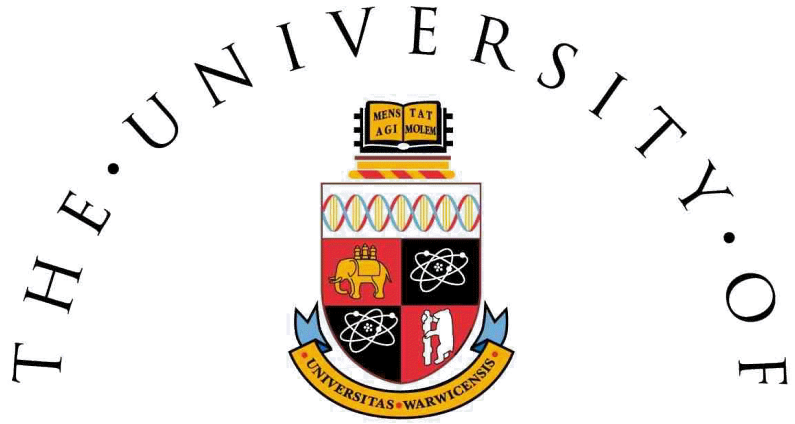


WARWICK

Peri-implantation Heparin Improves Implantation and the Clinical Pregnancy Rate and Live Birth Rate in Subfertile Women

Dr Muhammad Ahsan Akhtar MBBS DFFP MRCOG

University of Warwick

For the Degree Doctor of Medicine

March-May 2015

[redacted]

Table of Contents

Declaration

I, Dr Muhammad Ahsan Akhtar declare that:

1. My research has been conducted ethically and all of the work presented in this thesis, except where specifically stated, was original research performed by myself under the supervision of Professor Siobhan Quenby.
2. During my research I undertook a Cochrane systematic review and meta-analysis in collaboration with my colleagues at the University of Warwick and the University of Nottingham.
3. Following this, a Phase 1 study was designed with Professor Quenby. I wrote the protocol, obtained necessary approvals which allowed us to go ahead and perform the study.
4. Participants were recruited by myself and Professor Quenby. The interventions during the study were performed by myself with the support and help of my colleagues at the University of Warwick and University Hospitals Coventry and Warwickshire NHS Trust.
5. The data and the results presented are genuine and obtained during my research
6. I have appropriately acknowledged and referenced within my thesis, where I have drawn on the work, ideas and help of others.
7. The thesis submitted is within the required word limit as specified by the University of Warwick.

Acknowledgements

Professor S Quenby has been inspirational for me since I became a trainee in the Mersey Deanery. She is an exceptional supervisor who always supported and guided me patiently throughout this research. Her continued support and guidance has led to this unique project.

I would like to thank Professor J Brosens who also helped me enormously with this project.

I would like to thank Mr K Jayaprakasan, Mr N Raine-Fenning, Professor J Thornton and Dr S Sur (University of Nottingham) with whom I worked to complete the systematic review and meta-analysis. I especially thank Ms H Nagels and Ms J Clarke (Managing Editors), Ms J Marjoribanks, Ms M Showell (Trials Search Coordinator) and Professor C Farquhar, for their invaluable assistance, support and advice for the Cochrane review.

I would like to extend my thanks to Mr S James (University of Warwick) who has been a tremendous help with the laboratory work.

I would like to thank all the staff at the Centre for Reproductive Medicine at University Hospitals Coventry & Warwickshire especially Mr S Keay, Dr R Agrawal, Mr T Ghobara and the research team at the Biomedical Research Unit who have been great help.

I would like to thank the patients who participated in the research study as without them this study would not have been possible.

I am grateful to my colleagues Dr L Lacey and Dr K Birchnall for helping me in study recruitment and chaperoning me during the study interventions. Their presence and help was immense.

It was a pleasure to work with Ms C Jones (Head of Research and Development) and the Pharmacy Department at University Hospitals Coventry and Warwickshire NHS Trust.

I am thankful to my family who were patient during my research as they have to sacrifice time and encouraged me through the ups and downs during this period

Abstract

The clinical success of assisted reproductive technology (ART) is measured by the clinical pregnancies (implantation success) and the live births rates.

Following ART live birth rates vary from 20-40% and are dependent upon a variety of factors. Various adjunct therapies are being used with ART to improve implantation and pregnancy outcomes. The effectiveness of these adjuvant therapies remains unclear and requires further evaluation. One group of medical adjuvant therapies widely used in clinical practice are thromboprophylactic agents, including heparin. Heparin can potentially modulate many of the mechanisms of implantation including successful apposition, adhesion and penetration of the developing embryo into the endometrium. This is independent of its anticoagulant function for which it is used routinely in clinical practice.

Following completion of a literature review, it became evident that heparin could potentially improve decidualisation and implantation. It improves function of various growth factors and cytokines in the endometrium promoting and facilitating implantation in laboratory models. From this initial research, we postulated that heparin used as adjunct to ART should improve the clinical pregnancy and the live birth rates via these mechanisms described. Bleeding is a known side effect of systemic heparin due to its effect on the coagulation cascade.

A systematic review and meta-analysis protocol was devised and peer-reviewed to assess the published data. The aim of this was to establish whether using the currently available evidence, peri-implantation heparin improves pregnancy outcomes in women undergoing ART. A secondary aim was to determine if there were any significant side effects. The meta-analysis was performed in accordance with the protocol. This demonstrated that peri-implantation systematic heparin does improve clinical pregnancy rates and live birth rates in these women. Nevertheless, there were only three randomised control trials (RCTs) included in the review that met the inclusion criteria and there was significant heterogeneity amongst the participants in the included studies. Systemic side effects of heparin including bleeding and bruising were also identified in this review.

As the proposed mechanism of improving implantation by heparin is improvement of endometrial cytokines and growth factors. It was hypothesised that direct endometrial administration of heparin should improve decidualisation thus improving implantation. To confirm or refute this hypothesis, initially a phase 1 study is required to be undertaken for direct endometrial administration of heparin as currently it is only licenced as a systemic injectable formulation.

We developed a protocol to assess the feasibility of intrauterine flushing for direct endometrial administration of low molecular weight heparin (LMWH) with a prospective randomised placebo controlled pilot study. This novel study was approved by National Research Ethics Service (NRES), Medicine & Healthcare products Regulatory Authority (MHRA), UK. Sponsorship was obtained from the

University of Warwick and local Research & Development (R&D) approval was obtained. The study was undertaken at University Hospitals Coventry and Warwickshire NHS Trust (UHCW). It demonstrated the acceptability of intrauterine flushing of heparin to women. The concept of the trial was popular with patients making recruitment unproblematic. Minimal side effects were reported, no serious adverse events occurred. Most women recruited underwent ART following the study, with many achieving a clinical pregnancy and live birth. Our hypothesis for primary outcome measure, uterine natural killer (uNK) cell density, as a marker of decidualisation was refuted.

List of Abbreviations

AE - Adverse Event

ALP - Alkaline Phosphatase

ALT - Alanine Aminotransferase

APS - Antiphospholipid Syndrome

APTT - Activated Partial Thromboplastin Time

AR - Adverse Reaction

ART - Assisted Reproductive Technology

ASRM - American Society of Reproductive Medicine

BFS - British Fertility Society

BRU - Biomedical Research Unit

BMI - Body Mass Index

cAMP - Cyclic Adenosine Monophosphate

CD16 - Cluster of Differentiation molecule 16

CD56 - Cluster of Differentiation molecule 56

CEACAM1 – Carcinoembryonic Antigen-related Cell Adhesion Molecule 1

CENTRAL - The Cochrane Central Register of Controlled Trials

CERES – Consumers for Ethics in Research

cGH - Comparative Genomic Hybridisation

CI - Confidence Interval

CSRL - Clinical Sciences Research Laboratory

DARE - Database of Abstracts of Reviews of Effects

DNA - Deoxyribonucleic Acid

EGF - Epidermal Growth Factor

ESCs - Endometrial Stromal Cells

ESHRE - European Society of Human Reproduction and Embryology

eSET - Elective Single Embryo Transfer

ET - Embryo Transfer

FBC - Full Blood Count

FSH - Follicle Stimulating Hormone

GCP - Good Clinical Practice

GnRH - Gonadotrophin Releasing Hormone

GP - General Practitioner

HB-EGF - Heparin Binding Epidermal Growth Factor

hCG - Human Chorionic Gonadotrophin

HEFA - Human Embryo and Fertilisation Authority

HIT - Heparin Induced Thrombocytopenia

HLA - Human Leucocyte Antigen

HyCoSy - Hysterosalpingo-Contrast-Sonography

ICSI - Intracytoplasmic Sperm Injection

IGF - Insulin like Growth Factor

IGFBP - Insulin like Growth Factor Binding Protein

IL - Interleukin

IMP - Investigational Medicinal Product

IU - International Units

IUI - Intrauterine insemination

IV - Intravenous

IVF - In vitro Fertilisation

KDa - Kilo Daltons

LFTs - Liver Function Tests

LH - Luteinising Hormone

LIF - Leukaemia Inhibitory Factor

LMWH - Low Molecular Weight Heparin

LMP - Last Menstrual Period

mg - milligram

MHRA - Medicines and Healthcare products Regulatory Agency

mls - millilitres

mm - millimeter

MMP - Matrix Metalloproteinases

NaCl - Sodium Chloride

NHS - National Health Service

NICE - National Institute of Clinical Excellence

NK - Natural Killer

NRES - National Research Ethics Service

NSAIDS - Non Steroidal Anti-Inflammatory Drugs

OHSS- Ovarian Hyperstimulation Syndrome

OR - Odds Ratio

PG - Prostaglandin

PGS - Pre implantation Genetic Screening

PI - Principal Investigator

PRL - Prolactin

PT - Pregnancy Test

PTT - Prothrombin Time

R & D - Research and Development

RCOG - Royal College of Obstetricians and Gynaecologists

RCT - Randomised Controlled Trial

RNA - Ribonucleic Acid

RR - Risk Ratio

SAE - Serious Adverse Event

SD - Standard Deviation

SGK - Serum and Glucocorticoid regulated Kinase

STAT3 - Signal Transducer and Activator of Transcription 3

SmPC - Summary of Product Characteristics

SUSAR - Suspected Unexpected Serious Adverse Reaction

TGF - Transforming Growth Factor

TNF - Tumour Necrosis Factor

TVS - Transvaginal ultrasonography

UFH - Unfractionated Heparin

UHCW - University Hospitals of Coventry and Warwickshire

UK - United Kingdom

uNK - uterine Natural Killer

VEGF - Vascular Endothelial Growth Factor

WHO - World Health Organisation

List of Figures and Tables

Chapter 1

1.0 Introduction:

There were 698,512 births in England and Wales in 2013 providing joy to thousands of parents (Office for National Statistics, 2013). Many couples are not so fortunate, one in seven couples experience subfertility (NICE, 2013). Despite this, a large number of couples still fail to conceive following ART even with the transfer of good quality embryos (Polanski *et al*., 2014).

ART has revolutionised the treatment of subfertility in last three decades. ART including in-vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) are now employed widely in significant numbers to help some subfertile couples to achieve a pregnancy. 45,264 women had IVF treatment in 2010 and there were 12,714 babies born in 2009 as a result of IVF treatment (HEFA, 2010).

ART has significant physical, social, psychological and financial implications. Its success is determined by a clinical pregnancy and the live birth of a child. Live birth rates with ART vary from 20% to 40%. Thus, at present, more than 50% of women having ART will not achieve a successful implantation leading to pregnancy. These women are devastated by this outcome.

Whilst significant improvements have been made in the preparation of embryos (with blastocyst culture and selection of embryos with time lapse microscopy), little progress has been made in improving the endometrial environment. A successful pregnancy is dependent upon a favourable endometrial environment. Decidualisation is the process in which the endometrium prepares itself for an

implanting embryo. Failure of adequate decidualisation can lead to an abnormal maternal response to embryonic signals resulting in implantation failure and defective placentation (Salker *et al.,* 2010, Teklenburg *et al*., 2010).

Various medical adjuncts are used in conjunction with ART to improve the endometrial environment, implantation and therefore its overall success. Some of the widely used medical adjuncts to improve the success of ART include low dose aspirin and heparin (particularly LMWH).

In this chapter, I will summarise the current literature about the physiology of the menstrual cycle, the decidualisation process (before the presence of embryo to prepare for pregnancy and after the presence of embryo so to help in implantation), infertility and its management with ART, and heparin as a medical adjunct during ART. This chapter will conclude with a discussion about the gaps in the literature and provide an outline of my aims for this thesis.

1.1 Menstrual Cycle:

The menstrual cycle occurs as a consequence of the physiological changes in endometrium in women of reproductive age. The endometrium is under the influence of endocrine hormones (hypothalamic pituitary ovarian axis) and these changes transpire for the purposes of reproduction. It is divided into two phases, the proliferative phase and the luteal phase. The proliferative phase starts from

first day of menstruation and ends with ovulation. Secretion of gonadotrophin releasing hormone (GnRH) from the hypothalamus acts upon anterior pituitary resulting in the release of follicle stimulating hormone (FSH). FSH acts on the granulosa cells in the developing follicles within the ovaries leading to oestrogen secretion. This oestrogen causes endometrial proliferation. Ovulation is triggered following a surge in the release of luteinising hormone (LH) from the anterior pituitary which occurs as a consequence of high oestrogen levels. This happens once a dominant follicle is formed within the ovary.

After ovulation, the dominant follicle on the ovary forms the corpus luteum which produces progesterone. The luteal phase starts after ovulation and ends with the start of menstruation. Progesterone from corpus luteum causes secretory changes within the endometrium in preparation for the possible implantation of an embryo. When implantation of an embryo does not occur, the corpus luteum involutes with a decline in circulating oestrogen and progesterone, leading to menstruation. The menstrual cycle is illustrated in Figure 1.1.

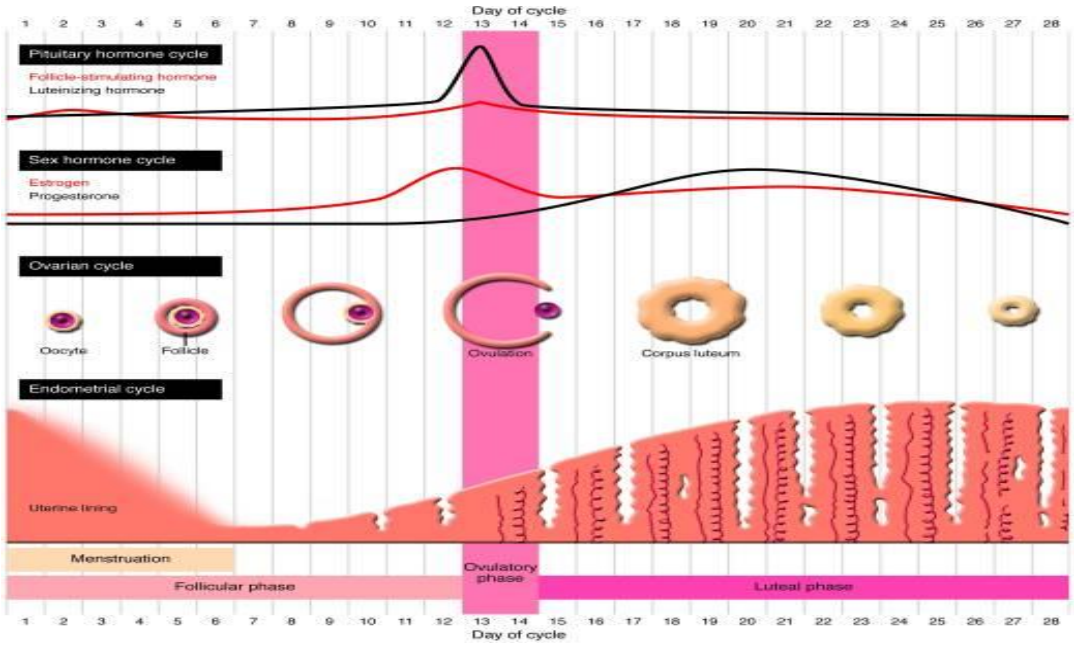


Figure 1.1: Human Female Menstrual Cycle (Aitken et al., 2008)

1.1.1 Decidualisation (prior to embryo presence):

Decidualisation is a post-ovulatory process within the endometrium under the influence of progesterone (Kuroda *et al.*, 2013). During decidualisation, the endometrial stromal cells (ESCs) transform in preparation for an implanting embryo. This process involves glycogen accumulation in the ESC cytoplasm, the influx of leucocytes including uNK cells, and spiral artery angiogenesis. Consequently, this promotes an increase in stromal vascularity and oedema within the endometrium (Vontver, 2008). It involves endometrial stromal fibroblasts becoming specialised secretory decidual cells (Kuroda *et al.*, 2013). The purpose of the process is to enable decidualising ESCs to regulate

trophoblast invasion and to resist both oxidative and inflammatory stresses. It is pivotal for successful implantation.

Unlike many animal species, in humans, decidualisation begins in the mid-luteal phase of the menstrual cycle regardless of the presence of an implanting embryo (Gellersen, Brosens & Brosens, 2007, Salamonsen *et al.,* 2003). A successful interaction between the implanting embryo and the endometrium can only take place in mid luteal phase described as the window of implantation (Gellersen, Brosens & Brosens, 2007).

In the endometrium, progesterone acts with cyclic AMP (cAMP) regulated pathways causing decidualisation (Brosens, Hayashi & White, 1999, Brosens, Pijenborg & Brosens, 2002). Various factors within the endometrium including metalloproteinases, cytokines and surface integrins are up and down regulated. ESCs become circular, showing structural similarities with epithelial cells and myofibroblasts (Oliver *et al.,* 1999). They produce growth factors to aid decidualisation including prolactin (PRL), relaxin and insulin-like growth factor binding protein-1 (IGFBP-1) (Salamonsen *et al.,* 2003, (Brosens, Hayashi & White, 1999, Brosens, Pijenborg & Brosens, 2002, King, 2000). In vitro studies have revealed that the decidualisation is associated with a change in sex steroid hormone receptor expression and the expression of growth factors, cytokines and chemokines. Remodelling of the extracellular matrix with induction of apoptosis modulators and transcription factors is also reported (Cooper *et al.,* 2001).

In the endometrium, various leukocytes are present. The most common leucocytes are uNK cells, macrophages and T cells. Uterine NK cells play an important role in embryo implantation as evidenced by their increase in number just after ovulation. Uterine NK cells are different to peripheral blood NK cells, both morphologically and functionally. Uterine NK cells have no cytolytic activity unlike peripheral blood NK cells (Cooper *et al.,* 2001). Human peripheral blood NK cells express various surface markers including CD16 and CD56. In contrast, uNK express CD56 but not CD16 (Poli *et al.,* 2009). It has been reported that uNK cells are important in early pregnancy, as their numbers increase from ovulation until the mid-luteal phase and in early pregnancy but decrease in the second trimester and become non-existent in the decidua at delivery (Poli *et al.,* 2009). Appropriate uNK cell recruitment depends upon presence of hormones including glucocorticoid and progesterone, but not on an implanting embryo (Ordi *et al.,* 2006). Glucorticoid steroid hormones decrease the number of uNK cells within the endometrium (Quenby & Farquharson, 2006). Maternal uNK cells directly interact with fetal trophoblast and this interaction is thought to influence migration and invasion of trophoblast thereby, regulating implantation (King, 2000, Parham, 2004).

In conclusion, decidualisation consists of many complex dynamic processes involving interactions among growth factors, cytokines and hormones in the endometrium and other many processes which are still not fully understood. The process of decidualisation continues under the influence of factors secreted by the embryo and endometrium.

1.1.2 Decidualisation (post embryo presence):

Human embryo implantation involves the apposition of the embryo to the endometrium, adhesion, subsequent breaching of the luminal epithelium and finally invasion into endometrium (Quenby & Brosens, 2013). Apposition is the initial contact between an embryo and the endometrium. Adhesion involves a series of interactions between the embryo and the decidualised endometrium via chemokine and cytokine signalling which facilitate invasion and penetration of the embryo into the endometrium. This is illustrated in Figure 1.2.

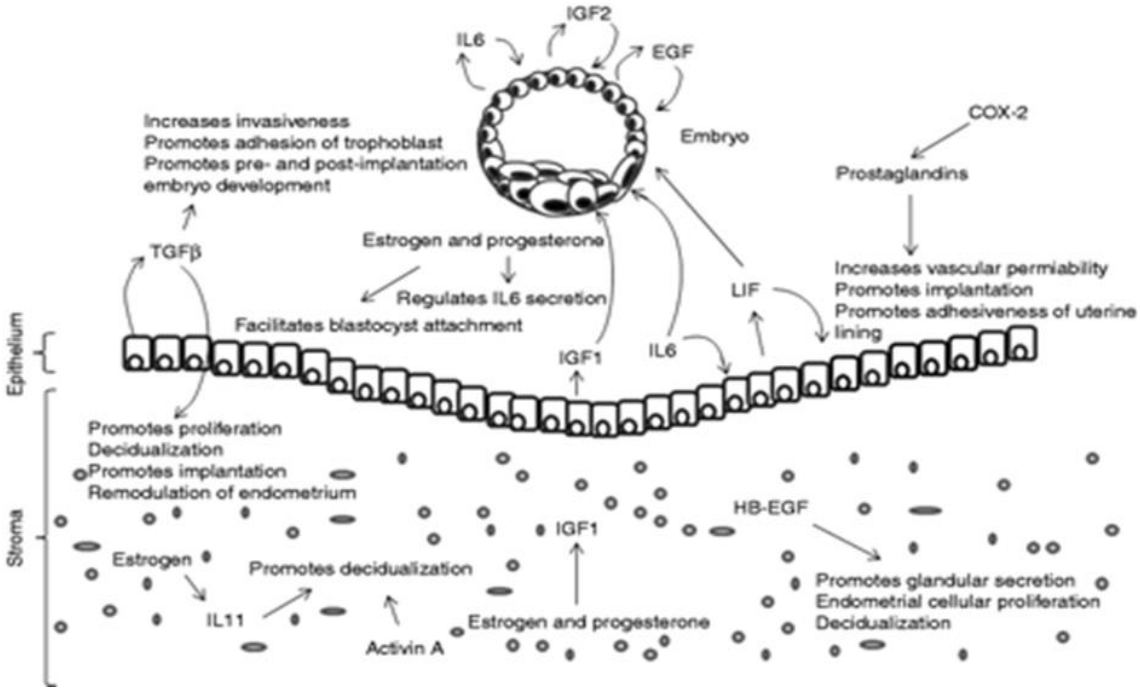


Figure 1.2: Summary of various growth factors, cytokines, and hormones involved in implantation process (Singh, Chaudhry & Asselin, 2011).

With the exception of the mid-luteal phase of the menstrual cycle, the apical surface of the endometrial epithelium is covered by a thick glycocalyx composed of mucin. Mucin is a transmembrane glycoprotein which prevents embryo attachment to the endometrium (Tathiah *et al.,* 2004). Desmosomes are present on the lateral surface of the luminal epithelium with mucin the apical surface in a pre-receptive endometrium (Aplin & Kimber, 2004). The human embryo can attach to a receptive endometrium as the embryo induces cleavage of this mucin at the specific site where implantation will occur (Meseguer *et al.,* 2001).

Adhesion molecules which include selectins, cadherins and integrins are present on the endometrial epithelium and trophoblast. These adhesions molecules play a vital role in facilitating embryo attachment to the endometrial lining. E-cadherin expression is up-regulated by oestradiol in the proliferative phase of menstrual cycle (Wada-Hiraike *et al*., 2006) and down regulated by progesterone in the luteal phase of menstrual cycle facilitating trophoblast invasion resulting in successful implantation (Jha *et al*, 2006). This adhesion interaction is strengthened further by the bridging ligands osteopontin and galectin-15. Endometrial glands produce these bridging ligands (Spencer *et al.,* 2004). Invasion of endometrial stroma is further facilitated by reduction in desmosome density (Aplin & Kimber, 2004).

An important growth factor involved in implantation is heparin-binding epidermal growth factor (HB-EGF). It is produced by leucocytes and chemotactic in nature (Iwamoto & Mekada, 2000). HB-EGF is vital to reduce apoptosis in ESCs

which is caused by transforming growth factor (TGF)-β or tumour necrosis factor (TNF)-α (Chobotova *et al*., 2005). HB-EGF expression within the endometrium is enhanced by sex steroid hormones particularly in the luteal phase of the endometrial cycle and continues to increase in early pregnancy (Leach *et al*., 2004). Pinopodes are epithelial cellular protrusions on the endometrium where attachment of embryo takes place. HB-EGF is present on the surface of these pinopodes (Stavreus-Evers *et al.*, 2002) suggesting the importance of role of HB-EGF in embryo implantation.

Insulin-like growth factors (IGF) type I and II are important for successful implantation (Fowden, 2003). In vitro studies found that IGF-I helps in the migration of the trophoblast aiding in implantation (Lacey *et al*., 2002). Increased expression of IGF-II is associated with trophoblastic invasion into the decidua (Hamilton *et al.,* 1998).

Transforming growth factors (TGF types β1-3) are present both in the endometrial and trophoblast cells. They are responsible for inhibition of proliferation and invasion of trophoblast leading to successful implantation (Lash *et al*., 2005).

Cytokines, in particular interleukins (IL) I and II act as immune regulators and growth factors. They play a positive role in decidualisation (Dimitriadis *et al*., 2005), trophoblast invasion (von Rango *et al*., 2004) leading to successful implantation (Dimitriadis *et al*., 2006). IL-I binding can lead to STAT-3 activation which is a transcription factor involved in trophoblast invasion (Corvinus *et al*.,

2003; Poehlmann *et al*., 2005). Lower levels of IL-II were found in endometrium of infertile women when compared with fertile women (Dimitriadis *et al*., 2006). Similarly, low levels of IL-II levels were found in the glands of non-viable tubal pregnancies compared with viable tubal and intrauterine pregnancies (von Rango *et al*., 2004). Another member of IL-6 family required for successful implantation is leukemia inhibitory factor (LIF) (Kimber, 2005).

Chemokines induce an inflammatory state in the endometrium by promoting leukocyte migration into the endometrium. This is manifested by an interaction between non-polymorphic HLA class I antigens with uNK cells (Pijnenborg, 2002). Prostaglandins (PGs) promote cytokine production resulting in an improvement in vascular permeability, which is necessary for invasion and implantation. Chemokines interacting with G protein-coupled receptors in the endometrium cause a structural change in integrins, promoting implantation (Bokoch, 1995). This is illustrated in Figure 1.3.

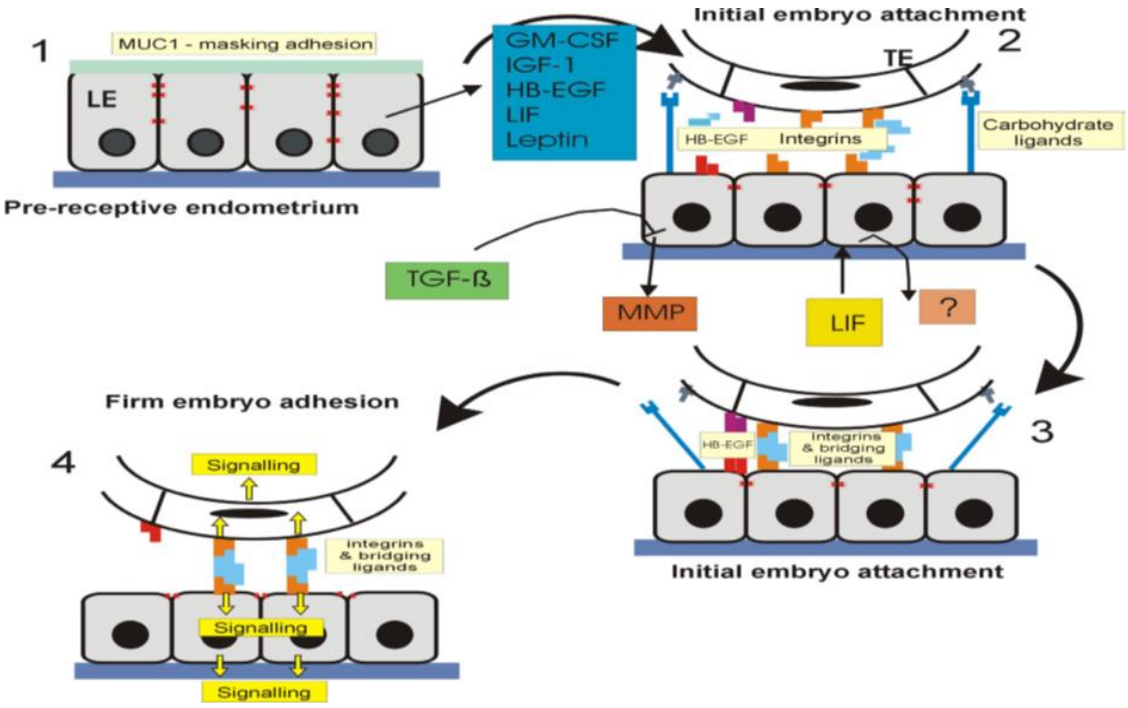


Figure 1.3: Human embryo adhesion interactions (Aplin & Kimber, 2004-published by BioMed Central)

Therefore embryo apposition, adhesion and invasion facilitating implantation is dependent upon ovarian steroid hormone, endometrial cell proliferation with glandular secretion, modulation of endometrial blood flow, interaction of chemokines, cytokines and growth factors within the endometrium.

Historically, implantation has been viewed as a passive process with regards to the endometrium requiring only receptivity and then an invading embryo. This required only an invading fetal trophoblast. However, more recent evidence indicates that human embryo implantation is more dynamically controlled by the

endometrium than was previously understood. The process involves the mutual attraction and interaction of maternal ESCs and the fetal trophoblast resulting in expansion of the trophoblast and ESC migration. This is a two way communication between the endometrium and the embryo dependent upon interactions between human ESCs and some of the growth factors discussed previously including HB-EGF, IGF and IL1 (Quenby & Brosens, 2013). This is shown in Figure 1.4.

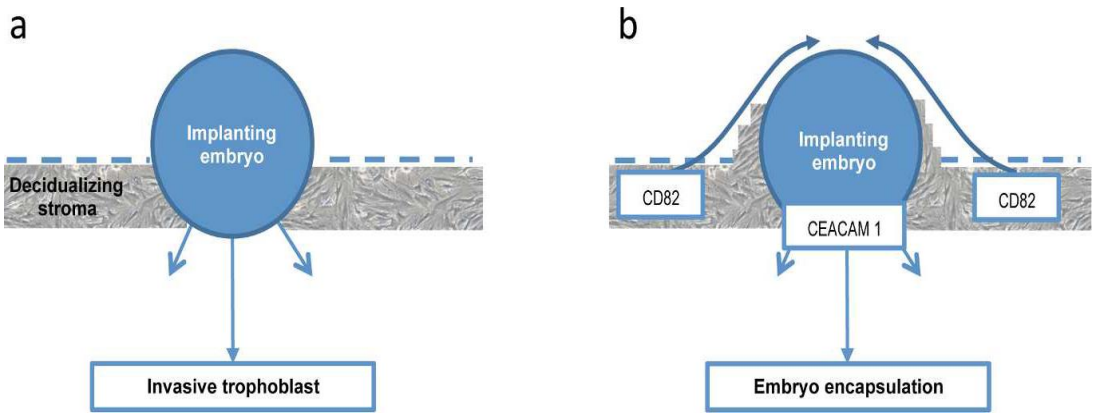


Figure 1.4: Models to explain human embryo implantation (Quenby & Brosens, 2013)

1.2 Subfertility:

In the UK, subfertility is defined as failure to conceive after regular unprotected sexual intercourse for one year in the absence of known reproductive pathology (NICE, 2013). It effects 15% of couples worldwide (Collins & Van Steirteghem, 2004) and can be due to female and/or male factors, or it can be unexplained.

Female causes of subfertility can be categorised as ovulation disorders (premature menopause, polycystic ovarian syndrome), hormonal disturbances (hypothalamic pituitary failure), genetic factors, tubal disorders (pelvic inflammatory disease), uterine disorders (adhesions, fibroids) and peritoneal disorders (endometriosis, previous pelvic surgery). Male causes of subfertility include defective sperm production, abnormal morphology and/or poor motility. These abnormalities can be genetic or acquired. In some couples no cause can be identified and this is categorised as unexplained subfertility.

Management options are offered to couples depending upon the cause of their subfertility. Options include treatment with medications (clomiphene, aromatase inhibitors and gonadotrophins), surgical treatments (laparoscopy or hysteroscopy, surgical sperm retrieval) and ART (NICE, 2013).

1.2.1 Thrombophilia and Subfertility:

Thrombophilias are associated with adverse pregnancy outcomes. It is thought that thrombophilias may also contribute to subfertility and recurrent implantation failure. This is because there is a higher prevalence of hereditary thrombophilias in women with recurrent implantation failure after ART (Grandone *et al*., 2001, Azem *et al*., 2004, Coulam *et al*., 2006).

The presence of acquired thrombophilias is reported to be 5-10 times higher in infertile women (Gleicher *et al*., 1994, Sher *et al*., 1994, Fisch *et al*., 1995, Nip *et al*., 1995, Birdsall *et al*., 1996, Denis *et al*., 1997, Kowalik *et al*., 1997, Kutteh *et al*., 1997) when compared with the general obstetric population (Lockwood *et al*., 1989). Despite the higher prevalence of thrombophilias in the ART population, they were not associated with poor pregnancy outcomes after ART as reported in meta-analyses (Hornstein, 2000, Hornstein *et al.,* 2000) and several reviews (Buckingham *et al*., 2006, Caccavo *et al*., 2007, Lee *et al*., 2007).

1.3 Assisted Reproduction Technology (ART):

ART involves all treatments or procedures or procedures requiring the in-vitro handling of human oocytes, sperm or embryos for the purpose of achieving a pregnancy as defined by World Health Organisation (WHO). ART includes IVF/ICSI but not intrauterine insemination (IUI) (Zegers-Hochschild *et al.,* 2009).

ART has provided an effective treatment for some infertile couples (Collins & Van Steirteghem, 2004). 50% of infertile couples require ART as their management option for infertility. 2-3% of all births in Europe are as a result of ART treatment and around 5 million babies have been born from ART since July 1978 (Kupka *et al.,* 2014). Choosing the most appropriate infertility management option is linked to the underlying cause and decision of individual couple. Couples make ethical considerations according to their social, cultural and religious belief (egg or sperm donation, surrogacy) before deciding about the treatment (Kamel, 2010).

1.3.1 Complications of ART:

Despite the benefits of ART, there are many reported complications experienced by women who have undergone ART in comparison to those who conceive spontaneously (Allen, Wilson & Cheung, 2006).

During ART there is a risk of ovarian hyperstimulation syndrome (OHSS) which can be a life threatening condition. It is caused by the release of oestradiol in response to ovarian hyperstimulation and compounded by human chorionic gonadotrophin (hCG) which is given to trigger ovulation. The hCG causes an increase in vascular endothelial growth factor (VEGF) leading to an increase in vascular permeability (Agrawal *et al.,* 1999). This then can lead to extravasation

of the intravascular fluid resulting in ascites, pleural effusions, abnormal liver function and renal failure.

Other complications of ART include the surgical risks which occur during oocyte retrieval (pelvic infection, haemorrhage and bowel, bladder and vascular injury). All fertility treatments can lead to multiple pregnancies including ART. The chances of a twin pregnancy occurring when a patient is taking clomiphene for ovulation induction is 10%, following IVF where two embryos are replaced is 20-30% and following intrauterine insemination (IUI) treatment is 10-20% (Kennedy, 2005).

Multiple pregnancies are associated with an increased risk of miscarriage, preterm labour, pre-eclampsia, antepartum haemorrhage, stillbirth, operative delivery and caesarean section (Kennedy, 2005). Due to these increased risks, a single embryo transfer policy has been introduced in UK practice with the aim to try to reduce the risks of multiple pregnancies (Cutting *et al.,* 2008).

In addition, if ART is successful in achieving a singleton pregnancy, there is still increased risk of maternal and fetal complications. Maternal complications include pregnancy induced hypertension, pre-eclampsia, gestational diabetes, placenta praevia and antepartum haemorrhage (Pinborg*,* 2005, Helmerhorst *et al*., 2004, Jackson *et al*., 2004). The pathophysiology of these conditions originates in early pregnancy and is thought to be due to abnormal trophoblastic invasion and implantation (Smith *et al.,* 1998, Smith *et al.,* 2002). Fetal complications include preterm delivery, fetal growth restriction and congenital

abnormalities. All of these cause an increase in perinatal mortality and morbidity (Katalinic, Rosch & Ludwig, 2004, Helmerhorst *et al*., 2004; Jackson *et al*., 2004). Unsuccessful ART treatment cycles which occur as a result of implantation failure have significant social, financial and psychological morbidity on couples (de Klerk *et al.,* 2007).

1.3.2 Progression in the Field of ART:

Since the birth of the first IVF baby on 25 July 1978, there has been a constant improvement in the pregnancy rates following ART as a result of progress and development in this field. There have been innovations in laboratory technologies (including in-vitro oocyte maturation, cryopreservation techniques, blastocyst culture) (Glujovsky *et al.,* 2012), pre-genetic testing (Sengupta & Delhanty, 2012) and more recently time lapse embryo imaging (embryoscope) which provides a better selection of embryos to transfer (Freour *et al.,* 2012).

There has been improvement in surgical treatments (laparoscopy, hysteroscopy), ultrasonography and ovarian stimulation pathways particularly focusing on integrated individualised management approach.

1.3.3 Measuring Success of ART:

As mentioned, the outcome of ART treatment is usually measured by clinical pregnancy and live birth rates. The European Society of Human Reproduction and Embryology (ESHRE) reported that the most important indicator of success of ART treatment is the birth of a single healthy child (Land & Evers, 2003). Offering an appropriate management plan for each infertile couple to improve the outcome of their ART treatment is dependent upon correct diagnosis for the cause of infertility, selection of the best ovarian stimulation protocol and transfer of the best quality embryo possible using a good transfer technique. Success should also take into consideration treatment complications including OHSS, treatment cycle failure and multiple pregnancy rates. This has encouraged clinicians to perform single embryo transfer, to consequently reduce some of the complications of assisted reproduction, primarily multiple pregnancies, thus reducing the cost and improving efficacy (Devroey, Fauser & Diedrich, 2009, Cutting *et al.,* 2008). An elective single embryo transfer (e-SET) policy will reduce the complications of ART particularly multiple pregnancies (Grady *et al.,* 2012, ASRM, 2012).

