trust*

Liverpool Women's NHS
NHS Foundation Trust

3D / 4D Ultrasound Guided Embryo Transfer vs. Clinical Touch: a randomised controlled trial

Participant Information Sheet

As part of your IVF treatment, you will require placement of your embryo(s) into your uterus (womb). This procedure is normally performed without ultrasound guidance and with the embryo being replaced approximately 6cm beyond the neck of your womb. This is often called a Clinical Touch technique.

This research study is looking at the process of replacing an embryo under ultrasound guidance which is performed trans-vaginally (this is where the ultrasound probe is inserted into the vagina which results in clearer images) and with the addition of 3D and 4D visualization of the uterus during the procedure. We want to find out whether this procedure improves the pregnancy rate and live birth rates compared with our standard practice.

We are aiming to recruit 314 participants to this research study who will be allocated to one of the two treatments. This is an entirely random process which we have no control over. If you decide not to take part in this research project your embryo transfer will be performed according our standard practice without the use of ultrasound, by clinical touch.

What would taking part involve?

If you wished to join the study you will be asked to sign a consent form which is essentially your permission. You will then be allocated a treatment intervention; either ultrasound guided or not ultrasound guided embryo transfer. Your embryo transfer will take place on the day that most suits your embryos development and is independent of the trial. Technically there will be very little difference in what happens next, although if you were to be assigned to the ultrasound group you would require an empty bladder rather than a full one.

Following the procedure you will be asked to complete a short questionnaire about your experience of the embryo transfer.

We obviously need to know whether you became pregnant and whether you go on to have a baby. This information will be collected from your hospital notes.

What are the potential benefits of taking part?

The study aims to discover whether there is any benefit to either procedure, so there may be no personal benefit to you taking part. The results of the trial will be published and may change how embryos are transferred for all patients undergoing IVF in the future.

What are the possible disadvantages and risks of taking part?

There is unlikely to be any disadvantage to taking part in the trial. If you choose not to take part, your embryo transfer will be performed without ultrasound guidance and consistent with our standard practice. We do not anticipate any risks to your health or chance of success from taking part in this trial.

What if something goes wrong?

In the event of something going wrong you would be entitled to make formal complaint to the hospital Trust who is acting as a sponsor for the study. The Trusts "Patient Advice and Liaison Advice" Service (PALS) can be contacted on 0151 702 4353 Monday to Friday between 08.30 and 4pm. Outside of these hours you will be able to contact the duty manager via the main hospital switchboard on 0151 708 9988.

What will happen if I change my mind about the study?

You will be under no pressure to continue with the study and you will be free to withdraw at any time without affecting your remaining treatment, which will continue according to our standard practice.

How will my information be kept confidential?

Your records are always confidential. Only members of the study team and members of the fertility team will have access to your records. Each patient in the study will be allocated a "study number" rather than using your personal details, meaning that your involvement is anonymised. The study information will be entered on a secure electronic database that complies with strict NHS Data Protection Standards.

Liverpool Women's NHS Foundation Trust is the sponsor for this study. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. We will keep identifiable information about you for one year after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already

obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You will not automatically be sent the results of the study, but you can request a lay summary of the results from one of the study team.

What will happen to the results of the study?

The results of the study will be published in a scientific journal. Your individual information will not be published or made available to anyone else.

Who is organising and funding this study?

The Hewitt Fertility Centre at the Liverpool Women's Hospital is organising and funding this study.

Who has reviewed this study?

All research involving people is looked at by an independent group of people, called a Research Ethics Committee, to protect the interests of the people taking part. This study has been reviewed and given favourable opinion by REC reference: 16/NW/0588 North West - Liverpool Central Research Ethics Committee.

Further Information & Contact Details

Mr Richard Russell Consultant Gynaecologist

The Hewitt Fertility Centre

Research Nurse

Tel: 0151 702 4346

Email: 4dtrial@lwh.nhs.uk

IRAS Project ID: 204885

Participant Information Sheet

Study Title

Determining the Optimal Embryo Implantation Site

Invitation and brief summary

As part of your IVF treatment, we will place an embryo (s) into your uterus (womb). This study is looking at whether there is a better position in your uterus where embryos are more likely to implant and continue as a successful pregnancy.

Explanation

The placement of an embryo is one of the most important steps in your IVF treatment. Embryo implantation is a complicated process that is not fully understood. We know from experience that where the embryo is placed in the uterus does not always mean is where it stays. This study will be looking at which area of the uterus the pregnancy has implanted that has led to your pregnancy. Using this information may help us better place an embryo in the first instance.

We are looking to recruit 200 participants to this research study.

What would taking part involve?

You will be asked to sign a consent form which gives your permission to join the study. At your routine pregnancy scan which is performed at approximately 7 weeks gestation, we will be looking more closely at the area of your uterus where the pregnancy is. This information will be recorded and subsequently analysed. Technically there will be very little difference in what happens compared with a "routine" pregnancy scan. We obviously need to know whether your pregnancy proceeds to you having a baby. This information will be collected from your hospital notes.

What are the potential benefits of taking part?

The study aims to discover where the best place is for an embryo to be placed that makes treatment more successful. There is likely no personal benefit to you taking part. The results of the trial may influence how IVF is performed in the future.

What are the possible disadvantages and risks of taking part?

There is unlikely to be any disadvantage to taking part in the trial. We do not anticipate any risks to your health or your pregnancy.

What if something goes wrong?

In the event of something going wrong you would be entitled to make formal complaint to the hospital trust who is acting as a sponsor for the study. The Trust's "Patient Advice and Liaison Service" (PALS) can be contacted on 0151 702 4353 Monday to Friday between 08.30 and 4pm. Outside of these hours you will be able to contact the duty manager via the main hospital switchboard on 0151 708 9988.

What will happen if I change my mind about the study?

You will be under no pressure to take part in the study and you will be free change your mind even after you have completed the consent form.

How will my information be kept confidential?

Your records are always confidential. Only members of the study team and members of the fertility team will have access to your records.

What will happen to the results of the study?

The results of the study will be published in a scientific journal. Your individual information will not be published or made available to anyone else.

Who is organising and funding this study?

The Hewitt Fertility Centre is organising and funding this study.

Who has reviewed this study?

The research study has been approved by the hospital's Research & Development team and also the Cheshire and the East Midlands – Leicester Central Research Ethics Committee (REC 16/EM/0392)

Further Information & Contact Details

Mr Richard Russell
Consultant Gynaecologist
The Hewitt Fertility Centre

Tel: 0151 702 4121
Email: **IVFTrial@lwh.nhs.uk**

Consent forms

Liverpool Women's NHS
NHS Foundation Trust

Affix patient hospital sticker here

Study Number:

Participant Identification Number:

CONSENT FORM

Title of Project: **3D / 4D Ultrasound Guided Embryo Transfer vs. Clinical Touch: a randomised controlled trial**

Name of Researcher: **Mr Mr Richard Russell**

Please initial box

1. I confirm that I have read the information sheet dated ..... (version .....) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study maybe looked at by individuals from the Hewitt Centre study team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes

Version 3.0, 20.02.2020 IRAS 202857

Liverpool Women's NHS
NHS Foundation Trust

Affix patient hospital sticker here

Centre Number: LWH

Study Number:

Participant Identification Number for this trial:

CONSENT FORM

Title of Project: **Determining the Optimal Embryo Implantation Site Study**

Name of Researcher: **Mr Richard Russell**

Please initial box

1. I confirm that I have read the information sheet dated …… (Version …….) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the Hewitt Fertility Centre study team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

Presentations and abstracts

Oral presentations

4D ultrasound guided embryo transfers statistically improve live birth rates - A randomised controlled trial- ESHRE Virtual Congress- July 2021

4D ultrasound guidance improves embryo transfer success rates: a randomised controlled trial. International Society of Ultrasound in Obstetrics and Gynaecology World Congress - Virtual-Oct 2020

Poster presentations

There is an urgent clinical need to standardise embryo transfer techniques to ensure best outcomes for patients. Fertility 2021 – Virtual - Jan 2021

4D-ultrasound guided embryo transfers improve live birth rates: a randomised controlled trial. RCOG world congress – Virtual - June 2021

4D ultrasound guided embryo transfers improve live birth rates: A randomised controlled trial. Fertility 2021 – Virtual- Jan 2021

Trophoblastic thickness and implantation site are associated with subsequent miscarriage: A prospective study. Fertility 2021 – Virtual- Jan 2021

An observational study: does pregnancy implantation site and trophoblastic ring thickness at early gestational scan predict outcomes of pregnancy? International Society of Ultrasound in Obstetrics and Gynaecology World Congress -Virtual -Oct 2020

Does pregnancy implantation site and trophoblastic ring thickness at early gestational scan predict outcomes of an intrauterine pregnancy? An observational study. ESHRE - Virtual conference- July 2020

Abstract publications

O-181 4D ultrasound guided embryo transfers statistically improve live birth rates - A randomised controlled trial- L Nancarrow, N Tempest, A Drakeley, R Homburg, K Ford, D

Hapangama, R Russell, *Human Reproduction*, Volume 36, Issue Supplement_1, July 2021, deab127.082, https://doi.org/10.1093/humrep/deab127.082

4D-ultrasound guided embryo transfers improve live birth rates: a randomised controlled trial- Nancarrow, L; Tempest, N; Drakeley, A; Homburg, R; Ford, K; Hapangama, D; Russell, R (2021), Category – Reproductive Medicine/Assisted Reproduction. BJOG: Int J Obstet Gy, 128: 230-242. https://doi.org/10.1111/1471-0528.18_16715

OC15.04: 4D ultrasound guidance improves embryo transfer success rates: a randomised controlled trial. Nancarrow, L., Ford, K., Drakeley, A., Hapangama, D., Homburg, R. and Russell, R. (2020),Ultrasound Obstet Gynecol, 56: 45-45. https://doi.org/10.1002/uog.22315

VP60.03: An observational study: does pregnancy implantation site and trophoblastic ring thickness at early gestational scan predict outcomes of pregnancy? Nancarrow, L., Vinayagam, S., Swanson, M., Drakeley, A., Ford, K., Homburg, R., Hapangama, D. and Russell, R. (2020), Ultrasound Obstet Gynecol, 56: 329-329. https://doi.org/10.1002/uog.23337

Publications

National Survey Highlights the Urgent Need for Standardisation of Embryo Transfer Techniques in the UK- Nancarrow L, Tempest N, Drakeley AJ, Homburg R, Russell R, Hapangama DK.. J Clin Med. 2021 Jun 27;10(13):2839. doi: 10.3390/jcm10132839. PMID: 34198995; PMCID: PMC8267796.

Endometriosis and the Fallopian Tubes: Theories of Origin and Clinical Implications. Hill CJ, Fakhreldin M, Maclean A, Dobson L, Nancarrow L, Bradfield A, Choi F, Daley D, Tempest N, Hapangama DK. J Clin Med. 2020 Jun 18;9(6):1905. doi: 10.3390/jcm9061905. PMID: 32570847; PMCID: PMC7355596.