1.3.4 Recurrent Implantation Failure:

In reproductive medicine, implantation failure is defined as failure of the embryo(s) to implant into the endometrium following ART. There is no consensus for a standardised definition of recurrent implantation failure (El-Toukhy & Tarinissi, 2006). In the UK, a commonly used definition after a survey of IVF clinicians defined recurrent implantation failure as not achieving a pregnancy after more than three cycles of ART (Tan *et al.,* 2005). Recently, a systematic review defined recurrent implantation failure as absence of implantation after two consecutive cycles of ART where at least two blastocysts or four cleavage stage embryos had been transferred (Polanski *et al.,* 2014).

Recurrent implantation failure could be a result of several factors including embryo quality, endometrial receptivity and embryo transfer techniques (Ola & Li, 2006). Other causes of implantation failure can be due to presence of endometriosis, hydrosalpnix, lesions within the endometrial cavity (tumours, polyps, fibroids, adhesions) and poor ovarian stimulation during ART resulting in immature oocytes (Margalioth *et al.,* 2006).

The quality of the transferred embryos could be affected by genetic disorders, difficult hatching (zona pellucida harding) and laboratory technical problems (poor cultural conditions). Assessment of the quality of the embryo relies upon morphological criteria and cleavage rates but is highly dependent on genetic composition. Many embryos are found to have genetic abnormalities despite

being morphologically normal. Pre-implantation genetic screening (PGS) and comparative genomic hybridisation (CGH) can be used to analyse the chromosomal or genetic abnormalities among the embryos before being transferred. Thus selecting a genetically normal embryo for embryo transfer during ART may improve implantation and pregnancy rates (Taranissi *et al.,* 2005). Its routine usage remains controversial. Time lapse embryo imaging will help us in choosing the best embryos and improve the success of ART (Conaghan *et al.,* 2013).

A receptive endometrium in the presence of a good quality embryo is vital to achieve successful implantation (Ly *et al.,* 2010). Defective endometrial receptivity and inadequate preparation for pregnancy can cause disruption in the normal physiological processes of implantation (Achache & Revel, 2006). Endometrial proliferation during ART resulting in an endometrial thickness of <8mm on the day of embryo transfer has been shown to lead to reduced live birth rates (Noyes *et al.,* 2001). A good embryo transfer technique has a positive impact on successful implantation (Schoolcraft, Surrey & Gardner, 2001, Frydman, 2004). There is a decline in implantation rates and live birth rates, with increasing miscarriage rates in women aged >38 year (Navot *et al.,* 1994).

It is believed that the implantation failure is the main cause for low fecundity after ART (Macklon, Geraedts & Fauser, 2002).

1.3.5 Medical Adjuncts in ART:

One of the many challenges for clinicians working in the field of reproductive medicine is to improve endometrial receptivity in preparation for pregnancy to increase clinical pregnancy and live birth rates. Various medical adjuncts have been used in ART to improve endometrial receptivity to increase embryo implantation and improve its success.

Low dose aspirin and heparin are the two most commonly used medical adjuncts during ART to improve outcomes. Both are used during ART particularly in women with recurrent implantation failure due to their extensive usage in women with recurrent miscarriages.

A Cochrane systematic review reported that use of low dose aspirin for women undergoing ART is not recommended as it does not improve the success of ART (Siristatidis, Dodd & Drakeley, 2011, Siristatidis, Dodd & Drakeley, 2012).

Currently, some controversy exists in the literature surrounding the benefits of heparin use during ART. A small non-randomised study (Kutteh *et al.,* 1997) showed that there was no difference in pregnancy outcomes with the use of heparin with low-dose aspirin in women with antiphospholipid positive antibodies undergoing ART. Conversely, a single centre non-randomised study (Sher *et al.,*1994) reported that heparin with low-dose aspirin during ART improved clinical pregnancy and live birth when given to women with positive antiphospholipid antibodies. Similar results were published by the same author

later in another non randomised study (Sher *et al.,* 1998). The American Society for Reproductive Medicine (ASRM, 2008) advises that assessment of antiphospholipid antibodies was not indicated among couples undergoing ART, and heparin therapy was not justifiable on the basis of existing data to improve pregnancy and live birth rates. A prospective cohort study suggested that in women who found to have thrombophilia after repeated implantation failure, peri-implantation heparin during ART could improve clinical pregnancy. However, no precise data were published (Sharif & Ghunaim, 2010). A review (Ricci *et al.,* 2010) suggested that heparin should not be used in women undergoing ART until its efficacy is reported in well-designed RCTs. A retrospective observational study (Lodigiani *et al.,* 2011) reported that heparin improved pregnancy rates when given to women with previous implantation failure during ART. Due to this conflicting evidence that heparin may improve success of ART. I wanted to look at the scientific and clinical evidence of the use of heparin as a medical adjunct during ART.

1.4 Heparin:

1.4.1 Historical Background:

Heparin as an anticoagulant was first identified by William Howell at John Hopkins University, USA in early 20th century shortly after the First World War.

The chemical structure of heparin was reported for the first time in the literature by Jorpes, 1935. Vitrum AB Sweden became the first manufacturer of this medication for intravenous (IV) use and the first ever study with use of IV heparin took place in 1935.

1.4.2 Heparin Structure:

Heparin is a glycosaminoglycan containing proteoglycans consisting of pyranosyluronic acid and glucosamine components (Comper, 1981). This is shown in Figure 1.5. The pyranosyluronic acid component consists of 90% L-iduronic acid and 10% D-glucuronic acid. The glucosamine component consists of an amino acid group which can be sulphated, acetylated or un-substituted (Gallagher & Walker, 1985). In practice, this is described as unfractionated heparin.

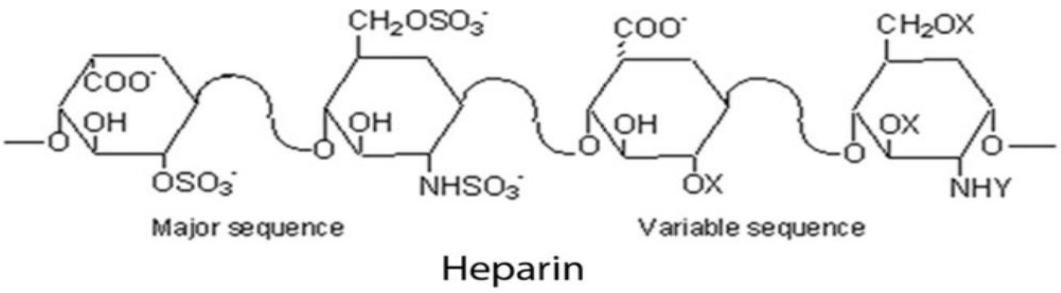


Figure 1.5: Structure of heparin (Nelson & Greer, 2008)

1.4.2.1 Heparin Binding:

Heparin binding sites contain positively charged proteins. Heparin contains negatively charged polysaccharide. Heparin binds with proteins via ionic bonding. This is strengthened by hydrogen bonding. Heparin can bind with proteins including anti-thrombin, various growth factors and their receptors, (particularly HB-EGF), viral proteins and extracellular matrix proteins (Nelson & Greer, 2008). The glycosaminoglycans, containing proteoglycans, on the surface of heparin bind with extracellular ligands. This binding activates the receptor leading to activation of a signalling cascade. These heparin binding proteoglycans are found extensively throughout the reproductive tract an play an important role in the regulation of folliculogenesis during the menstrual cycle, (Rodgers *et al.,* 2003), endometrial cycling and remodelling (Potter & Morris, 1992, Kelly, Tawia & Rogers, 1995, Germeyer *et al.,* 2007, Lai *et al.,* 2007, Xu *et al.,* 2007).

1.4.3 Heparin Types:

Heparin can be either

1. Unfractionated heparin (UFH)
2. Fractionated (commonly called LMWH)

1.4.3.1 Unfractionated Heparin:

UFH are short acting anticoagulants (plasma half-life 30 minutes) with a molecular weight of 10,000-15,000 Kilo Daltons (KDa). UFH is obtained from animal intestines or lungs (Warda *et al.,* 2003). It is less expensive than LMWH. Careful intense monitoring is required during its administration with activated partial thromboplastin time (APTT) (Bick *et al.,* 2005). UFH excretion is via the kidneys. It is safe to treat venous thromboembolism during pregnancy. However, it is not used routinely except in special circumstances as it requires intense monitoring.

1.4.3.2 Low Molecular Weight Heparin:

Fractionated or more commonly called, LMWHs are manufactured as they cannot be obtained from natural sources (Warda et al., 2003). Various LMWHs (weight <10,000 KDa) are manufactured by pharmaceutical companies by depolymerisation of UFH (10.000-15,000 KDa). This process is either achieved with the use of enzymes, for example tinzaparin (molecular weight of 6500 KDa), or with the use of chemicals, for example dalteparin (molecular weight of 5000 KDa) or enoxaparin (molecular weight of 4500 KDa). Careful intense monitoring of these medications is not required. Anti-Xa levels can be used to monitor the activity of LMWHs but this is not done routinely unless clinically indicated to ensure a patient is receiving an appropriate therapeutic dose. LMWHs are long

acting anticoagulants with a more predictable response when compared to UFH. This allows administration of fixed adjusted dosages for LMWH without requirement of routine anti-Xa level monitoring (Bick *et al.,* 2005) The plasma half-life of LMWH is around 3-6 hours (Nelson & Greer*,* 2008). LMWH clearance is by the kidneys. LMWHs are safe and effective in preventing and treating venous thromboembolism during pregnancy. Due to its longer half-life and a more predictable antithrombotic response, it can be administered to patients in fixed-weight adjusted doses without the need for laboratory monitoring. It has a reduced risk of heparin induced thrombocytopenia (HIT) (Warkentin et al., 1995, Warkentin & Greinacher, 2004).

1.4.4 Effects of Heparin on the Coagulation System:

Heparin prophylactically is used to prevent clots and therapeutically to treat already formed clots (deep venous thrombosis, pulmonary embolism, myocardial infarction or cerebral infarcts). Its action is primarily on coagulation cascade. The effect of heparin on the coagulation cascade is illustrated in Figure 1.6.

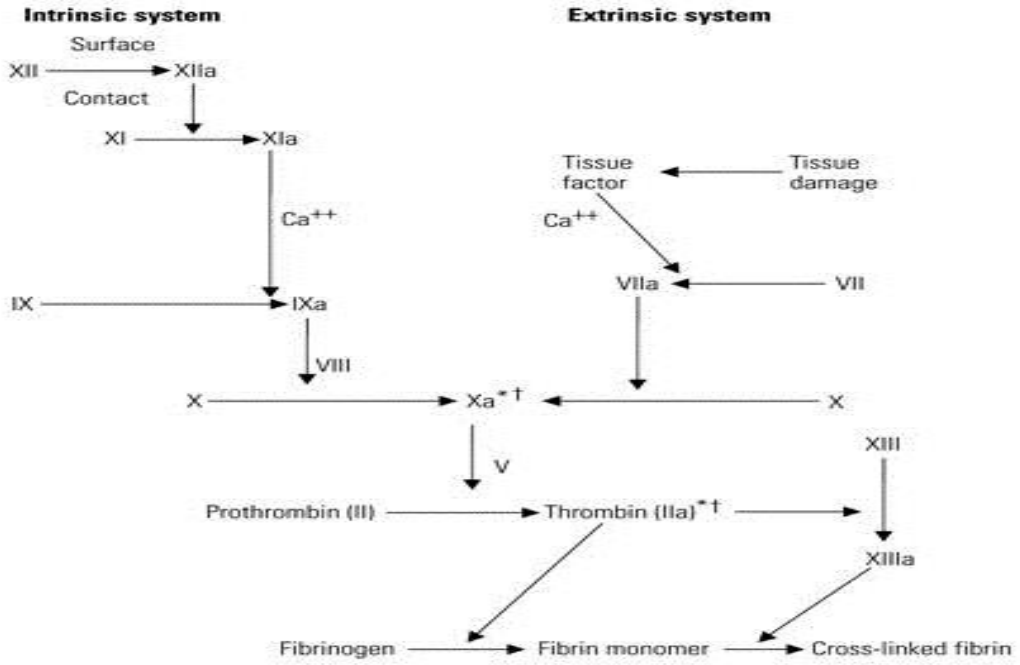


Figure 1.6: The coagulation cascade (Hovanessian, 1999)

" *Site of action of unfractionated heparin (Xa/IIa inhibition 1:1). †Site of action of LMWH (Xa/IIa inhibition 2:1 to 4:1)"

Heparin (UFH & LMWH) potentiates the anti-thrombin effect of anticoagulation in the coagulation cascade. LMWHs exhibit lower anti-IIa activity when compared to UFH. Due to their low molecular weight, they cannot attach simultaneously with thrombin and anti-thrombin to make complexes which are important for inhibition of thrombin by anti-thrombin. However, the interaction between anti-thrombin and factor Xa are not important for anti-Xa activity, thus the LMWH inactivates Xa with similar efficiency as UFH. Anti-Xa levels are therefore used to monitor LMWH activity (Bick *et al.,* 2005).

1.4.5 Routes of Administration:

UFH and LMWH are licenced to be administered either intravenously or subcutaneously. Practically UFH is commonly given by the intravenous route and LMWH by the subcutaneous route. These routes of administrations result in a systemic effect of heparin.

1.4.6 Adverse Effects of Heparin:

Adverse effects of heparin treatment include bleeding, bruises, haematomas, injection site sensitivity reactions, skin necrosis, thrombocytopenia, abnormal liver function tests (LFTs), osteoporosis (prolonged used) and HIT (prolonged use). All of these adverse effects are more common with use of UFH compared to LMWHs, particularly HIT (Warkentin & Greinacher, 2004) and heparin induced osteoporosis (Murray *et al.,* 1995). Protamine sulphate reverses the effects of UFH and partially reverses the effects of LMWHs and could be used in life threatening haemorrhage (Nelson & Greer, 2008).

1.4.7 Heparin in the Treatment of Miscarriages:

Heparin has been given as medical adjunct to improve the outcomes of women with recurrent miscarriages.

Heparin and aspirin treatment in early pregnancy were found to be effective in preventing pregnancy loss in women with antiphospholipid syndrome (APS) by a Cochrane systematic review (Empson *et al.,* 2005). However, another Cochrane systematic review reported that aspirin and heparin do not provide effective treatment for women suffering with idiopathic recurrent miscarriages (Kaandorp *et al.,* 2009).

1.4.8 Role of Heparin in Decidualisation and Implantation:

As described previously, heparin can interact with various proteins in the endometrium which are involved in the physiological processes of decidualisation and implantation. Heparin may improve embryo apposition and adhesion to the endometrium. Therefore heparin could potentially have an important role in early pregnancy and ART. This is illustrated in Figure 1.7.

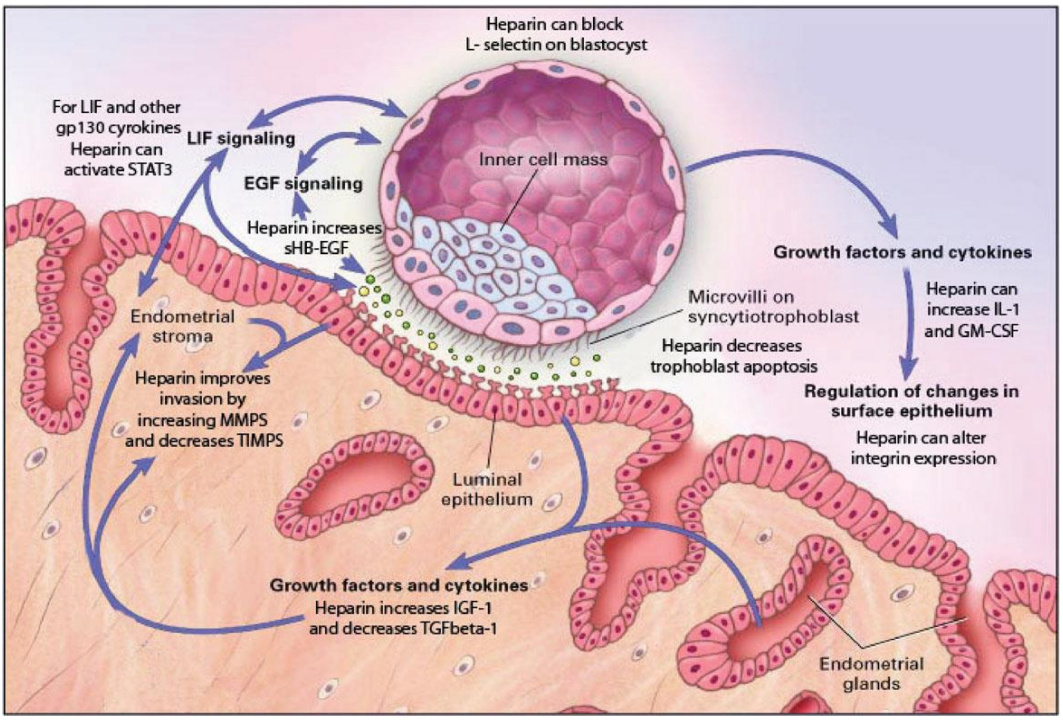


Figure 1.7: Potential actions of heparin on implantation (Nelson & Greer, 2008)

Adhesion molecules, particularly selectins play an important role in promoting implantation. Interestingly, certain heparins can block this selectin mediated embryo adhesion (Wang *et al*., 2002) but this is dependent upon the molecular weight of the heparin. Higher molecular weight heparins can block the selectin mediated ligand adhesion. UFH and tinzaparin (which has the highest molecular weight in LMWH, 6500KDa) block this selectin mediated ligand binding of the embryo to the endometrium (Nelson & Greer, 2008). But all other LMWHs with a smaller molecular weights than tinzaparin, for example, dalteparin (molecular weight 5000 KDa) and enoxaparin (molecular weight 4500 KDa) do not block this selectin binding and do not impair adhesion of the embryo to the endometrium.

Consequently, all LMWH except tinzaparin should not be detrimental to implantation.

E-cadherin expression is down regulated by progesterone in the luteal phase of menstrual cycle promoting implantation (Jha *et al*, 2006). It has been reported that UFH and LMWH (enoxaparin) down regulate endometrial E-cadherin expression (Erden *et al*., 2006), thus promoting implantation. It has been reported that UFH and LMWH help in extravillous trophoblast differentiation (Quenby *et al.,* 2004) further aiding in successful implantation.

HB-EGF binding to epidermal growth factor (EGF) receptor is essential step for embryo adhesion. This HB-EGF binding can only occur in the presence of heparin (Aviezer & Yayon*,* 1994).

Matrix metalloproteinases (MMP) are capable of degrading extracellular matrix proteins. HB-EGF cleavage by these matrix metalloproteinases may be an important step in the prevention of trophoblast apoptosis prior to trophoblast invasion (Armant *et al*., 2006). Laboratory studies have revealed that LMWH induces transcription of these trophoblastic MMPs (2 & 9) (Di Simone *et al.,* 2007). This results in HB-EGF up regulation and improves binding with EGF receptor.

Heparin increases the presence of free IGF-I thus helping promote the trophoblastic migration (Arai *et al*., 1994, Møller *et al*., 2006).

As previously mentioned, TGF-β (1-3) modulates trophoblastic proliferation and invasion (Lash *et al*., 2005). LMWH inhibits expression of TGF-β1 (Weigert *et al*., 2001) and decreases the TGF-β presence (Pecly *et al*., 2006) this further aids in promoting trophoblast invasion.

Heparin synergistically interacts with IL-II signalling potentiating its effects resulting in activation of STAT3 transcription (Walton *et al*., 2002) which is also a beneficial signalling pathway for successful implantation.

In conclusion, interaction of heparins with various factors involved in implantation and early pregnancy development include E-cadherin down regulation, HB-EGF and its binding, increase in IGF-I, interleukins (IL-1, IL-6), IL-11-induced STAT3 activation and induction of MMP (2 & 9) (Arai *et al.,* 1994, Aviezer & Yayon*,* 1994, Call & Remick*,* 1994, Di Simone *et al.,* 2007, Erden *et al.,* 2006, Liang *et al.,* 2006, Møller *et al.,* 2006, Rajgopal *et al.,* 2006, Stavreus-Evers *et al.,* 2002). Heparin has been found to modulate decidualisation in in vitro studies (Fluhr *et al.,* 2010, Fluhr *et al.,* 2011a) and pro-inflammatory cytokine signalling (Fluhr *et al.,* 2011b) in human ESC culture. UFH and LMWH are commonly known and used for their anticoagulant activity but their biological properties may also be vital for improvement of decidualisation and implantation (Fluhr *et al.,* 2010. Fluhr *et al.,* 2011a, Fluhr *et al.,* 2011b). Heparin can enhance the activity of several growth factors including IGF, ILs, EGF and HB-EGF . All of these properties of heparin may enhance embryo-endometrial interactions,

stimulate decidualisation and improve implantation (Bohlmann, 2011, Nelson & Greer, 2008).

1.5 Intrauterine Flushing:

Currently, medical adjuncts used in ART are not effective at improving the pregnancy and live birth rates (Nardo, Granne & Stewart, 2009). One possible explanation could be that all medical adjuncts used are administered systemically. Very few of these adjuncts have been given locally; there is a possibility that a novel technique of endometrial administration could be the future.

It has been reported that uterine flushing is a safe and simple procedure (Li, Mackenna & Roberts, 1993). Uterine flushing was initially performed to recover sperm and analyse the endometrial secretions for assessment of cytokines (Williams *et al.,* 1993). Endometrial flushing analysis prior to embryo transfer had been used in the past to study the role of cytokine profile within the endometrium with the aim of improving implantation (Boomsma *et al.,* 2009a, Boomsma *et al.,* 2009b). To improve the success of ART, endometrial secretions were aspirated prior to embryo transfer but this intervention did not improve outcomes (Van der Gaast *et al.,* 2003). Uterine flushing at oocyte retrieval to detect LIF has been performed and it was not associated with adverse pregnancy rates (Olivennes *et al.,* 2003). A RCT during which uterine flushing with embryo

culture media was done at embryo transfer, did not report any beneficial outcome (Berkkanoglu *et al.,* 2006). More recently, a RCT reported that flushing of embryo culture media supernatant before blastocyst transfer could improve implantation and pregnancy rates (Goto *et al.,* 2009).

In the literature, there are few publications which report the use of a medication by the intrauterine route. Ogasawara & Aoki*,* 2000 (case report) reported intrauterine administration of prednisolone before ovulation in a woman with previous ten unexplained miscarriages and led to a successful pregnancy. A recent prospective randomised study reported that intrauterine injection of human gonadotrophic hormone (hCG) prior to embryo transfer improves implantation and clinical pregnancy rates (Santibañez *et al.,* 2014).

Flushing mice uteri with culture media from decidualising ESCs had a profound effect on the endometrium and the success rate of embryo transfer (Brosens *et al.,* 2014).

Thus, there is a body of evidence suggesting medications could be delivered by endometrial flushing to directly treat the endometrium and achieve improved pregnancy outcome.

1.6 Evidence Based Medicine:

The medical profession needs to provide the most suitable care in light of available best evidence. Scientific and clinical evidence is formulated from studies. Clinical studies are categorised into two groups, descriptive or analytical. Descriptive studies can be cross-sectional or qualitative. Analytical studies can be observational (cohort, case control) or experimental (randomised or non-randomised). Results from individual studies of any type should be interpreted with caution due to the possibility of different biases. Bias can occur at any time during a study (planning, data collection, analysis and publication) (Pannucci & Wilkins, 2010). Results from multiple studies can be reviewed in a systematic review.

Clinical reviews are of two types, narrative or systematic. Narrative reviews are descriptive in nature and do not include a systematic search of the available literature. Systematic reviews are based on a detailed search plan prior to the review. This aims to minimise bias. Results of a systematic review can be presented in meta-analysis which formulates a quantitative summary effect size from multiple studies data (Uman, 2011). In a systematic review, outcome measures are prespecified. In this thesis the systematic review protocol (Chapter 3) was peer-reviewed by the Cochrane Menstrual Disorder and Subfertility Group.

In the era of evidence based medicine and practice (Sackett *et al.,* 1996, Straus & Sackett, 1998), levels of evidence had been defined in literature and level 1 evidence (highest level) comprises of systematic reviews with meta-analysis of randomised controlled studies.

1.7 Aims:

1.7.1 Gaps in Our Knowledge:

In this literature review I have identified that there is a clinical need to improve the ART success rates. I have identified that there is a lack of effective interventions currently available to improve the endometrial environment, decidualisation, embryo-endometrial interactions and implantation. I have provided evidence that heparin has the potential to improve the endometrial environment. I have also identified that intrauterine treatment may be a good alternative route of administration to directly treat the endometrium. In this thesis I aim to explore the role of heparin (particularly LMWH) in improving ART success in a series of projects.

1.7.2 Specific Aims:

1. I aim to assess the current clinical data available to assess the efficacy of peri-implantation heparin in ART. A systematic review and meta-analysis was undertaken with the guidance of Cochrane Menstrual disorder and Subfertility group. This included a protocol "Heparin for Assisted Reproduction" (Chapter 2) and the completed review with results "Heparin for Assisted Reproduction" (Chapter 3).
2. I aim to assess the feasibility of intrauterine heparin administration to improve the peri-implantation endometrial environment. Initially a phase 1 study is required for direct administration of heparin as currently only licensed as a systematic injectable formulation. The aim of this prospective phase 1 study is to demonstrate safety, acceptability, feasibility and the potential of this delivery method to effect implantation. We designed and carried out this single blinded, randomised, placebo control study of intrauterine LMWH flushing in non-conception cycle. This involved writing a protocol and obtaining ethical approval from NRES, MHRA and local R&D. Sponsorship was obtained from the University of Warwick. The study was undertaken at UHCW. Study protocol (Chapter 4) and results analysed and reported (Chapter 5).

Chapter 2

2.0 Heparin for Assisted Reproduction (Cochrane Review Protocol):

2.1 Introduction:

The systematic review and meta-analysis was performed under the well-established “The Cochrane Collaboration”. Cochrane is an international, independent organisation which aids clinicians and the public with evidence-based health decision-making. It produces high-quality, relevant, accessible systematic reviews. The Cochrane Collaboration has more than 50 review groups which look at individual subspecialties of medicine and surgery one of which is the Menstrual Disorders and Subfertility Cochrane Review Group. This is led by Professor C Farquhar (Department of Obstetrics and Gynaecology, University of Auckland).

Cochrane reviews are standardised. Each review addresses a specific well formulated question. Anybody who would like to undertake a review needs to submit a research question to the Chair of the Cochrane review group. Once accepted, a protocol for the systematic review and meta-analysis is submitted which is only approved by the Cochrane review group committee after careful discussion. The review can then be undertaken and submitted to the Cochrane review group. It is carefully scrutinised, checked and cross referenced before acceptance. Training to perform Cochrane reviews in UK is provided at the UK Cochrane Centre, Oxford.

Following completion of the systematic review protocol workshop at the UK Cochrane centre in May 2011, in collaboration with colleagues at the University of Warwick and University of Nottingham, I submitted a protocol to the Cochrane Menstrual Disorder and Subfertility Review Group to address the question "Does peri-implantation heparin improves clinical pregnancy and live birth rate in women undergoing ART?" The published protocol is included in Other Appendices.

2.2 Objectives:

This review assessed the benefits and risks of peri-implantation heparin (unfractionated or low molecular weight) in subfertile women undergoing ART.

2.3 Methods:

2.3.1 Types of studies, Participants and Interventions to be Included in the Review:

Only RCTs were included in the review. The method of randomisation was explained. No prospective or retrospective observational/case control/cohort studies were included. Quasi-randomised studies were excluded as per Cochrane recommendation. Participants were subfertile women having ART. WHO

definition of ART does not include intrauterine insemination (IUI) (Zegers-Hochschild *et al.,* 2009). For this reason, women having IUI were excluded. Studies were included in which UFH or LMWH was compared to no treatment, placebo or aspirin during peri-implantation period at ART (from oocyte retrieval or embryo transfer until pregnancy test two weeks later).

2.3.2 Outcomes to be assessed:

The primary outcomes were the live birth rate per woman and the adverse effects of heparin. Secondary outcomes were the clinical pregnancy rate per woman, on-going pregnancy rate per woman and multiple pregnancy rates per woman. In addition, we reported any maternal or fetal pregnancy complications documented in the randomised control studies included in the review.

Following outcomes were not pooled for statistical analysis (implantation rate, incidence of miscarriage and multiple pregnancies). However this data was reported into the 'table of comparisons' in the review.

2.3.3 Search Methodology:

A thorough search was performed in conjunction with Trials Search Coordinator of the Cochrane Menstrual Disorders and Subfertility Group at University of

Auckland and according to the Cochrane Handbook for Systematic Reviews of Interventions (Version 5.1.0) (Higgins & Green, 2011).

These databases searched were The Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, PsycINFO, The Cochrane library, Database of Abstracts of Reviews of Effects (DARE), Current Controlled Trials and The WHO International Trials Registry and Grey literature.

2.3.4 Statistical Analysis:

This was done according to the Cochrane Handbook for Systematic Reviews of Interventions version 5.1.0 (Higgins & Green, 2011). Revman software was used to input and analyse the data. Grade Pro software was used to make the summary of findings table in the systematic review.

2.3.5 Study Selection and Data Collection:

For all records identified which met the search criteria were further explored separately by two authors, any discrepancies between these two authors was resolved by consultation with a third author.

Study characteristics	Patient data	Intervention	Outcome
• Randomisation method • Allocation concealment • Study design • Screening log for eligibility, patients randomised, excluded, and finally analysed • Duration of study • Timing of study • Location of study • Source of funding	• Age of patient • Fertility history • IVF or ICSI	• Type • Dosage • Regimen	• Definition • How it is measured • Timing

Table 2.1: Information collected for each included study. This will be reported in the table 'Characteristics of included studies' in the published review (Akhtar et al., 2013)

2.3.6 Bias Assessment:

This was assessed by two authors autonomously according to modification of the quality criteria specified by the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0.

Any differences between two authors were resolved by consultation with a third author.

Risk of bias assessment was performed for the following:

- Selection bias- this refers to selection of population where adequate randomisation or allocation had not been achieved. So the population sample analysed is not representative of the intended population which needs to be analysed.
- Performance bias- this involves non blinding of participants and researchers in a study which attributes to behavioural reactions and responses in view of which study group the subjects are.
- Detection bias- refers to the systematic differences between groups and how the outcome had been assessed. Blinding of the outcome assessors reduces the risk of this bias. For example, ultrasound scan for clinical pregnancy to be performed by a radiologist or sonographer who are not part of the research team.
- Attrition bias- refers to differences between groups in withdrawal from the study. This could result in incomplete outcome data.

- Reporting bias- refers to selective reporting where negative results, for example side effects, are not reported.
- Any other bias

When required, we used funnel plot to report potential publication bias, however it should be used with caution if there are fewer number of studies (less than 10) (Egger *et al.,* 1997). Funnel plot looked at precision comparing the effect size against sample size. This helped identify any publication bias.

2.3.7 Data Synthesis and Statistical Analysis:

Meta-analyses were performed, as appropriate. A fixed-effect model was used in accordance with methods recommended in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins & Green, 2011). An increase in the odds of a positive or negative outcome was shown graphically.
Odds ratios (OR) with 95% confidence intervals (CI) were calculated in both groups. Data per woman randomised was used for analysis. Data per cycle, per pregnancy or per embryo transfer (ET) were not appropriate for statistical pooling because of 'unit of analysis errors'. It meant that the use of multiple observations per woman leads to unpredictable bias in the estimate of treatment difference (Vail & Gardener, 2003). Multiple births were represented as one live birth event.

Authors were contacted to obtain any missing information or resolve any queries.

We planned to perform subgroup analysis only if enough data is available (including heparin with different ART (fresh or frozen), regimen of heparin, age of women, previous implantation failure following ART, women with or without thrombophilias).

2.3.7.1 Heterogeneity Assessment*:*

The similarity of the studies with regards to participants, interventions and outcomes were assessed for suitability for pooling in the meta-analysis. Chi^2 test was performed to ascertain statistical heterogeneity in the pooled data, with significance level of $P < 0.1$. The variation across the studies as a consequence of heterogeneity was assessed by I^2 statistic (< 25% is low-level, 25-50% is moderate-level, and > 50% is high-level heterogeneity). Sensitivity analysis was performed if high levels of heterogeneity are noted. It was also done to evaluate study quality.

Chapter 3

3.0 Heparin for Assisted Reproduction (Cochrane Review with Results):

3.1 Introduction:

A literature search, data collection, analyses and assessment of risk of bias were performed by two authors autonomously in accordance with the protocol described in chapter 2.

No open discussion or consultation with the third author was required to settle any differences between the two review authors.

The published Cochrane review and the published summary of Cochrane review are included in the other appendices.

3.2 Results of Literature Search:

Seven studies met the pre-set criteria from the search performed. This is shown in Figure 3.1.

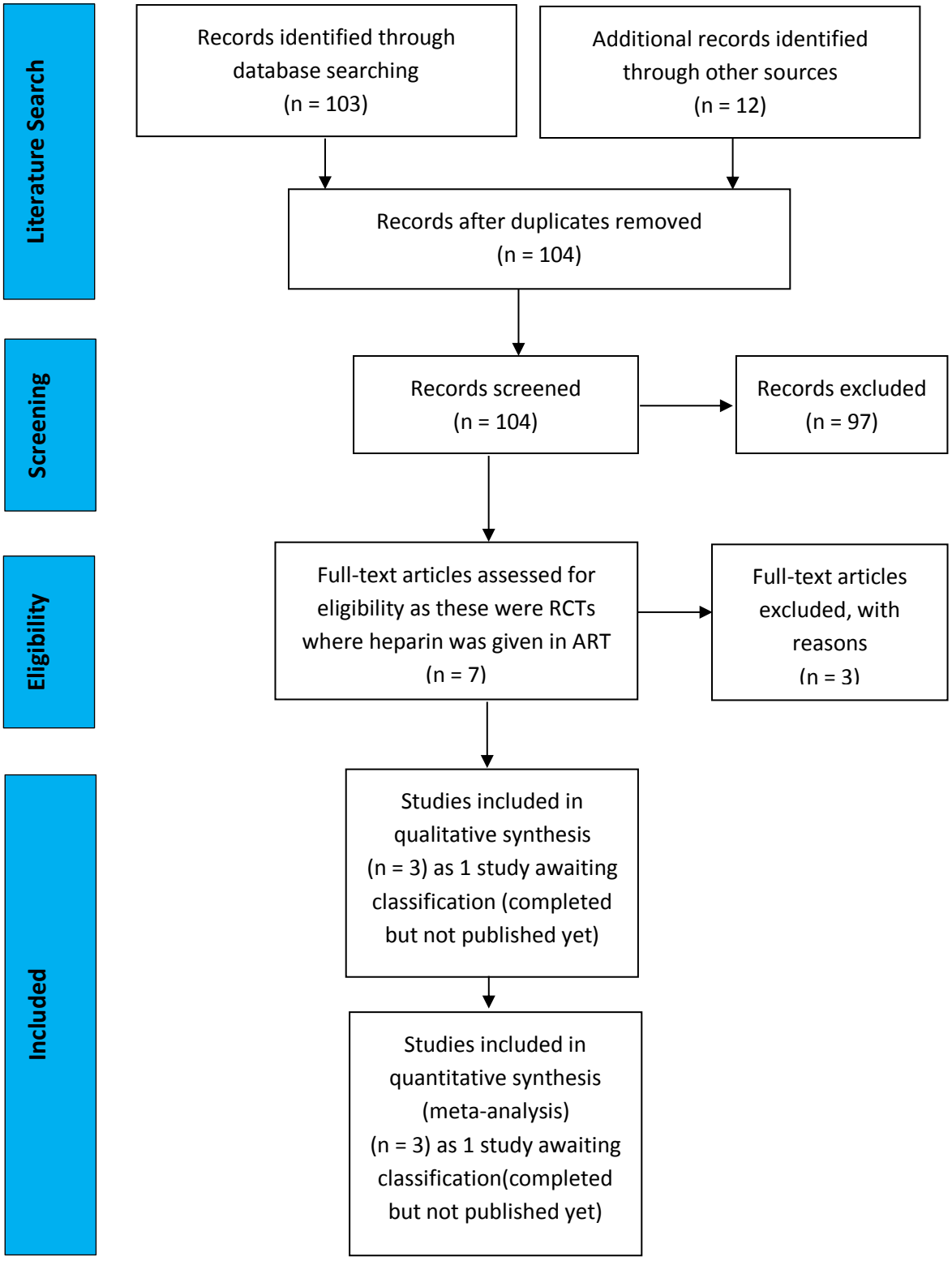


Figure 3.1: PRISMA flow chart

3.2.1 Included Studies:

However, on further assessment only three of these studies were eligible for the review. These three included studies in the review were Qublan *et al.,* 2008; Urman *et al.,* 2009; Noci *et al.,* 2011. It is presented in the table 'Characteristics of included studies' in the published review (Akhtar *et al*., 2013).

3.2.2 Excluded Studies:

Three studies identified failed to meet all of the inclusion criteria and this is summarised in Table 3.1.

Study	Reason for exclusion
Berker *et al.,* 2011	This was a quasi-randomised study.
Colicchia *et al.,* 2011	LMWH was used in addition to prednisolone
Stern *et al.,* 2003	Unfractionated heparin was used with low-dose aspirin

Table 3.1: Excluded studies with reasons for exclusion

Further information about these studies is available in the characteristics of excluded studies section in the published review (Akhtar *et al*., 2013).

3.2.3 Study completed but not published:

We cannot include this study (Mashayekhy, 2011) as it has not yet been published. Only abstract published in Iranian Journal of Reproductive Medicine, spring 2011. Further information was sought from the authors of this study but no response received.

3.3 Results (Included Studies):

Results from these three included studies Qublan *et al.,* 2008, Urman *et al.,* 2009 and Noci *et al.,* 2011 were utilised for systematic review and meta-analysis.

3.3.1 Participants:

There were 386 trial participants in total. Participants in the included studies were less than 40 years old.

3.3.2 Interventions and Comparisons:

All participants were undergoing single IVF/ICSI cycles only. During the cycle LMWH was given to the participants either from oocyte retrieval or from embryo transfer. The methods used in each study is summarised in Table 3.2.