Journal of
Clinical Medicine

MDPI

Article

National Survey Highlights the Urgent Need for Standardisation of Embryo Transfer Techniques in the UK

Lewis Nancarrow [1,2,*], Nicola Tempest [1,2,3], Andrew J. Drakeley [2], Roy Homburg [4], Richard Russell [2] and Dharani K. Hapangama [1,3]

[1] Centre for Women's Health Research, Department of Women's and Children's Health, Institute of Life Course and Medical Sciences, University of Liverpool, Member of Liverpool Health Partners, Liverpool L8 7SS, UK; ntempest@liverpool.ac.uk (N.T.); dharani@liv.ac.uk (D.K.H.)
[2] Hewitt Centre for Reproductive Medicine, Liverpool Women's NHS Foundation Trust, Liverpool L8 7SS, UK; andrew.drakeley@lwh.nhs.uk (A.J.D.); Richard.Russell@lwh.nhs.uk (R.R.)
[3] Liverpool Women's NHS Foundation Trust, Liverpool Health Partners, Liverpool L8 7SS, UK
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Abstract: Embryo transfer (ET) is one of the vital steps in the in vitro fertilisation (IVF) process, yet there is wide variation in ET technique throughout the UK, without a nationally approved standardised approach. The aim of this study was to gain contemporaneous information regarding the current clinical ET practice in the UK. Method: A 38-question electronic survey was distributed to the 79 UK Human Fertilisation and Embryology Authority (HFEA) registered clinics performing ETs. Results: In total, 59% (47/79) of units responded, 83% (39/47) performing ultrasound-guided transfers, with 42% (20/47) of units using a tenaculum; 22% (10/45) would proceed with transfer regardless of fluid in the endometrial cavity. In 91% (43/47) of units, embryos were deposited in the upper/middle portion of the uterine cavity, but interpretation of this area ranged from 0.5 to >2 cm from the fundus, with 68% (32/47) allowing patients to mobilise immediately after transfer. In 60% (27/45) of clinics, success rates were based on clinical pregnancy rates (CPR). Conclusion: Within the UK there is a wide range of variability in ET techniques, with >70% of discordance in survey-responses between clinics. Whilst there are areas of good practice, some disadvantageous techniques continue to persist. This survey emphasises the importance of developing a standardised, evidence-based approach to improve ET success rates.

Keywords: embryo transfer; survey; standardisation; IVF; in vitro fertilisation

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1. Introduction

Transferring a good quality embryo in to an appropriately prepared uterine cavity is an integral part of the in vitro fertilisation (IVF) process and a fundamental step in conception [1]. Reproductive medicine as a speciality, and the IVF process in particular, have seen significant changes over the past 40 years, with many developments in both clinical practice and laboratory procedures [2]. However, during this time, there has been little change in the embryo transfer (ET) technique originally developed by Steptoe et al. [3,4] other than ultrasound guidance and the use of catheters specific for ET [2].

The best ET technique would deliver the embryo to the optimum location within the uterine cavity in the least traumatic way without disturbing the primed uterine environment [4]. The first described ET technique introduced and delivered a preloaded embryo with a soft catheter into the uterine cavity via the cervical canal [3]. The intrauterine position of the catheter tip for embryo deposition was either determined by measuring 6 cm from the external cervical os or by measuring the cavity length with a dummy transfer prior to the actual ET [1]. The first ultrasound-guided ET was reported in 1985 [5], and 30 years

later a Cochrane review concluded that ultrasound guidance should be the recommended and preferred method for ET [1]. Despite this Cochrane guidance, a lack of universal implementation exists, demonstrated by two recent surveys showing wide variation in ET techniques [4,6]. The reason for this is thought to be multifactorial, with most of the published data on efficacy of ET techniques being conflicting, inconclusive or affected by confounding variables dependent on either the practitioner or the technique [1,4,7–10]. This is an important issue in IVF research. For example, studies using different embryo deposition points of 1, 1.5 or 2 cm from the fundus, and measuring the outcome of clinical pregnancy are confounded by the embryo deposition site [1,10–13]. Another example of conflicting evidence is the removal of cervical mucus prior to ET. Some studies recommend removal [14–17], whilst others, including a meta-analysis, failed to show any significant benefit [18,19]. Use of a patient relaxant, direction of the removal of the ET catheter and duration of bedrest following transfer are some of the other discordances between studies [4]. Such differences could also impact the outcomes between trials [20], resulting in misinterpretation of the available evidence. It is estimated that up to 30% of all cycle failures can be considered due to poor practice used in the transfer technique [21], and it has been shown that pregnancy rates can differ depending on the clinician performing the transfer [17], which emphasises the expertise required for this often-overlooked component of the IVF process [2].

The lack of consensus that exists at the present time may also be due to the apparent absence of a robust, specific guideline highlighting the practice of the ET technique. Such guidelines from professional organisations such as the British Fertility Society (BFS) or the European Society of Human Reproduction and Embryology (ESHRE) may facilitate standardisation of best evidence-based practice, which is a fundamental first step towards improving clinical outcomes in IVF.

The last UK survey on ET was conducted nearly two decades ago, and their main recommendation was the need for a standardised national protocol to be implemented for ET [22]. Since then, new evidence has found that subtle differences between individual practitioners can significantly affect ET success rates despite using a similar technique [22–24]. Examples for these include two separate Cochrane reviews recommending the use of ultrasound guidance, as well as the use of soft catheters for ETs [1,25]. However, a universally available, standardised, national guideline or protocol for practitioners in IVF units in the UK is yet to be produced. Our aim, therefore, was to evaluate and gain insight into the current clinical practice regarding ET in the UK. Our data aims to provide the basis for future attempts to harmonise the practice in the UK with the formulation of a standardised protocol.

2. Materials and Methods

2.1. The Survey

Items in the survey were identified based on a literature review and expert clinical opinion. Expert clinical opinion was sought initially from local practitioners (reproductive medicine specialists and embryologists) at the Hewitt Fertility Centre, Liverpool, which is one of the larger National Health Service (NHS) IVF units in the UK with approximately 1800 fresh IVF/ICSI cycles being performed per annum. The initial survey questions were formulated in August 2018 after reviewing current ET techniques and by considering the practice pertinent to individual practitioners. The initial 33 question survey was subsequently modified after being peer reviewed by five other fertility specialists who were directly contacted by the authors, from IVF units around the country, before a final 38 question national survey was finalized and distributed to all IVF units in the country (Supplementary Figure S1).

The survey questions were informed by current evidence relating to different aspects of the ET technique. The questions in the final survey included demographic information on the unit (type of practice, number of ETs per year, location) and important outcome measurements (including biochemical pregnancy rate (BPR), clinical pregnancy rates (CPR)

and live birth rates (LBR)). We also included questions relevant to the ET technique (such as the type of catheter used, the use of ultrasound guidance, how practitioners clean the cervix) and questions relevant to the practitioners involved during the ET (which professionals were involved and their experience). Previous evidence suggested that the use of ultrasound guidance [1,7], soft catheters [25,26] and removal of cervical mucus [16] can improve ET success rates, and physician-associated factors also play an important role [27], thus these were included. Our data, therefore, provides evidence for heterogeneity in practice that may affect outcomes of clinical trials in this area, as well as highlighting existing uncertainties to focus on in future research efforts.

The final electronic survey was emailed on the 16 December 2018 through SurveyHero (www.surveyhero.com) to all clinical leads in the 79 Human Fertilisation and embryology authority (HFEA) registered units that perform ETs in the UK. SurveyHero is an online anonymous survey tool, and no patient-identifiable data were collected. Electronic reminders were sent out in the interim six-month period when they were requested to respond. When there was no response from clinical leads, other consultants within the same unit were contacted requesting a response to the survey. To remove duplication or inaccuracy of responses from a particular unit, the name of the organisation was included. If multiple responses were received from the same unit, the first response from that unit (after confirming concordance with duplicate responses) was used in the analysis.

As this was an anonymous survey with no patient-identifiable data, ethical approval was not required.

2.2. Statistical Analysis

This survey was not designed as a comparative study or powered to detect differences. Therefore, in line with our research aims of the current national practice in the UK, we report summary statistics of the data obtained from the survey. Where possible, the Statistical package for the Social Sciences (SPSS) for Windows (Version 26; IBM Corporation, Chicago, IL, USA) was used to analyse categorical data using the χ^2 test or the Student's paired *t*-test for continuous data.

3. Results

Sixty-one out of the 79 clinics responded, fourteen responses were excluded (seven incomplete and seven duplicate), leaving the final number of responses analysed as 47 (Figure 1).

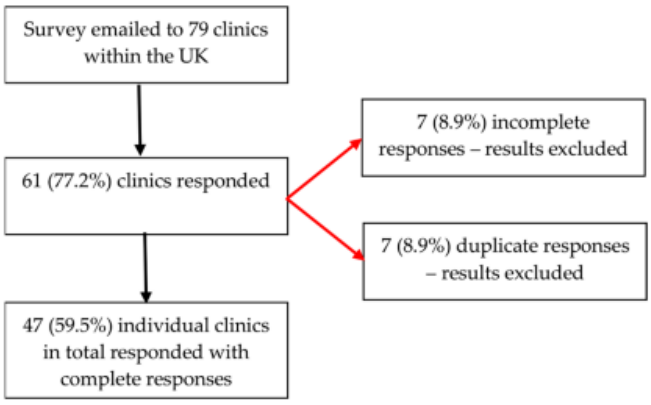

Figure 1. Flowchart of survey respondents.

3.1. Demographics of the Units

Table 1 outlines the demographic data of the units that responded to the survey. It demonstrates that the majority of practices treat both NHS and privately funded patients (36, 77%), base their ET success rate on CPR (27, 57%) and estimate their LBR to be between 30 and40% (28, 60%).

Table 1. Unit demographics.

Types of IVF Practice *n* (%)	
NHS	2 (4)
NHS and Private	36 (77)
Private	9 (19)
Basis of ET success *n* (%)	
Positive pregnancy test	13 (28)
Clinical pregnancy rate	27 (57)
Live birth rate	5 (11)
No response	2 (4)
Persons performing the ET *n* (%)	
Consultant only	18 (38)
Consultant and nurse	14 (30)
Consultant, registrar and nurse	7 (15)
Consultant and registrar	6 (13)
Nurse only	2 (4)
Estimated clinical pregnancy rates per ET *n* (%)	
20–30	3 (6)
30–40	18 (38)
40–50	23 (49)
50–60	1 (2)
60–70	0 (0)
>70	1 (2)
No response	1 (2)
Estimated Live birth rate per ET *n* (%)	
20–30	13 (28)
30–40	28 (60)
40–50	3 (6)
50–60	0 (0)
60–70	1 (2)
No response	2 (4)

3.2. Embryo Transfers

Seven clinics (15%) allowed individuals to utilise their preferred ET technique. No zygote intrafallopian transfers were performed by any of the clinics (Table 2).

Table 2. Number of transfers performed by units.

Presence of Standardised Technique within the Unit *n* (%)	
Standard technique	40 (85)
Technique based on individual preference	7 (15)
Number of ETs per year *n* (%)	
<500 *n* (%)	7 (15)
500–1000 *n* (%)	20 (43)
1000–1500	10 (21)
1500–2000	2 (4)
>2000	8 (17)
Number of transmyometrial transfers per year *n* (%)	
10	1 (2)
5	2 (4)
3	1 (2)
2	7 (15)
1	6 (13)
0	30 (64)

When the published HFEA clinic success rates were considered, those clinics performing more transfers appeared to have better LBR than those performing less ET's (Table 3).

Table 3. Number of ETs relating to average HFEA LBR.

Number of ETs	Number of Clinics	Average HFEA LBR (%)
<500	7	20.1
500–1000	20	22.8
1000–1500	10	22.2
1500–2000	2	28.5
>2000	8	24.3

3.3. ET Preparation

Most units did not use sedation for ET (94%), with one unit (2%) using sedation when required (when a patient was unable to tolerate the procedure without sedation). Forty-three (91%) of the clinics cleaned the cervix prior to ET and 33 (72%) removed cervical mucus with a cotton wool swab (Table 4). Most units (78%) would abandon the ET if there was fluid within the endometrial cavity on ultrasound. Thirty-nine (83%) of the clinics performed ultrasound-guided ET with nursing staff performing the majority of the ultrasound scanning (92%).