Study	Type of LMWH	LMWH started	LMWH stopped	Control
Noci *et al.*, 2011	Dalteparin 2500 IU daily	Day of oocyte retrieval	At 9 weeks of pregnancy with a positive pregnancy test	No LMWH
Qublan *et al.*, 2008	Enoxaparin 40 mg daily	At embryo transfer (ET)	If two weeks after ET β-hCG was less than 425 IU/ml or if fetal demise or until delivery	Placebo
Urman *et al.*, 2009	Enoxaparin 1mg/kg daily	Day after oocyte retrieval	At 12 weeks of pregnancy with a positive pregnancy test	No LMWH

Table 3.2: Included studies with intervention used

3.3.3 Outcomes:

All three studies reported the primary outcome as the live birth rate.

Other outcomes reported in all studies:

- Adverse effects
- Clinical pregnancy rate per woman
- Multiple pregnancy rate per woman
- Implantation rate per woman
- Miscarriage rate per woman

3.3.3.1 Primary Outcomes:

3.3.3.1.1 Live Birth Rate per Woman:

Results pooled in meta-analysis (fixed-effect model) (Akhtar *et al.,* 2013) showed that there was a statistically significant improvement in live birth rate with the use of LMWH (odds ratio (OR) 1.77, 95% confidence interval (CI) 1.07, 2.90 P=0.03, I^2 = 51%, three studies, 386 women) in comparison to placebo or no LMWH. See Figure 3.2.

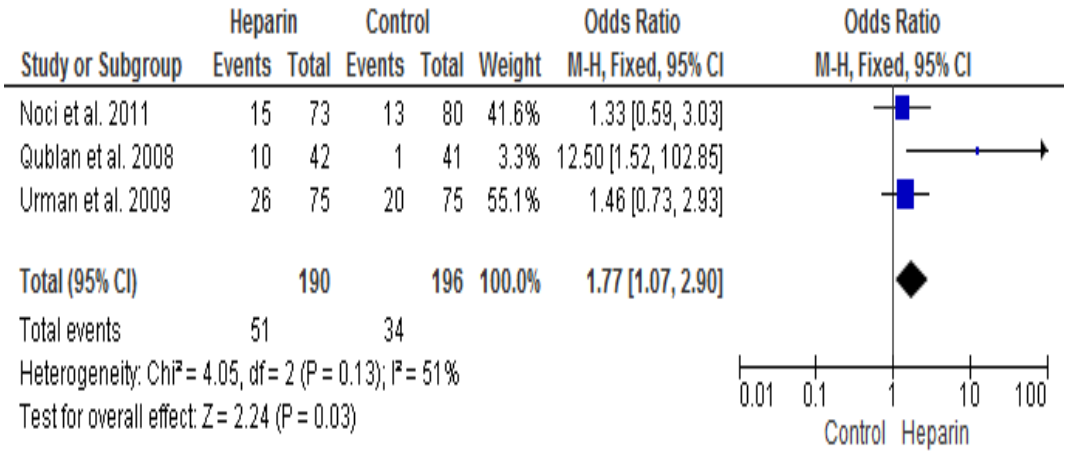


Figure 3.2 Forest plot of comparison: Live Birth Rate per woman (Fixed effect model) (Akhtar et al., 2013)

Sensitivity analysis performed with a random-effects model (Akhtar *et al.,* 2014) showed that there was no statistically significant improvement in live birth rate with the use of LMWH (OR 1.85, 95% CI 0.80, 4.24 P=0.15, I^2=51%, three studies, 386 women) in comparison to placebo or no LMWH. See Figure 3.3.

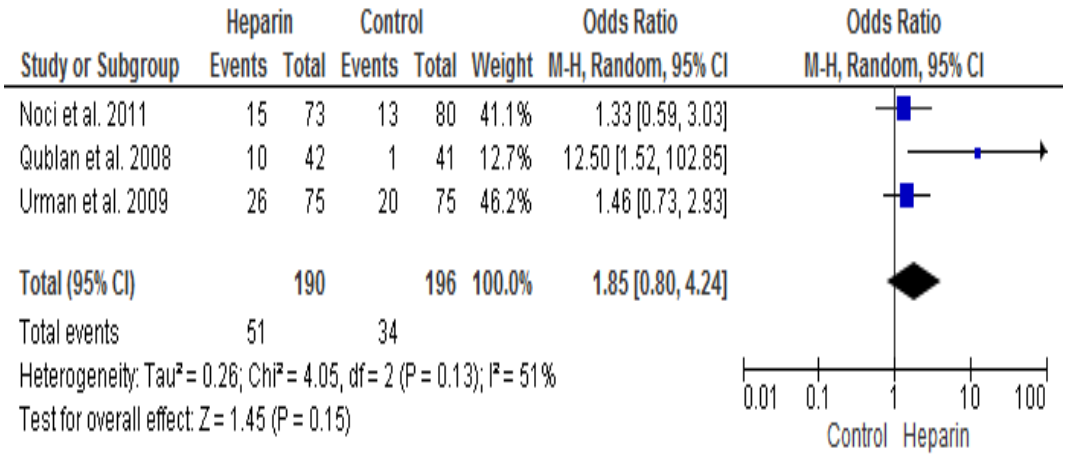


Figure 3.3 Forest plot of comparison: Live Birth Rate per woman (Random effect model) (Akhtar et al., 2014)

This finding should be viewed with extreme caution due to high heterogeneity and sensitivity to choice of statistical model. The evidence was of very low quality as shown in summary of findings for the main comparison (Akhtar *et al.,* 2013).

3.3.3.1.2 Adverse Effects:

Table 3.3 summarises the reported side effects in each of the studies.

Study	Adverse effect	Number of patients affected
Noci *et al.*,2011	Minimal bruising around injection site	Not quantified
Qublan *et al.*, 2008	Bleeding	3/42 (7.1%)
	Thrombocytopenia	2/42 (4.8%)
	Allergic reaction	1/42 (2.4%)
Urman *et al.*, 2009	Small ecchymosis around injection site	Not quantified

Table 3.3: Adverse effects of heparin reported in each study

When heparin was given over a longer duration, the reported side effects increased as shown in Qublan *et al.,* 2008.

3.3.3.2 Secondary Outcomes:

3.3.3.2.1 Clinical Pregnancy Rates per Woman:

This was reported in all included studies. Results pooled in meta-analysis (fixed-effect model) (Akhtar *et al.,* 2013) showed a statistically significant improvement in clinical pregnancy rate with the use of LMWH (OR 1.61 95% CI 1.03, 2.53 P = 0.04, I^2 = 29%, three studies, 368 women) in comparison to placebo or no LMWH. See Figure 3.4.

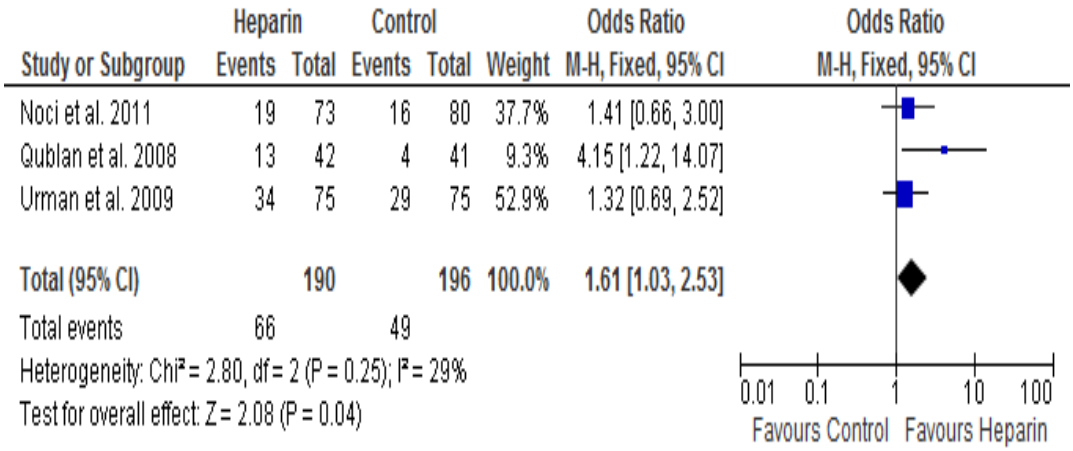


Figure 3.4 Forest plot of comparison: Clinical Pregnancy Rate per woman (Fixed effect model) (Akhtar et al., 2013)

Sensitivity analysis performed with a random-effects model showed no statistically significant improvement in clinical pregnancy rate with the use of LMWH (OR 1.66, 95% CI 0.94, 2.90, I^2 = 29%, three studies, 368 women) in comparison to placebo or no LMWH. See Figure 3.5.

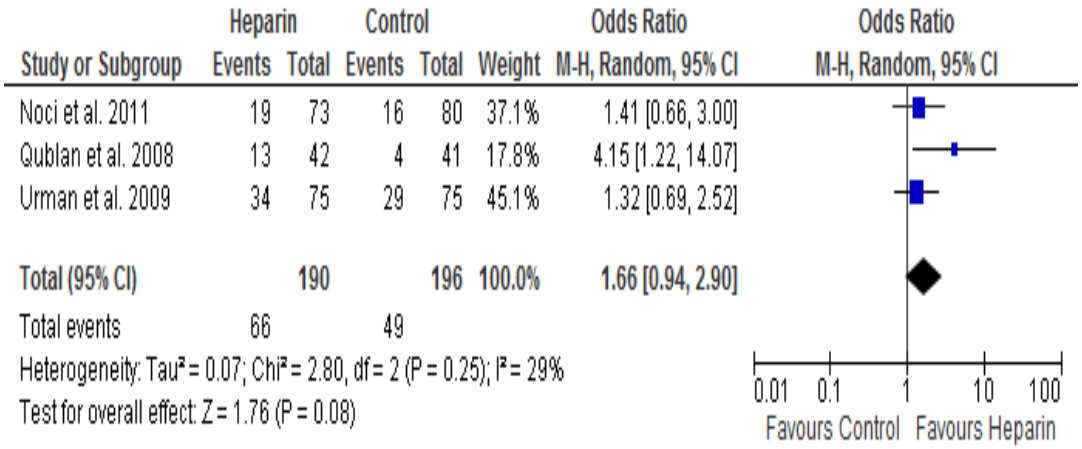


Figure 3.5 Forest plot of comparison: Clinical Pregnancy Rate per woman (Random effect model).

3.3.3.2.2 Multiple Pregnancy Rates per Woman:

'Multiple pregnancy rates per woman' were not reported in any of the included studies. "Multiple pregnancy rates per total number of pregnancies" was reported in all studies but cannot be pooled for meta-analysis due to unit of analysis errors as described in Chapter 2.

3.3.3.2.3 Maternal Pregnancy Complications:

Study	Complication	Patient group	Details
Noci *et al.*, 2011	No maternal complications reported		
Qublan *et al.*, 2008	Placental abruption	LMWH	One patient
	Pre-eclampsia	Control	Two patients
Urman *et al.*, 2009	Preterm delivery	LMWH	Nine patients - At 32 weeks gestation: 3 patients, one singleton, one set of twins and one set of quadruplets - At 34 weeks gestation: two sets of twins - At 35 weeks gestation: three sets of twins - At 36 weeks gestation: one singleton
		Control	Six patients - At 33 weeks gestations: one singleton - At 34 weeks gestation: two sets of twins - At 35 weeks gestation: one set of twins - At 36 weeks gestation: two sets of twins

Table 3.4 Summary of maternal pregnancy complications described in the included studies

3.3.3.2.4 Fetal Complications during Pregnancy:

Study	Fetal complication
Noci *et al.*, 2011	No complications described
Qublan *et al.*, 2008	Two intrauterine deaths in LMWH group
Urman *et al.*, 2009	One baby with unilateral undescended testis in LMWH group. One baby who delivered at 32 weeks underwent surgery due to necrotising enterocolitis in LMWH group

Table 3.5 Summary of fetal complications during pregnancy described in the included studies

3.3.3.2.5 Other Analyses:

We were unable to perform any subgroup analyses due to the small number of included studies. It was also not possible to create a funnel plot to assess publication bias.

Implantation rate, incidence of miscarriage and multiple pregnancy data which was not appropriate for statistical pooling for meta-analysis is shown in Tables 3.6-3.8.

Study ID	Heparin group	Control group
Noci *et al.*, 2011	15%	12%
Qublan *et al.*, 2008	19.8%	6.1%
Urman *et al.*, 2009	24.5%	19.8%

Table 3.6: Implantation rate per embryos transferred

Study ID	Heparin group per pregnancy	Control group per pregnancy	Heparin group per woman	Control group per woman
Noci et al., 2011	4/19	3/16	4/73	3/80
Qublan *et al.*, 2008	1/13 *IUFD 2/13	2/4 *IUFD 0/4	1/42 *IUFD 2/42	2/41 *IUFD 0/41
Urman *et al.*, 2009	n/a	n/a	n/a	n/a

*IUFD-intrauterine fetal death

Table 3.7: Incidence of miscarriage per total number of pregnancies

Study ID	Heparin group	Control group
Noci *et al.*, 2011	(6/19) 31.5%	(2/16) 12.5%
Qublan *et al.*, 2008	(3/13) 23.1%	(1/4) 25%
Urman *et al.*, 2009	(12/34) 35.3%	(10/29) 34.5%

Table 3.8: Incidence of multiple pregnancies per total number of pregnancies

3.4 Bias Assessment:

In accordance to the protocol, all included studies were assessed for bias. This is represented in the form of a graph (Figure 3.6) and summary (Figure 3.7)

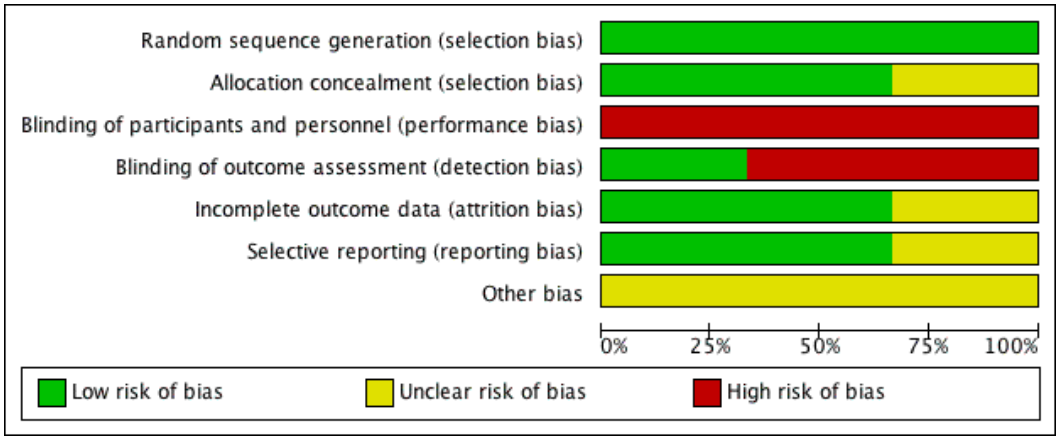


Figure 3.6 Risk of bias graph presented as percentages across all included studies (Akhtar et al., 2013)

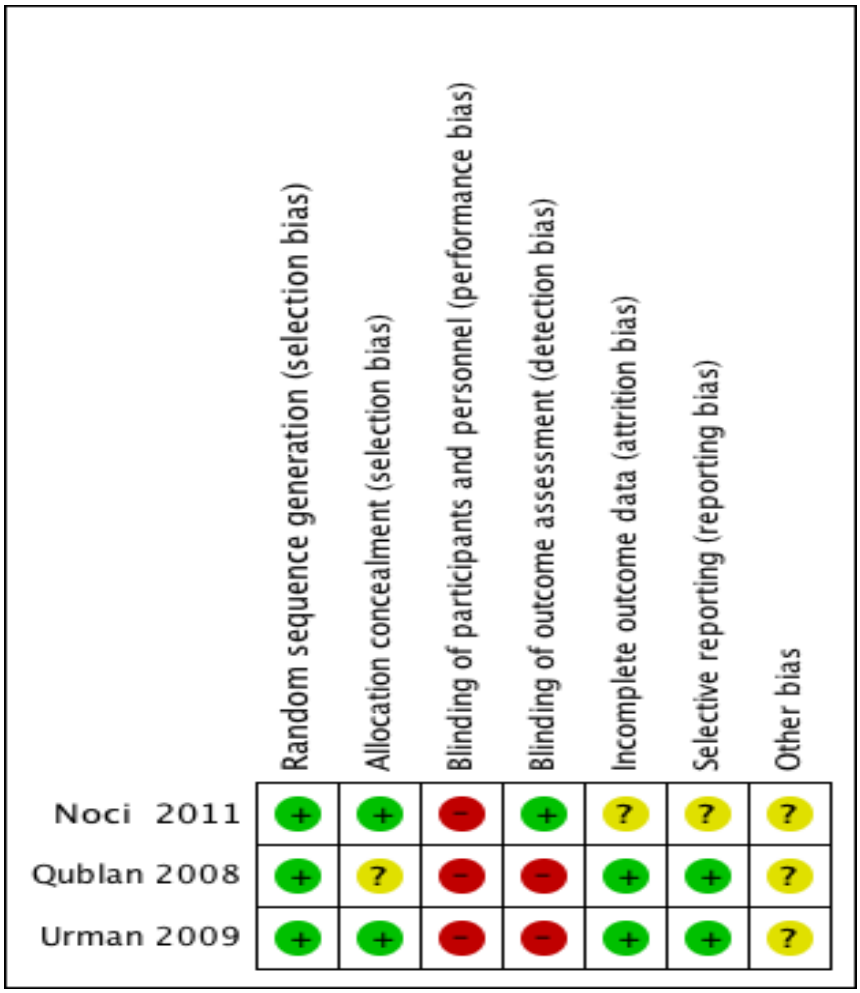


Figure 3.7 Risk of bias summary for each risk of bias item for each included study (Akhtar et al., 2013)

3.4.1 Selection Bias:

3.4.1.1 Random Sequence Generation:

Noci *et al.,* 2011 and Urman *et al.,* 2009 randomised participants following an adequate computer generated randomisation method. Qublan *et al.,* 2008 randomised women from table of random numbers.

3.4.1.2 Allocation Concealment:

Noci *et al.,* 2011 and Urman *et al.,* 2009 reported allocation concealment. However, Qublan *et al.,* 2008 did not describe allocation concealment so the bias risk is assessed as unclear.

3.4.2 Performance Bias:

Noci *et al.,* 2011 and Urman *et al.,* 2009 did not provide any information regarding blinding of participants and clinicians. However, Qublan *et al.,* 2008 reported blinding of participants but not clinicians. Thus, all were assessed as at high risk of performance bias.

3.4.3 Detection Bias:

Outcome assessors were blinded in Noci *et al.,* 2011. Blinding of outcome assessors was not performed in Qublan *et al.,* 2008 and Urman *et al.,* 2009 so categorised as high risk of detection bias.

3.4.4 Attrition Bias:

Incomplete outcome data is classified as attrition bias. Qublan *et al.,* 2008 study reported all outcome data. Urman *et al.,* 2009 compensated for participant dropouts lost to follow up (five women) by using negative outcomes. Noci *et al.,* 2011 recruited 210 women. Thirty eight were excluded on the day of oocyte retrieval. The remaining one hundred and seventy two women were divided into two groups (intervention and control). Thirteen women in the intervention group and six women in the control group were excluded as they did not have did not have any embryos to transfer. It was therefore rated as at unclear risk of attrition bias.

3.4.5 Reporting Bias:

Qublan *et al.,* 2008 and Urman *et al.,* 2009 were considered at low risk of reporting bias. As Noci *et al.,* 2011 did not report any adverse events, so it was as at unclear risk of selective reporting.

3.4.6 Any Other Bias:

No other obvious potential sources of bias were found in any of the included studies. But all of this was unclear.

3.5 Overall Quality of Evidence:

Overall quality of body of evidence for main outcomes are summarised in accordance with Cochrane review in a 'Summary of findings' table (Akhtar *et al*., 2013). This was generated using GRADEPRO software. The criteria used for grading quality of evidence are based upon risks of bias, consistency and imprecision of effect.

3.6 Conclusions:

386 participants from only three studies were eligible to be included in the systematic review and meta-analysis. All the included studies characteristics were heterogeneous. One study was multi-centred (Noci *et al.,* 2011), the other two were single centre studies (Qublan *et al.,* 2008, Urman *et al.,* 2009). There was no regimen uniformity of LMWH among studies. Only one study was placebo controlled (Qublan *et al.,* 2008). Performance bias was high amongst all studies due to the lack of blinding.

Statistical analysis of these studies revealed that the current evidence suggests that LMWH usage during peri-implantation period prior to ART may improve the clinical pregnancy rate and the live birth rate.
However, this result should be interpreted with caution due to small number of heterogeneous studies with few participants. Most importantly, the result of this meta-analysis is sensitive to the choice of statistical model (fixed effect model or random effect model).

Adverse effects (bruising, ecchymosis, bleeding, thrombocytopenia and allergic reactions) were associated with subcutaneous use of LMWH and as would be expected these adverse effects occurred more commonly with prolonged use of the drug.

In conclusion, the current available evidence is not robust enough to justify the clinical use of peri-implantation LMWH in subfertile women undergoing ART. However, its efficacy should be further explored in good quality randomised controlled studies with no other medical adjuncts used additionally.
Studies should be undertaken where local (uterine) heparin unlike systemic heparin is assessed for its efficacy and to ascertain its effect on decidualisation. As heparin does not have a medicinal licence to be given locally (intrauterine), a Phase 1 study will be required to establish its feasibility in the first instance.

Chapter 4

4.0 Study Protocol: Endometrial Flushing of Low Molecular Weight Heparin Improves Decidualisation- A Prospective Randomised Controlled Pilot Study

Trial Registration: Current Controlled Trials ISRCTN78466363

4.1 Background:

Successful implantation is the result of a favourable embryo-endometrial interaction. Recent advances in laboratory techniques in ART focussing on the embryo have led to improvements in pregnancy rates. However, currently there are few treatments to improve the endometrium in this critical interaction. Heparin has been used as an adjunct to ART and some studies suggest that it may improve implantation in women both with and without thrombophilias (Fiedler & Wurfel, 2004). In contrast, four recent high quality trials of heparin administration in early pregnancy failed to demonstrate efficacy in preventing miscarriage (RCOG, 2011). Nevertheless, heparin given earlier, around the time of implantation may be beneficial to subfertile couples. We performed a Cochrane meta-analysis of RCTs which suggests that subcutaneous LMWH may improve clinical pregnancy and live birth rates during ART (Akhtar *et al.,* 2013). One problem with these RCTs was that they gave LMWH from the day of oocyte retrieval. Haemorrhage is a recognised risk during oocyte retrieval for ART. Administration of systemic LMWH at this time may increase the bleeding risk

further. Heparin has also been associated with other side effects including female genital tract bleeding, swelling and itching at the injection sites, osteopenia and potentially HIT (Bohlmann, 2011). Hence, local administration of LMWH is likely to be safer and reduce these risks and side effects as systemic absorption should be less. Intrauterine LMWH could be equally or more beneficial for improving implantation. Furthermore this pre-conception method of administration of heparin may prevent miscarriages when post conception method has not shown any benefit because it has the potential to influence decidualisation directly.

4.1.1 Pharmacology of Enoxaparin:

Enoxaparin (Clexane- Sanofi Aventis ATC code B01A B05) has a molecular weight of 4500 KDa. Compared to UFH it has a higher ratio of antithrombotic activity to anticoagulant activity. It does not influence platelet aggregation or their binding to fibrinogen and it also does not influence clotting tests (APTT and prothrombin time (PTT)). As shown in Figure 1.6, enoxaparin leads to the inhibition of coagulation factors IIa and Xa (Hovanessian, 1999). The maximum anti-Xa effect following subcutaneous injection of enoxaparin occurs 1 to 4 hours later. This anti-Xa level can range from 0.16 IU/ml to 0.38 IU/ml after doses of 20 mg or 40 mg respectively. The half-life of enoxaparin is 4 to 5 hours in a healthy non-pregnant adult. Following a 40 mg dose, anti-Xa activity could be detected for up

to 24 hours. This is presented in the Other Appendices as the Summary of Product Characteristics (SmPC) of enoxaparin.

4.1.2 Rationale for the Study:

In humans, the endometrium is receptive to the embryo only during the implantation window. The implantation window occurs 5-7 days from ovulation. Ovulation can be detected by an ovulation kit testing for urinary LH surge. The ability of the endometrium to support implantation depends upon adequate development of the endometrium prior to implantation, during the implantation and during early pregnancy. This process is known as decidualisation. As discussed in Chapter 1, in-vitro studies have shown that that LMWH promotes decidualisation (Arai *et al.,* 1994, Aviezer & Yayon, 1994, Call & Remick, 1994, Di Simone *et al.,* 2007, Erden *et al.,* 2006, Liang *et al.,* 2006, McBride, Armstrong & McMurray, 1996, Møller *et al.,* 2006, Rajgopal *et al.,* 2006, Stavreus-Evers *et al.,* 2002). We postulate that LMWH promotes decidualisation in-vivo and that this explains the clinical improvement in implantation demonstrated by the meta-analysis. We would like to assess a different route of administration of LMWH, endometrial flushing. This will enable us to administer LMWH locally to the endometrium and then to assess acceptability and its influence if any upon endometrial decidualisation. Following this, improvement in decidualisation is anticipated to improve the quality of implantation and may assist in the future with treating patients who experience infertility or recurrent miscarriage. Local

application is expected to minimise the dose received systemically, while targeting the appropriate tissue for efficacy. In this study, we will obtain an endometrial biopsy 24 hours after LMWH or normal saline 0.9% application and test for markers of decidualisation.

4.1.3 Markers of Decidualisation:

As discussed in Chapter 1, Professor Brosens at the University of Warwick has established in-vitro models of stromal cell decidualisation (Teklenburg *et al.,* 2010, Salker *et al.,* 2010, Salker *et al.,* 2011). More recently this decidualisation model has been found to correlate well with uNK cell density established by Professor Quenby at the University of Warwick (Kuroda et al., 2013).

4.2 Methods:

Eligible participants will be women aged 18-45 years old, who had previous one unsuccessful ART cycle or with history of recurrent miscarriages and able to provide informed consent.

During a non-conception cycle, participants will be randomised to have endometrial flushing with either LMWH (treatment) or normal saline 0.9% (control) 5-7 days after ovulation (during the implantation window). All women will have serum anti-Xa levels performed four hours post endometrial flushing.

All participants will have an endometrial biopsy taken 24 hours post endometrial flushing. Full blood count (FBC) and liver function tests (LFTs) will be obtained at endometrial biopsy. Side effect diaries will be provided to all participants and a telephone follow up will be done two weeks after the endometrial biopsy. Participating women will then go ahead with fertility treatments including ART if they wish to after the study.

An overview of the methods is shown in Figure 5.1. Patient Information Sheet, Consent Form, Participant Invitation Letter and GP Letter are shown in the Appendices 1-4 respectively.

4.2.1 Design:

This will be a phase 1 prospective randomised, single-blind, placebo controlled trial of LMWH (enoxaparin) to assess feasibility of recruitment, integrity of trial procedures and to generate data to base future power calculations. This will enable us to obtain data about participant acceptability for this novel route of administration.

The duration of the study will be 12 months from ethical approval to collect samples from patients. A further 12 months will be required to analyse the data. Recruitment will start upon approval of the study. Participants will be recruited from the implantation, reproductive medicine and recurrent miscarriage clinics, at Centre of Reproductive Medicine at UHCW. The Implantation clinic is already established and run by Professor Quenby and Professor Brosens.

This is a pilot study to assess efficacy and safety. Based upon the ART cycles at Centre of Reproductive Medicine and the availability of patients, we anticipate around 40 participants would be recruited over 12 months period (Expected to be from 1 January 2013 to 31 December 2013).

4.2.2 Aims:

We wish to develop a different method of administration of LMWH to improve decidualisation, hence reducing implantation failure and miscarriage. The aim of this phase 1 study is to demonstrate feasibility, safety, acceptability and the potential of this delivery method to effect decidualisation.

4.2.3 Primary Outcome:

Density of uNK cells as a surrogate marker of effective decidualisation will be looked as primary outcome. Previous work by Professor Quenby (Kuroda *et al.,* 2013) has shown that elevated uNK cell density corresponds to impaired induction of decidual markers (Prolactin, IGFBP1) in vitro. Unfractionated heparin and LMWH has shown to improve markers of decidualisation (Prolactin, IGFB1) in in vitro studies (Fluhr *et al.,* 2010). So uNK cell density as a surrogate marker of decidualisation is appropriate. But this surrogate marker predicts improved decidualisation but not representative of clinical pregnancy and live birth.

Density of uNK cell is measured by immunohistochemistry as described previously by Professor Quenby (Quenby *et al.,* 2009, Drury *et al.,* 2011, Kuroda *et al.,* 2013).

4.2.4 NK Cell Density Count Method:

The endometrial biopsy tissue obtained 24 hours post intrauterine flushing will be fixed in formalin at room temperature, processed and embedded in paraffin. Slides will be prepared for immunohistochemistry with the help of research histopathologist Mr S James at the University of Warwick. Slides will be stained for CD56 and subsequently photographed for analysis. Image J analysis (free java based software) (Schneider, Rasband & Eliceiri*,* 2012) with point picker tool (within image J software) was used for uNK cell counting. This highly reproducible method was first utilised to count uNK cells by Professor Quenby (Drury *et al.,* 2011). CD56 cells were counted within the stroma closer to the epithelial edge in five high power fields. The formula for uNK cell density is below (Drury *et al.,* 2011)

$$\%uNK = \frac{uNK\ cell\ count\ \times 100}{Stromal\ cell\ count}$$

Uterine NK cell density is classified as normal if <5% and classified as high if >5% (Quenby *et al.,* 2005, Tang *et al.,* 2013, Kuroda *et al.,* 2013).

4.2.5 Secondary Outcomes:

- Side effects of endometrial administration of LMWH. For example, bleeding, pain, discomfort and infection.
    - Venous blood will be sampled after the flushing of the endometrium with LMWH or normal saline 0.9%. Anti-Xa level will be checked 4 hours after administration. FBC and LFTs will be checked 24 hours after intrauterine flushing at the time of endometrial biopsy.
- Patient acceptability will be assessed with a questionnaire. Participants will undergo further fertility treatment if they wish to.
- Endometrial samples will be divided into three (one frozen in liquid nitrogen (for future analysis), second into RNA later and the third is fixed in formalin for immunohistochemistry). These samples will be available for the laboratory team to ascertain which will be the most appropriate outcome measure for decidualisation in further studies. DNA, RNA and protein for PRL, L-selectin, HB-EGF, EGF and SGK1 will be assessed. These analyses are beyond the scope of this thesis and results will be published later.

4.2.6 Side Effect Monitoring:

Women will be given a diary to record any side effects. They will also have a telephone consultation after enoxaparin or control administration when they will be asked about side effects and subsequent menstrual period.

4.2.7 Inclusion Criteria:

- Women attending the Centre for Reproductive Medicine, UHCW who have had one unsuccessful ART cycle, or women with recurrent miscarriages.
- Women aged 18-45 years
- Able to give informed consent.

Women included in the study were heterogeneous as there were two distant groups, subfertile women, and women with recurrent miscarriages. This could have resulted in selection bias and confound the results. Both groups of women were included in the study to improve recruitment, there were fears that we may not be able to recruit women for this novel study in which there is no known direct beneficial effect upon patient outcomes.

4.2.8 Exclusion Criteria:

- Currently pregnant
- Currently breastfeeding
- Women who have unprotected sexual intercourse the within the month when endometrial flushing is planned
- Women with body weight of < 45 kg (due to higher risk of bleeding)
- Women with history of medical disorders (bleeding disorders, severe hypertension, known renal or liver disease, diabetes mellitus, peptic ulcer disease, recent stroke)
- Women taking the following medications on a regular basis (warfarin, systemic steroids, acetylsalicylic acid, non-steroidal anti-inflammatory drugs (NSAIDS), dextran, clopidogrel or any immunosuppressant medications
- Women with any known hypersensitivity to heparin, pork, beef or other animal products
- Women having tubal patency testing, hysteroscopy or laparoscopy at the time when endometrial flushing is planned
- Women being treated for a current genital tract infection (these women will be eligible for inclusion once treatment of the infection has been completed).

Some of the exclusion criteria were to ensure that we do not cause harm to an on-going pregnancy or exacerbate genital tract infection. Other exclusion criteria

were developed for patient safety using summary of products characteristics of enoxaparin (see other appendices).

4.2.9 Withdrawal Criteria:

- Women may voluntarily withdraw from the study at any stage.
- Women may be withdrawn if they should have been excluded from the study initially.

4.2.10 Randomisation:

Patients will be allocated study number on a sequential basis. Computer software will be used to generate random numbers which will allocate each study number to a treatment group.

4.2.11 Allocation Concealment:

This will be done with opaque numbered envelopes. This method will be used for allocation concealment in accordance to study numbers.

4.2.12 Blinding:

Patients and outcome assessors will be unaware of the treatment group. However, the clinicians will be aware of treatment allocation in the interests of

patient safety. The effects of LMWH can be partially reversed with protamine sulphate, hence the clinicians administrating the drug will be unblinded so that prompt action can be taken in the case of bleeding. Laboratory staff as the outcome assessors will be blinded to the intervention or control groups.

4.2.13 Study Centre:

The study will be conducted at University of Warwick. The patients will be seen in the outpatients department at Centre of Reproductive Medicine at UHCW, UK.

4.2.14 Study Sponsor:

University of Warwick (Sponsor Study ID: HEP001QUEN).

4.2.15 Study Funder:

Biomedical Research Unit (BRU), University of Warwick

4.2.16 Interventions:

All women were offered oral analgesia (paracetamol or codeine) 1 hour prior to interventions (endometrial flushing and endometrial biopsy).

4.2.17 Stability Data for Enoxaparin:

Enoxaparin will be diluted with 4ml of sodium chloride 0.9% (normal saline) prior to administration for immediate use prior to administration. The supporting stability data from Sanofi-Aventis (manufacturer of Enoxaparin-Clexane) is provided. See Appendix 5.

4.2.18 Administration Method of Treatment Arm:

Pharmacy will supply enoxaparin (as per the sample drug label – see Appendix 6) as the 20mg/0.2ml undiluted pre-filled syringe.

Using an aseptic technique the clinician will add the contents of an enoxaparin 20mg pre-filled syringe to an empty sterile 5ml syringe and then dilute this by drawing up 4ml of sterile sodium chloride 0.9%. The prepared solution is for immediate use and will be administered into the endometrial cavity under ultrasound guidance with hysterosalpingo-contrast-sonograpghy (HyCoSy) catheter (Rocket Medical Ltd).

4.2.19 Administration Method of Control Arm:

Pharmacy will supply sodium chloride 0.9% (as per the sample drug label – see Appendix 6), which is commercially available as 10ml plastic ampoules. Using an aseptic technique the clinician will draw 4ml of sterile sodium chloride 0.9% into

an empty sterile 5ml syringe. The prepared solution is for immediate use and will be administered into the endometrial cavity under ultrasound guidance with HyCoSy catheter (Rocket Medical Ltd).

4.2.20 Treatment for Heparin Toxicity:

If any adverse events or serious adverse events occur, these will be reported to the chief investigator, see Appendix 7.

Specifically, if haemorrhage occurs, the anticoagulant effects of enoxaparin can be largely reversed by intravenous protamine sulphate. The available data suggest that in the first 8 hours after enoxaparin administration 1 mg of protamine sulphate should neutralise the effects of 1mg of enoxaparin. The clinicians will consider that in a non-pregnant woman the amount of enoxaparin in the body is reduced to 50% after 8 hours and 33% or less after 12 hours. The dose of protamine sulphate should be adjusted depending upon the length of time since the enoxaparin was administered. If protamine sulphate is required, all treatments will be discussed with the Consultant Haematologist at UHCW.

4.2.21 Dispensing and Accountability:

Dispensing will be done using a trial specific prescription (see Appendix 8). For this study enoxaparin and sodium chloride 0.9% as placebo are both being used as an investigational medicinal product (IMP) (see Appendix 6).

Dispensing of enoxaparin 20 mg and sodium chloride 0.9% will be done by pharmacy department of UHCW. Both enoxaparin and sodium chloride 0.9% will be taken from commercially available stock. The pharmacy department will maintain accountability logs for enoxaparin and sodium chloride 0.9% used in the trial. Accountability logs will record the manufacturer, batch number, expiry date and the patient's trial number, to allow traceability of the stock issued within the trial. Pharmacy will be responsible for the labelling of the IMP.

The labelling of IMP will be in accordance with Volume 4 of Good Manufacturing Practices, Annex 13 (Manufacture of investigational medicinal products) (Eudralex, 2003).

All records will be maintained in accordance with current Good Clinical Practice (GCP) and in line with the Medicines for Human Use (Clinical Trials) Regulations 2004.

4.3 Assessment and Follow Up:

All participants will be assessed 4 hours and 24 hours after endometrial flushing of LMWH or normal saline 0.9%. Anti-Xa levels will be checked at 4 hours. FBC and LFTs will be checked 24 hours after endometrial flushing. Participants in both groups will have an endometrial biopsy obtained 24 hours after the endometrial flushing procedure. All endometrial biopsies will be performed by study clinicians. Participants will have a telephone follow up 14 days after obtaining

endometrial biopsy and asked to complete a non-validated participant questionnaire (see Appendix 9). The participants can contact the research study team 24 hours a day 7 days a week by telephone. We will seek information from the participants regarding any future treatment outcomes once participated in the study.

4.3.1 Loss to Follow Up:

If participants do not arrive for their follow up, letters will be sent and telephone calls will be made. If we are still unable to contact them, we will contact their General Practitioner (GP) by telephone and letter. Participants' GPs will already be aware about their patients' participation in the study as per the GP letter (See Appendix 4).

4.4 Trial Closure:

Trial will be closed after the recruitment of 40 participants in total. The trial will be closed early if there is any mortality or significant morbidity due to the treatment.