Table 4. Patient and practitioner preparation prior to ET.

Patient Relaxant *n* (%)	
None	44 (94)
Voltarol	1 (2)
Sedation when required	1 (2)
Sedation	1 (2)
Sterility of Procedure *n* (%)	
Sterile gloves after handwashing	27 (57)
Aseptic technique	18 (38)
Scrubbed and gowned	2 (4)
Warmed speculum *n* (%)	
Yes	11 (23)
No	36 (77)
Lubrication on speculum *n* (%)	
None	10 (21)
Culture media	1 (2)
Normal Saline	23 (49)
Sterile water	11 (23)
Ultrasound gel	2 (4)
What is used to clean the cervix *n* (%)	
Normal Saline	34 (72)
Media from lab	7 (15)
Not cleaned	4 (9)
Sterile water	2 (4)
Instrumentation used to clean the cervix *n* (%)	
Cotton wool	23 (50)
Gauze sponge on forceps	19 (41)
Cotton wool and Gauze	2 (4)
Pipette	1 (2)
N/A	1 (2)
Removal of endocervical mucous *n* (%)	
Cotton wool	29 (63)
Aspirate	4 (9)
Cotton wool and flush	4 (9)
Flush	2 (4)
Not removed	7 (15)

Table 4. *Cont.*

Embryo transfer technique *n* (%)	
2D ultrasound guidance	38 (81)
3D ultrasound guidance	1 (2)
Clinical touch technique	7 (15)
Dummy ET and measurement of cavity length	1 (2)
Person performing the ultrasound scan *n* (%)	
HCA	8 (17)
Embryologist	1 (2)
Nurse	36 (77)
Doctor	4 (9)
Ultrasound technician	1 (2)
Approach to fluid within the endometrial cavity *n* (%)	
Abandon the transfer	35 (74)
Aspirate the fluid and continue with transfer	7 (15)
Continue with the transfer	3 (6)
No response	2 (4)
Use of a routine mock transfer *n* (%)	
For specific indication	27 (57)
Not routinely done	10 (21)
Immediately before transfer	4 (9)
At oocyte retrieval	2 (4)
Before cycle begins	4 (9)

3.4. ET Technique

The most common ET technique was the afterload technique (53%), with 100% of respondents using soft catheters (Table 5). Clinics generally used (72%) a stylet for less than 25% of their transfers and the routine use of tenaculum was uncommon. Most (91%) reported deposition of the embryo in the upper or middle portion of the uterine cavity, although exact deposition points from the uterine fundus varied from 0.5 cm to over 2 cm. Embryo retention following transfer was <5% in all clinics, with 31 respondents (66%) re-transferring the embryo in a new catheter when this occurred.

Table 5. ET technique.

Embryo Transfer Technique (*n*%)	
Afterload technique	24 (53)
Trial with transfer technique	12 (27)
Direct technique	9 (20)
ET catheter preference *n* (%)	
Wallace	29 (62)
Cook	22 (47)
Kitazato	6 (13)
Surepro	2 (4)
Labotect	1 (2)
Use of stylet *n* (%)	
All the time	1 (2)
>50% of transfers	6 (13)
25–50% of transfers	5 (11)
<25% of transfers	34 (72)
Never	1 (2)
Use of a tenaculum *n* (%)	
Never	9 (19)
Several times in career	18 (38)
<10% of transfers	18 (38)
<30% of transfers	2 (4)
Approximate location of catheter tip in uterine cavity *n* (%)	
Upper third	18 (38)
Middle third	25 (53)
Lower third	4 (9)

Table 5. *Cont.*

Approximate distance embryo is is deposited (cm) from uterine fundus *n* (%)	
0.5	1 (2)
1	10 (21)
1.5	12 (26)
2	5 (11)
>2	4 (9)
Don't measure	15 (32)
Who depresses the plunger once the catheter is in place *n* (%)	
Clinician	34 (72)
Embryologist	13 (28)
Speed and process of embryo deposit *n* (%)	
As slowly as possible	7 (15)
Slow pace with steady pressure	29 (62)
Moderately fast with steady pressure	11 (23)
As quick as possible	1 (2)
Approach to retained embryos *n* (%)	
Retransfer in same catheter	19 (40)
Retransfer in new catheter	31 (66)
Frequency of retained embryos *n* (%)	
<1% of ET	35 (74)
1–5%	12 (26)
Presence of blood or mucus on catheter tip *n* (%)	
<5%	22 (47)
5–10%	18 (38)
10–20%	5 (11)
20–30	2 (4)
Duration catheter left inside cavity following embryo deposition *n* (%)	
Immediately removed	6 (13)
5–10 s	18 (38)
10–20 s	17 (36)
30 s	5 (11)
1 min	3 (6)
Direction catheter removed *n* (%)	
Straight	21 (45)
Rotate as removed	25 (53)
Both	1 (2)
Patient remaining supine after transfer *n* (%)	
Get up immediately	32 (68)
5–10 min	15 (32)

Clinics were asked to rank how they would deal with a difficult transfer and what steps they would take (Figure 2). When faced with a difficult transfer, the majority responded claiming to use a stylet. Use of cervical dilators was the most infrequent response.

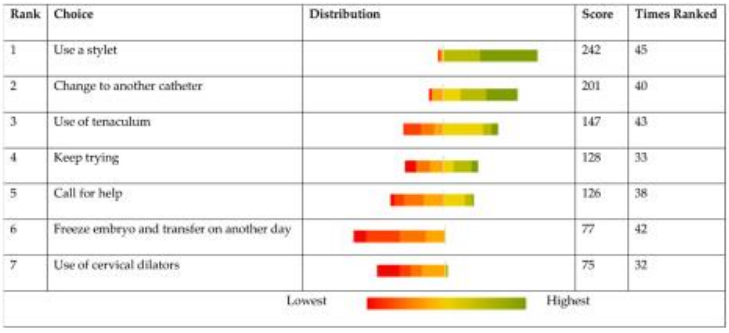

Figure 2. If there is difficulty in ET, what would be your preferred options in order 1–7.