4.5 Safety Considerations:

1. Patients will be able to contact the hospital 24 hours a day 7 days a week. A member of the team will be available. Protamine sulphate will be available 24 hours 7 days a week.
2. Anti-Xa level, FBC and LFTs will be reviewed by the study team and appropriate action will be taken if any abnormalities are detected. Dr Chapman, Consultant Haematologist at UHCW will review these results if there are any concerns.
3. The administration and sampling techniques which will be performed in the study are used regularly by investigators at the Centre of Reproductive Medicine with minimal complications. These comprise of ultrasonography, endometrial flushing with the use of HyCoSy catheter and performing an endometrial biopsy.
4. We are using a thromboprophylactic (enoxaparin 20 mg- 2000IU) rather than therapeutic dose of LMWH, even if it is systemically absorbed completely, it is unlikely to cause bleeding.
5. Endometrial flushing will be performed in non-pregnant women in a cycle when they are not trying to conceive. All patients will be advised to use non spermicidal barrier contraception and a pregnancy test will be performed prior to any procedure.

4.6 Sample Size Calculation:

For this pilot study the sample size was determined following discussion with the statistician Professor N Stallard (Professor and Head of Statistics and Epidemiology Department, University of Warwick). He calculated that 95% Confidence interval widths for two-arm randomised study in subgroup with 20 patients per arm

Endpoint	95% Confidence interval width
Uterine NK cells	9.642
IGFBP1	0.05960
PRL	0.01525

In terms of power for a statistical test, for the two arm comparative study, a test will have 90% power to detect a difference between the two groups of one half of the confidence interval width. Thus we will have 90% power to detect a difference between the two groups of 4.82% in the uNK cell density test.

4.7 Data Analysis:

Analysis will proceed in the following steps:

1. Summary statistics for demographic information relating to the allocation groups will be tabulated. The data will be examined to determine the extent to which the treatment and placebo groups are similar.
2. The primary outcome will be assessed in each group. This will be expressed in a table and graph format. It will include the median and range of each group.
3. All adverse events will be reported according to allocation group.
4. Secondary outcomes including anti-Xa level, blood haematology and biochemistry and side effect reporting (telephone follow up and side effect diaries) will be presented in a tabulated form.

Data and all appropriate documentation will be stored for a minimum of ten years after the completion of the study, including the follow up period. Graph pad and Microsoft Excel software will be used to interpret results when required.

4.8 Reporting Procedure:

All adverse events (AEs) will be reported to the chief investigator. She will then forward these to the MHRA, Trust R&D Office, and the Research Ethics and Governance Manager within Warwick Medical School, University of Warwick. See Appendix 7.

4.9 Ethical and Regulatory Issues:

Approval to conduct the trial has been granted from the West Midlands Research Ethics Committee (NRES Reference: 12/WM/0347) and MHRA (24637/0004/001-0001). See Other Appendices. The trial is registered with European and international Clinical trials database (EUDRACT No: 2012-003682-18). The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

4.10 Patient Acceptability and Consent

Women who meet the inclusion criteria will be informed about the study objectives and they will be given a written patient information sheet (Appendix 1-3). All participants will have voluntarily consented prior to enrolment. Consent to participate in the study will be sought from each participant after a full

explanation has been given, an information leaflet offered and sufficient time allowed for consideration (minimum 24 hours). Signed participant consent will be obtained by the trial investigators prior to participation in the study. The right of the participant to refuse to participate without giving reasons must be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment. Potential participants will be made aware by the research team and the information sheet that participation is entirely voluntary and that their decision whether or not to participate in the research has no bearing upon their treatment or care.

4.11 Data Monitoring:

The chief investigator will be responsible for adhering to the protocol. As this is a single site trial, direct supervision by the chief investigator will ensure a high standard of care for the participants.

The day to day management of the trial will be coordinated by the investigators based at the Clinical Sciences Research Laboratories, University of Warwick including review of AEs, serious adverse events (SAE) and serious unexpected serious adverse reactions (SUSAR). They will also look at the safety data (side effect diaries, anti- Xa levels, FBCs, LFTs).

4.12 Trial Status:

The study started in January 2013 and recruitment of all 42 participants was completed in July 2013. A flow diagram of the study is illustrated in Figure 4.1. This explains the process each study participant will undertake if they consent to participate in the trial.

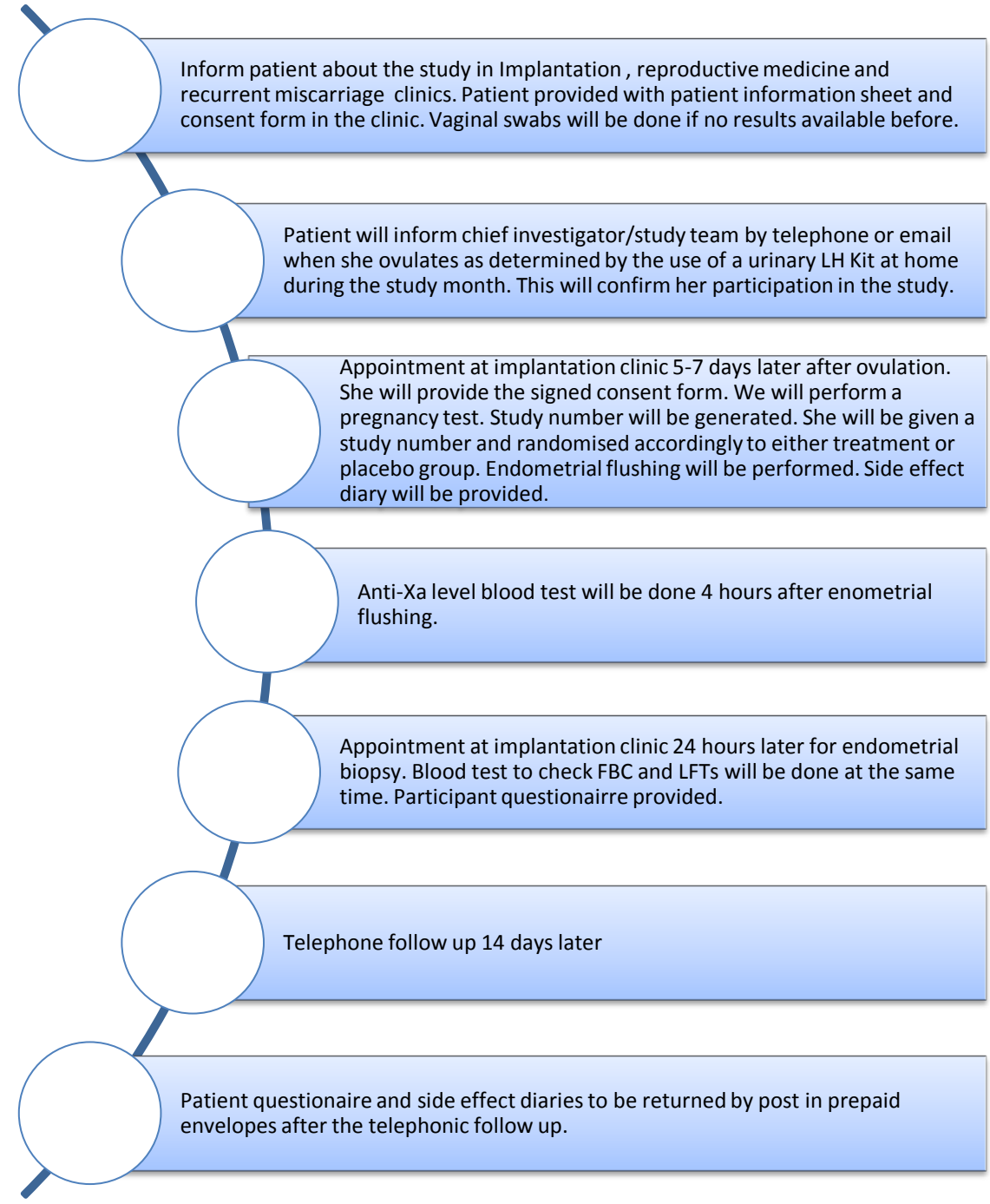


Figure 4.1: Flow Diagram of the study

Chapter 5

5.0 Results of Endometrial Flushing of Low Molecular Weight Heparin to Improve Decidualisation- A Prospective Randomised Controlled Pilot Study:

5.1 Introduction:

As discussed in Chapter 3, a Cochrane systematic review (Akhtar *et al.,* 2013) reported that the administration of LMWH during ART may improve clinical pregnancy and live birth rates. The suggested biological mechanism for this improvement is modulation and optimisation of embryo apposition, adherence and implantation, and trophoblast differentiation and invasion (Nelson & Greer, 2008). Daily LMWH administration is associated with side effects that include patient dissatisfaction with injecting for long periods of time, genital tract bleeding, and swelling, bruising and itching at the injection site (Bohlmann, 2011). There is one case reported in the literature about a patient who died following cerebral haemorrhage who was taking aspirin and heparin in order to improve pregnancy outcome (Bohlmann, 2011).

In view of this, it would be desirable to develop a treatment in which the positive effects on endometrial receptivity of LMWH could be utilised without the deleterious effects of daily systemic injections. Therefore, our group decided to investigate an alternative mode of administration of LMWH. The mode of administration was based upon the already well-defined HyCoSy technique. The HyCoSy technique is performed to help to determine the patency of the fallopian

tubes (NICE, 2013). A radio-opaque solution is injected into the uterine cavity at high pressure while visualising the uterus and fallopian tubes using transvaginal ultrasonography (TVS) (see Figure 5.1 & 5.2). If the fallopian tubes are patent, the fluid is seen to pass through them into the peritoneal cavity. HyCoSy is well tolerated by patients (Marci *et al.,* 2013).

For our study, we hypothesised that we could improve decidualisation by administering a single dose of LMWH directly into the uterine cavity. Figure 5.1 shows the HyCoSy catheter used and Figure 6.2 illustrates the HyCoSy technique.

We successfully developed a method for human intrauterine flushing, allowing targeted treatment to the endometrium during the implantation window, whilst reducing the potential for side effects seen with systemic administration. This method was assessed in terms of patient acceptability of the technique, including any reported side-effects. Endometrial samples were collected and the primary outcomes measure of NK cell density assessed.

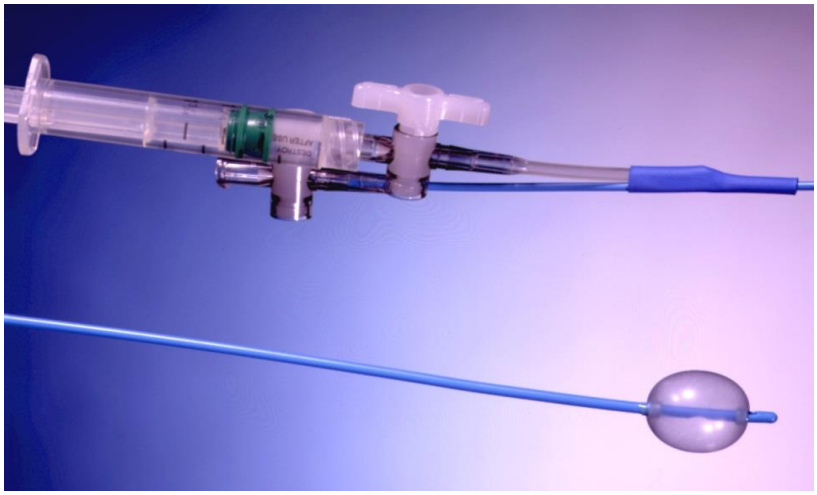


Figure 5.1: Hycosy Catheter (Rocket Medical plc. used with permission)

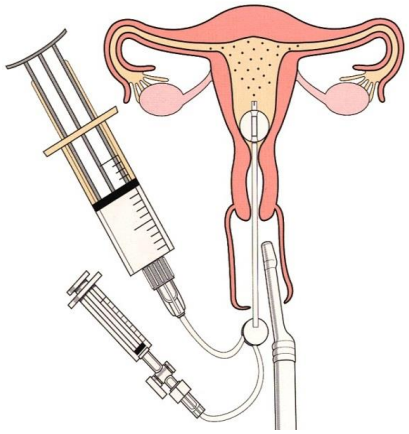


Figure 5.2: Hycosy Technique Illustration (Rocket Medical plc. used with permission)

5.2 Methods:

Forty two participants with either, one or more unsuccessful ART cycles, or a history of recurrent miscarriage were recruited from the UHCW implantation, reproductive medicine and recurrent miscarriage clinics, between January 2013 and July 2013. Written information in the form of patient information sheets were provided to all patients (see Appendix 1). Those who wanted to take part and thought that they met the eligibility criteria then had high vaginal and endocervical swabs to exclude asymptomatic pelvic infections and completed an eligibility questionnaire.

Eligible women were randomly allocated to the control or treatment group and followed the protocol described in chapter 4.

Participants were asked to monitor their LH levels daily with an ovulation kit and to contact us by telephone or email when the test became positive. The flushing procedure was scheduled 5-7 days after the LH surge in order to occur within the implantation window. All women signed a written consent form (see Appendix 2) to participate in the study.

As mentioned, our technique was based on the already established HyCoSy procedure (Figure 5.1 & 5.2). An important difference was that the fluid (enoxaparin or placebo) was administered into the uterus slowly by an assistant with minimal force so that the flush did not enter the fallopian tubes but remained within the uterine cavity for 4 minutes. Each intrauterine flushing procedure was monitored using TVS.

A clinician and an assistant were present for each intrauterine flushing. Each flush was 4ml, drawn up in a 5ml syringe as discussed in Chapter 4.2.18 and 4.2.19. The participant was positioned in the lithotomy position, sedation was not required. TVS was performed prior to the flushing for several reasons. Firstly, to determine the flexion, size and shape of the uterus, secondly to measure the endometrial thickness and thirdly to rule out any obvious uterine pathology such as endometrial polyp, submucosal fibroid and adenomyosis. The cervix was visualised using a Cusco's vaginal speculum and cleaned with 0.9% sodium chloride using a cotton wool ball or gauze swab mounted on a Rampleys sponge

holding forceps. A Hycosy catheter (Rocket Medical Ltd- see Figure 5.1) was passed through the cervix to the 5cm mark, using a sterile Rampleys sponge holding forceps to gently guide its passage if required. The catheter balloon of the HyCoSy catheter was inflated with 1ml of 0.9% normal saline and the Cusco's speculum carefully removed. A TVS was used to confirm the correct position of the catheter and to monitor administration of the flush to ensure it did not pass through the fallopian tubes and remained in the uterine cavity. The flush was then slowly and gently injected into the uterine cavity over 2 minutes (rate of 2mls/minute). Using this direct visualisation technique, the fluid was seen to be absorbed from the uterine cavity but not to enter the pelvis via fallopian tubes. Figures 5.3-5.6 illustrate two participants' images prior and during the intrauterine flushing procedure.

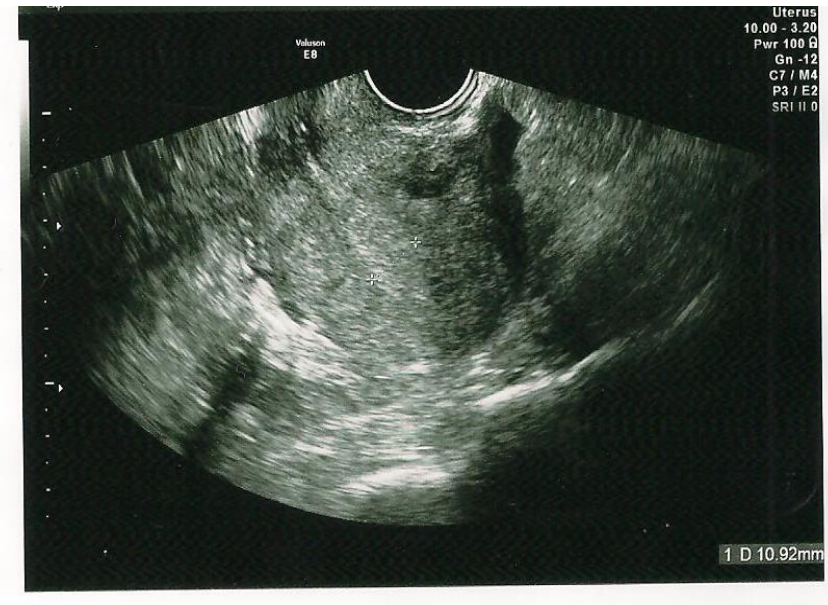


Figure 5.3: Endometrium prior to flushing procedure (participant 1)

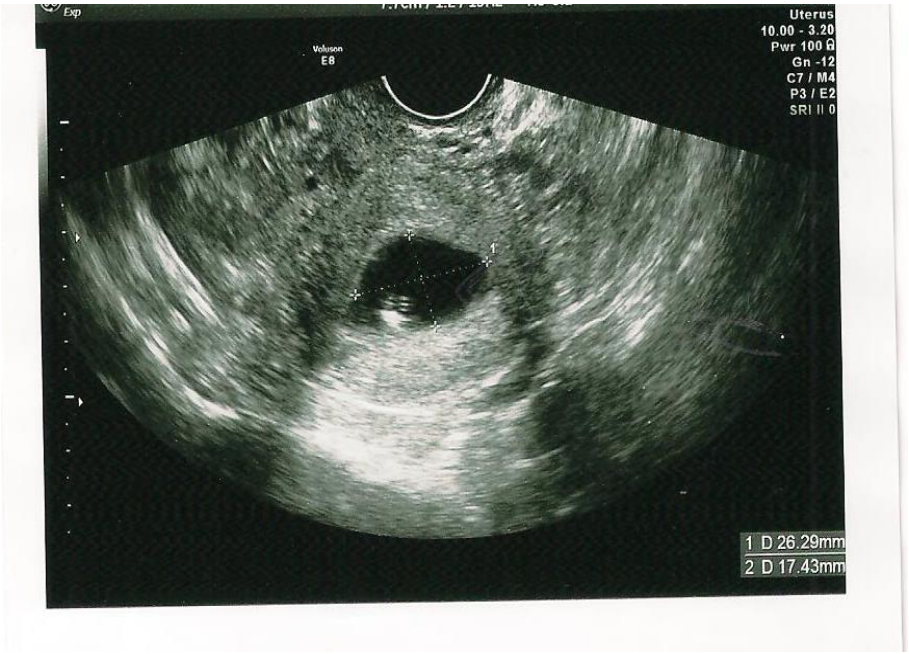


Figure 5.4: Endometrium showing presence of hycosy catheter balloon and flushing fluid (participant 1)

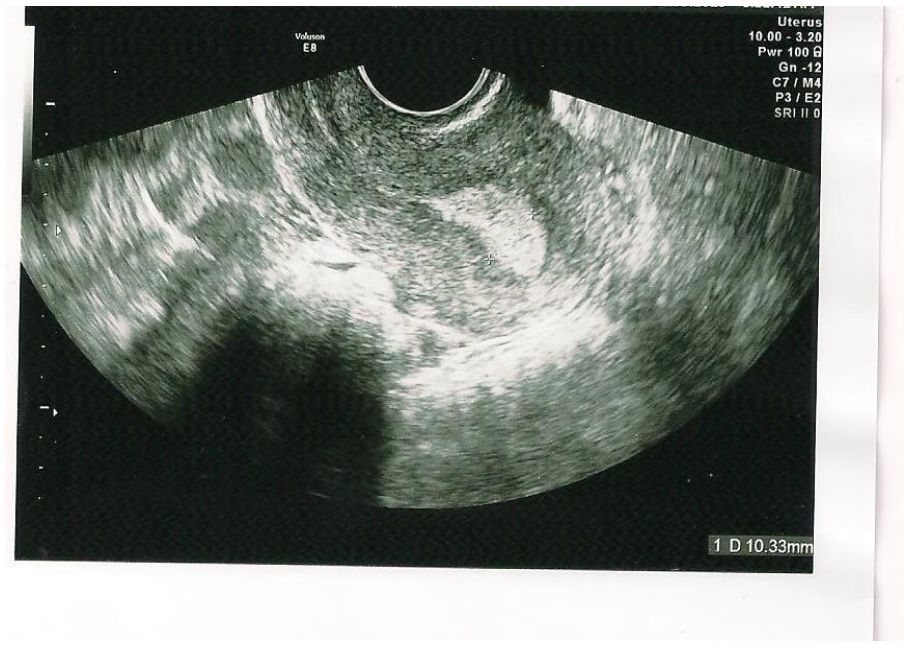


Figure 5.5: Endometrium prior to flushing procedure (participant 2)

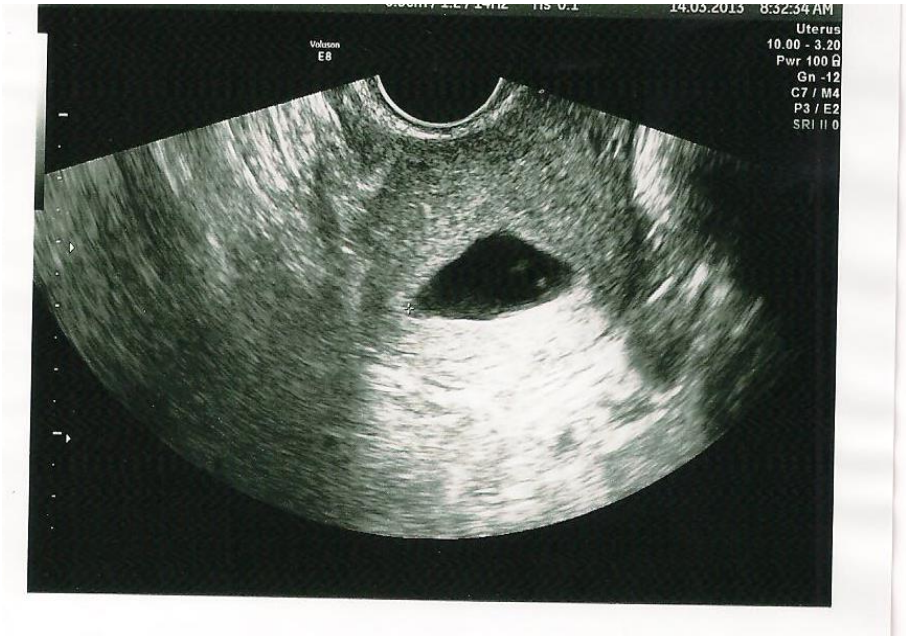


Figure 5.6: Endometrium showing presence of flushing fluid after removal of Hycosy catheter (participant 2)

Following intrauterine flushing, the TVS probe was removed, the catheter balloon deflated and the HyCoSy catheter removed.

Participants remained in the Centre for Reproductive Medicine, UHCW for 5-20 minutes following the procedure and left once comfortable. Side effects diaries and questionnaires with pre-paid envelopes were provided to all participants on the day of intrauterine flushing. As mentioned in Chapter 2, all women had an anti-Xa level blood test four hours after the endometrial flushing. Participants attended 24 hours later for endometrial biopsy which was obtained in presence of chaperone by Wallace catheter. FBC and LFTs were checked at that visit. A follow up telephone consultation occurred two weeks later to collect information

about period heaviness, duration and pain, any other side effects noted and patient acceptability. Participant questionnaire (see Appendix 9) and side effects diaries were sent back to the study team in the prepaid envelope provided. Participating women then underwent fertility treatments including ART if they wish to after the study.

5.3 Results:

We screened 66 women to participate in the study. Forty two women were eligible to participate. Forty two participating women were randomised. Two women were excluded. It was not possible to pass the HyCoSy catheter through the cervix due to cervical stenosis in one participant. The other excluded participant had adenomyosis within uterus so decided not to proceed due to risk of bleeding after intrauterine flushing of heparin.

5.3.1 Participant's Demography:

Participants' age, parity, BMI and reproductive history in both control and the treatment groups are shown in Table 5.1. A student t test was undertaken for statistical analysis. All groups were similar for other demographic data except women with recurrent miscarriages in both groups. Women in control group had a higher number of miscarriages compared to the treatment group. However,

the total number of women in both groups was five (3 in control and 2 in treatment group). BMI was also raised in women with recurrent miscarriages compared to women with subfertility.

	Control group (normal saline 0.9%) (n=20)	Treatment group (enoxaparin) (n=20)	
Previous unsuccessful IVF/ICSI	17	18	
Recurrent miscarriage	3	2	
Age (years): mean+- SD (range)	33.7 +- 5.80 (24-43)	33.5+-4.88 (25-42)	p<0.96
Parity: mean+- SD (range)	0.25 +- 0.71 (0 – 3)	0.2 +- 0.41 (0 - 1)	p<0.78
BMI (Kg/m^2): mean +- SD	26.74 +- 5.96	25.74 +-5.87	p<0.59
(range all patients)	(17-43)	(20-45)	
(range for IVF patients)	(17-36)	(20-33)	
Duration of subfertility (years): mean +- SD (range)	2.91+- 1.54 (1-7)	3.47 +- 1.73 (2-8)	p<0.38
Previous number of IVF/ICSI attempts: mean+- SD (range)	2+- 1.45 (1-6)	1.58 +- 1.06 (1-4)	p<0.35
Embryos transferred per cycle: mean +- SD (range)	1.82 +- 0.39 (1-2)	1.65 +- 0.58 (1-2)	p<0.30
Number of miscarriages in IVF/ICSI patients : Mean +- SD (range)	0.35 +- 0.60 (0-2)	0.27 +- 0.46 (0-1)	p<0.68
Number of miscarriages recurrent miscarriage patients: mean +- SD (range)	7 +- 1 (6-8)	3.5 +- 0.70 (3-4)	p<0.02
Endometrial flushing day (LH+): mean +- SD (range)	5.85 +- 0.74 (5-7)	6.2 +- 0.95 (4-7)	p<0.20
Endometrial biopsy timing (hours after flushing): mean +- SD (range)	24.17 +- 1.54 (20.27)	24.05 +- 2.08 (20-30)	p<0.83

Table 5.1: Participants demographic Table

5.3.2 Primary Outcome:

The uNK cell density obtained after the intrauterine flushing in both groups is shown in Table 5.2 with a graph. There was no difference in uNK cell density count density in both groups.

	Control group (normal saline 0.9%) (n=20)	Treatment group (enoxaparin) (n=20)
Uterine Natural Killer cells (%) median (range)	5.34 (2.13-19.73)	4.61 (1.17-16.33)

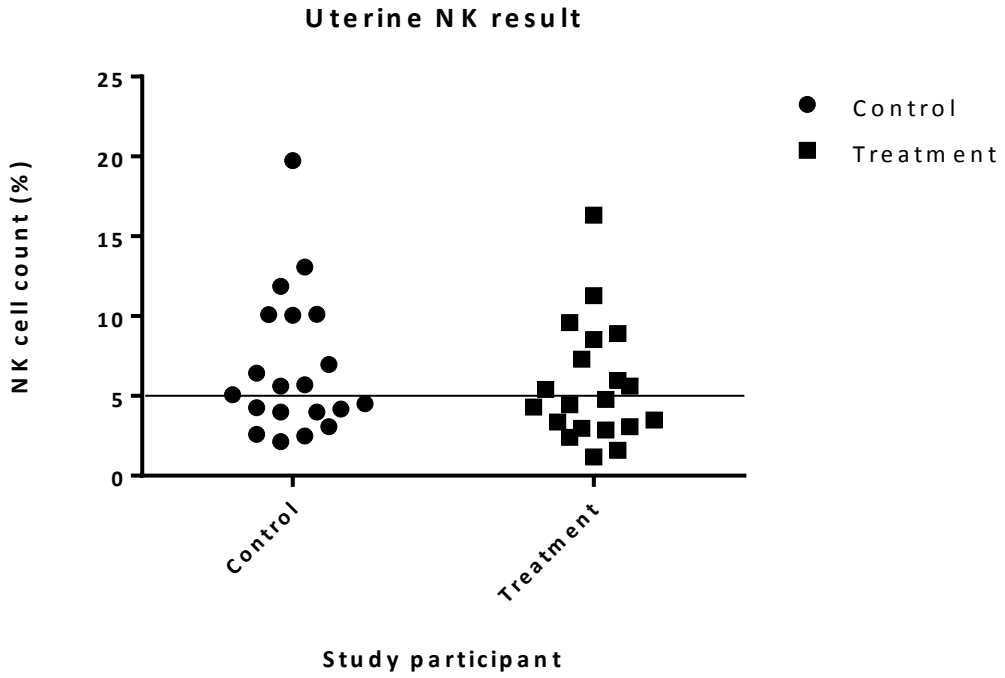


Table 5.2 with graph: showing Uterine NK cell levels in control and intervention group

5.3.3 Secondary Outcomes:

5.3.3.1 Adverse Events

There were no AEs reported during or after the study by participants.

5.3.3.2 Anti-Xa Assay Level Results:

Anti-Xa assay levels obtained 4 hours after the intrauterine flushing in both groups are shown in Table 6.3 with the graph below.

	Control group (normal saline 0.9%) (n=20)	Treatment group (enoxaparin) (n=20)
Anti-Xa level (IU/ml) median (range) Mean	0.10 (0.02-0.014)	0.14 (0.03-0.33)

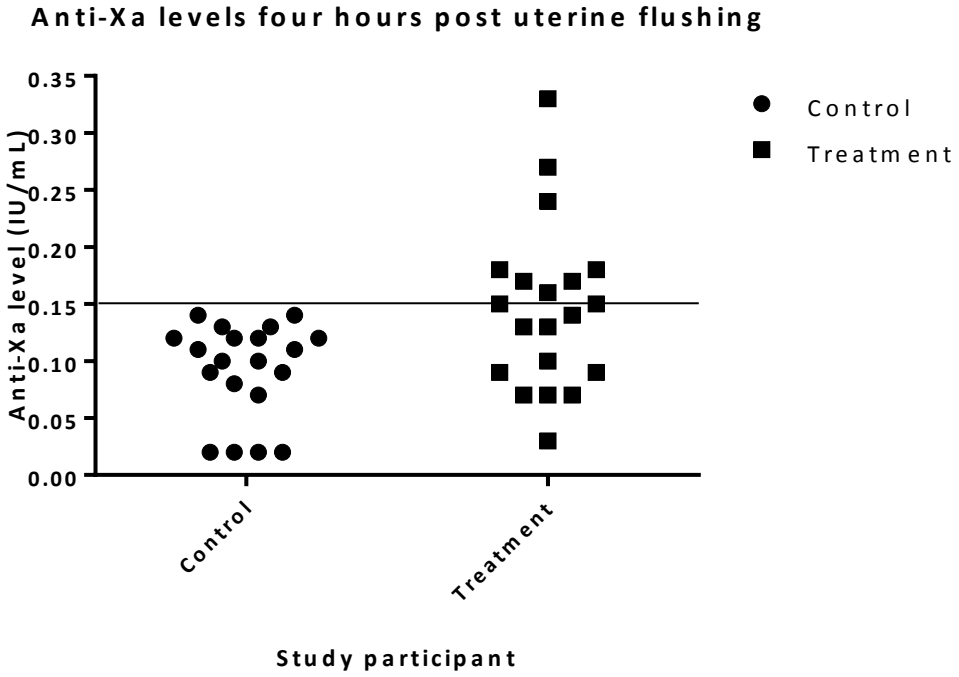


Table 5.3 with graph: showing anti-Xa levels in control and intervention group

Anti-Xa levels were significantly higher in the treatment group when compared to control group.

5.3.3.3 Blood Haematology and Biochemistry Results:

Blood haematology and biochemistry results including FBC and LFTs obtained 24 hours after intrauterine flushing for safety of LMWH administration are shown in Table 5.4.

Test	Control group (normal saline 0.9%) (n=20)	Treatment group (enoxaparin) (n=20)
Haemoglobin (g/dL): median (range)	13.25 (9.8-14.7)	13.65 (12.3-14.8)
White cell count (x 10^9/L): median (range)	7.71 (5.61-12.14)	7.45 (4.83-12.94)
Platelet count(x 10^9/L): median (range)	277 (206-420)	265.5 (123-345)
Bilirubin (µmol/L): median (range)	8 (5-24)	8.5 (3-18)
Albumin (g/L): median (range)	47 (42-49)	46 (43-52)
Alkaline phosphatase (ALP) (U/L): median (range)	58 (42-109)	62 (39-98)
Alanine aminotransferase (ALT) (U/L): median (range)	17 (8-35)	15 (8-27)

Table 5.4: Blood biochemistry results in the study groups.

There were no differences in blood platelet count or liver function tests after single intrauterine flushing with a dose of 20 mg enoxaparin.

5.3.3.4 Participant Questionnaire and Side Effect Diary Outcome:

Participant reporting of pain, vaginal spotting, effect on next period and other side-effects were collected at the two-week telephone review, from the side effect diaries and the patient questionnaire. A summary of these are shown in Tables 5.5 and 5.6.

	Control group (normal saline 0.9%) (n=20)	Treatment group (enoxaparin) (n=20)
Diaries/questionnaires returned	n=15	n=11
Pain following flushing?		
Yes (mild, significant)	6 (4,2)	4 (2,2)
No	9	7
Pain following biopsy?		
Yes (mild, significant)	13 (6,7)	9 (5,4)
No	2	2
Spotting?		
Yes	11	7
No	4	4

Table 5.5: Table showing patient recorded outcomes from side-effect diaries and participant questionnaire

	Control group (normal saline 0.9%) (n=20)	Treatment group (enoxaparin) (n=20)
Telephone follow-up (two weeks post endometrial biopsy)	n=20	n=20
Bruising or bleeding other than vaginal	Nil	Nil
Pain		
Yes	13	7
No	7	13
Median pain score (range)	0 (0-5)	0 (0-6)
Analgesia required		
Yes	6	6
No	14	14
Period length		
Shorter	4	1
Normal	14	8
Longer	2	11
Period strength		
Lighter	2	0
Normal	14	4
Heavier	4	16

Table 5.6: Table showing patient recorded outcomes from telephone follow-up (two weeks after intrauterine flushing and endometrial biopsy)

All patients had a two week telephone follow-up interview, during which they were asked about spotting, pain and the length and heaviness of their period following the procedures. From these interviews, the majority of participants in both the treatment and the control groups reported no pain following the

flushing procedure. Most of the treatment group and most of the control group reported spotting following the procedures. There was no significant difference between the reported pain scores or vaginal spotting between the control and treatment groups.

As shown in Table 5.5 and 5.6, participants recorded greater levels of pain in their side effect diaries when compared with the telephone consultation. However, the side effect diaries were returned by a smaller number of participants, but from these diaries we were better able to distinguish between pain following flushing and pain related to the endometrial biopsy. The majority of pain reported was associated with the endometrial biopsy rather that the intrauterine flushing. No significant side effects were reported and no AEs occurred. Comments in the side effects diaries included: "was made to feel very special" "no negative comments" "no pain" "mild spotting for a few days after" “flushing: slight stomach cramps” Spotting after biopsy” "only pain during biopsy" "straight forward and easy".

35/40 endometrial flushing procedures were technically easy (one attempt), 4/40 difficult (two attempts) and 1/40 was very difficult with more than two attempts.

5.3.3.5 Decidualisation Markers:

Further work which is beyond the scope of this thesis will be performed on the stored samples for DNA, RNA and protein studies examining PRL, L-selectin, HB-EGF, EGF, IGFBP1 and SGK1. Endometrial stromal cell cultures are required to test for majority of these markers.

5.3.4 Outcome of Participants after Study Participation:

Figures 5.7 and 5.8 show the outcomes collected for both the control and treatment groups within one year of participating in the study.

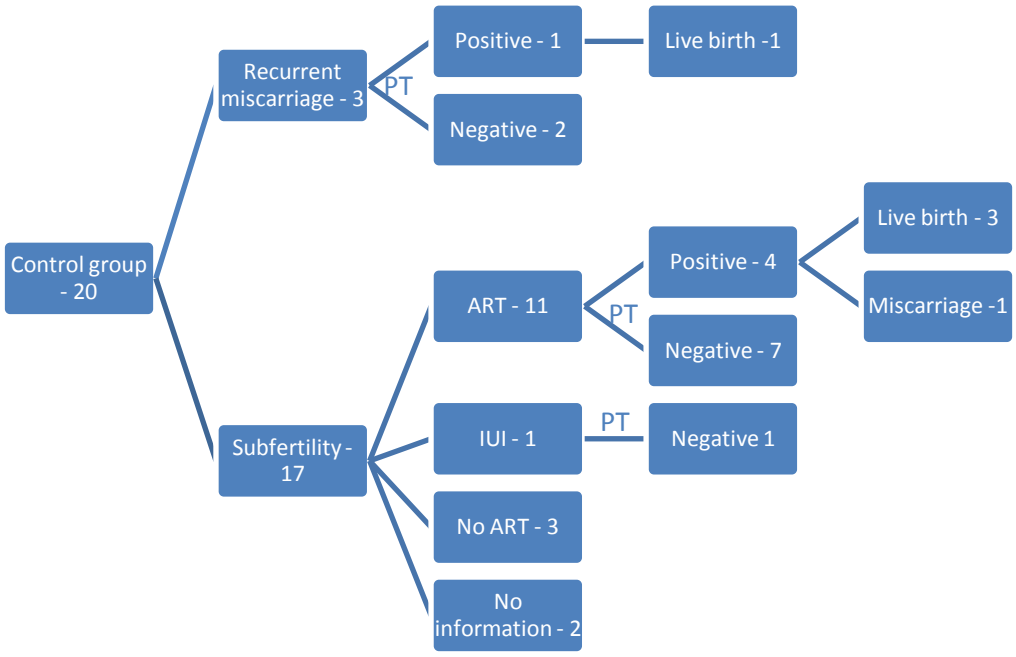


Figure 5.7: Control group: Outcome of study participants within 1 year of taking part in study. 20 patients in total, 3 patients with recurrent miscarriages and 17 with subfertility. PT: pregnancy test

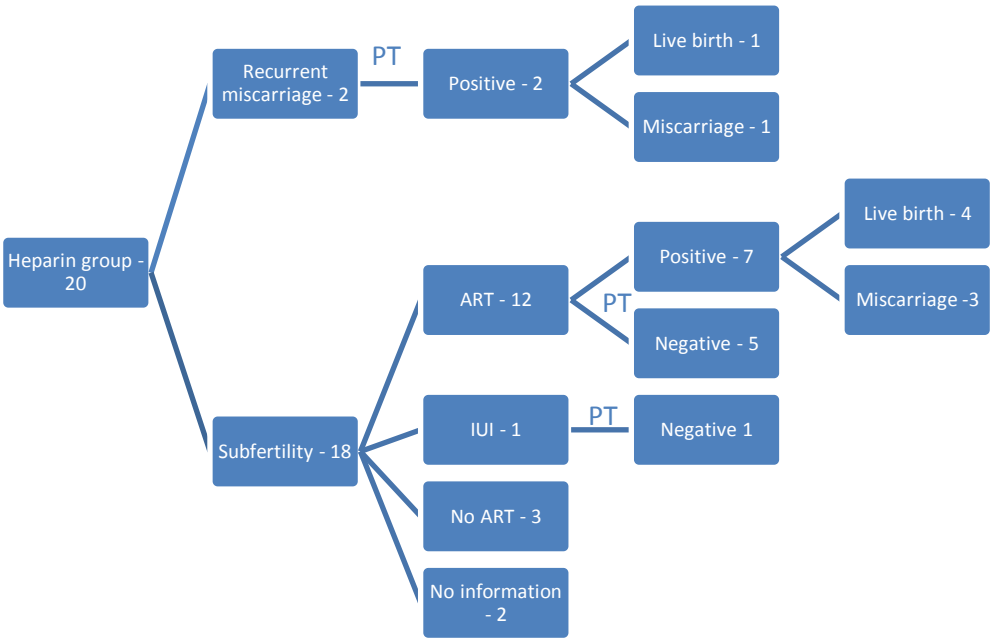


Figure 5.8: Heparin group: Outcome of study participants within 1 year of taking part in study. 20 patients in total, 2 patients with recurrent miscarriage and 18 with subfertility. PT: pregnancy test

5.4 Discussion:

Overall, the intrauterine flushing procedure was well tolerated by participants. This is highlighted by the positive comments recorded in the side effects diaries. Where pain was reported, it was mainly associated with the endometrial biopsy which is known to be uncomfortable and would not necessarily be performed if intrauterine flushing was used as a treatment. Therefore, the procedure itself

could be said to be well tolerated. The difference in reporting of side effects when comparing the two week telephone consultation with the side effect diaries may be due to recall bias or the participants not feeling comfortable making negative comments to the research team. This highlights the importance of the side effect diaries.

Importantly, those in the treatment group had higher anti-Xa levels 4 hours post intrauterine flushing compared to the control group, indicating that the enoxaparin was absorbed via the endometrium which was surprising.

There is a clear biological effect seen, significantly more of the participants in the treatment group reported a longer and heavier period than normal following the intrauterine flushing. This again supports the suggestion that the enoxaparin had a direct effect on the endometrium. It also suggests that the LMWH was absorbed by the endometrium even though exposure to the drug was only 4 minutes. This suggests that during the implantation window, the endometrium has the ability to absorbed drugs very rapidly and is thus a sensible therapeutic target.

There was no difference observed in the primary outcome measure of uNK cell density between the two groups. The uNK cell density count was performed as described by Professor Quenby (Kuroda *et al.,* 2013). There are several possible reasons for this; enoxaparin may not effect decidualisation, selection bias, the included study population with extremes of BMI could have influenced the result, the study was not adequately powered to detect subtle differences, there

was only 24 hours between intrauterine flushing and obtaining the endometrial biopsy and this may not have been a sufficient time to measure any effect.

Endometrial flushing has been used in the past to assess improvement in implantation and pregnancy rates. Li, Mackenna & Roberts, 1993 reported a case series of 90 patients suggesting that uterine flushing is a safe, simple procedure and causes less discomfort than endometrial biopsy. Lédée-Bataille *et al.,* 2002 analysed uterine flushing for LIF and TNF to predict pregnancy outcomes. Olivennes *et al.,* 2003 reported in a prospective study that uterine flushing at the time of egg collection is not associated with adverse pregnancy rates. A prospective randomised study Berkkanoglu *et al.,* 2006 reported that direct flushing of endometrial cavity with culture media just after cervical irrigation at the time of embryo transfer had no beneficial effects on implantation and pregnancy rates. During this study 0.4 mls of embryo culture media was flushed into the endometrial cavity with an embryo transfer catheter under ultrasound guidance. Embryo transfer was performed soon afterwards. There was no improvement in outcome with this intervention. A case report Ogasawara & Aoki, 2000 reported uterine steroid therapy before ovulation for a woman with previous ten unexplained miscarriages led to a successful pregnancy. Thus suggesting medications could be delivered by endometrial flushing to achieve improved pregnancy outcomes. Mostly published studies focussing upon uterine flushing are used to analyse cytokine presence in the uterine cavity to predict pregnancy outcomes.

It is becoming increasingly clear that successful implantation is dependent upon a favourable embryo-endometrial interaction. However, there are few interventions available to improve the endometrial part of this critical interaction with the exception of the increasing utilised endometrial biopsy prior to ART (Nastri *et al.,* 2011). We have described the potential for a novel way to administer drugs directly to the endometrium. This method does result in biological and clinical effects, allowing for potential administration of a single dose of a drug, prior to ART. This is distinct from systemic drug administration which has the potential to reduce side effects of the drug.

5.5 Conclusion:

This is one of the few RCTs to evaluate intrauterine flushing of a medicinal product. Our study findings show that endometrial flushing is a safe, simple feasible, well tolerated technique which can be used to deliver medications directly to the endometrium. The procedure is well tolerated by patients. There were no adverse events in the study. Minimal side effects were reported.

The primary outcome measure (uNK cell density) was observed to be no different in both groups, however a larger sample size study (Phase 2) is needed to confirm or refute this. There could be various possible reasons for this result mainly relating to the methodology.

Firstly, included women in the study were heterogeneous as it included two distant groups, subfertile women, and women with recurrent miscarriages. This could have led to selection bias may have attributed to a negative result. Both of these groups of women were included in the study to improve recruitment for the study, there were concerns that we may not be able to recruit women for this novel method in which there is no direct beneficial effects upon patient outcomes. However, the study recruitment was completed within six months.

Secondly, uNK cell density was counted as described by Professor Quenby previously (Kuroda *et al.,* 2013). UNK cell density assessment by Professor Quenby's method did correlate with laboratory markers of decidualisation (Kuroda *et al.,* 2013). There are other methods of counting NK cell density and there is no consensus about the best method of counting uNK cell density (in numbers or percentages) as described in this systematic review and meta-analysis (Sheshadri *et al.,* 2014). This also suggested that there is not a significant difference in live birth rates in women with increased NK cells compared with women without increased NK cells but the test should be offered in context of the research settings (Sheshadri *et al.,* 2014). However, Professor Quenby's method of uNK density assessment did appear to predict pregnancy outcomes (Tang *et al.*, 2013). But the use of a surrogate marker for decidualistaion (uNK cell count) cannot replace the clinical outcomes of implantation, clinical pregnancy and live birth.

Thirdly, the endometrial biopsy was obtained 24 hours after intrauterine flushing suggesting that there may not been enough time to see an effect in the uNK cell density or stromal cell decidualisation. Endometrial biopsy for uNK cell density was done 5-8 days after ovulation (around day 19-22). It has been established that uNK cell count increases from 5% in early secretory phase of the menstrual cycle to more than 35% in the premenstrual endometrium. So the optimal time to count uNK cell density is before 23 of the menstrual cycle as from day 23 uNK density continue to increase (Russell *et al.,* 2011). Endometrial biopsy was taken at the appropriate time but there was only a 24 hours interval from intrauterine flushing to endometrial biopsy, this may have contributed to the result of no difference in the primary outcome.

Fourthly, in this pilot study we compared intrauterine flushing of LMWH with intrauterine flushing of normal saline 0.9%. Both interventions caused a mechanical disruption of the endometrium regardless of any pharmacological effect. This mechanical disruption might have attributed to this negative result in the primary outcome. On further consideration, there should have been three groups compared. The third group of participants would have no intervention of intrauterine flushing prior to endometrial biopsy. The result from those participants in the third group would have informed us if any mechanical disruption of the endometrium with intrauterine flushing was influencing the primary outcome.

The recruitment for the study was achieved with in six months. Women were willing to participate and there were no major adverse events during the study. Intrauterine flushing of LMWH has a pharmacological and biological effect. The majority of participants underwent ART after taking part in the study and of those, there have been nine live births.

Chapter 6

6.0 Conclusion:

Worldwide, most couples can achieve pregnancy naturally but some are unable to do so. In the UK it is estimated that one in seven couples experience subfertility at some point in their reproductive life. This causes a great deal of stress, anxiety and unhappiness. Human fecundity is poor; at best natural conception per cycle is 25 %. This reduces further with increasing female age. Additionally, 20-25 % of pregnancies end with miscarriage. In the UK, the number of children born per family has decreased over the recent few decades. The number of live births in the UK decreased by 4.3% from 2012 to 2013 with 729 674 live births in 2012 and only 698 512 in 2013 (Office of National Statistics, 2013). Society is adapting with changes in life styles. As we all are working for more years and living longer. Life has become more sedentary with use of technology, more focus on our careers and changing eating and exercise habits. This has led to increase in male and female subfertility in the developed world including the UK. All women will lose the ability to have their own biological child as they reach the menopause. Currently, there are no tests to confirm fertility and fertility treatments fail more often than they succeed.

Since the birth of the first IVF baby in 1978, ART has revolutionised the management of the subfertile couple. Many innovations in the field of reproductive medicine have led to improvements in embryo culture, selection of good quality embryos (usage of time-lapse microscopy) and blastocyst transfer. This has led to improvements in the success rates of ART. There have been

improvements in the diagnosis and management of conditions associated with male and female subfertility. Improving controlled ovarian stimulation during ART by individualising each treatment cycle has led to a better yield of oocytes. ICSI treatment has enhanced the fertilisation rate in couples where the male partner has poor quality sperm. Surgical interventions in women (salpingectomy for hydrosalpinx, removal of submucosal fibroid or endometrial polyp) and men (surgical sperm retrieval) prior to ART has contributed to the continued improvement in outcomes.

However, despite these innovations, the success rates of ART are around 20-40% although this depends upon the couple's clinical situation. This means that ART fails more often than it is successful. One of the most important factors which remains a challenge in improving ART outcomes is to improve endometrial receptivity.

The endometrium is one of the most fascinating tissues in the human body as it regenerates each month. The sole purpose of the endometrium is to implant and support an early embryo during a small window of implantation (Revel, 2012).

Implantation is steered by physical, physiological and biochemical contact between an embryo and the endometrium. The process of implantation involves complex signalling between the embryo and endometrium leading to the processes of apposition, adhesion, attachment and penetration. Successful implantation involves a harmony in these interactions in the presence of a competent embryo and a receptive endometrium. An appropriately receptive

and selective endometrium will pave the way for the successful embryo intrusion within the endometrial stroma. This is achieved by the interaction between trophoblast cells and the endometrium, allowing penetration through the luminal epithelium and basal lamina into the stroma. Embryo attachment to the luminal epithelium is followed by continued decidualisation of the endometrial stroma. So a functional harmonious communication involving embryo, endometrial epithelial and stromal cells is necessary for decidualisation, thus paving way for successful implantation (Cha, Sun & Dey*.,* 2012).

Implantation involves various cytokines, chemokines and ovarian steroids. One important signalling pathway is HB-EGF mediated, this pathway known to be important in the process of decidualisation (Das*,* 2009). HB-EGF is expressed in increasing amounts in the secretory phase endometrium on the surface of the pinopodes. The expression of HB-EGF in luminal and glandular epithelium is highest when fully developed pinopodes are present. These findings suggest that HB-EGF may play a role in both the attachment and penetration steps in the human implantation process (Stavreus-Evers *et al.,* 2002).

Much of the current focus is on improving endometrial receptivity so as to improve pregnancy outcomes (Revel*,* 2012). Medical adjuncts including low dose aspirin, LMWH, corticosteroids, immunoglobulins and intralipids have been used with the aim of improving endometrial receptivity (Fatemi & Popovic-Todorovic*,* 2013). Heparin is the second most commonly used medical adjunct in ART. It is believed that heparin improves implantation rates leading to a positive

pregnancy test, not by its anticoagulant effect but with by its effect upon endometrial decidualisation and embryo apposition, adhesion, attachment and penetration.

A systematic review and meta-analysis of randomised controlled studies was undertaken to find the best currently available evidence with the use of LMWH in ART. This level 1 evidence (systematic review and meta-analysis) “Heparin for Assisted Reproduction” was carried out following the pre-specified, published methodology by Cochrane Review Group (Akhtar *et al.,* 2013). We reported that peri-implantation LMWH used in ART cycles may improve the clinical pregnancy and the live birth rates. However, with only a small number of studies (three studies with 386 participants), extrapolation of these findings is difficult. In the included studies there was no uniformity of dose and timing or duration of the intervention. There was heterogeneity among included studies. Only one study used a placebo control. There was performance bias in all studies and detection bias in two studies. There were reported side effects of systematic usage of heparin with bleeding. The use of heparin in this patient group needs to be further investigated with adequately powered, large scale double-blind, randomised, placebo-controlled, multicentre trials.

Previously endometrial secretions obtained from the uterus have been analysed for cytokine profiling to assess endometrial receptivity (Boomsma *et al.,* 2009a, Boomsma *et al.,* 2009b, Mikolajczyk, Wirstlein & Skrzypczak, 2007). Intrauterine flushing of embryo culture media prior to embryo transfer does not adversely

affect the outcome as reported by this prospective randomised controlled study (Berkkanoglu *et al.,* 2006). Intrauterine injection of hCG with the embryo culture media prior to embryo transfer during ART improves the success of ART cycles (Mansour *et al.,* 2011, Santibañez *et al.,* 2014). Our study differed from those adding hCG to embryo culture media. We developed an intrauterine flushing technique with LMWH as a novel method of administration, to improve endometrial preparation for pregnancy. This method resulted in the local administration of the drug. Currently, heparin is only licensed for intravenous or subcutaneous usage. It is not licensed for intrauterine use. We therefore needed to do a Phase 1 feasibility and patient acceptability study to determine if it is safe and acceptable to use this method of administration. To enable this study to be carried out a protocol was written. The study required sponsorship and approval by the MHRA UK, NRES and local R&D. Obtaining these approvals was a lengthy process. This required individual training (Good Clinical Practice course, Principle Investigator course) to undertake this study. This study required multidisciplinary collaboration with haematology, R&D, statistician, pharmacy, histopathology, gynaecology, reproductive medicine and the manufacturer of LMWH (enoxaparin). The study required support from colleagues, administration team, healthcare assistants and nursing staff. I wrote all the forms including protocol for the study with Professor Quenby and obtained approval from NRES, MHRA UK and Local R&D. Following these approvals recruitment to the study was not difficult and achieved within six months. As this was a feasibility study, there were 42 participants. Study participants were randomised in to two groups

(control and treatment). Both interventions were performed in women during a non-conception cycle. Women underwent ART if they wished to after the study. Our endometrial flushing technique was based on the HyCoSy technique. An endometrial biopsy was obtained 24 hours later. Most women described mild pain during endometrial biopsy as expected but found intrauterine flushing tolerable.

Anti-Xa assay levels measured the anticoagulant activity of LMWH. Anti-Xa levels were higher in the treatment group when compared to the control group but remained in the thromboprophylactic range rather than in the therapeutic range for the LMWH. Prior to the study, the research team had previously found that uNK cell density could act as a marker of adequate decidualisation (Kuroda *et al.,* 2013). Hence the primary outcome of the study was the uNK cell density. This was found not to be different in the two groups. The women who were in the intervention group had a heavier period following flushing than control group suggesting a biological effect of enoxaparin. Following the study nine women have given birth. Other secondary outcomes included the measurement of the implantation markers PRL, HB-EGF and SGK-1 which will be done in the future. If evidence of a positive effect of heparin on decidualisation emerges then a phase 2 study could be undertaken in the future.

We have established that endometrial flushing is an acceptable mode of delivery of a medication to treat the endometrium. The recruitment to the study was successful. Intrauterine flushing is a feasible, well tolerated procedure. No

adverse events occurred during the study suggesting intrauterine flushing of LMWH is safe. There was pharmacological and biological effects with elevated Anti- Xa levels and subsequent heavy periods seen in participants who had intrauterine flushing with LMWH compared to those participants who had intrauterine flushing with normal saline. The most interesting phenomenon witnessed in the study was how quickly the intrauterine flushing fluid was absorbed (within 2 minutes of flushing). Unfortunately, we did not take consent for videos in our study so are unable to show this. This could suggest that a luteal phase endometrium may be targeted for therapeutic intervention in future.

I have not obtained evidence that heparin administered this way is beneficial to endometrial preparation for pregnancy. This could be due to heterogeneity of the study participants comprised of women with recurrent miscarriages and subfertile women. Even subfertile women had only at least one unsuccessful ART only. This could have attributed to non-significance between the drug (LMWH) and placebo (normal saline) of the primary outcome. Changes in markers of decidualistaion does not equate to improvement in live birth rate, so this study looked at improving endometrial receptivity for improving implantation. Study methodology had only two groups with interventions and both groups had intrauterine flushing either with LMWH or placebo. This might have contributed to the non-significance of primary outcome. Mechanical disruption of endometrium rather than any pharmacological effect could have led to these results. We should have another group in the study with no intrauterine flushing intervention so to compare with the other two groups. We also could have

performed intrauterine flushing one day earlier than we did so that we had 48 hrs before obtaining endometrial biopsy. This could have given more time to detect any effect in the endometrial stroma.

In future, a multicentre RCT should be undertaken. The RCT (350 participants in each group) should compare LMWH with placebo during ART. Subcutaneous daily LMWH should be given day after oocyte retrieval until pregnancy test and continued until a clinical pregnancy is visualised on ultrasonography. The study population for this study should be women with previous unsuccessful ART with at least 4 cleavage stage embryos transferred or 2 blastocyst embryos transferred).

In future, intrauterine flushing of LMWH or any other medication (for example hCG) should be undertaken in three groups (LMW, placebo and no intervention) in a phase 1 study to look at markers of decidualisation. The study population for this study should be homogeneous comprised of women with previous unsuccessful ART with at least 4 cleavage stage embryos transferred or 2 blastocyst embryos transferred. There should be at least 48 hrs between intrauterine flushing and endometrial biopsy in the study.

References

Achache, H. & Revel, A. (2006) Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update*, 12 (6): 731-746.

Agrawal, R., Tan, S. L., Wild, S., Sladkevicius, P., Engmann, L., Payne, N., Bekir, J., Campbell, S., Conway, G. & Jacobs, H. (1999) Serum vascular endothelial growth factor concentrations in in vitro fertilization cycles predict the risk of ovarian hyperstimulation syndrome. *Fertil Steril*, 71 (2): 287-293.

Aitken, R. J., Baker, M. A., Doncel, G. F., Matzuk, M. M., Mauck, C. K. & Harper, M. J. (2008) As the world grows: contraception in the 21st century. *J Clin Invest*, 118 (4): 1330-1343.

Akhtar, M. A., Sur, S., Raine-Fenning, N., Jayaprakasan, K., Thornton, J. G. & Quenby, S. (2013) Heparin for assisted reproduction. *Cochrane Database Syst Rev*, (8): CD009452.

Akhtar, M. A., Sur, S., Raine-Fenning, N., Jayaprakasan, K., Thornton, J., Quenby, S. & Marjoribanks, J. (2014) Heparin for assisted reproduction: summary of a Cochrane review. *Fertil Steril*, *in Press*

Allen, V. M., Wilson, R. D. & Cheung, A. (2006) Pregnancy outcomes after assisted reproductive technology. *J Obstet Gynaecol Can*, 28 (3): 220-250.

Aplin, J. D. & Kimber, S. J. (2004) Trophoblast-uterine interactions at implantation. *Reprod Biol Endocrinol*, 2: 48.

Arai, T., Parker, A., Busby, W., Jr. & Clemmons, D. R. (1994) Heparin, heparan sulfate, and dermatan sulfate regulate formation of the insulin-like growth factor-I and insulin-like growth factor-binding protein complexes. *J Biol Chem*, 269 (32): 20388-20393.

Armant, D. R., Kilburn, B. A., Petkova, A., Edwin, S. S., Duniec-Dmuchowski, Z.M., Edwards, H. J., Romero, R. & Leach, R. E. (2006) Human trophoblast survival at low oxygen concentrations requires metalloproteinase-mediated shedding of heparin-binding EGF-like growth factor. Development, 133 (4):751-759.

ASRM. Practice Committee of American Society of Reproductive Medicine. (2008) Anti-phospholipid antibodies do not affect IVF success. *Fertil Steril*, 90 (5 suppl): S172-173.

ASRM. Practice Committee of American Society for Reproductive Medicine Committee (2012) Elective single-embryo transfer. *Fertil Steril*, 97 (4): 835-842.

Aviezer, D. & Yayon, A. (1994) Heparin-dependent binding and autophosphorylation of epidermal growth factor (EGF) receptor by heparin-binding EGF-like growth factor but not by EGF. *Proc Natl Acad Sci U S A*, 91 (25): 12173-12177.

Azem, F., Many, A., Ben Ami, I., Yovel, I., Amit, A., Lessing, J. B. & Kupferminc, M. J. (2004) Increased rates of thrombophilia in women with repeated IVF failures. *Hum Reprod*, 19 (2): 368-370.

Berker, B., Taskin, S., Kahraman, K., Taskin, E. A., Atabekoglu, C. & Sonmezer, M. (2011) The role of low-molecular-weight heparin in recurrent implantation failure: a prospective, quasi-randomized, controlled study. *Fertil Steril*, 95 (8): 2499-2502.

Berkkanoglu, M., Isikoglu, M., Seleker, M. & Ozgur, K. (2006) Flushing the endometrium prior to the embryo transfer does not affect the pregnancy rate. *Reprod Biomed Online*, 13 (2): 268-271.

Bick, R. L., Frenkel, E. P., Walenga, J., Fareed, J. & Hoppensteadt, D. A. (2005) Unfractionated heparin, low molecular weight heparins, and pentasaccharide: basic mechanism of actions, pharmacology, and clinical use. *Hematol Oncol Clin North Am*, 19 (1): 1-51.

Birdsall, M. A., Lockwood, G. M., Ledger, W. L., Johnson, P. M. & Chamley, L. W. (1996) Antiphospholipid antibodies in women having in-vitro fertilization. *Hum Reprod*, 11 (6): 1185-1189.

Bohlmann, M. K. (2011) Effects and effectiveness of heparin in assisted reproduction. *J Reprod Immunol*, 90 (1): 82-90.

Bokoch, G. M. (1995) Chemoattractant signaling and leukocyte activation. *Blood*, 86 (5): 1649-1660.

Boomsma, C. M., Kavelaars, A., Eijkemans, M. J., Amarouchi, K., Teklenburg, G., Gutknecht, D., Fauser, B. J., Heijnen, C. J. & Macklon, N. S. (2009a) Cytokine profiling in endometrial secretions: a non-invasive window on endometrial receptivity. *Reprod Biomed Online*, 18 (1): 85-94.

Boomsma, C. M., Kavelaars, A., Eijkemans, M. J., Lentjes, E. G., Fauser, B. C., Heijnen, C. J. & Macklon, N. S. (2009b) Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. *Hum Reprod*, 24 (6): 1427-1435.

Brosens, J. J., Hayashi, N. & White, J. O. (1999) Progesterone receptor regulates decidual prolactin expression in differentiating human endometrial stromal cells. *Endocrinology*, 140 (10): 4809-4820.

Brosens, J. J., Pijnenborg, R. & Brosens, I. A. (2002) The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *Am J Obstet Gynecol*, 187 (5): 1416-1423.

Brosens, J. J., Salker, M. S., Teklenburg, G., Nautiyal, J., Salter, S., Lucas, E. S., Steel, J. H., Christian, M., Chan, Y. W., Boomsma, C. M., Moore, J. D., Hartshorne, G. M., Sućurović, S., Mulac-Jericevic, B., Heijnen, C. J., Quenby, S., Koerkamp, M. J., Holstege, F. C., Shmygol, A. & Macklon, N.S. (2014) Uterine selection of human embryos at implantation. *Sci Rep,* 4: 3894.

Buckingham, K. L., Stone, P. R., Smith, J. F. & Chamley, L. W. (2006) Antiphospholipid antibodies in serum and follicular fluid--is there a correlation with IVF implantation failure? *Hum Reprod*, 21 (3): 728-734.

Caccavo, D., Pellegrino, N. M., Lorusso, F., Capotorto, M., Vacca, M., Vimercati, A. & Depalo, R. (2007) Anticardiolipin antibody levels in women undergoing first in vitro fertilization/embryo transfer. *Hum Reprod*, 22 (9): 2494-2500.

Call, D. R. & Remick, D. G. (1998) Low molecular weight heparin is associated with greater cytokine production in a stimulated whole blood model. *Shock*, 10 (3): 192-197.

Cha, J., Sun, X. & Dey, S. K. (2012) Mechanisms of implantation: strategies for successful pregnancy. *Nat Med*, 18 (12): 1754-1767.

Chobotova, K., Karpovich, N., Carver, J., Manek, S., Gullick, W. J., Barlow, D. H. & Mardon, H. J. (2005) Heparin-binding epidermal growth factor and its receptors mediate decidualization and potentiate survival of human endometrial stromal cells. *J Clin Endocrinol Meta,* 90: 913–919.

Colicchia, A., Pergolini, I., Gilio, B., Rampini, M., Alfano, P. & Marconi, D. (2011) Role of heparin in embryo implantation in women without thrombophilia: A pilot study. 27th Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE) Conference: Conference Publication, pp i157.

Collins, J. A. & Van Steirteghem, A. (2004) Overall prognosis with current treatment of infertility. *Hum Reprod Update*, 10 (4): 309-316.

Comper, W. D. (1981) *Heparin (and related polysaccharides) structural and functional properties.* Gordon and Breach Science Publishers Ltd.

Conaghan, J., Chen, A. A., Willman, S. P., Ivani, K., Chenette, P. E., Boostanfar, R., Baker, V. L., Adamson, G. D., Abusief, M. E., Gvakharia, M., Loewke, K. E. & Shen, S. (2013) Improving embryo selection using a computer-automated time-lapse image analysis test plus day 3 morphology: results from a prospective multicenter trial. *Fertil Steril*, 100 (2): 412-419.

Cooper, M. A., Fehniger, T. A., Turner, S. C., Chen, K. S., Ghaheri, B. A., Ghayur, T., Carson, W. E. & Caligiuri, M. A. (2001) Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. *Blood*, 97 (10): 3146-3151.

Corvinus, F. M., Fitzgerald, J. S., Friedrich, K. & Markert, U. R. (2003) Evidence for a correlation between trophoblast invasiveness and STAT3 activity. *Am J Reprod Immunol*, 50 (4): 316-321.

Coulam, C. B., Jeyendran, R. S., Fishel, L. A. & Roussev, R. (2006) Multiple thrombophilic gene mutations are risk factors for implantation failure. *Reprod Biomed Online*, 12 (3): 322-327.

Cutting, R., Morroll, D., Roberts, S. A., Pickering, S. & Rutherford, A. (2008) Elective single embryo transfer: guidelines for practice British Fertility Society and Association of Clinical Embryologists. *Hum Fertil (Camb)*, 11 (3): 131-146.

Das, S. K. (2009) Cell cycle regulatory control for uterine stromal cell decidualization in implantation. *Reproduction*, 137 (6): 889-899.

de Klerk, C., Macklon, N. S., Heijnen, E. M., Eijkemans, M. J., Fauser, B. C., Passchier, J. & Hunfeld, J. A. (2007) The psychological impact of IVF failure after two or more cycles of IVF with a mild versus standard treatment strategy. *Hum Reprod*, 22 (9): 2554-2558.

Denis, A. L., Guido, M., Adler, R. D., Bergh, P. A., Brenner, C. & Scott, R. T., Jr. (1997) Antiphospholipid antibodies and pregnancy rates and outcome in in vitro fertilization patients. *Fertil Steril*, 67 (6): 1084-1090.

Devroey, P., Fauser, B. C. & Diedrich, K. (2009) Approaches to improve the diagnosis and management of infertility. *Hum Reprod Update*, 15 (4): 391-408.

Di Simone, N., Di Nicuolo, F., Sanguinetti, M., Ferrazzani, S., D'Alessio, M. C., Castellani, R., Bompiani, A. & Caruso, A. (2007) Low-molecular weight heparin induces in vitro trophoblast invasiveness: role of matrix metalloproteinases and tissue inhibitors. *Placenta*, 28 (4): 298-304.

Dimitriadis, E., Stoikos, C., Baca, M., Fairlie, W. D., McCoubrie, J. E. & Salamonsen, L.A. (2005) Relaxin and prostaglandin E2 regulate interleukin 11 during human endometrial stromal cell decidualization. *J Clin Endocrinol Metab,* 90: 3458–3465.

Dimitriadis, E., Stoikos, C., Stafford-Bell, M., Clark, I., Paiva, P., Kovacs, G. & Salamonsen, L.A. (2006) Interleukin-11, IL-11 receptor[alpha] and leukemia inhibitory factor are dysregulated in endometrium of infertile women with endometriosis during the implantation window. *J Reprod Immunol*, 69: 53–64.

Drury, J. A., Nik, H., van Oppenraaij, R. H., Tang, A. W., Turner, M. A. & Quenby, S. (2011) Endometrial cell counts in recurrent miscarriage: a comparison of counting methods. *Histopathology*, 59 (6): 1156-1162.

Egger, M., Davey Smith, G., Schneider, M. & Minder, C. (1997) Bias in meta-analysis detected by a simple, graphical test. *BMJ*, 315 (7109): 629-634.

El-Toukhy, T. & Taranissi, M. (2006) Towards better quality research in recurrent implantation failure: standardizing its definition is the first step. *Reprod Biomed Online*, 12 (3): 383-385.

Empson, M., Lassere, M., Craig, J. & Scott, J. (2005) Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. *Cochrane Database Syst Rev*, (2): CD002859.

Erden, O., Imir, A., Guvenal, T., Muslehiddinoglu, A., Arici, S., Cetin, M. & Cetin, A. (2006) Investigation of the effects of heparin and low molecular weight heparin on E-cadherin and laminin expression in rat pregnancy by immunohistochemistry. *Hum Reprod*, 21 (11): 3014-3018.

EudraLex (2003) Volume 4 Good manufacturing practice (GMP) Guidelines, Commission Directive 2003/94/EC [On-line]: Available: http://www.euvaccine.eu/sites/default/files/uploads/docs/EC_-_GMP_Annex_13_final_24-02-05.pdf Accessed: 1 December 2014

Fatemi, H. M. & Popovic-Todorovic, B. (2013) Implantation in assisted reproduction: a look at endometrial receptivity. *Reprod Biomed Online*, 27 (5): 530-538.

Fiedler, K. & Wurfel, W. (2004) Effectivity of heparin in assisted reproduction. *Eur J Med Res*, 9 (4): 207-214.

Fisch, B., Fried, S., Manor, Y., Ovadia, J., Witz, I. P. & Yron, I. (1995) Increased antiphospholipid antibody activity in in-vitro fertilization patients is not treatment-dependent but rather an inherent characteristic of the infertile state. *Am J Reprod Immunol*, 34 (6): 370-374.

Fluhr, H., Spratte, J., Ehrhardt, J., Steinmuller, F., Licht, P. & Zygmunt, M. (2010) Heparin and low-molecular-weight heparins modulate the decidualization of human endometrial stromal cells. *Fertil Steril*, 93 (8): 2581-2587.

Fluhr, H., Spratte, J., Heidrich, S., Ehrhardt, J., Greinacher, A. & Zygmunt, M. (2011a) The molecular charge and size of heparins determine their impact on the decidualization of human endometrial stromal cells. *Mol Hum Reprod*, 17 (6): 354-359.

Fluhr, H., Spratte, J., Heidrich, S., Ehrhardt, J., Steinmuller, F. & Zygmunt, M. (2011b) Heparin inhibits interferon-gamma signaling in human endometrial stromal cells by interference with the cellular binding of interferon-gamma. *Fertil Steril*, 95 (4): 1272-1277.

Fowden, A. L. (2003) The insulin-like growth factors and feto-placental growth. *Placenta,* 24 (8-9): 803-12.

Freour, T., Lammers, J., Splingart, C., Jean, M. & Barriere, P. (2012) Time lapse Embryoscope as a routine technique in the IVF laboratory: A useful tool for better embryo selection? *Gynecol Obstet Fertil*, 40 (9): 476-480.

Frydman, R. (2004) Impact of embryo transfer techniques on implantation rates. *J Gynecol Obstet Biol Reprod (Paris)*, 33 (1 Pt 2): S36-9.

Gallagher, J. T. & Walker, A. (1985) Molecular distinctions between heparan sulphate and heparin. Analysis of sulphation patterns indicates that heparan sulphate and heparin are separate families of N-sulphated polysaccharides. *Biochem J*, 230 (3): 665-674.

Gellersen, B., Brosens, I. A. & Brosens, J. J. (2007) Decidualization of the human endometrium: mechanisms, functions, and clinical perspectives. *Semin Reprod Med*, 25 (6): 445-453.

Germeyer, A., Klinkert, M. S., Huppertz, A. G., Clausmeyer, S., Popovici, R. M., Strowitzki, T. & von Wolff, M. (2007) Expression of syndecans, cell-cell interaction regulating heparan sulfate proteoglycans, within the human endometrium and their regulation throughout the menstrual cycle. *Fertil Steril*, 87 (3): 657-663.

Gleicher, N., Liu, H. C., Dudkiewicz, A., Rosenwaks, Z., Kaberlein, G., Pratt, D. & Karande, V. (1994) Autoantibody profiles and immunoglobulin levels as predictors of in vitro fertilization success. *Am J Obstet Gynecol,* 170 (4): 1145-1149.

Glujovsky, D., Blake, D., Farquhar, C. & Bardach, A. (2012) Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. *Cochrane Database Syst Rev*, (7): CD002118.

Goto, S., Kadowaki, T., Hashimoto, H., Kokeguchi, S. & Shiotani, M. (2009) Stimulation of endometrium embryo transfer can improve implantation and pregnancy rates for patients undergoing assisted reproductive technology for the first time with a high-grade blastocyst. *Fertil Steril*, 92 (4): 1264-1268.

Grady, R., Alavi, N., Vale, R., Khandwala, M. & McDonald, S. D. (2012) Elective single embryo transfer and perinatal outcomes: a systematic review and meta-analysis. *Fertil Steril*, 97 (2):324-331.

Grandone, E., Colaizzo, D., Lo Bue, A., Checola, M. G., Cittadini, E. & Margaglione, M. (2001) Inherited thrombophilia and in vitro fertilization implantation failure. *Fertil Steril*, 76 (1): 201-202.

Hamilton, G. S., Lysiak, J.,J., Han, V.K. & Lala, P. K. (1998) Autocrine-paracrine regulation of human trophoblast invasiveness by insulin-like growth factor (IGF)-II and IGF-binding protein (IGFBP)-1. *Exp Cell Res,* 244 (1):147-56.

Helmerhorst, F. M., Perquin, D. A., Donker, D. & Keirse, M. J. (2004) Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ*, 328 (7434): 261.

Higgins, J.P.T. & Green, S. (2011) Cochrane Handbook for Systematic Reviews of Intervention. [On-line] Available: http://www.cochrane.org/training/cochrane-handbook Accessed: 1 December 2014

Hornstein, M. D. (2000) Antiphospholipid antibodies in patients undergoing IVF: the data do not support testing. *Fertil Steril*, 74 (4): 635-636.

Hornstein, M. D., Davis, O. K., Massey, J. B., Paulson, R. J. & Collins, J.A. (2000) Antiphospholipid antibodies and in vitro fertilization success: a meta-analysis. *Fertil Steril,* 73 (2): 330-3.

Hovanessian, H. C. (1999) New-generation anticoagulants: the low molecular weight heparins. *Ann Emerg Med*, 34 (6): 768-779.

Human Fertilisation and Embryo Authority (HEFA). (2010) *Fertility Treatment in 2010*. [online] Available: http://www.hfea.gov.uk/docs/2011-11-16_-_Annual_Register_Figures_Report_final.pdf Accessed: 1 December 2014

Iwamoto, R. & Mekada, E. (2000) Heparin-binding EGF-like growth factor: a juxtacrine growth factor. *Cytokine Growth Factor Rev,* 11:335–344.

Jackson, R. A., Gibson, K. A., Wu, Y. W. & Croughan, M. S. (2004) Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol*, 103 (3): 551-563.

Jha, R.K., Titus, S., Saxena, D., Kumar, P.G. & Laloraya, M. (2006) Profiling of E-cadherin, beta-catenin and Ca(2+) in embryo-uterine interactions at implantation. *FEBS Lett*, 580 (24): 5653-60.

Jorpes, E. (1935). The chemistry of heparin. *The Biochemical Journal* 29 (8): 1817-1830.

Kaandorp, S., Di Nisio, M., Goddijn, M. & Middeldorp, S. (2009) Aspirin or anticoagulants for treating recurrent miscarriage in women without antiphospholipid syndrome. *Cochrane Database Syst Rev*, (1): CD004734.

Kamel, R. M. (2010) Management of the infertile couple: an evidence-based protocol. *Reprod Biol Endocrinol*, 8: 21.

Katalinic, A., Rosch, C. & Ludwig, M. (2004) Pregnancy course and outcome after intracytoplasmic sperm injection: a controlled, prospective cohort study. *Fertil Steril*, 81 (6): 1604-1616.

Kelly, F. D., Tawia, S. A. & Rogers, P. A. (1995) Immunohistochemical characterization of human endometrial microvascular basement membrane components during the normal menstrual cycle. *Hum Reprod*, 10 (2): 268-276.

Kennedy, R. (2005) Risks and Benefits of Assisted Reproduction. British Fertility Society Fact sheet [On-line]: UK: Available:

http://www.britishfertilitysociety.org.uk/public/factsheets/docs/BFS-risks%20and%20complications%20of%20assisted%20conception%20.pdf
Accessed: 1 December 2014

Kimber, S. J. (2005) Leukaemia inhibitory factor in implantation and uterine biology. *Reproduction*, 130 (2): 131-145.

King, A. (2000) Uterine leukocytes and decidualization. *Hum Reprod Update*, 6 (1): 28-36.

Kowalik, A., Vichnin, M., Liu, H. C., Branch, W. & Berkeley, A. S. (1997) Midfollicular anticardiolipin and antiphosphatidylserine antibody titers do not correlate with in vitro fertilization outcome. *Fertil Steril*, 68 (2): 298-304.

Kupka, M. S., Ferraretti, A. P., de Mouzon, J., Erb, K., D'Hooghe, T., Castilla, J. A., Calhaz-Jorge, C., De Geyter, C. & Goossens, V. (2014) Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. *Hum Reprod,* 29 (10):2099-113.

Kuroda, K., Venkatakrishnan, R., James, S., Sucurovic, S., Mulac-Jericevic, B., Lucas, E. S., Takeda, S., Shmygol, A., Brosens, J. J. & Quenby, S. (2013) Elevated periimplantation uterine natural killer cell density in human endometrium is associated with impaired corticosteroid signaling in decidualizing stromal cells. *J Clin Endocrinol Metab*, 98 (11): 4429-4437.