When the respondents were asked what they thought was the most important aspect with regard to ET, the majority of responses suggested guidance with ultrasound and

good consistent technique (Figure 3). Interestingly, there were three responses stating that a slow steady transfer improves chances of success, whilst three other responses urged speedier transfers.

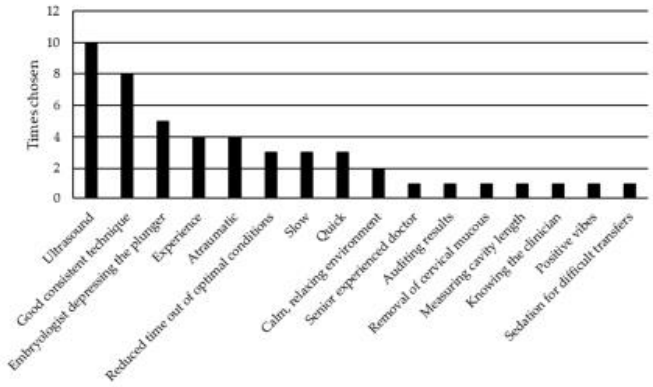

Figure 3. Most important aspects of embryo transfer.

When comparing the LBR published by the HFEA for units, very similar results were observed between those units that used ultrasound guidance and those which used clinical touch technique (CTT). For the CTT, the LBR was 22.8% (SD ± 3.06) compared to 22.4% (SD ± 5.4) for the ultrasound-guided group ($p = 0.873$).

4. Discussion

This contemporary national survey updates the 16-year-old previous survey on ET technique in the UK and highlights the existing wide variation in practice with no standardised approach to the procedure prevailing in the UK. It therefore emphasises the urgent need for a standardised national protocol to ensure best outcomes for women undergoing IVF in the UK [22].

Over the years there have been many changes in ET techniques in general, with new evidence demonstrating the benefit of particular practices to improve outcome, such as the use of ultrasound guidance [1], soft catheters [14,17,26] and avoiding prolonged bed rest following transfer [28]. Reassuringly, the majority of units that responded, appeared to acknowledge the new evidence in their practice (83% ultrasound guidance, 100% soft catheters and 68% immediate mobilisation). Interestingly, we unexpectedly found no significant difference in LBR between clinics regardless of the use of ultrasound guidance.

Positioning of the embryo catheter in the upper or middle third of the cavity was the practice in 91% of the units, in line with the systematic reviews [14,17]. However, this apparently excellent practice should be considered with caution since some survey responders appear to have different interpretations of the terms upper, middle and lower third of the cavity (Figure 4). They determined the upper third of the cavity as 0.5→ 2 cm, middle third as 1 → 2 cm and the lower third as 1.5 → 2 cm from the fundus. Among those respondents who measured the distance from the fundus, 85% placed the catheter 1–2 cm from the fundus of the uterus. Frequency of depositing the embryo at the upper third of the cavity increased to 97% if we included those who transfer at >2 cm in keeping with the recommendations from the Cochrane reviews [14,17,29]. This draws attention to the need for clarity in a future guideline/study protocol in which embryo deposition is described.

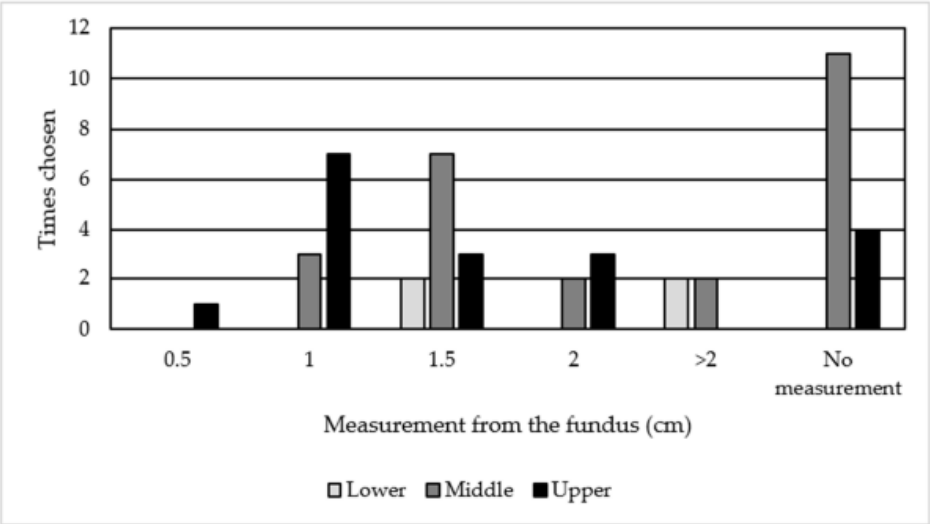

Figure 4. Units' interpretation on distance from fundus to location in uterine cavity.

Despite the available evidence supporting immediate withdrawal of the catheter following embryo expulsion [14,17,30,31] only six units (13%) adhered to this, with the remaining units allowing a delay prior to removal. There was no significant difference in pregnancy rates between the groups regardless of this practice [30,31]. However this practice may unnecessarily prolong the uncomfortable procedure for the patient without conferring any benefit.

All units reported embryo retention rates at <5% in keeping with previously quoted incidence rates [8]. Maintaining a low retention rate would help reduce patient anxiety and reduce the time that the embryo is outside of the incubator optimal conditions. Prolonged transfer times are known to have a detrimental effect on pregnancy rates [32,33], although the retransfer of retained embryos has not shown to be detrimental [34–38].