Kutteh, W. H., Yetman, D. L., Chantilis, S. J. & Crain, J. (1997) Effect of antiphospholipid antibodies in women undergoing in-vitro fertilization: Role of heparin and aspirin. *Hum Reprod*, 12 (6): 1171-1175.

Lacey, H., Haigh, T., Westwood, M. & Aplin, J.D. (2002) Mesenchymally-derived insulin-like growth factor 1 provides a paracrine stimulus for trophoblast migration. *BMC Dev Biol,* 2: 5.

Lai, T. H., King, J. A., Shih Ie, M., Vlahos, N. F. & Zhao, Y. (2007) Immunological localization of syndecan-1 in human endometrium throughout the menstrual cycle. *Fertil Steril*. 87 (1): 121-126.

Land, J. A. & Evers, J. L. (2003) Risks and complications in assisted reproduction techniques: Report of an ESHRE consensus meeting. *Hum Reprod*, 18 (2): 455-457.

Lash, G.E., Otun, H. A., Innes, B. A., Bulmer, J. N., Searle, R. F. & Robson, S. C. (2005) Inhibition of trophoblast cell invasion by TGFB1, 2, and 3 is associated with a decrease in active proteases. *Biol Reprod*, 73: 374–381.

Leach, R. E., Kilburn, B., Wang, J., Liu, Z., Romero, R. & Armant, D. R. (2004) Heparin-binding EGF-like growth factor regulates human extravillous cytotrophoblast development during conversion to the invasive phenotype. *Dev Biol*, 266 (2): 223-237.

Ledee-Bataille, N., Lapree-Delage, G., Taupin, J. L., Dubanchet, S., Frydman, R. & Chaouat, G. (2002) Concentration of leukaemia inhibitory factor (LIF) in uterine flushing fluid is highly predictive of embryo implantation. *Hum Reprod*, 17 (1): 213-218.

Lee, S. R., Park, E. J., Kim, S. H., Chae, H., Kim, C. H. & Kang, B. M. (2007) Influence of antiphospholipid antibodies on pregnancy outcome in women undergoing in vitro fertilization and embryo transfer. *Am J Reprod Immunol*, 57 (1): 34-39.

Li, T. C., MacKenna, A. & Roberts, R. (1993) The techniques and complications of out-patient uterine washing in the assessment of endometrial function. *Hum Reprod*, 8 (3): 343-346.

Liang, A., Du, Y., Wang, K. & Lin, B. (2006) Quantitative investigation of the interaction between granulocyte-macrophage colony-stimulating factor and heparin by capillary zone electrophoresis. *J Sep Sci*, 29 (11): 1637-1641.

Lockwood, C. J., Romero, R., Feinberg, R. F., Clyne, L. P., Coster, B. & Hobbins, J. C. (1989) The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. *Am J Obstet Gynecol*, 161 (2): 369-373.

Lodigiani, C., Di Micco, P., Ferrazzi, P., Librè, L., Arfuso, V. & Polatti, F. (2011) Low-molecular-weight heparin in women with repeated implantation failure. *Womens Health (Lond Engl)* 7 (4):425– 31.

Ly, K., Aziz, N., Safi, J. & Agarwal, A. (2010) Evidence-Based Management of Infertile Couples. *Current Women's Health Reviews*, 6: 200-218.

Macklon, N. S., Geraedts, J. P. & Fauser, B. C. (2002) Conception to ongoing pregnancy: the 'black box' of early pregnancy loss. *Hum Reprod Update*, 8 (4): 333-343.

Mansour, R., Tawab, N., Kamal, O., El-Faissal, Y., Serour, A., Aboulghar, M. & Serour, G. (2011) Intrauterine injection of human chorionic gonadotropin before embryo transfer significantly improves the implantation and pregnancy rates in in vitro fertilization/intracytoplasmic sperm injection: a prospective randomized study. *Fertil Steril*, 96 (6): 1370-1374.

Marci, R., Marcucci, I., Marcucci, A. A., Pacini, N., Salacone, P., Sebastianelli, A., Caponecchia, L., Lo Monte, G. & Rago, R. (2013) Hysterosalpingocontrast sonography (HyCoSy): evaluation of the pain perception, side effects and complications. *BMC Med Imaging*, 13: 28.

Margalioth, E. J., Ben-Chetrit, A., Gal, M. & Eldar-Geva, T. (2006) Investigation and treatment of repeated implantation failure following IVF-ET. *Hum Reprod*, 21 (12): 3036-3043.

Mashayekhy, M., Dehghani, F. & Ghasemi, N. (Spring 2011) The effect of heparin in treatment of recurrent IVF-ET failure. *Iranian Journal of Reproductive Medicine*, 9 (Suppl 2): 30-30.

McBride, W. T., Armstrong, M. A. & McMurray, T. J. (1996) An investigation of the effects of heparin, low molecular weight heparin, protamine, and fentanyl on the balance of pro- and anti-inflammatory cytokines in in-vitro monocyte cultures. *Anaesthesia*, 51 (7): 634-640.

Meseguer, M., Aplin, J. D., Caballero-Campo, P., O'Connor, J. E., Martin, J. C., Remohi, J., Pellicer, A. & Simon, C. (2001) Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biol Reprod*, 64 (2): 590-601.

Mikolajczyk, M., Wirstlein, P. & Skrzypczak, J. (2007) The impact of leukemia inhibitory factor in uterine flushing on the reproductive potential of infertile women--a prospective study. *Am J Reprod Immunol*, 58 (1): 65-74.

Møller, A. V., Jorgensen, S. P., Chen, J. W., Larnkjaer, A., Ledet, T., Flyvbjerg, A. & Frystyk, J. (2006) Glycosaminoglycans increase levels of free and bioactive IGF-I in vitro. *Eur J Endocrinol*, 155 (2): 297-305.

Murray, W. J., Lindo, V. S., Kakkar, V. V. & Melissari, E. (1995) Long-term administration of heparin and heparin fractions and osteoporosis in experimental animals. *Blood Coagul Fibrinolysis*, 6 (2): 113-118.

Nardo, L. G., Granne, I. & Stewart, J. (2009) Medical adjuncts in IVF: evidence for clinical practice. *Hum Fertil (Camb)*, 12 (1): 1-13.

Nastri, C. O., Gibreel, A., Raine-Fenning, N., Maheshwari, A., Ferriani, R. A., Bhattacharya, S. & Martins, W. P. (2012) Endometrial injury in women undergoing assisted reproductive techniques. *Cochrane Database Syst Rev*, (7): CD009517.

National Institute for Health and Care Excellence (NICE) guideline CG 156 (2013) *Fertility: Assessment and Treatment for People with Fertility Problems*. [On-line]: UK: Available: https://www.nice.org.uk/guidance/cg156 Accessed: 1 December 2014

Navot, D., Drews, M. R., Bergh, P. A., Guzman, I., Karstaedt, A., Scott, R. T., Jr., Garrisi, G. J. & Hofmann, G. E. (1994) Age-related decline in female fertility is not due to diminished capacity of the uterus to sustain embryo implantation. *Fertil Steril*, 61 (1): 97-101.

Nelson, S. M. & Greer, I. A. (2008) The potential role of heparin in assisted conception. *Human Reprod Update*, 14 (6): 623-645.

Nip, M. M., Taylor, P. V., Rutherford, A. J. & Hancock, K. W. (1995) Autoantibodies and antisperm antibodies in sera and follicular fluids of infertile patients; relation to reproductive outcome after in-vitro fertilization. *Hum Reprod*, 10 (10): 2564-2569.

Noci, I., Milanini, M. N., Ruggiero, M., Papini, F., Fuzzi, B. & Artini, P. G. (2011) Effect of dalteparin sodium administration on IVF outcome in non-thrombophilic young women: a pilot study. *Reprod Biomed Online*, 22 (6): 615-620.

Noyes, N., Hampton, B. S., Berkeley, A., Licciardi, F., Grifo, J. & Krey, L. (2001) Factors useful in predicting the success of oocyte donation: a 3-year retrospective analysis. *Fertil Steril*, 76 (1): 92-97.

Office for National Statistics (2013) *Births in England and Wales 2013* [On-line]: UK: Available: http://www.ons.gov.uk/ons/rel/vsob1/birth-summary-tables--england-and-wales/2013/info-births-2013.html Accessed: 1 December 2014

Ogasawara, M. & Aoki, K. (2000) Successful uterine steroid therapy in a case with a history of ten miscarriages. *Am J Reprod Immunol*, 44 (4): 253-255.

Ola, B. & Li, T. C. (2006) Implantation failure following in-vitro fertilization. *Curr Opin Obstet Gynecol*, 18 (4): 440-445.

Olivennes, F., Ledee-Bataille, N., Samama, M., Kadoch, J., Taupin, J. L., Dubanchet, S., Chaouat, G. & Frydman, R. (2003) Assessment of leukemia inhibitory factor levels by uterine flushing at the time of egg retrieval does not adversely affect pregnancy rates with in vitro fertilization. *Fertil Steril*, 79 (4): 900-904.

Oliver, C., Montes, M. J., Galindo, J. A., Ruiz, C. & Olivares, E. G. (1999) Human decidual stromal cells express alpha-smooth muscle actin and show ultrastructural similarities with myofibroblasts. *Hum Reprod*, 14 (6): 1599-1605.

Ordi, J., Casals, G., Ferrer, B., Creus, M., Guix, C., Palacin, A., Campo, E. & Balasch, J. (2006) Uterine (CD56+) natural killer cells recruitment: association with decidual reaction rather than embryo implantation. *Am J Reprod Immunol*, 55 (5): 369-377.

Pannucci, C. J. & Wilkins, E. G. (2010) Identifying and avoiding bias in research. *Plast Reconstr Surg*, 126 (2): 619-625.

Parham, P. (2004) NK cells and trophoblasts: partners in pregnancy. *J Exp Med*, 200 (8): 951-955.

Pecly, I. M., Goncalves, R. G., Rangel, E.P., Takiya, C.M., Taboada, F. S., Martinusso, C. A., Pavao, M. S. G. & Leite, M. (2006) Effects of low molecular weight heparin in obstructed kidneys: decrease of collagen, fibronectin and TGF-beta, and increase of chondroitin/dermatan sulfate proteoglycans and macrophage infiltration. *Nephrol Dial Transplant,* 21 (5): 1212-1222.

Pijnenborg, R. (2002) Implantation and immunology: maternal inflammatory and immune cellular responses to implantation and trophoblast invasion. *Reprod Biomed Online*, 4 (suppl 3): 14-17.

Pinborg, A. (2005) IVF/ICSI twin pregnancies: risks and prevention. In: eds. *Hum Reprod Update*, 11 (6): 575-593.

Poehlmann, T. G., Fitzgerald, J. S., Meissner, A., Wengenmayer, T., Schleussner, E., Friedrich, K. & Markert, U. R. (2005) Trophoblast invasion: tuning through LIF, signalling via Stat3. In: eds. *Placenta*, 26 (suppl A): S37-41.

Polanski, L. T., Barbosa, M. A., Martins, W. P., Baumgarten, M. N., Campbell, B., Brosens, J., Quenby, S. & Raine-Fenning, N. (2014) Interventions to improve reproductive outcomes in women with elevated natural killer cells undergoing assisted reproduction techniques: a systematic review of literature. *Hum Reprod*, 29 (1): 65-75.

Polanski, L. T., Baumgarten, M. N., Quenby, S., Brosens, J., Campbell, B.K. & Raine-Fenning, N. J. (2014) What exactly do we mean by 'recurrent implantation failure'? A systematic review and opinion. *Reprod Biomed Online,* 28 (4): 409-23.

Poli, A., Michel, T., Theresine, M., Andres, E., Hentges, F. & Zimmer, J. (2009) CD56 bright natural killer (NK) cells: an important NK cell subset. *Immunology*, 126 (4): 458-465.

Potter, S. W. & Morris, J. E. (1992) Changes in histochemical distribution of cell surface heparan sulfate proteoglycan in mouse uterus during the estrous cycle and early pregnancy. *Anat Rec*, 234 (3): 383-390.

Qublan, H., Amarin, Z., Dabbas, M., Farraj, A. E., Beni-Merei, Z., Al-Akash, H., Bdoor, A. N., Nawasreh, M., Malkawi, S. & Diab, F. (2008) Low-molecular-weight heparin in the treatment of recurrent IVF-ET failure and thrombophilia: a prospective randomized placebo-controlled trial. *Human Fertility*, 11 (4): 246-253.

Quenby, S. & Brosens, J. J. (2013) Human implantation: a tale of mutual maternal and fetal attraction. *Biol Reprod*, 88 (3): 81.

Quenby, S. & Farquharson, R. (2006) Uterine natural killer cells, implantation failure and recurrent miscarriage. *Reprod Biomed Online*, 13 (1): 24-28.

Quenby, S., Kalumbi, C., Bates, M., Farquharson, R. & Vince, G. (2005) Prednisolone reduces preconceptual endometrial natural killer cells in women with recurrent miscarriage. *Fertil Steril,* 84 (4): 980-4.

Quenby, S., Mountfield, S., Cartwright, J. E., Whitley, G. S. & Vince, G. (2004) Effects of low-molecular-weight and unfractionated heparin on trophoblast function. *Obstet Gynecol*, 104 (2): 354-361.

Quenby, S., Nik, H., Innes, B., Lash, G., Turner, M., Drury, J. & Bulmer, J. (2009) Uterine natural killer cells and angiogenesis in recurrent reproductive failure. *Hum Reprod*, 24 (1): 45-54.

Rajgopal, R., Butcher, M., Weitz, J. I. & Shaughnessy, S. G. (2006) Heparin synergistically enhances interleukin-11 signaling through up-regulation of the MAPK pathway. *J Biol Chem*, 281 (30): 20780-20787.

Revel, A. (2012) Defective endometrial receptivity. *Fertil Steril*, 97 (5): 1028-1032.

Ricci, G., Giolo, E. & Simeone, R. (2010) Heparin's potential to improve pregnancy rates and outcomes is not evidence based. *Human Reprod Update* 16 (2) :225–227.

Rodgers, R. J., Irving-Rodgers, H. F. & Russell, D.L. (2003) Extracellular matrix of the developing ovarian follicle. *Reproduction,* 126 (4): 415–424.

Royal College of Obstetricians and Gynaecologists (RCOG), London Scientific Impact Paper (2011) The use of Antithrombotics in the Prevention of Recurrent Pregnancy Loss. [On-line]: UK: Available: https://www.rcog.org.uk/globalassets/documents/guidelines/sip_no_26.pdf Accessed: 1 December 2014

Russell, P., Anderson, L., Lieberman, D., Tremellen, K., Yilmaz, H., Cheerala, B., Sacks, G. (2011) The distribution of immune cells and macrophages in the endometrium of women with recurrent reproductive failure I: Techniques. *J Reprod Immunol,* (1-2): 90-102.

Sackett, D. L., Rosenberg, W. M., Gray, J. A., Haynes, R. B. & Richardson, W. S. (1996) Evidence based medicine: what it is and what it isn't. *BMJ*, 312 (7023): 71-72.

Salamonsen, L. A., Dimitriadis, E., Jones, R. L. & Nie, G. (2003) Complex regulation of decidualization: a role for cytokines and proteases--a review. *Placenta*, 24 (suppl A): S76-85.

Salker, M., Teklenburg, G., Molokhia, M., Lavery, S., Trew, G., Aojanepong, T., Mardon, H. J., Lokugamage, A. U., Rai, R., Landles, C., Roelen, B. A., Quenby, S., Kuijk, E. W., Kavelaars, A., Heijnen, C. J., Regan, L., Macklon, N. S. & Brosens, J. J. (2010) Natural selection of human embryos: impaired decidualization of endometrium disables embryo-maternal interactions and causes recurrent pregnancy loss. *PLoS One*, 5 (4): e10287.

Salker, M. S., Christian, M., Steel, J. H., Nautiyal, J., Lavery, S., Trew, G., Webster, Z., Al-Sabbagh, M., Puchchakayala, G., Foller, M., Landles, C., Sharkey, A. M., Quenby, S., Aplin, J. D., Regan, L., Lang, F. & Brosens, J. J. (2011) Deregulation of the serum- and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. *Nat Med*, 17 (11): 1509-1513.

Santibañez, A., Garcia, J., Pashkova, O., Colin, O., Castellanos, G., Sanchez, A. P. & De la Jara, J. F. (2014) Effect of intrauterine injection of human chorionic gonadotropin before embryo transfer on clinical pregnancy rates from in vitro fertilisation cycles: a prospective study. *Reprod Biol Endocrinol*, 12 9.

Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*, 9 (7): 671-675.

Schoolcraft, W. B., Surrey, E. S. & Gardner, D. K. (2001) Embryo transfer: techniques and variables affecting success. *Fertil Steril*, 76 (5): 863-870.

SenGupta, S. B. & Delhanty, J. D. (2012) Preimplantation genetic diagnosis: recent triumphs and remaining challenges. *Expert Rev Mol Diagn*, 12 (6): 585-592.

Sharif, K. W. & Ghunaim, S. (2010) Management of 273 cases of recurrent implantation failure: results of a combined evidence-based protocol. *Reproductive Bio Med Online* 21: 373–80.

Sher, G., Feinman, M., Zouves, C., Kuttner, G., Maassarani, G., Salem, R., Matzner, W., Ching, W. & Chong, P. (1994) High fecundity rates following in-vitro fertilization and embryo transfer in antiphospholipid antibody seropositive women treated with heparin and aspirin. *Hum Reprod*, 9 (12): 2278-2283.

Sher, G., Matzner, W., Feinman, M., Maassarani, G., Zouves, C. & Chong, P. (1998) The selective use of heparin/aspirin therapy, alone or in combination with intravenous immunoglobulin G, in the management of antiphospholipid antibody positive women undergoing in vitro fertilization. *Am J Reprod Immunol* 40 (2):74–82.

Seshadri, S., Sunkara, S. K. (2014) Natural killer cells in female infertility and recurrent miscarriage: a systematic review and meta-analysis. *Hum Reprod Update,* 20 (3): 429-38.

Singh, M., Chaudhry, P. & Asselin, E. (2011) Bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors. *J Endocrinol*, 210 (1): 5-14.

Siristatidis, C. S., Dodd, S. R. & Drakeley, A. J. (2011) Aspirin for in vitro fertilisation. *Cochrane Database Syst Rev*, (8): CD004832.

Siristatidis, C. S., Dodd, S. R. & Drakeley, A. J. (2012) Aspirin is not recommended for women undergoing IVF. *Hum Reprod Update*, 18 (3): 233.

Smith, G. C., Smith, M. F., McNay, M. B. & Fleming, J. E. (1998) First-trimester growth and the risk of low birth weight. *N Engl J Med*, 339 (25): 1817-1822.

Smith, G. C., Stenhouse, E. J., Crossley, J. A., Aitken, D. A., Cameron, A. D. & Connor, J. M. (2002) Early-pregnancy origins of low birth weight. *Nature*, 417 (6892): 916.

Spencer, T. E., Johnson, G. A., Bazer, F. W. & Burghardt, R. C. (2004) Implantation mechanisms: insights from the sheep. *Reproduction*, 128 (6): 657-668.

Stavreus-Evers, A., Aghajanova, L., Brismar, H., Eriksson, H., Landgren, B. M. & Hovatta, O. (2002) Co-existence of heparin-binding epidermal growth factor-like growth factor and pinopodes in human endometrium at the time of implantation. *Mol Hum Reprod*, 8 (8): 765-769.

Stern, C., Chamley, L., Norris, H., Hale, L. & Baker, H. (2003a) A randomized, double-blind, placebo-controlled trial of heparin and aspirin for women with in vitro fertilization implantation failure and antiphospholipid or antinuclear antibodies. *Fertil Steril*, 80 (2): 376-383.

Straus, S. E. & Sackett, D. L. (1998) Using research findings in clinical practice. *BMJ*, 317 (7154): 339-342.

Tan, B. K., Vandekerckhove, P., Kennedy, R. & Keay, S. D. (2005) Investigation and current management of recurrent IVF treatment failure in the UK. *BJOG*, 112 (6): 773-780.

Tang, A. W., Alfirevic, Z., Turner, M. A., Drury, J. A., Small, R. & Quenby, S. (2013) A feasibility trial of screening women with idiopathic recurrent miscarriage for high uterine natural killer cell density and randomizing to prednisolone or placebo when pregnant. *Hum Reprod,* 28 (7): 1743-52.

Taranissi, M., El-Toukhy, T., Gorgy, A. & Verlinsky, Y. (2005) Influence of maternal age on the outcome of PGD for aneuploidy screening in patients with recurrent implantation failure. *Reprod Biomed Online*, 10 (5): 628-632.

Teklenburg, G., Salker, M., Molokhia, M., Lavery, S., Trew, G., Aojanepong, T., Mardon, H. J., Lokugamage, A. U., Rai, R., Landles, C., Roelen, B. A., Quenby, S., Kuijk, E. W., Kavelaars, A., Heijnen, C. J., Regan, L., Brosens, J. J. & Macklon, N. S. (2010) Natural selection of human embryos: decidualizing endometrial stromal cells serve as sensors of embryo quality upon implantation. *PLoS One*, 5 (4): e10258.

Thathiah, A., Brayman, M., Dharmaraj, N., Julian, J. J., Lagow, E. L. & Carson, D. D. (2004) Tumor necrosis factor alpha stimulates MUC1 synthesis and ectodomain release in a human uterine epithelial cell line. *Endocrinology*, 145 (9): 4192-4203.

The Medicines for Human Use (Clinical Trials) Regulations (2004) [On-line]: UK: Available:http://www.mhra.gov.uk/home/groups/l-unit1/documents/websiteresources/con2022633.pdf Accessed: 1 December 2014

Uman, L. S. (2011) Systematic reviews and meta-analyses. *J Can Acad Child Adolesc Psychiatry*, 20 (1): 57-59.

Urman, B., Ata, B., Yakin, K., Alatas, C., Aksoy, S., Mercan, R. & Balaban, B. (2009) Luteal phase empirical low molecular weight heparin administration in patients with failed ICSI embryo transfer cycles: a randomized open-labeled pilot trial. *Hum Reprod*, 24 (7): 1640-1647.

Vail, A. & Gardener, E. (2003) Common statistical errors in the design and analysis of subfertility trials. *Hum Reprod*, 18 (5): 1000-1004.

Van der Gaast, M. H., Beier-Hellwig, K., Fauser, B. C., Beier, H. M. & Macklon, N. S. (2003) Endometrial secretion aspiration prior to embryo transfer does not reduce implantation rates. *Reprod Biomed Online*, 7 (1): 105-109.

von Rango, U., Alfer, J., Kertschanska, S., Kemp, B., Muller-Newen, G., Heinrich, P. C., Beier, H.M, & Classen-Linke I. (2004) Interleukin-11 expression: its significance in eutopic and ectopic human implantation. *Mol Hum Reprod,* 10 (11): 783–792.

Vontver, L. A. (2008) *Lange Q&A: Obstetrics and Gynaecology*. 8th edn. USA, McGraw-Hill.

Wada-Hiraike, O., Hiraike, H., Okinaga, H., Imamov, O., Barros, R.P., Morani, A., Omoto, Y., Warner, M. & Gustafsson, J.A. (2006) Role of estrogen receptor beta in uterine stroma and epithelium: Insights from estrogen receptor beta-/- mice. *Proc Natl Acad Sci U S A,*103 (48):18350-5.

Walton, K. J., Duncan, J. M., Deschamps, P. & Shaughnessy, S. G. (2002) Heparin acts synergistically with interleukin-11 to induce STAT3 activation and invitro osteoclast formation. *Blood,* 100 (7): 2530-2536.

Wang, L., Brown, J. R., Varki, A. & Esko, J. D. (2002) Heparin's anti-inflammatory effects require glucosamine 6-O-sulfation and are mediated by blockade of L- and P-selectins. *J Clin Invest*, 110 (1): 127-136.

Warda, M., Gouda, E. M., Toida, T., Chi, L. & Linhardt, R. J. (2003) Isolation and characterization of raw heparin from dromedary intestine: evaluation of a new source of pharmaceutical heparin. *Comp Biochem Physiol C Toxicol Pharmacol,* 136 (4) :357-365.

Warkentin, T. E. & Greinacher, A. (2004) Heparin-induced thrombocytopenia: recognition, treatment, and prevention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest*, 126 (3 suppl): 311S-337S.

Warkentin, T. E., Levine, M. N., Hirsh, J., Horsewood, P., Roberts, R. S., Gent, M. & Kelton, J. G. (1995) Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *N Engl J Med*, 332 (20): 1330-1335.

Weigert, C., Brodbeck, K., Haring, H. U., Gambaro, G. & Schleicher, E. D. (2001) Low-molecular-weight heparin prevents high glucose- and phorbol ester-induced TGF-beta 1 gene activation. *Kidney Int,* 60 (3): 935-943.

Williams, M., Thompson, L. A., Li, T. C., Mackenna, A., Barratt, C. L. & Cooke, I. D. (1993) Uterine flushing: a method to recover spermatozoa and leukocytes. *Hum Reprod*, 8 (6): 925-928.

Xu, X., Ding, J., Rao, G., Shen, J., Prinz, R. A., Rana, N. & Dmowski, W. P. (2007) Estradiol induces heparanase-1 expression and heparan sulphate proteoglycan degradation in human endometrium. *Hum Reprod*, 22 (4): 927-93.

Zegers-Hochschild, F., Adamson, G. D., de Mouzon, J., Ishihara, O., Mansour, R., Nygren, K., Sullivan, E. & van der Poel, S. (2009) The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. *Hum Reprod*, 24 (11): 2683-2687.

Appendices

Appendix 1: Patient Information Sheet

Version 5: 27/10/2012

Participant Information Sheet

Endometrial Flushing of Low Molecular Weight Heparin Improves decidualisation- a prospective randomised control pilot study

Project Reference No: HEP001QUEN
Eudra CT Reference No: 2012-003682-18
ISRCTN78466363

Principal Investigator: Prof Siobhan Quenby (University of Warwick)

You are being invited to take part in a research study. Before you decide whether you wish to participate or not, it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and discuss it with relatives, friends and your GP, if you wish. If there is anything that is not clear, or if you have any further questions, please do not hesitate to ask. Do take time to decide whether you would like to take part or not. You may also like to have a copy of a leaflet published by Consumers for Ethics in Research (CERES), entitled 'Medical Research and You', which we can provide on request. This leaflet gives more information about medical research and looks at some questions you may want to ask.

If you do decide to participate, please let us know beforehand if you are currently involved in another study.

What is the purpose of the study?
Every year in England and Wales there are 700,000 births and thus hundreds of thousands of delighted parents. However, many couples are not so fortunate, in the same period of time: 15% of couples experience subfertility; 25% (500,000) of conceptions fail to implant; 15% (300,000) of pregnancies end in early miscarriage; 2% of couples suffer recurrent miscarriages (three or more losses). We have shown previously that failure of the lining of the womb (endometrium) to prepare adequately for pregnancy, a process known as decidualisation, is an underlying factor in these clinical problems. During decidualisation, the womb lining prepares to accept the embryo.

The blood thinning agent, Heparin, has been used in attempts to improve reproductive success. Heparin is currently given by daily subcutaneous injections and when given by this route causes bruising, pain and occasional bleeding.
As well as thinning blood, Heparin also has the potential to improve decidualisation by acting on factors in the lining of the womb in a way that could improve pregnancy outcome.
We would like to study the effect of flushing heparin directly into the womb prior to pregnancy, as this new method of administration should enhance its beneficial effects and minimise its adverse effects.

Do I have to take part?
No. It is up to you to decide whether or not to take part. You need to read this information sheet in detail and ask any further questions to the research team. You are

still free to withdraw at any time, without giving a reason. A decision to withdraw or a decision not to take part will not affect the standard of care you receive. You may find it helpful to discuss your decision with someone else.

What will happen to me if I take part?
Your treatment will be the same, whether or not you decide to take part. You do not need to do anything differently. You will be asked to sign a consent form.

You will be required to have the results of vaginal swabs prior to the study. If any infection is found on the vaginal swabs appropriate treatment will be commenced prior to any intervention.

If you are eligible and voluntarily agree to participate in the study, you will be asked:

- To use **barrier methods of contraception** for the month in which the study occurs.
- To email Principal Investigator Prof Quenby (s.quenby@warwick.ac.uk) or phone 02476967528 once you have detected ovulation (as assessed by home ovulation kit).
- To attend the implantation clinic 5 to 7 days after ovulation (as assessed by a home ovulation kit).
    - At the clinic, you will be randomly allocated to either flushing of your womb with low molecular weight heparin OR saline.
    - You will be asked to have a blood test 4 hours after the flushing to assess any heparin activity in your blood.
- To fill in a questionnaire and side effect diary at home.
- To re-attend clinic 24 hours later for an endometrial biopsy (sampling of lining of the womb) together with further blood tests.
- To take part in a telephone consultation 14 days later to ask about period heaviness and duration.
- To return a questionnaire and side effects diaries in a prepaid envelope back to principal investigator.

The lining of the womb, which has been in contact with heparin during the flushing, will be shed when you will have your next period. Once the treatment cycle is complete, you do not need to use contraception any more. This study does not restrict your lifestyle and you can carry on with all your normal activities.

What interventions will take place during flushing and sampling of lining of the womb during the study?
The study will have two groups. You will not be aware that which group you are allocated to.
A small plastic tube (Hycosy catheter) will be passed into the neck of your womb. Four mls of normal saline will be gently flushed into the womb cavity for less than two minutes with minimal pressure under ultrasound guidance. The ultrasound will enable us to see if the infusion has been given slowly and is contained in the womb.
In the intervention group: The saline will contain the blood thinning agent (low molecular weight heparin- Enoxaparin 20 mg/0.2mls)
In the control group: Saline alone will be used

Do I require pain relief for these visits?
You do not have to take pain relief but if you wish, you could take Paracetamol 1g orally alone or with Codeine Phosphate 30 mg orally, 1 hour prior to the endometrial flushing or sampling appointments.

What happens to the womb sample you obtained during the study?
We will analyse your endometrial sample in order to study markers of decidualisation (the lining of the womb's preparation for pregnancy). The decidualisation tests will be done in laboratories at the University of Warwick. The researchers working on the samples will not know your identity, because the samples will have been coded.

Who would be able take part in this study?
You will be able to participate if:
You are 18 years or older but younger than 45 years
You are able to give informed consent
You had previous unsuccessful IVF treatment (no live birth after transfer of two good quality embryos) or had recurrent miscarriages (3 or more miscarriages) in the past

Who would not be able take part in this study?
You will not be able to participate if:
You have had unprotected sexual intercourse during the month when the study is taking place.
You have an untreated vaginal infection.
You are pregnant or Breast feeding
Your body weight is below 45 kg
You have any bleeding disorders or you are taking any other blood thinning agents (injections or tablets e.g. warfarin)
You have high blood pressure, stroke, stomach ulcers, diabetes, kidney or liver disease
You are taking steroid tablets, systemic salicylates, acetylsalicylic acid, NSAIDS including Ketorolac, dextran and clopidogrel or any immunosuppressant medications
You had any allergic reaction previously to any blood thinning agents, Pork, Beef or other Animal products

Will my taking part in the study be kept confidential?
All information that is collected about you during the course of the research will be kept strictly confidential and accessible only to authorised staff who already have access to your medical records in connection with your clinical care. All research information about you will have your name removed so that you cannot be recognised by it. We will store all information collected about you in locked cabinets or on password protected computers (even if you at any time withdraw from the study) for a length of 10 years. We will also ask for your consent to inform your GP that you are taking part in the study.

What are the possible risks and disadvantages of taking part?
There is a risk to pregnancy so if it is possible that you are pregnant you should not take part in this study. A urine pregnancy test will be taken to check you are not pregnant.
Flushing and sampling the lining of the womb may be uncomfortable, but leaves no long term effects because your womb lining regenerates every month.
There is a very small risk of bleeding with the use of heparin. For this reason, we will take a blood test 4 and 24 hours after administration. We have an antidote against the blood-thinning agent that will reverse its effect should bleeding occur.
There is a very small risk of infection.

What are the possible benefits of taking part?
There is no immediate medical benefit to taking part, however, the information that we gain from this study may help us to treat women in the future. We do not expect any commercially significant results to be gained from this research. It is possible that taking an endometrial biopsy may improve the chance of a live birth in a future pregnancy however, the medical evidence for this is currently limited and there is no guarantee that the biopsy may help.

Although there are no benefits to you, the research will lead to new knowledge and we expect that this may be of benefit to others in future. This new knew knowledge may help us develop new treatments for problems of infertility, recurrent miscarriage and failures in in- vitro fertilisation (IVF).

Would any kind of treatment be available once this study has finished?
This is a preliminary or pilot study so no treatment will be available for participants after completion of the study.

Expenses and payments
The study incurs some extra visits but you will be reimbursed for any extra traveling expenses for these visits.

Do I need to make any extra hospital visits?
Yes. You are expected to make two hospital visits. You will also receive a follow up telephone call.

Do I need to have any extra blood tests or scans?
Yes. You need to have extra blood tests and pelvic scans.

Where is the study conducted?
The study will be conducted at University of Warwick. The patients will be seen in outpatients at Centre of Reproductive Medicine at University Hospitals Coventry and Warwickshire NHS Trust.

What if something goes wrong?
Complaints:
If you have any concerns or complaints about this research or staff, please contact Ms Nicola Owen, Deputy Registrar, University of Warwick, Research support Services, University House, Kirby Corner Road, Coventry CV4 8UW
Telephone: 02476522785
Fax: 02476524751
Email: Nicola.Owen@warwick.ac.uk

For general advice and guidance for NHS, non-staff, Participants:
Patient Advice and Liaison Service, PALS
Local contact details can be found on **http://www.pals.nhs.uk/**

Any Concern or Harm:
We do not anticipate that this study will cause any harm to you. However, in the event of any adverse effects, please contact us immediately. We are available 24 hours a day, 7 days a week. You can contact us
On weekdays from 8 am till 5pm
Either Professor S Quenby on 02476967528
Or Dr M Akhtar on 02476964000 Bleep 2730.
If there are any concerns outside these hours, please contact Gynaecology Emergency Room at University Hospitals Coventry and Warwickshire NHS Trust at 02476967000.

The University of Warwick, which is the research sponsor for this study, also holds vicarious liability insurance and provides professional indemnity insurance for its researchers.

How long the study is expected to run for?
The duration of the study will be 12 months starting from the ethics approval and ending at collecting samples from patients. A further 12 months will be required to analyse the data.

How will I be informed of the results of the study?
You will be informed of the results by letter and you will be invited to a patient information evening at the end of study.

What happens when the research study stops?
Any tissue samples collected will be kept for use in this project. The data will be analysed and results will be published.
Only the researchers and specific technical staff at the University will be authorised to access the tissue. All the samples will be anonymous and their labels will be coded so you would never be identifiable. Tissue that is no longer needed for research will be destroyed through the normal clinical waste procedures of the University.

It is possible that any stored or fixed tissue might turn out to be useful in other research projects, not currently envisaged. If you agree, any such tissue could be kept for use in such future projects. The tissue would only be used in future projects if permission had been granted by a Research Ethics Committee and the future use of samples will be limited to research in this field of medicine only. Please indicate on your consent form if you agree to this. Occasionally samples may be moved between different universities or research sites to enable new research to be undertaken in collaboration with experts in different disciplines. Ethics Committee approval would be required before any such move was undertaken.

What will happen to the results of the research study?
This is an academic study and we expect to publish the results. They may be published in a medical or scientific journal. However, please be reassured that you will not be identified in any such publication.
We will also need to submit results for scrutiny by grant giving bodies, ethics committees and other experts, for example, at conferences. Please be reassured that you will not be identified in any report or publication.

Who is organising and funding the research?
This work is funded by Biomedical Research Unit, University Hospitals Coventry and Warwickshire NHS Trust. No member of staff, including researchers, doctors or nursing staff is being paid for including you in this study. The research is organised and will be conducted according to the legal framework for use of human tissues in research embodied in the Human Tissue Act. This trial will adhere to the principles outlined in the Medicines for Human use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the international conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines; it will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Who has approved the study?
This study has been approved by NRES-West Midlands Edgbaston, Ethics Committee, Medicines and Healthcare Products Regulatory Agency (MHRA), University Hospitals of Coventry and Warwickshire NHS Trust's Research and Development department.

Contact Details:
If you would like further information or have any concerns about the study, please contact

Professor Siobhan Quenby B. Sc., MBBS, MD, FRCOG
Professor in Obstetrics, Division of Reproductive Health
Clinical Sciences Research Laboratory
Warwick Medical School, University of Warwick
Coventry CV2 2DX
Tel: 02476967528
E-mail: s.quenby@warwick.ac.uk

Thank you for taking the time to read this information sheet.

Appendix 2: Consent form

Hospital Number:
Patient Code Number for this project:

Study number: HEP001QUEN Consent form version 5: 27 October 2012

CONSENT FORM

Title of Project: Endometrial flushing of low molecular weight heparin improves decidualisation- a prospective randomised control pilot study

Name of Researchers: Professor Siobhan Quenby, Professor Jan J Brosens, Dr Muhammad Akhtar

	Please initial box
I confirm that I have read and understand the information sheet dated 27 October 2012 (Version 5) for the above study; I have been given the opportunity to ask questions and discuss this study. I have received satisfactory answers to all my questions	☐
I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, and without my medical care or legal rights being affected.	☐
I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from University of Warwick, from regulatory authorities or from University Hospitals Coventry and Warwickshire 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.	☐
I agree to material collected and stored in this study being used in future studies limited to research in this field, subject to Ethics Committee approval.	☐
I agree to take part in the above study voluntarily and I have received enough information about the study.	☐
I agree to material collected and stored in this study being used in future studies limited to research in this field, subject to Ethics Committee approval.	☐

Signed.....Date.....

(NAME IN BLOCK CAPITALS).....

The study has been explained to me by:

Investigator's signature.....…Date: .....

(NAME IN BLOCK CAPITALS).....