Conversely, there are areas with room for improvement. Amongst them, of concern is how clinics approach fluid within the endometrial cavity, since 21% of respondents claimed that they would either aspirate (15%) or would proceed with transfer (6%) when there was fluid identified within the endometrial cavity, despite available advice to the contrary [8,39]. We appreciate that fluid in the endometrial cavity is not an absolute contraindication to ET, and that in cases where embryos need to be refrozen this may have a negative impact on subsequent implantation and LBR [40]. Other studies have also found that those with transient, small amounts of fluid within the endometrial cavity (<3.5 mm) are not associated with poorer outcomes [41,42]. However, those with known hydrosalpinx, or with persistent endometrial fluid in the cavity, continue to have poorer outcomes compared to those without fluid in the endometrial cavity [42]. These cases need to be dealt with on an individual basis, taking into account patient preference whilst weighing the risks and benefits of continuing with the ET. The recommendation from our survey would be to abandon the ET if endometrial fluid is found in the cavity and freeze the embryo for transfer in a subsequent cycle, particularly since emerging evidence is showing no detrimental effect when embryos are refrozen [43,44].

The frequent use of a tenaculum in some units is another concern. The use of a tenaculum is not only painful but can also have a negative impact on embryo implantation rates due to increased uterine contractions due to stimulating oxytocin release [45,46]. With this in mind, the use of a tenaculum for ET should only be used once all other options are

exhausted, yet surprisingly, it was the third most popular option to be used for difficult transfers. When 57% of respondents reported having never used a tenaculum or only having used one several times in their career, this raises the question how much their technique differs to those who use the tenaculum on a more frequent basis.

One other interesting feature identified in our survey was that the majority of respondents estimated their LBR to be between 30 and 40%. However, the 2017 HFEA data reported most of the clinics having a LBR between 20 and 30% [47]. Although it is possible that this is due to the HFEA data being two years older than when the clinics responded to our survey, this may also be relevant to personal perception versus actual figures, and further highlights the important impact such discrepancies may have when patients are counselled by the clinicians in these units. Relevant to this, CPR was the preferred marker of success for the responders, since presumably it is an easily and relatively rapidly attained marker of success, with the majority of clinics performing the initial scan themselves to confirm a pregnancy, and thereby acquiring this data [48]. Subsequently, patients may be lost to follow up, and accurate LBR data is more difficult to collate [49]. Importantly, LBR is a mandatory outcome to be reported in the UK, and possibly the most relevant data for patients. However, publicizing the CPR, which is naturally higher than the LBR, may be more attractive to patients [48].

Whilst there are a number of questions where concordance was observed in this survey, there were more responses that differed than were similar. This lack of standardisation amongst units can be one of the reasons why LBR between clinics range from 11 to 34% [47]. We appreciate that there are numerous other steps involved in the ART technique that impact overall success rates, including type of ovarian stimulation cycles, oocyte retrieval and laboratory techniques. However, if standardisation of ET techniques were to occur, it could potentially highlight other imperfect areas in the above-mentioned steps of the IVF process that also have an impact on the LBR.

Standardization could also reduce research bias, which has previously been noted by Gambadauro et al. [50]. When reviewing published trials in IVF there was very little information about the methods and execution involved in the ET and this could potentially be a source of performance bias [50,51].

Our findings are in agreement with a previous survey conducted by the ASRM [4], which also highlighted the need for standardization. They also demonstrated a highly diverse approach to the ET technique, with multiple areas of discordance including use of a patient relaxant at the time of ET, direction of catheter removal and duration of bed rest following transfer [4]. As a consequence of their survey, the ASRM have been able to produce a protocol for ET suitable for North American practice [4,17,52]. We anticipate our survey should facilitate the launch of a similar national/European protocol following discussion with representative bodies such as the British fertility society (BFS) and/or the European society of human reproduction and embryology (ESHRE).

5. Recommendation

The previously mentioned ASRM survey [4], as well as the review by Saravelos et al. [14], made recommendations based on their literature reviews. These can be seen in Table 6.

Based on the findings of this survey, and the above evidence, we propose the following approach to embryo transfer:

1. No routine use of anaesthesia or analgesia.
2. Use sterile gloves.
3. No use of warmed speculum.
4. Use sterile water or normal saline for speculum lubrication.
5. Clean the cervix with normal saline or laboratory media.
6. Use cotton wool or gauze to clean the cervix and remove mucus.
7. Use ultrasound guidance for embryo transfer.
8. Abandon transfer if fluid is within the endometrial cavity.

9. Perform mock transfer for specific indication.
10. Afterload technique.
11. Deposit the embryo in the upper/middle portion of the endometrial cavity.
12. Use a stylet when required or anticipated difficulty.
13. Avoid the use of tenaculum/vulsellum.
14. Slow and steady pressure of plunger.
15. Remove the catheter either straight or rotational immediately following transfer.
16. Immediate ambulation.

The main limitation of this survey was that we did not achieve full coverage of all UK IVF units. The response rate was reasonably high (59%), but we accept that this survey is not necessarily representative of universal practice within the UK. The main instrument utilised to gather information in our study was a questionnaire. We specifically developed this questionnaire with the involvement of a number of specialists and experts from around the UK to provide a snapshot of current practice, and it was not for general use among the public. Therefore, although we acknowledge that not validating this questionnaire as a limitation of our work, we followed similar pathways to other previous surveys [4,6,53,54] in this field, and the involvement of multiple experts in the field in its development improves its validity. The data obtained is qualitative and should be interpreted as such, but it is meant to highlight the variations in current practice within the UK and to prompt conversations on how standardisation could be achieved in ET techniques.

Table 6. ET recommendations.