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

Appendix 3: Participant Invitation Letter

University Hospitals Coventry and Warwickshire NHS Trust

Letter of Invitation to participants

Version 5: Dated 27/10/12

Prof Siobhan Quenby
Professor of Obstetrics
University of Warwick
Department of Obstetrics and Gynaecology
University Hospitals of Coventry and Warwickshire
Clifford Bridge Road
Coventry CV2 2DX
Email: s.quenby@warwick.ac.uk
Telephone: 02476968657

Dear

Title: Endometrial Flushing of Low Molecular Weight Heparin Improves decidualisation- a prospective randomised control pilot study
(Study number: HEP001QUEN)

We are inviting you to take part in the above-mentioned study at University of Warwick and Centre of Reproductive Medicine, University Hospitals Coventry and Warwickshire NHS Trust. We are aiming to find out whether flushing a blood-thinning agent (Heparin) directly into the inside of a womb has a beneficial effect on the womb lining.

This project involves two visits one 5-7 days after ovulation to have your womb flushed with either heparin or saline and one the following day to have your womb lining sampled. We will also test your blood to ensure that the heparin has no side effects.

It is important that you are not pregnant when we do this.

- We hope that information from this study will enable us to design new treatments to increase the chance of a live birth in women with fertility problems.
- I enclose the patient Information Sheet. Please read carefully before making your decision.
- You have the right to agree or not to take part in the study.
- Your care will remain entirely unchanged whatever decision you make.

Yours sincerely,

Professor S Quenby,
Principal Investigator

Appendix 4: GP Letter

University Hospitals Coventry and Warwickshire NHS Trust

Version 4: Dated 09/09/12

Prof Siobhan Quenby
Professor of Obstetrics
University of Warwick
Department of Obstetrics and Gynaecology
University Hospitals of Coventry and Warwickshire
Clifford Bridge Road
Coventry CV2 2DX
Email: s.quenby@warwick.ac.uk
Telephone: 02476968657

Dear Doctor

Subject: Endometrial Flushing of Low Molecular Weight Heparin Improves decidualisation- a prospective randomised control pilot study

As you know, the above-named patient is currently being seen at the Centre for Reproductive Medicine at University Hospitals Coventry and Warwickshire NHS Trust for investigation of sub-fertility or miscarriages.

I am writing to inform you that she has agreed to participate in a research study which is being conducted at University of Warwick and University Hospitals Coventry and Warwickshire NHS Trust.

We are aiming to test the effects of endometrial flushing with low molecular weight heparin in a non-conception menstrual cycle upon markers of decidualisation during the potential implantation window. In future this will enable us to improve implantation for patients experiencing problems with fertility or miscarriages.

Her care will otherwise be entirely unchanged, and we will keep you posted with her progress.

I enclose the Information Sheet that we have given her.

Yours sincerely,

Professor S Quenby
Principal Investigator

Appendix 5: Stability data of Clexane-Enoxaparin (Sanofi-Aventis)

Reference: 0014-7546

14th August 2012

Dr Muhammad Akhtar
Walsgrave General Hospital
Clifford Bridge Road
Coventry
United Kingdom

Dear Dr Akhtar,

Thank you for your enquiry requesting the following information on Clexane - enoxaparin, received via my colleague Deborah Woods:

Clexane - Dilution

As you are aware from the Summary of Product Characteristics (SPC) for Clexane:

Subcutaneous Injection:
Clexane should not be mixed with any other injections or infusions.

Intravenous (Bolus) Injection for acute STEMI indication only: Enoxaparin: sodium may be safely administered with normal saline solution (0.9%) or 5% dextrose in water.

Having carried out a search of the literature, I have located the following studies which have investigated the stability of enoxaparin when diluted.

Patel et al [1] investigated the stability of enoxaparin (100 mg/mL) diluted with sterile water or 4% glucose to a concentration of 20 mg/mL over a 31-day period under different temperature storage conditions. A pooled batch of enoxaparin (100 mg/mL) was used to make up a solution diluted with preservative free sterile water for injection, and another solution diluted with sterile 4% glucose solution. An undiluted sample served as a control. The samples were stored for up to 31 days at 3 different temperatures (4°C, –12°C, or –80°C). The control solution lost a significant percentage of its AFXa activity when stored at –12°C and –80°C ($p<0.05$). The solution diluted with 4% glucose, however, retained >99.0% of its original activity when stored for 31 days at 4°C. This solution lost some activity when frozen at –12°C after 31 days. The authors concluded that the results indicated that the inclusion of glucose prevented the loss of AFXa activity of diluted enoxaparin when stored at 4°C or –12°C for up to 31 days.

In a similar study, Dager et al[2] found that the storage of enoxaparin diluted with sterile water (end concentration 20mg/ml), in either 1ml plastic tuberculin syringes or a glass vial at room temperature or under refrigeration did not result in a significant loss of anti-Xa activity over a 4 week period. Those diluted samples stored in the glass vials, both at room temperature and in the fridge had trending decreases in loss of anti-Xa activity at weeks 3 and 4. Overall, the decreases in activity did not reach significance.

In a third study, Mewborn and colleagues [3] conducted a study to evaluate the stability of enoxaparin when diluted in 0.9% sodium chloride solution (final concentration 1.2mg/ml) and stored in polyvinyl chloride containers for up to 48 hours at 20-22°C. Samples from the solutions were assessed for physical compatibility and anti-Xa activity at the time of preparation (t_0) and then at 0.25, 0.5, 0.75, 1, 4, 12, 16, 24 and 48 hours. The enoxaparin was found to be compatible with the 0.9% sodium chloride solution (no colour change or precipitation) and the pharmacologic activity at 48 hours was >94% of the activity measured at t_0.

Marketing Authorisation

I can confirm, from the current SPC for clexane, the following:

MARKETING AUTHORISATION HOLDER

Sanofi-aventis
One Onslow Street
Guildford
Surrey, GU1 4YS
UK

MARKETING AUTHORISATION NUMBER(S)

PL 04425/0187

DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

Date of first authorisation: 22 October 1990
Date of latest renewal: 8 August 2002

Unfortunately we are unable to provide you with an Investigational Medicinal Product Dossier (IMPD) for Clexane as this is a strictly confidential document. If you are wishing to obtain this IMPD in order to set a clinical trial application within licence to the MHRA or IMB, the SPC is enough to do this.

If you have any more queries regarding the clinical trial you wish to pursue, please contact our scientific advisor Debbie Woods who may be able to help you further as the only documentation Medical information could supply you with is the SPC. Please find this attached for your reference.

If you have any further enquiries regarding Clexane - enoxaparin then please contact us.

Yours sincerely

Damilola Surakatu BSc
Medical Information Officer

References

1. Patel RP, Narkowicz C, Jacobson GA. In Vitro Stability of Enoxaparin Solutions (20 mg/mL) Diluted in 4% Glucose.. Clinical Therapeutics, 2008 Vol 30 (10) p1880-1885 2008;

2. Dager WE, Gosselin RC, King JH et al. Anti-Xa stability of diluted enoxaparin for use in pediatrics. Annals of Pharmacotherapy, 2004 Vol 38 (4) p569-73 2004;

3. Mewborn AL, Kessler JM, Joyner KA. Compatibility and activity of enoxaparin sodium in 0.9-percent sodium chloride injection for 48 hours. American Journal of Health-Systems Pharmacy, 1996 Vol 53 p167-169 1996;

Appendix 6: Sample Drug Labels

Sample trial drug Label Enoxaparin

For Clinical Trials Use Only

Endometrial Flushing of Low Molecular weight Heparin study

EudraCT Number: 2012-003682-18

Enoxaparin 20mg/0.2 mls Pre-Filled Syringe

For Intrauterine use but to be given as directed in the study protocol

Keep out of reach of children. Do not store above 25◦C. Do not refrigerate or Freeze.

Batch Number: ……

Expiry: MM/YYYY

Patient Name:

Patient Hospital Number:

Patient Trial Number:

Study Sponsor: University of Warwick, Coventry CV4 8UW

Tel: *+44(0)2476523716*

Principal Investigator: Prof S Quenby, University Hospitals Coventry and Warwickshire NHS Trust

Tel: *+44(0)2476967528*

Trial Site:

University Hospitals Coventry and Warwickshire NHS Trust, UK

Sample trial drug Label for Sodium Chloride 0.9%

For Clinical Trials Use Only

Endometrial Flushing of Low Molecular weight Heparin study

EudraCT Number: 2012-003682-18

Sodium Chloride 0.9% in 10 ml ampoule

For Intrauterine use but to be given as directed in the study protocol

Keep out of reach of children. Do not refrigerate or Freeze.

Batch Number: ……

Expiry: MM/YYYY

Patient Name:

Patient Hospital Number:

Patient Trial Number:

Study Sponsor: University of Warwick, Coventry CV4 8UW

Tel: *+44(0)2476523716*

Principal Investigator: Prof S Quenby, University Hospitals Coventry and Warwickshire NHS Trust

Tel: *+44(0)2476967528*

Trial Site:

University Hospitals Coventry and Warwickshire NHS Trust, UK

Appendix 7: Serious Adverse Event Form (HEP001QUEN)

PATIENT IDENTIFICATION

Consultant: _____

Hospital: _____

Study Number ☐☐☐☐

Participant's Full Name: _____

Hospital Number: ☐☐☐☐☐☐☐☐☐☐

Date of birth: DD / MM / YYYY

Patient in Intervention group: Yes / No

Patient in Control group: Yes/ No

Date of endometrial flushing: DD / MM/ YYYY

Date of Endometrial Biopsy: DD / MM/ YYYY

SAE TYPE

Is this an initial or follow-up report? Initial Report ☐ Follow-up report ☐

Is this the final report Yes ☐ No ☐

REASON FOR REPORTING	Yes	No	
Death?	☐	☐	Date of death:
Life-threatening event?	☐	☐	
In-patient hospitalisation or prolongation of existing hospitalisation?	☐	☐	If yes, no of days? ☐
Persistent or significant disability/ incapacity?	☐	☐	
Congenital anomaly/ birth defect?	☐	☐	
Other pertinent medical reason for reporting?	☐	☐	

If other, please specify: _____

SAE DESCRIPTION

Date Event Started: DD / MM / YYYY

Date Event Ceased: DD / MM / YYYY

Details of Adverse Event (please attach copies of relevant reports):

_____

_____

TRIAL TREATMENT

This section must be completed by a clinician

Date last dose administered	**Causality Assessment** **(Please assume the mother was prescribed levothyroxine)** 1 Probably unrelated to treatment 2 Possibly related to treatment 3 Probably related to treatment 4 Definitely related to treatment	**Action taken due to SAE** 1 None 2 Treatment stopped 3 Treatment delayed
DD / MM / YYYY		

Please give reasons if you consider the event to be treatment related:

_____

Was the SAE **unexpected**, i.e. of a **type** or **severity** which is NOT consistent with the up-to-date SPC of low molecular weight Heparin **This section must be completed by a clinician**

Unexpected ☐ **Expected** ☐

Please give reasons if you consider the event to be unexpected:

_____

CONCOMITANT MEDICATION

Has the patient taken any other medication within the last week? Yes ☐ No ☐

If yes, please complete below:

Drug	Start date	Tick if continuing or specify stop date	Dose (mg)	Indication
_____	DD / MM / YYYYY	☐ DD / MM / YYYY		
_____	DD / MM / YYYY	☐ DD / MM/ YYYY		
_____	DD / MM / YYYY	☐ DD / MM / YYYY		
_____	DD / MM / YYYY	☐ DD / MM / YYYY		
_____	DD / MM / YYYY	☐ DD / MM / YYYY		

OUTCOME OF SAE

Outcome: Fatal ☐ Recovered ☐ Continuing ☐

Please describe final outcome if event continuing at time of faxing initial report:

_____

_____

Signature of Person Reporting: _____ Date: DD / MM / YYYY

You must have signed the Site Delegation Log

Name: _____ Position: _____

Telephone No: _____

Signature of Investigator: _____ Date: DD / MM / YYYY

If not completed by Investigator

SUSAR Reporting – Warwick University R&D USE ONLY

SAE reference number: ☐☐☐

Date reported to WU? DD / MMM / YYYY

Date reported to CI? DD / MM / YYYY Date reply received from CI? DD / MM / YYYY

Is this event a SUSAR? Yes ☐ If yes: 7 day report ☐ OR 15 day report ☐

No ☐ If NO, is this an SAE? Yes ☐ No ☐

PI comments:_____

_____

PI Signature:_____ PRINT Name:_____

Date due to be reported to MHRA and MREC: DD / MM / YYYY

Appendix 8: Trial Prescription

University Hospitals Coventry and Warwickshire NHS Trust Clinical Trial Prescription

CLINICAL TRIAL PRESCRIPTION

Clinical Area	
Consultant	

Patient LABEL:
Patient Trial No:

Endometrial Flushing of LMWH

A randomised pilot trial evaluating to assess the effects of Enoxaparin on endometrial decidualisation in a non-conception menstrual cycle.

Sponsor Study Number: HEP001QUEN

EudraCT Number: 2012-003682-18

Sponsor: University of Warwick

Treatment Prescription

Treatment Group:Enoxaparin 20 mg in 4 mls of Sodium Chloride 0.9%

Control Group (placebo): 4 mls of Sodium Chloride 0.9% only

CHECK LIST

1. Patient has read and understood the participant information leaflet.
2. Patient has signed the consent form to participate in the study.
3. Patient has been randomised and allocated to either treatment or control group.
4. Negative pregnancy test for all participants in this study group.
5. She has used barrier methods of contraception during this month of intervention.
6. Patient fulfils the inclusion criterion which includes women who had previous one unsuccessful IVF treatment (defined as failure to achieve live birth after transfer of two good quality embryos) or women with recurrent miscarriages (defined as 3 or more unexplained miscarriages), aged 18 years of age or older but younger than 45 years old who are able and willing to give informed consent
7. Patient fulfils the exclusion criterion which includes Women who had unprotected sexual intercourse during the month when endometrial flushing is planned, Women having a vaginal infection which is not treated, Women who are pregnant or Breastfeeding, Women with weight of < 45 kg (due to higher risk of bleeding), Women with bleeding disorders, severe hypertension, Diabetes Mellitus, Peptic Ulcers, Renal or Liver Diseases and who had recent stroke, Women taking Warfarin for any medical condition or taking systemic steroids, systemic salicylates, acetylsalicylic acid, NSAIDS including Ketorolac, dextran and clopidogrel or any immunosuppressant medications, Women with hypersensitivity to Heparin, Pork, Beef or other Animal products and if she is undergoing Tubal Patency testing, Hysteroscopy or Laparoscopy at the time when endometrial flushing is planned

Patient Group	**Prescribed By:**	**Date:**
Treatment or Control (please delete as necessary)	**Signature of Prescriber:**	

Results/date	**BP** (<150/100)	*Pregnancy Test*	*HVS*	*Other Pelvic Swabs*	**Height (cm)**	**Weight (kg)**	**BMI**

LMP
Allergies

	DRUG	**ACTUAL DOSE**	**Administration ROUTE**	**Time given**	**Given by**	**Checked by**
DATE ……	**Enoxaparin**	**20 mg**	Intrauterine			
	Sodium Chloride 0.9%	**4 mls**	Intrauterine			

Appendix 9: Participant Questionnaire (Non Validated)

University Hospitals Coventry and Warwickshire NHS Trust

Version 4: 09/09/2012

STUDY: Endometrial Flushing of Low Molecular Weight Heparin Improves decidualisation- a prospective randomised control pilot study

Project Reference No: HEP001QUEN

Name of researcher Prof S Quenby, Prof J Brosens, Dr M Akhtar

PATIENT NAME:

Date of completion of the questionnaire: DD / MM/ YYYY

Date of Intervention: DD / MM/ YYYY

We would like to know your opinions of this study and we also need to find out about any problems that might arise.

This questionnaire has to be completed once you have finished your first period after the procedure of flushing and sampling of the lining of your womb during the above mentioned study.

Please send your completed questionnaire back to us using the prepaid addressed envelope provided. Thank you very much for your participation in the study and your time and effort in completing this form.

1. How did you find out about the study?

2. Was the information provided helpful? Was there anything else that you would have liked to know?

3. Please tell us your experience of the study?

4. What could we do better to improve it?

5. Did you encounter any unpleasant situation during the study? If so, please tell us about it.

6. Was the member of staff helpful during the study?

7. Did you encounter any side effects or complications during the study?
    a. Bleeding Yes/ No, If yes where was the bleeding? …….
    b. Infection Yes/ No, If yes where was the infection? …….
    c. Bruising of skin Yes/ No
    d. Allergic reaction Yes/No
    e. Pain Yes/No
       If yes when did you experience pain? Can you describe the pain?

       _____

    f. Any other ……..
    g. Next period after intervention Normal/ Heavy/ Light
    h. Duration of next period after intervention Normal/ Prolong/ Short
       (Please specify duration in days……)

8. Are there any other comments that you wish to make?

9. If you would like us to reply to your comments, please give us your contact details below.

Much Appreciated.

Other Appendices:

SUMMARY OF PRODUCT CHARACTERISTICS

1 NAME OF THE MEDICINAL PRODUCT

Clexane® Syringes

2 QUALITATIVE AND QUANTITATIVE COMPOSITION

Pre-filled syringes:

20 mg Injection	Enoxaparin sodium 20 mg (equivalent to 2,000 IU anti-Xa activity) in 0.2 mL Water for Injections
40 mg Injection	Enoxaparin sodium 40 mg (equivalent to 4,000 IU anti-Xa activity) in 0.4 mL Water for Injections
60 mg Injection	Enoxaparin sodium 60 mg (equivalent to 6,000 IU anti-Xa activity) in 0.6 mL Water for Injections
80 mg Injection	Enoxaparin sodium 80 mg (equivalent to 8,000 IU anti-Xa activity) in 0.8 mL Water for Injections
100 mg Injection	Enoxaparin sodium 100mg (equivalent to 10,000 IU anti-Xa activity) in 1.0 mL Water for Injections

For full list of excipients, see section 6.1

3 PHARMACEUTICAL FORM

Solution for injection.
Clear, colourless to pale yellow solution.

4 CLINICAL PARTICULARS

4.1 Therapeutic indications

The prophylaxis of thromboembolic disorders of venous origin, in particular those which may be associated with orthopaedic or general surgery.

The prophylaxis of venous thromboembolism in medical patients bedridden due to acute illness.

The treatment of venous thromboembolic disease presenting with deep vein thrombosis, pulmonary embolism or both.

The treatment of unstable angina and non-Q-wave myocardial infarction, administered concurrently with aspirin.

Treatment of acute ST-segment Elevation Myocardial Infarction (STEMI) including patients to be managed medically or with subsequent Percutaneous Coronary Intervention (PCI) in conjunction with thrombolytic drugs (fibrin or non-fibrin specific).

The prevention of thrombus formation in the extracorporeal circulation during haemodialysis.

4.2 Posology and method of administration

Adults:

Prophylaxis of venous thromboembolism:
In patients with a low to moderate risk of venous thromboembolism the recommended dosage is 20 mg (2,000 IU) once daily by subcutaneous injection for 7 to 10 days, or until the risk of thromboembolism has diminished. In patients undergoing surgery, the initial dose should be given approximately 2 hours pre-operatively. In patients with a higher risk, such as in orthopaedic surgery, the dosage should be 40 mg (4,000 IU) daily by subcutaneous injection with the initial dose administered approximately 12 hours before surgery.

Prophylaxis of venous thromboembolism in medical patients:
The recommended dose of enoxaparin sodium is 40 mg (4,000 IU) once daily by subcutaneous injection. Treatment with enoxaparin sodium is prescribed for a minimum of 6 days and continued until the return to full ambulation, for a maximum of 14 days.

Treatment of venous thromboembolism:
Clexane should be administered subcutaneously as a single daily injection of 1.5 mg/kg (150 IU/kg). Clexane treatment is usually prescribed for at least 5 days and until adequate oral anticoagulation is established.

Dosage chart for 1.5mg/kg SC treatment of DVT, PE or both				
Patient weight	**Kg**	**Syringe label**	**Dose (mg)**	**Injection volume (ml)**
100mg/ml Solution for Injection CLEXANE syringes	40	60mg / 0.6ml	60 od	0.60
	45	80mg / 0.8ml	67.5 od	0.675
	50	80mg / 0.8ml	75 od	0.75
	55	100mg / 1ml	82.5 od	0.825
	60	100mg / 1ml	90 od	0.90
	65	100mg / 1ml	97.5 od	0.975
150mg/ml Solution for Injection CLEXANE Forte syringes	70	120mg / 0.8ml	105 od	0.70
	75	120mg / 0.8ml	112.5 od	0.76
	80	120mg / 0.8ml	120 od	0.80
	85	150mg / 1ml	127.5 od	0.86
	90	150mg / 1ml	135 od	0.90
	95	150mg / 1ml	142.5 od	0.96
	100	150mg / 1ml	150 od	1.00

Please be aware that in some cases it is not possible to achieve an exact dose due to the graduations on the syringe and so some of the volumes recommended in this table have been rounded up to the nearest graduation.

Treatment of unstable angina and non-Q-wave myocardial infarction
The recommended dose is 1 mg/kg Clexane every 12 hours by subcutaneous injection, administered concurrently with oral aspirin (100 to 325mg once daily).

Treatment with Clexane in these patients should be prescribed for a minimum of 2 days and continued until clinical stabilisation. The usual duration of treatment is 2 to 8 days.

Dosage chart for 1mg/kg SC treatment of UA or NSTEMI				
Patient weight	**Kg**	**Syringe label**	**Dose (mg)**	**Injection volume (ml)**
100mg/ml Solution for Injection CLEXANE syringes	40	40mg / 0.4ml	40 bd	0.40
	45	60mg / 0.6ml	45 bd	0.45
	50	60mg / 0.6ml	50 bd	0.50
	55	60mg / 0.6ml	55 bd	0.55
	60	60mg / 0.6ml	60 bd	0.60
	65	80mg / 0.8ml	65 bd	0.65
	70	80mg / 0.8ml	70 bd	0.70
	75	80mg / 0.8ml	75 bd	0.75
	80	80mg / 0.8ml	80 bd	0.80
	85	100mg / 1ml	85 bd	0.85
	90	100mg / 1ml	90 bd	0.90
	95	100mg / 1ml	95 bd	0.95
	100	100mg / 1ml	100 bd	1.00
150mg/ml Solution for Injection CLEXANE Forte syringes	105	120mg / 0.8ml	105 bd	0.70
	110	120mg / 0.8ml	110 bd	0.74
	115	120mg / 0.8ml	115 bd	0.78
	120	120mg / 0.8ml	120 bd	0.80
	125	150mg / 1ml	125 bd	0.84
	130	150mg / 1ml	130 bd	0.88
	135	150mg / 1ml	135 bd	0.90
	140	150mg / 1ml	140 bd	0.94
	145	150mg / 1ml	145 bd	0.98
	150	150mg / 1ml	150 bd	1.00

Please be aware that in some cases it is not possible to achieve an exact dose due to the graduations on the syringe and so some of the volumes recommended in this table have been rounded up to the nearest graduation.

Treatment of acute ST-segment Elevation Myocardial Infarction
The recommended dose of enoxaparin sodium is a single IV bolus of 30mg plus a 1mg/kg SC dose followed by 1mg/kg administered SC every 12 hours (max 100mg for the first two doses only, followed by 1mg/kg dosing for the remaining doses). For dosage in patients ≥75 years of age, see section 4.2 Posology and method of administration: *Elderly*.

Dosage chart for 1mg/kg SC treatment of STEMI				
Patient weight	**Kg**	**Syringe label**	**Dose (mg)**	**Injection volume (ml)**
100mg/ml Solution for Injection CLEXANE syringes	40	40mg / 0.4ml	40 bd	0.40
	45	60mg / 0.6ml	45 bd	0.45
	50	60mg / 0.6ml	50 bd	0.50
	55	60mg / 0.6ml	55 bd	0.55
	60	60mg / 0.6ml	60 bd	0.60
	65	80mg / 0.8ml	65 bd	0.65
	70	80mg / 0.8ml	70 bd	0.70
	75	80mg / 0.8ml	75 bd	0.75
	80	80mg / 0.8ml	80 bd	0.80
	85	100mg / 1ml	85 bd	0.85
	90	100mg / 1ml	90 bd	0.90
	95	100mg / 1ml	95 bd	0.95
	100	100mg / 1ml	100 bd	1.00
150mg/ml Solution for Injection CLEXANE Forte syringes	105	120mg / 0.8ml **(1)**	105 bd **(1)**	0.70 **(1)**
	110	120mg / 0.8ml **(1)**	110 bd **(1)**	0.74 **(1)**
	115	120mg / 0.8ml **(1)**	115 bd **(1)**	0.78 **(1)**
	120	120mg / 0.8ml **(1)**	120 bd **(1)**	0.80 **(1)**
	125	150mg / 1ml **(1)**	125 bd **(1)**	0.84 **(1)**
	130	150mg / 1ml **(1)**	130 bd **(1)**	0.88 **(1)**
	135	150mg / 1ml **(1)**	135 bd **(1)**	0.90 **(1)**
	140	150mg / 1ml **(1)**	140 bd **(1)**	0.94 **(1)**
	145	150mg / 1ml **(1)**	145 bd **(1)**	0.98 **(1)**
	150	150mg / 1ml **(1)**	150 bd **(1)**	1.00 **(1)**

(1) Not to be given for the first two doses - (maximum 100mg for the first two doses only, followed by 1mg/kg dosing for the remaining doses)
Please be aware that in some cases it is not possible to achieve an exact dose due to the graduations on the syringe and so some of the volumes recommended in this table have been rounded up to the nearest graduation.

When administered in conjunction with a thrombolytic (fibrin specific or non-fibrin specific) enoxaparin sodium should be given between 15 minutes before and 30 minutes after the start of fibrinolytic therapy. All patients should receive acetylsalicylic acid (ASA) as soon as they are identified as having STEMI and maintained under (75 to 325mg once daily) unless contraindicated.

The recommended duration of enoxaparin sodium treatment is 8 days or until hospital discharge, whichever comes first.

For patients managed with Percutaneous Coronary Intervention (PCI): If the last enoxaparin sodium SC administration was given less than 8 hours before balloon inflation, no additional dosing is needed. If the last SC administration was given more than 8 hours before balloon inflation, an IV bolus of 0.3mg/kg of enoxaparin sodium should be administered.

Prevention of extracorporeal thrombus formation during haemodialysis:
A dose equivalent to 1 mg/kg (100 IU/kg) introduced into the arterial line at the beginning of a dialysis session is usually sufficient for a 4 hour session. If fibrin rings are found, such as after a longer than normal session, a further dose of 0.5 to 1mg/kg (50 to 100 IU/kg) may be given. For patients at a high risk of haemorrhage the dose should be reduced to 0.5 mg/kg (50 IU/kg) for double vascular access or 0.75 mg/kg (75 IU/kg) for single vascular access.

Elderly:
For treatment of acute ST-segment Elevation Myocardial Infarction in elderly patients ≥75 years of age, do not use an initial IV bolus. Initiate dosing with 0.75mg/kg SC every 12 hours (maximum 75mg for the first two doses only, followed by 0.75mg/kg dosing for the remaining doses).

For other indications, no dosage adjustments are necessary in the elderly, unless kidney function is impaired (see also section 4.2 Posology and method of administration: *Renal impairment*; section 4.4 Special warnings and precautions for use: *Haemorrhage in the elderly*; *Renal impairment, and Monitoring*; section 5.2 Pharmacokinetic properties).

Dosage chart for 0.75mg/kg SC treatment of STEMI (elderly patients aged ≥75 years only)

Patient weight	Kg	Syringe label	0.75mg/kg Dose (mg)	Adjusted dosing (mg)	Injection volume (ml)
100mg/ml Solution for Injection CLEXANE syringes	40	60mg / 0.6ml	30 bd	30 bd	0.30
	45	60mg / 0.6ml	33.75 bd	35 bd	0.35
	50	60mg / 0.6ml	37.5 bd	37.5 bd	0.375
	55	60mg / 0.6ml	41.25 bd	42.5 bd	0.425
	60	60mg / 0.6ml	45 bd	45 bd	0.45
	65	60mg / 0.6ml	48.75 bd	50 bd	0.5
	70	60mg / 0.6ml	52.5 bd	52.5 bd	0.525
	75	60mg / 0.6ml	56.25 bd	57.5 bd	0.575
	80	60mg / 0.6ml	60 bd	60 bd	0.60
	85	80mg / 0.8ml	63.75 bd	65 bd	0.65
	90	80mg / 0.8ml	67.5 bd	67.5 bd	0.675
	95	80mg / 0.8ml	71.25 bd	72.5 bd	0.725
	100	80mg / 0.8ml	75 bd	75 bd	0.75
	105	80mg / 0.8ml	78.75 bd **(1)**	80 bd **(1)**	0.80 **(1)**
	110	100mg / 1ml	82.5 bd **(1)**	82.5 bd **(1)**	0.825 **(1)**
	115	100mg / 1ml	86.25 bd **(1)**	87.5 bd **(1)**	0.875 **(1)**
	120	100mg / 1ml	90 bd **(1)**	90 bd **(1)**	0.90 **(1)**
	125	100mg / 1ml	93.75 bd **(1)**	95 bd **(1)**	0.95 **(1)**
	130	100mg / 1ml	97.5 bd **(1)**	97.5 bd **(1)**	0.975 **(1)**
150mg/ml Solution for Injection CLEXANE Forte syringes	135	120mg / 0.8ml	101.25 bd **(1)**	102 bd **(1)**	0.68 **(1)**
	140	120mg / 0.8ml	105 bd **(1)**	105 bd **(1)**	0.7 **(1)**
	145	120mg / 0.8ml	108.75 bd **(1)**	111 bd **(1)**	0.74 **(1)**
	150	120mg / 0.8ml	112.5 bd **(1)**	114 bd **(1)**	0.76 **(1)**

(1) not to be given for the first two doses - (maximum 75mg for the first two doses only, followed by 0.75mg/kg dosing for the remaining doses)

Please be aware that in some cases it is not possible to achieve an exact dose due to the graduations on the syringe and so some of the volumes recommended in this table have been rounded up to the nearest graduation.

Children: Not recommended, as dosage not established.

Renal impairment: (See also section 4.4 Special warnings and precautions for use: *Renal impairment and Monitoring*; section 5.2 Pharmacokinetic properties).

Severe renal impairment:
A dosage adjustment is required for patients with severe renal impairment (creatinine clearance < 30 ml/min), according to the following tables, since enoxaparin sodium exposure is significantly increased in this patient population:

Dosage adjustments for therapeutic dosage ranges

	Standard dosing	**Severe renal impairment**	
	1 mg/kg SC twice daily	1 mg/kg SC once daily	
	1.5 mg/kg SC once daily	1 mg/kg SC once daily	
For treatment of acute STEMI in patients <75 years of age			
	30mg-single IV bolus plus a 1mg/kg SC dose followed by 1mg/kg twice daily. (Max 100mg for each of the first two SC doses)	30mg-single IV bolus plus a 1mg/kg SC dose followed by 1mg/kg once daily. (Max 100mg for first SC dose only)	
For treatment of acute STEMI in elderly patients ≥75 years of age			
	0.75mg/kg SC twice daily without initial bolus. (Max 75mg for each of the first two SC doses)	1mg/kg SC once daily without initial bolus. (Max 100mg for first SC dose only)	

Dosage adjustments for prophylactic dosage ranges

	Standard dosing	**Severe renal impairment**	
	40 mg SC once daily	20 mg SC once daily	
	20 mg SC once daily	20 mg SC once daily	

The recommended dosage adjustments do not apply to the haemodialysis indication.

Moderate and mild renal impairment:
Although no dosage adjustments are recommended in patients with moderate renal impairment (creatinine clearance 30-50 ml/min) or mild renal impairment (creatinine clearance 50-80 ml/min), careful clinical monitoring is advised.

Hepatic impairment: In the absence of clinical studies, caution should be exercised.

Body weight:
No dosage adjustments are recommended in obesity or low body weight (see also section 4.4 Special warnings and precautions for use: *Low body weight and Monitoring*; section 5.2 Pharmacokinetic properties).

Clexane is administered by subcutaneous injection for the prevention of venous thromboembolic disease, treatment of deep vein thrombosis or for the treatment of unstable angina, non-Q-wave myocardial infarction and acute ST elevation myocardial infarction (STEMI); through the arterial line of a dialysis circuit for the prevention of thrombus formation in the extra-corporeal circulation during haemodialysis; and via intravenous (bolus) injection through an intravenous line only for the initial dose of acute STEMI indication and before PCI when needed. It must not be administered by the intramuscular route.

To avoid accidental needle stick after injection, the prefilled syringes are fitted with an automatic safety device

Subcutaneous injection technique
The prefilled disposable syringe is ready for immediate use. Clexane should be administered when the patient is lying down by deep subcutaneous injection. The administration should be alternated between the left and right anterolateral or posterolateral abdominal wall. The whole length of the needle should be introduced vertically into a skin fold held between the thumb and index finger. The skin fold should not be released until the injection is complete.
Once the plunger is fully pressed down the safety device is activated automatically. This protects the used needle.
Note: The plunger has to be pressed down all the way for the safety device to be activated.
Do not rub the injection site after administration.

Intravenous (Bolus) Injection Technique (for acute STEMI indication only):
For intravenous injection, either the Multidose Vial or 60mg, 80mg or 100mg prefilled syringes can be used. Enoxaparin sodium should be administered through an intravenous line. It should not be mixed or co-administered with other medications. To avoid the possible mixture of enoxaparin sodium with all other drugs, the intravenous access chosen should be flushed with a sufficient amount of saline or dextrose solution prior to and following the intravenous bolus administration of enoxaparin sodium to clear the port of drug. Enoxaparin sodium may be safely administered with normal saline solution (0.9%) or 5% dextrose in water.

- **Initial 30mg bolus**

For the initial 30mg bolus, using an enoxaparin sodium graduated prefilled syringe (60, 80 or 100mg), expel the excessive volume to retain only 30mg (0.3ml) in the syringe. The 30mg dose can then be directly injected into an injection site in the intravenous line.

- **Additional bolus for PCI when last SC administration was given more than 8 hours before balloon insertion**

For patients being managed with Percutaneous Coronary Intervention (PCI), an additional IV bolus of 0.3mg/kg is to be administered if last SC administration was given more than 8 hours before balloon inflation (see section 4.2 Posology and method of administration: *Treatment of acute ST-segment Elevation Myocardial Infarction*).

In order to assure the accuracy of the small volume to be injected, it is recommended to dilute the drug to 3mg/ml.
To obtain a 3mg/ml solution, using a 60mg enoxaparin sodium prefilled syringe, it is recommended to use a 50ml infusion bag (i.e. using either normal saline solution (0.9%) or 5% dextrose in water) as follows:
Withdraw 30ml from the infusion bag with a syringe and discard the liquid.
Inject the complete contents of the 60mg enoxaparin sodium prefilled syringe into the 20ml remaining in the bag. Gently mix the contents of the bag.
Withdraw the required volume of diluted solution with a syringe for administration into the intravenous line (using an appropriate injection site or port).

After dilution is completed, the volume to be injected can be calculated using the following formula [Volume of diluted solution (ml) = Patient weight (kg) x 0.1] or using the table below. It is recommended to prepare the dilution immediately before use and to discard any remaining solution immediately after use.

Volume to be injected through intravenous line after dilution is completed

Weight [Kg]	Required dose (0.3 mg/kg) [mg]	Volume to inject when diluted to a final concentration of 3 mg/ml [ml]
45	13.5	4.5
50	15	5
55	16.5	5.5
60	18	6
65	19.5	6.5
70	21	7
75	22.5	7.5
80	24	8
85	25.5	8.5
90	27	9
95	28.5	9.5
100	30	10
105	31.5	10.5
110	33	11
115	34.5	11.5
120	36	12
125	37.5	12.5
130	39	13
135	40.5	13.5

140	42	14
145	43.5	14.5
150	45	15

4.3 Contraindications

Contraindicated in patients with acute bacterial endocarditis, active major bleeding and conditions with a high risk of uncontrolled haemorrhage, including recent haemorrhagic stroke, thrombocytopenia in patients with a positive in-vitro aggregation test in the presence of enoxaparin; active gastric or duodenal ulceration; hypersensitivity to either enoxaparin sodium, heparin or its derivatives including other Low Molecular Weight Heparins; in patients receiving heparin for treatment rather than prophylaxis, locoregional anaesthesia in elective surgical procedures is contraindicated.

4.4 Special warnings and precautions for use

Low Molecular Weight Heparins should not be used interchangeably since they differ in their manufacturing process, molecular weights, specific anti Xa activities, units and dosage. This results in differences in pharmacokinetics and associated biological activities (e.g. anti-IIa activity, and platelet interactions). Special attention and compliance with the instructions for use specific to each proprietary medicinal product are therefore required.

Enoxaparin is to be used with extreme caution in patients with a history of heparin-induced thrombocytopenia with or without thrombosis.

As there is a risk of antibody-mediated heparin-induced thrombocytopenia also occurring with low molecular weight heparins, regular platelet count monitoring should be considered prior to and during therapy with these agents. Thrombocytopenia, should it occur, usually appears between the 5th and the 21st day following the beginning of therapy. Therefore, it is recommended that the platelet counts be measured before the initiation of therapy with enoxaparin sodium and then regularly thereafter during the treatment. In practice, if a confirmed significant decrease of the platelet count is observed (30 to 50 % of the initial value), enoxaparin sodium treatment must be immediately discontinued and the patient switched to another therapy.

Enoxaparin injection, as with any other anticoagulant therapy, should be used with caution in conditions with increased potential for bleeding, such as: impaired haemostasis, history of peptic ulcer, recent ischaemic stroke, uncontrolled severe arterial hypertension, diabetic retinopathy, recent neuro- or ophthalmologic surgery.
As with other anticoagulants, bleeding may occur at any site (see section 4.8 Undesirable effects). If bleeding occurs, the origin of the haemorrhage should be investigated and appropriate treatment instituted.

Heparin can suppress adrenal secretion of aldosterone leading to hyperkalaemia, particularly in patients such as those with diabetes mellitus, chronic renal failure, pre-existing metabolic acidosis, a raised plasma potassium or taking potassium sparing drugs. The risk of hyperkalaemia appears to increase with duration of therapy but is usually reversible. Plasma

potassium should be measured in patients at risk before starting heparin therapy and monitored regularly thereafter particularly if treatment is prolonged beyond about 7 days.