Recommendation	ASRM Guideline [4]	Saravelos et al. [14]
Removal of cervical mucous	Grade B evidence	Grade B evidence
Use soft ET catheters	Grade A evidence	Grade A evidence
Abdominal ultrasound guidance	Grade A evidence	Grade A evidence
Embryo transfer to central or upper cavity	Grade B evidence	Grade B evidence
Immediate catheter withdrawal	Grade B evidence	Grade B evidence
Immediate ambulation	Grade A evidence	Grade A evidence
Immediate retransfer of retained embryo	Grade B evidence	Grade B evidence

The strengths of this survey are that it is the first of its kind in the UK, and comprehensively and systematically dissects out the practice of ET procedures. It emphasized the concordance, discordance and areas of improvement required in certain practices involved in the ET process, identifying the areas in need of a standardized approach. Areas of improvement should aim to abandon ET when fluid is seen in the endometrial cavity and only use tenaculums when all other options have been exhausted.

ET techniques have been shown to have a significant impact on pregnancy rates [24,27,55] and the variation between practices could have an influence (along with other factors of the IVF process) on a unit's success rate. In a field of medicine where every percentage point counts, slight changes could result in significant improvement in success rates and patient satisfaction. Therefore, we have a responsibility to ensure that all patients receive best evidence-based care, and this survey brings to light that this may not be the case, at least in some aspects of the ET process in the UK.

6. Conclusions

This is the first survey that sheds light on contemporary practice and attitudes among different units regarding ET in the UK. It highlights the urgent need for standardisation in ET, a process that is vital for IVF success rates. Such standardisation of practice will facilitate practitioner training, research and ultimately IVF success rates. The lack of evidence for best practices that prevails in many areas of the ET procedure will need to be overcome with a consensus expert meeting and review of all literature. We believe that areas of discordance identified in our survey, where there is insufficient evidence for the most favourable method, will guide future research to fill the gaps in our current knowledge.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10132839/s1, Figure S1: Final Survery.

Author Contributions: Conceptualization, L.N., R.R., R.H., D.K.H.; distribution and first analysis of survey results, L.N.; writing and editing, L.N., N.T., R.R., A.J.D., R.H. and D.K.H.; figures, L.N., N.T.; funding acquisition, N.T. and D.K.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Embryo transfer technique survey

Final Survey

Demographics

Name of Organisation- _____
Types of IVF practice- NHS / Private / NHS and private
How many embryo transfers do you do each year - <500 / 500<1000 / 1000<1500 / 1500<2000 / >2000
How many ZIFT transfers do you do per year? _____
How many transmyometrial transfers do you do each year? _____
Basis of ET success rate - Positive hCG / Clinical pregnancy rate / Live birth rate
Estimated Success rate (%)- <20 / 20-30 / 30-40 / 40-50 / 50-60 / 60-70 / >70
Estimated clinical pregnancy rate (%)- <20 / 20-30 / 30-40 / 40-50 / 50-60 / 60-70 / >70
Estimated live birth rate (%)- <20 / 20-30 / 30-40 / 40-50 / 50-60 / 60-70 / >70
Who performs the embryo transfer (ET) – Nurse / Registrar / Consultant

Patient and practitioner preparation

Presence of a standard technique/operating procedure (SOP) for ET practice in unit- Standard technique / Technique based on individual preference
Use of patient relaxant- No / Gas and air / Sedation / Nifedipine / Ritodrine / Other
Sterility of the procedure - Sterile gloves after handwashing / Aseptic technique / Scrubbed and gowned
Use of warmed speculum – Yes / No
Lubrication used on speculum – None / Culture media / Normal saline / Sterile water / Ultrasound gel
What do you use to clean the cervix before transfer - Normal saline / Media from lab / Other / NA(don't cleanse)
What Instruments do you use to clean cervix? Cotton swab / Gauze sponge on forceps / NA / Other
How do you remove mucous from the endocervical canal - Cotton swab / Flush / Cotton swab and flush / Aspirate / NA
What technique is used in your clinic for embryo transfer? Clinical touch technique / 2D Abdominal ultrasound / 3D Abdominal ultrasound / Other (please specify)
Person performing US guidance - US technician / Nurse / Doctor / NA / Other
Approach to fluid in the cavity- Cancel transfer / Aspirate fluid / Continue with transfer
Routine performance of mock transfer - Before cycle begins / During stimulation / At oocyte retrieval / Immediately before transfer / Not routinely done / For specific indication

Embryo transfer technique

Predominant technique - Trial with transfer technique / Afterload technique / Direct technique
ET catheter preference – Cook / Wallace / Other
If there is difficulty in ET, what would you be your preferred options in order 1-7

- Use a stylet
- Change to another catheter
- Use of tenaculum
- Freeze embryo and transfer on another day
- Call for help
- Keep trying
- Use of cervical dilators

Use of stylet - All the time / >50% / 25-50% / <25% / Never
Frequency of transfers using a tenaculum – Never / Several times in career / <10%/ <30% / <50%

Approximate location of endometrial cavity where tip of catheter is aimed - Upper third / Middle third / Lower third

Distance from uterine fundus embryo deposited - 0.5cm / 1cm / 1.5cm / 2cm / >2cm / Don't measure

Who pushes the plunger once the embryo catheter is in place – Clinician / Embryologist

Speed and process of plunge - As slowly as possible / Slow with steady pressure / Moderately fast with steady pressure / As quick as possible

Approach to retained embryos - Retransfer in same catheter / Reload into new catheter

Frequency of retained embryos (%) - <1 / 1-5 / 5-15 / 15-20 / >20

Frequency of blood or mucous on end of catheter(%) - <5/ 5-10 / 10-20 / 20-30 / 30-40 / >40

Time after embryos expelled into cavity before removing catheter - Immediate removal / 5-10sec /10-20sec / 30 sec / 1min / Other

Direction for catheter removal – Straight / Rotate as removed

Patient remains supine after transfer - Gets up immediately / 5-10 min / 10-15 min / 15-30 min / >30min

Please highlight those areas that you think are the most relevant to your practice.

_____

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