As with other anti-coagulants, there have been cases of intra-spinal haematomas reported with the concurrent use of enoxaparin sodium and spinal/epidural anaesthesia or spinal puncture resulting in long term or permanent paralysis. These events are rare with enoxaparin sodium dosage regimens 40 mg od or lower. The risk is greater with higher enoxaparin sodium dosage regimens, use of post-operative indwelling catheters or the concomitant use of additional drugs affecting haemostasis such as NSAIDs (see section 4.5 Interaction with other medicinal products and other forms of interaction). The risk also appears to be increased by traumatic or repeated neuraxial puncture or in patients with a history of spinal surgery or spinal deformity.

To reduce the potential risk of bleeding associated with the concurrent use of enoxaparin sodium and epidural anaesthesia/analgesia, the pharmacokinetic profile of the drug should be considered (see section 5.2 Pharmacokinetic properties). Placement and removal of the catheter is best performed when the anticoagulation effect of enoxaparin is low.

Placement or removal of a catheter should be delayed for 10 - 12 hours after administration of DVT prophylactic doses of enoxaparin sodium, whereas patients receiving higher doses of enoxaparin sodium (1.5 mg/kg once daily) will require longer delays (24 hours). The subsequent enoxaparin sodium dose should be given no sooner than 4 hours after catheter removal.

Should the physician decide to administer anticoagulation in the context of epidural/spinal anaesthesia, extreme vigilance and frequent monitoring must be exercised to detect any signs and symptoms of neurological impairment such as midline back pain, sensory and motor deficits (numbness or weakness in lower limbs), bowel and/or bladder dysfunction. Patients should be instructed to inform their nurse or physician immediately if they experience any of the above signs or symptoms. If signs or symptoms of spinal haematoma are suspected, urgent diagnosis and treatment including spinal cord decompression should be initiated.

Percutaneous coronary revascularisation procedures:
To minimise the risk of bleeding following vascular instrumentation during the treatment of unstable angina, non-Q-wave myocardial infarction and acute ST-elevation myocardial infarction, adhere precisely to the intervals recommended between enoxaparin sodium doses. It is important to achieve homeostasis at the puncture site after PCI. If a closure device is used, the sheath can be removed immediately. If a manual compression method is used, sheath should be removed 6 hours after the last IV/SC enoxaparin sodium injection. If treatment is to be continued, the next scheduled dose should be given no sooner than 6 to 8 hours after sheath removal. The site of the procedure should be observed for signs of bleeding or haematoma formation.

For some patients with pulmonary embolism (e.g. those with severe haemodynamic instability) alternative treatment such as thrombolysis or surgery may be indicated.

Prosthetic Heart Valves
There have been no adequate studies to assess the safe and effective use of enoxaparin sodium in preventing valve thrombosis in patients with prosthetic heart valves. Prophylactic doses of enoxaparin are not sufficient to prevent valve thrombosis in patients with prosthetic heart valves. Confounding factors, including underlying diseases and insufficient clinical data, limit the evaluation of these cases. Therapeutic failures have been reported in pregnant women with prosthetic heart valves on full anti-coagulant doses (see section 4.6 Pregnancy and lactation). The use of enoxaparin sodium cannot be recommended for this purpose.

Haemorrhage in the elderly: No increased bleeding tendency is observed in the elderly within the prophylactic dosage ranges. Elderly patients (especially patients aged eighty years and above) may be at an increased risk for bleeding complications within the therapeutic dosage ranges. In the treatment of acute ST-segment Elevation Myocardial Infarction (STEMI), an increase in bleeding events was observed in patients aged 65-75 years suggesting these patients might be at particular risk of bleeding. Careful clinical monitoring is advised (see also section 4.2 Posology and method of administration: Elderly; section 5.2 Pharmacokinetic properties).

Renal impairment: In patients with renal impairment, there is an increase in enoxaparin exposure which increases the risk of bleeding. Since enoxaparin exposure is significantly increased in patients with severe renal impairment (creatinine clearance < 30 ml/min) dosage adjustments are recommended in therapeutic and prophylactic dosage ranges. Although no dosage adjustments are recommended in patients with moderate (creatinine clearance 30-50 ml/min) and mild (creatinine clearance 50-80 ml/min) renal impairment, careful clinical monitoring is advised (see also section 4.2 Posology and method of administration: Renal impairment; section 5.2 Pharmacokinetic properties). In the treatment of acute ST-segment Elevation Myocardial Infarction (STEMI), the data are limited in patients with creatinine levels above 220 and 175 µmol/L for males and females respectively.

Low body weight: In low-weight women (< 45 kg) and low-weight men (< 57 kg), an increase in enoxaparin exposure has been observed within the prophylactic dosage ranges (non-weight adjusted), which may lead to a higher risk of bleeding. Therefore, careful clinical monitoring is advised in these patients (see also section 5.2 Pharmacokinetic properties).

Monitoring: Risk assessment and clinical monitoring are the best predictors of the risk of potential bleeding. Routine anti-Xa activity monitoring is usually not required. However, anti-Xa activity monitoring might be considered in those patients treated with LMWH who also have either an increased risk of bleeding (such as those with renal impairment, elderly and extremes of weight) or are actively bleeding.

Laboratory tests:
At doses used for prophylaxis of venous thromboembolism, enoxaparin sodium does not influence bleeding time and global blood coagulation tests significantly, nor does it affect platelet aggregation or binding of fibrinogen to platelets. At higher doses, increases in APTT (activated partial thromboplastin time) and ACT (activated clotting time) may occur. Increases in APTT and ACT are not linearly correlated with increasing enoxaparin sodium antithrombotic activity and therefore are unsuitable and unreliable for monitoring enoxaparin sodium activity.

4.5 Interaction with other medicinal products and other forms of interaction

It is recommended that agents which affect haemostasis should be discontinued prior to enoxaparin therapy unless their use is essential, such as: systemic salicylates, acetylsalicylic acid, NSAIDs including ketorolac, dextran, and clopidogrel, systemic glucocorticoids, thrombolytics and anticoagulants. If the combination cannot be avoided, enoxaparin should be used with careful clinical and laboratory monitoring.

4.6 Pregnancy and lactation

Pregnancy: Animal studies have not shown any evidence of foetotoxicity or teratogenicity. In the pregnant rat, the transfer of ^{35}S-enoxaparin across the maternal placenta to the foetus is minimal.

In humans, there is no evidence that enoxaparin crosses the placental barrier during the second trimester of pregnancy. There is no information available concerning the first and the third trimesters.

As there are no adequately powered and well-controlled studies in pregnant women and because animal studies are not always predictive of human response, this drug should be used during pregnancy only if the physician has established a clear need.

Pregnant women with mechanical prosthetic heart valves
The use of enoxaparin for thromboprophylaxis in pregnant women with mechanical prosthetic heart valves has not been adequately studied. In a clinical study of pregnant women with mechanical prosthetic heart valves given enoxaparin (1 mg/kg bid) to reduce the risk of thromboembolism, 2 of 8 women developed clots resulting in blockage of the valve and leading to maternal and foetal death. There have been isolated postmarketing reports of valve thrombosis in pregnant women with mechanical prosthetic heart valves while receiving enoxaparin for thromboprophylaxis. Pregnant women with mechanical prosthetic heart valves may be at higher risk for thromboembolism. Enoxaparin sodium is not recommended for use in pregnant women with prosthetic heart valves (see section 4.4 Special warnings and precautions for use: Prosthetic heart valves).

Lactation: In lactating rats, the concentration of ^{35}S-enoxaparin or its labelled metabolites in milk is very low.

It is not known whether unchanged enoxaparin is excreted in human breast milk. The oral absorption of enoxaparin is unlikely. However, as a precaution, lactating mothers receiving enoxaparin should be advised to avoid breast-feeding.

4.7 Effects on ability to drive and use machines

Enoxaparin has no effect on the ability to drive and operate machines

4.8 Undesirable effects

The adverse reactions observed in clinical studies and reported in post-marketing experience are detailed below.
Frequencies are defined as follows: very common (≥ 1/10); common (≥ 1/100 to < 1/10); uncommon (≥ 1/1000 to < 1/100); rare (≥ 1/10,000 to <1/1,000); and very rare (< 1/10,000).
Adverse events which have not been observed in clinical trials, but were reported in post-marketing experience are ranked under the frequency "Rare".

Haemorrhages
In clinical studies, haemorrhages were the most commonly reported reaction. These included major haemorrhages, reported at most in 4.2 % of the patients (surgical patients[1]). Some of these cases have been fatal.

As with other anticoagulants, haemorrhage may occur during enoxaparin therapy in the presence of associated risk factors such as: organic lesions liable to bleed, invasive procedures or the concomitant use of medications affecting haemostasis (see section 4.5 Interaction with other medicinal products and other forms of interaction). The origin of the bleeding should be investigated and appropriate treatment instituted.

MedDRA system organ class	Prophylaxis in surgical patients	Prophylaxis in medical patients	Treatment in patients with DVT with or without PE	Treatment in patients with unstable angina and non-Q-wave MI	Treatment in patients with acute STEMI
Vascular disorders	*Very common*: Haemorrhage * *Rare:* Retroperitoneal haemorrhage	*Common:* Haemorrhage *	*Very common:* Haemorrhage * *Uncommon:* Intracranial haemorrhage, Retroperitoneal haemorrhage	*Common:* Haemorrhage * *Rare:* Retroperitoneal haemorrhage	*Common:* Haemorrhage * *Uncommon:* Intracranial haemorrhage, Retroperitoneal haemorrhage

*: such as haematoma, ecchymosis other than at injection site, wound haematoma, haematuria, epistaxis and gastro-intestinal haemorrhage.

In addition, in post marketing experience:
Rare: Cases of spinal haematoma (or neuraxial haematoma) have been reported with the concurrent use of enoxaparin sodium as well as spinal/epidural anaesthesia or spinal puncture and post operative indwelling catheters. These reactions have resulted in varying degrees of neurologic injuries including long-term or permanent paralysis
(see section 4.4 Special warnings and precautions for use).

[1] In surgical patients, haemorrhage complications were considered major: (1) if the haemorrhage caused a significant clinical event, or (2) if accompanied by an haemoglobin decrease ≥ 2 g/dL or transfusion of 2 or more units of blood products. Retroperitoneal and intracranial haemorrhages were always considered
major.

Thrombocytopenia and thrombocytosis

MedDRA system organ class	Prophylaxis in surgical patients	Prophylaxis in medical patients	Treatment in patients with DVT with or without PE	Treatment in patients with unstable angina and non-Q-wave MI	Treatment in patients with acute STEMI
Blood and lymphatic system disorders	*Very common*: Thrombocytosis* *Common:* Thrombocytopenia	*Uncommon:* Thrombocytopenia	*Very common*: Thrombocytosis * *Common:* Thrombocytopenia	*Uncommon:* Thrombocytopenia	*Common:* Thrombocytosis* Thrombocytopenia *Very rare:* Immuno-allergic thrombocytopenia

*: Platelet increased > 400 G/L

In addition, in post marketing experience:
Rare: Cases of immuno-allergic thrombocytopenia with thrombosis; in some of them thrombosis was complicated by organ infarction or limb ischaemia (see section 4.4 Special warnings and precautions for use: Monitoring).

Other clinically relevant adverse reactions

These reactions are presented below, whatever the indications, by system organ class, frequency grouping and decreasing order of seriousness.

MedDRA system organ class	All indications
Immune system disorders	*Common:* Allergic reaction *Rare:* Anaphylactic / anaphylactoid reaction
Hepatobilary disorders	*Very common:* Hepatic enzymes increase (mainly transaminases **)
Skin and subcutaneous tissue disorders	*Common:* Urticaria, pruritus, erythema, *Uncommon:* Bullous dermatitis
General disorders and administration site conditions	*Common:* Injection site haematoma, injection site pain, other injection site reaction* *Uncommon:* Local irritation; skin necrosis at injection site
Investigations	*Rare:* Hyperkaliemia

*: such as injection site oedema, haemorrhage, hypersensitivity, inflammation, mass, pain, or reaction (NOS)
**: transaminases levels > 3 times the upper limit of normality

In addition, in post marketing experience:
• Skin and subcutaneous disorders
Rare:
- Cutaneous vasculitis, skin necrosis usually occurring at the injection site (these phenomena have been usually preceded by purpura or erythematous plaques, infiltrated and painful). Treatment with enoxaparin sodium must be discontinued.
- Injection site nodules (inflammatory nodules, which were not cystic enclosure of enoxaparin). They resolve after a few days and should not cause treatment discontinuation.

Valve thrombosis in patients with prosthetic heart valves have been reported rarely, usually associated with inadequate dosing (see section 4.4 Special warnings and precautions for use).

Long term treatment with heparin has been associated with a risk of osteoporosis. Although this has not been observed with enoxaparin the risk of osteoporosis cannot be excluded.

Heparin products can cause hypoaldosteronism which may result in an increase in plasma potassium. Rarely, clinically significant hyperkalaemia may occur particularly in patients with chronic renal failure and diabetes mellitus (see section 4.4 Special warnings and precautions for use).

4.9 Overdose

Orally administered enoxaparin is poorly absorbed and even large oral doses should not lead to any serious consequences. This may be checked by plasma assays of anti-Xa and anti-IIa activities.

Accidental overdose following parenteral administration may produce haemorrhagic complications. The anticoagulant effects can be largely neutralised by the slow intravenous injection of Protamine, but even with high doses of Protamine, the anti-Xa activity of enoxaparin sodium is never completely neutralised (maximum about 60%). The initial dose of Protamine depends on the dose of enoxaparin given and also consideration of the maximum recommended Protamine dose (50mg). Data on Protamine dosing in humans for enoxaparin overdose is extremely limited. The available data suggest that in the first 8 hours after enoxaparin administration 1mg Protamine should neutralise the effects of 1mg of enoxaparin. Where the dose of enoxaparin has exceeded 50mg, an initial dose of 50mg Protamine would be appropriate, based on the maximum recommended single protamine dose. Decisions regarding the necessity and dose of subsequent Protamine injections should be based on clinical response rather than measurement of anti Xa or anti XIIa results. The physician should also consider that the amount of enoxaparin in the body drops to 50% after 8 hours and 33% or less after 12 hours. The dose of Protamine should be adjusted depending on the length of time since enoxaparin was administered.

5 PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Antithrombotic agent, heparin group. ATC code B01A B05

Enoxaparin is a low molecular weight heparin with a mean molecular weight of approximately 4,500 daltons. The drug substance is the sodium salt. The molecular weight distribution is:
<2000 daltons ≤20%
2000 to 8000 daltons ≥68%
>8000 daltons ≤18%

Enoxaparin sodium is obtained by alkaline depolymerization of heparin benzyl ester derived from porcine intestinal mucosa. Its structure is characterized by a 2-O-sulfo-4-enepyranosuronic acid group at the non-reducing end and a 2-N,6-O-disulfo-D-glucosamine at the reducing end of the chain. About 20% (ranging between 15% and 25%) of the enoxaparin structure contains an 1,6 anhydro derivative on the reducing end of the polysaccharide chain.

Enoxaparin sodium is characterised by a higher ratio of antithrombotic activity to anticoagulant activity than unfractionated heparin. At recommended doses, it does not significantly influence platelet aggregation, binding of fibrinogen to platelets or global blood clotting tests such as APTT and prothrombin time.

Enoxaparin binds to anti-thrombin III leading to inhibition of coagulation factors IIa and Xa.

Enoxaparin has been shown to increase the blood concentration of Tissue Factor Pathway Inhibitor in healthy volunteers.

5.2 Pharmacokinetic properties

Enoxaparin is rapidly and completely absorbed following subcutaneous injection. The maximum plasma anti-Xa activity occurs 1 to 4 hours after injection with peak activities in the order of 0.16 IU/ml and 0.38 IU/ml after doses of 20 mg or 40 mg respectively. The anti-Xa activity generated is localised within the vascular compartments and elimination is characterised by a half life of 4 to 5 hours. Following a 40 mg dose, anti-Xa activity may persist in the plasma for 24 hours.

A 30mg IV bolus immediately followed by a 1mg/kg SC every 12 hours provided initial peak anti-Factor Xa levels of 1.16IU/ml (n=16) and average exposure corresponding to 88% of steady state levels.

A linear relationship between anti-Xa plasma clearance and creatinine clearance at steady-state has been observed, which indicates decreased clearance of enoxaparin sodium in patients with reduced renal function. In patients with severe renal impairment (creatinine clearance < 30 ml/min), the AUC at steady state is significantly increased by an average of 65% after repeated, once daily subcutaneous doses of 40mg.

Hepatic metabolism by desulphation and depolymerisation also contributes to elimination. The elimination half life may be prolonged in elderly patients although no dosage adjustment is necessary.

A study of repeated, once daily subcutaneous doses of 1.5 mg/kg in healthy volunteers suggests that no dosage adjustment is necessary in obese subjects (BMI 30-48 kg/m^2) compared to non-obese subjects.

Enoxaparin, as detected by anti-Xa activity, does not cross the placental barrier during the second trimester of pregnancy.

Low Body Weight
When non-weight adjusted dosing was administered, it was found after a single-subcutaneous 40 mg dose, that anti-Xa exposure is 52% higher in low-weight women (<45 kg) and 27% higher in low-weight men (<57 kg) when compared to normal weight control subjects (see section 4.4 Special warnings and precautions for use: Low Body Weight).

Pharmacokinetic interactions
No pharmacokinetic interactions were observed between enoxaparin and thrombolytics when administered concomitantly.

5.3 Preclinical safety data

No long-term studies in animals have been performed to evaluate the carcinogenic potential of enoxaparin.

Enoxaparin was not mutagenic in *in vitro* tests, including the Ames test, mouse lymphoma cell forward mutation test, and human lymphocyte chromosomal aberration test, and the *in vivo* rat bone marrow chromosomal aberration test.

Enoxaparin was found to have no effect on fertility or reproductive performance of male and female rats at SC doses up to 20 mg/kg/day. Teratology studies have been conducted in pregnant rats and rabbits at SC doses of enoxaparin up to 30 mg/kg/day. There was no evidence of teratogenic effects or fetotoxicity due to enoxaparin.

Besides the anticoagulant effects of enoxaparin, there was no evidence of adverse effects at 15 mg/kg/day in the 13-week subcutaneous toxicity studies both in rats and dogs and at 10 mg/kg/day in the 26-week subcutaneous and intravenous toxicity studies both in rats and monkeys.

6 PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Water for Injections

6.2 Incompatibilities

Subcutaneous Injection
Clexane should not be mixed with any other injections or infusions.

Intravenous (Bolus) Injection for acute STEMI indication only
Enoxaparin sodium may be safely administered with normal saline solution (0.9%) or 5% dextrose in water.

6.3 Shelf life

36 months

6.4 Special precautions for storage

Do not store above 25°C. Do not refrigerate or freeze.
Clexane pre-filled syringes are single dose containers - discard any unused product

6.5 Nature and contents of container

Solution for injection in Type I glass pre-filled syringes fitted with injection needle and an automatic safety device in packs of 10.

6.6 Special precautions for disposal

See section 4.2 Posology and method of administration.

7 MARKETING AUTHORISATION HOLDER

Sanofi-aventis
One Onslow Street
Guildford
Surrey, GU1 4YS
UK

8 MARKETING AUTHORISATION NUMBER(S)

PL 04425/0187

9 DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

Date of first authorisation: 22 October 1990
Date of latest renewal: 8 August 2002

10 DATE OF REVISION OF THE TEXT

19 December 2011

LEGAL STATUS

POM

Warwick
Medical School

Muhammad Akhtar
Centre for Reproductive Medicine
Clinical Sciences Research Laboratory
Warwick Medical School

31 July 2012

Endometrial flushing of low molecular weight heparin study

Dear Muhammad

I am writing to confirm that I have discussed with you your planned study of endometrial flushing of heparin. In particular, I have given advice regarding the study design and statistical power.

I wish you every success in your research.

Yours sincerely

Nigel Stallard
Professor of Medical Statistics

Nigel Stallard
Professor of Medical Statistics

Head, Statistics and Epidemiology
Division of Health Sciences
Warwick Medical School
The University of Warwick
Coventry CV4 7AL, United Kingdom

Tel: +44 24 7657 5130
Fax: +44 24 7652 8375
Email: n.stallard@warwick.ac.uk

THE UNIVERSITY OF
WARWICK

NHS
Health Research Authority

NRES Committee West Midlands - Edgbaston
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839368
Facsimile: 0115 8839294

05 November 2012

Professor Siobhan Quenby
Biomedical Research Institute
University Hospitals Coventry & Warwickshire NHS Trust
Clifford Bridge Road
Coventry
CV2 2DX

Dear Professor Quenby

Study title:	**Endometrial Flushing of Low Molecular Weight Heparin Improves Decidualisation- a prospective randomised control pilot study**
REC reference:	**12/WM/0347**
IRAS project reference:	**112835**
Protocol number:	**HEP001QUEN**
EudraCT number:	**2012-003682-18**

Thank you for your letter of 31 October 2012, 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.

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.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites listed in the application, 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 been notified of the outcome of 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 one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

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

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

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System 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 approvals from host organisations

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

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).

Approved documents

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

Document	*Version*	*Date*
Covering Letter	1	28 September 2012
Evidence of insurance or indemnity		
GP/Consultant Information Sheets	4	09 September 2012
Investigator CV		
Letter from Sponsor		
Letter from Statistician		
Letter of invitation to participant	5	27 October 2012
Participant Consent Form	5	27 October 2012
Participant Information Sheet	5	27 October 2012
Protocol	5	27 October 2012
Questionnaire: Non-Validated Questionnaire	4	09 September 2012
REC application	IRAS 3.4	28 September 2012
Response to Request for Further Information		31 October 2012
Summary/Synopsis	SmPC	

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

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 NRES 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 National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/WM/0347	**Please quote this number on all correspondence**

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

Yours sincerely

pp L Gregory

Mr Paul Hamilton
Chair

Email: lisa.gregory@nottspct.nhs.uk

Enclosures: "After ethical review – guidance for researchers" *[SL-AR1]*

Copy to: *Dr Peter Hedges*
Ceri Jones, University Hospitals Coventry And Warwickshire NHS Trust

Dr P Hedges
UNIVERSITY OF WARWICK
UNIVERSITY HOUSE
KIRBY CORNER ROAD
COVENTRY
CV4 8UW
UNITED KINGDOM

19/10/2012

Dear Dr P Hedges

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our reference:	24637/0004/001-0001
Eudract Number:	2012-003682-18
Product:	CLEXANE SYRINGES 100MG/ML
Protocol number:	HEP001QUEN

NOTICE OF ACCEPTANCE

I am writing to inform you that the Licensing Authority accepts your request for a clinical trial authorisation (CTA), received on 08/10/2012.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed.

Yours sincerely,

Clinical Trials Unit
MHRA

Heparin for assisted reproduction (Protocol)

Akhtar M, Sur S, Raine-Fenning N, Jayaprakasan K, Thornton JG, Quenby S

THE COCHRANE COLLABORATION®

This is a reprint of a Cochrane protocol, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2011, Issue 11

http://www.thecochranelibrary.com

WILEY
Publishers Since 1807

TABLE OF CONTENTS

[Intervention Protocol]

Heparin for assisted reproduction

Muhammad Akhtar[1], Shyamaly Sur[2], Nick Raine-Fenning[2], Kannamannadiar Jayaprakasan[2], Jim G Thornton[3], Siobhan Quenby[4]

[1]Clinical Reproductive Medicine Unit, University Hospitals, Coventry & Warwickshire NHS Trust, Coventry, UK. [2]Division of Obstetrics and Gynaecology, School of Clinical Sciences, University of Nottingham, Nottingham, UK. [3]Department of Obstetrics and Gynaecology, University of Nottingham, Nottingham, UK. [4]Clinical Sciences Research Institute, University of Warwick, Coventry, UK

Contact address: Muhammad Akhtar, Clinical Reproductive Medicine Unit, University Hospitals, Coventry & Warwickshire NHS Trust, Clifford Bridge Road, Coventry, UK. drmakh@hotmail.com.

Editorial group: Cochrane Menstrual Disorders and Subfertility Group.
Publication status and date: New, published in Issue 11, 2011.

Citation: Akhtar M, Sur S, Raine-Fenning N, Jayaprakasan K, Thornton JG, Quenby S. Heparin for assisted reproduction. *Cochrane Database of Systematic Reviews* 2011, Issue 11. Art. No.: CD009452. DOI: 10.1002/14651858.CD009452.

ABSTRACT

This is the protocol for a review and there is no abstract. The objectives are as follows:

To evaluate the risks and benefits of periconceptual heparin in women undergoing an ART cycle.

BACKGROUND

Description of the condition

Infertility is the failure of a couple of reproductive age to conceive after having regular unprotected sexual intercourse for a period of 12 months or more. Primary infertility refers to couples who have never conceived, and secondary infertility refers to couples who have previously conceived but are unable to do so again after a year of trying.

Infertility affects 15% of couples and is becoming increasingly common. Of these couples, 70% will have primary and 30% secondary infertility. Assisted reproduction techniques (ART) have been employed to help some of these couples achieve a pregnancy. Assisted reproduction has significant physical, social, psychological and financial implications. The success of assisted reproductive treatment is determined by clinical pregnancy and the live birth of a child. Live birth rates with ART vary from 5% to 60%; hence various adjuncts have been employed during assisted reproduction to increase the likelihood of pregnancy and live birth. The effectiveness of these adjuncts remains to be determined in many cases. Heparin, given as an adjunct to women with or without a known thrombophilia, is one such therapy and has been suggested as being efficacious in improving implantation (attachment of the fertilised egg to the wall of the uterus) and achieving pregnancy.

This Cochrane review will provide evidence-based knowledge of the efficacy of heparin given in the periconceptual period (around the time of conception) to reduce implantation failure in women who have a history of infertility and are undergoing assisted reproduction treatments. In this review we will not be assessing the efficacy of heparin as an antithrombophilic agent (preventing blood clots) later in pregnancy or in women with a history of recurrent miscarriage.

Heparan sulphates have an important role in conception and early pregnancy events. The role of heparin (a structural analogue of Heparan) in assisted conception is, however, not clear. Heparin is a linear polydisperse polysaccharide consisting of 1-4 linked pyranosyluronic acid and 2-amino-deoxyglucopyranose (glucosamine) residues (Comper 1981). Owing to their highly anionic nature, heparin and heparan sulphate have high binding affinity to antithrombin, growth factors, growth factor receptors, viral envelope proteins and extracellular matrix molecules.

Heparan sulphate proteoglycans (HSPGs) are expressed throughout the reproductive tract and are involved in the regulation of endometrial cycling (Potter et al 1992; Kelly et al 1995, San Martin et al 2004; Germeyer et al 2007; Lai et al 2007; Xu et al 2007).

Description of the intervention

When heparin is used as an adjunct treatment during assisted reproduction, there is no consensus regarding the optimum type of heparin, either unfractionated heparin or low molecular weight heparin, or the dose. This is an area which will be considered in the review.

Low molecular weight heparins (LMWH) are derived from heparin by enzymatic (for example tinzaparin) or chemical (for example dalteparin, nadroparin and enoxaparin) depolymerization of unfractionated heparin (UFH), which in itself cannot be synthesized in vitro.

UFH and LMWHs facilitate the anticoagulant effect of antithrombin (Bick et al 2005) but, compared with UFH, LMWH has reduced antifactor IIa activity leading to inefficient inhibition of thrombin by antithrombin. However, the smaller LMWH fragments inactivate factor Xa with equal efficacy. LMWH has a longer half-life, a more predictable antithrombotic response, and a substantially lower risk of heparin-induced thrombocytopenia (HIT) (Warkentin et al 1995; Warkentin and Greinacher et al 2004) and osteoporosis (Murray et al 1995), which has obvious clinical benefits.

LMWHs have a mean molecular weight of 4300 to 5000 kDa (range 1000 to 10 000 kDa), compared to 15,000 kDa for UFH (Nelson and Greer et al 2008).

How the intervention might work

Implantation is a complex, dynamic process which involves coordination of various interactions at an intra and intercellular level. The interaction between the developing embryo and the endometrium is still not fully understood; however heparin can potentially modulate many of the known mechanisms that underlie the successful implantation of the developing embryo.

Traditionally, the role of heparin in early pregnancy was believed to be in the prevention of blood clotting during implantation and placentation in women with inherited and acquired thrombophilias. However, more recent work suggests a possible therapeutic role for heparin in other mechanisms fundamental to implantation. UFH as well as LMWH are able to modulate the process of decidualisation, whereby the cells in the lining of the womb prepare for pregnancy. This positive effect on decidualisation is a potential mechanism by which heparin improves implantation in ART (Corvinus et al 2003, Poehlmann et al 2005, Fluhr H et al 2010).

Heparin also has the ability to bind with and modulate a wide variety of proteins, which can alter a number of physiological processes that are involved in implantation and trophoblastic development. These processes include adhesion of the blastocyst to the endometrial surface (Wang et al 2002), trophoblastic differentiation and invasion (Erden et al 2006, Quenby et al 2004, Di Simone et al 2007a, Leach et al 2004, Arai et al 1994,; Moller et al 2006, Weigert et al 2001, Nelson and Greer et al 2008, d'Souza et al 2007)

Why it is important to do this review

Heparin is often offered to couples as an adjunct in an attempt to improve live birth rates, its presumed effect being to improve implantation. Clinicians may be using heparin as an adjunct based on biological plausibility rather than evidence of efficacy.
A systematic review is required to determine the efficacy of heparin to increase pregnancy and live birth rates and reduce adverse perinatal outcomes for all women undergoing assisted reproduction.

OBJECTIVES

To evaluate the risks and benefits of periconceptual heparin in women undergoing an ART cycle.

METHODS

Criteria for considering studies for this review

Types of studies

Truly randomised controlled trials (RCTs).
Only trials that are either clearly randomised or claim to be randomised and do not have evidence of inadequate sequence generation such as date of birth or hospital number will be included.

Types of participants

All women undergoing assisted reproduction treatment (ART) with a history of infertility. Women undergoing stimulated or unstimulated intrauterine insemination (IUI) will not be included. Women with a previously known thrombophilia will not be excluded.

Types of interventions

1. Heparin versus no treatment
2. Heparin versus placebo
3. Heparin versus aspirin
4. Heparin versus heparin and aspirin
5. Unfractionated heparin (UFH) versus low molecular weight heparin (LMWH)

Studies will be included if heparin was administered in the periconceptual period (from the day of egg collection or embryo transfer to 14 days later).

Types of outcome measures

Primary outcomes

1. Live birth rate per woman
- Number of live births divided by the number of randomised women (live birth is defined as delivery of one or more live infants)
2. Adverse effects of heparin e.g. any bleeding, bruising, heparin-induced thrombocytopenia (HIT), anaphylaxis and any other unexpected side effects

Secondary outcomes

1. Clinical pregnancy rate per woman
- Number of clinical pregnancies divided by the number of randomised women

The presence of a gestational sac with fetal heart beat on ultrasound scan defines a clinical pregnancy.

2. Pregnancy rate per woman

Number of women achieving a pregnancy divided by the number of randomised women

3. On-going pregnancy rate per woman
- Number of women achieving an on-going pregnancy divided by the number of randomised women (pregnancies going beyond 12 weeks duration)
4. Multiple pregnancy rate per woman
- Incidence of multiple pregnancy per randomised women

The demonstration of more than one sac with a fetal pole on ultrasound scan defines multiple pregnancies.

5. Maternal pregnancy complications including first trimester miscarriage, second trimester miscarriage, preterm delivery, pre-eclampsia, pregnancy-induced hypertension, any maternal bleeding
6. Fetal complications during pregnancy including intrauterine growth restriction, placenta previa, placental abruption

Additional outcomes not appropriate for statistical pooling

Data per cycle, per pregnancy or per embryo transfer (ET) are not appropriate for pooling because of what statisticians refer to as 'unit of analysis errors'. Simple group comparison tests for categorical data require that observations are statistically independent. The use of multiple observations per woman leads to unpredictable bias in the estimate of treatment difference (Vail et al 2003). However, due to the frequency with which this form of data is reported in subfertility research it will be entered into the 'table of comparisons' for the following outcomes:

• implantation rate, the number of fetal sacs divided by the number of embryos transferred;
• incidence of miscarriage per total number of pregnancies;
• incidence of multiple pregnancies per total number of pregnancies.

Search methods for identification of studies

A comprehensive and exhaustive search strategy has been developed in consultation with the Trials Search Coordinator of the Cochrane Menstrual Disorders and Subfertility Group. The strategy will be used in an attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, and in progress). Relevant trials will be identified from both electronic databases and other resources.

Completion of the review is expected within one year of publication of the protocol on *The Cochrane Library*. It is also the intention of the review authors that a new search for RCTs will be performed every two years. When an important study is published we will update the review accordingly.

Electronic searches

We will search the following electronic databases, from inception to the present with the Cochrane highly sensitive search strategy for identifying randomised trials, which appears in the *Cochrane Handbook for Systematic Reviews of Interventions* (version 5.1.0; chapter 6, 6.4.11) (Higgins 2011).

1. The Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* latest issue) (see Appendix 1).
2. English language electronic databases: MEDLINE, EMBASE and PsycINFO (see Appendix 2, Appendix 3, Appendix 4).
3. *The Cochrane Library* (www.cochrane.org/index.htm) for DARE, the Database of Abstracts of Reviews of Effects (reference lists from non-Cochrane reviews on similar topics).
4. Current Controlled Trials (www.controlled-trials.com).
5. The World Health Organization International Trials Registry Platform search portal (www.who.int/trialsearch/Default.aspx).

Searching other resources

We will search the references lists of all included studies and relevant reviews to identify further relevant articles.

If required, we will contact authors and experts in the relevant field for potential studies.

We will do a search for grey literature.

Data collection and analysis

We will perform statistical analysis in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). Review Manager 5.1 will be used for input of data.

Selection of studies

The title, abstract, and keywords of every record retrieved will be scrutinized independently by two review authors to determine which studies require further assessment. The full text will be retrieved when the information given in the titles, abstracts, and keywords suggest that the randomised controlled study intervention is heparin as an adjunct to assisted reproduction therapy.

If there are any doubts regarding these criteria, from scanning the titles and abstracts, the full article will be retrieved for clarification. Disagreements will be resolved by discussion with a third review author (Professor S Quenby), if necessary. The authors of trials will be contacted to provide missing data, if required.

Data extraction and management

The following information will be extracted from the studies included in the review. It will be presented in the table 'Characteristics of included studies'.

Trial characteristics

These will include:

1. method of generating randomisation sequence;
2. allocation concealment;
3. trial design;
4. number of women screened for eligibility then randomised, excluded, and finally analysed;
5. duration, timing, and location of the trial;
6. source of funding.

Baseline characteristics of the studied groups

1. Age of the women
2. Duration of infertility
3. Type of ART
4. Previous fertility treatments

Intervention

1. Type of intervention and control group
2. Dose regimen and timing

Outcomes

1. Outcomes
2. How outcomes were defined
3. How outcomes were measured
4. Timing of outcome measurement

All data will be extracted independently by two review authors using forms designed according to Cochrane guidelines. Additional information will be sought from the authors on trial methodology and trial data for trials that appear to meet the eligibility criteria but have aspects of methodology that are unclear or data in an unsuitable form for meta-analysis.

Differences of opinion are to be noted and resolved by consensus.

Assessment of risk of bias in included studies

Assessment of risk of bias in the included studies will be independently performed by two review authors; disagreements will be noted and resolved by a third review author.

The risk of bias table will be included in the table 'Characteristics of included studies'.

The following risk of bias domains will be assessed according to modification of the quality criteria specified by the *Cochrane Handbook for Systematic Reviews of Interventions* 5.1.0.

1. Random sequence generation method (e.g. computer generated, random number tables, or drawing lots)
2. Allocation concealment: adequate (e.g. third party, sealed envelopes); inadequate (e.g. open list of allocation codes); not clear (e.g. not stated).
3. Blinding of participants, personnel, and outcome assessors.
4. Whether an intention-to-treat analysis was performed or not.
5. Incomplete outcome data.
6. Selective outcome reporting.
7. Any other sources of bias not included in this protocol.

Measures of treatment effect

All outcomes are expected to be dichotomous. We will use the numbers of events in the control and intervention groups of each study to calculate odds ratios (OR) with 95% confidence intervals (CI).

Unit of analysis issues

The primary analysis will be per woman randomised. Reported data that do not allow valid analysis (for example, 'per cycle' rather than 'per woman', where women contribute more than one cycle) will be briefly summarised in an additional table and will not be used in meta-analysis. Multiple live births (for example, twins or triplets) will be counted as one live birth event.

In cross-over trials, only first cycle data will be included in the analysis.

Dealing with missing data

We will contact the authors of the RCTs to source any missing data or to resolve any queries that may arise.

If required we will extract data to allow an intention-to-treat analysis (this analysis will include all the participants in the original randomly assigned groups). If the participant numbers randomised and the numbers analysed are inconsistent then the percentage loss to follow up will be calculated and reported in an additional table.

Assessment of heterogeneity

The review authors will check to see if the participants, interventions, and outcomes in the included studies are similar enough to consider pooling in a meta-analysis.

Tests for statistical heterogeneity in pooled data will be carried out using the Chi^2 test, with significance set at $P < 0.1$. The I^2 statistic will be used to estimate the total variation across studies that is due to heterogeneity, where < 25% is considered as low-level, 25% to 50% as moderate-level, and > 50% as high-level heterogeneity. If high levels of heterogeneity (I^2 > 50%) are seen for primary outcomes, we will explore possible sources of heterogeneity using sensitivity and subgroup analyses described below.

Assessment of reporting biases

Potential publication bias will be assessed using a funnel plot, or other corrective analytical methods, depending on the number of included studies (Egger et al 1997).

Data synthesis

Meta-analyses will be performed, as appropriate, where data are available from multiple studies investigating the same treatment, and where the outcome has been measured in a standard way between the studies. A fixed-effect model will be used. We will undertake this meta-analysis according to methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). An increase in the odds of a particular outcome, which may be beneficial (for example, live birth) or detrimental (for example, adverse effects), will be displayed graphically in the meta-analyses to the right of the centre-line and a decrease in the odds of an outcome to the left of the centre-line.

Subgroup analysis and investigation of heterogeneity

Where data are available, we will conduct subgroup analyses to investigate the following.

1. Efficacy of heparin with different ART excluding IUI.
2. Efficacy of adjunct therapy of heparin with or without thrombophilia for women undergoing ART.
3. Duration, dose, timing and type of heparin therapy during ART.
4. Any other adjunct therapy used in addition with heparin during ART.
5. Efficacy of heparin during ART according to age.
6. Efficacy of heparin during ART according to number of implantation failures.
7. Efficacy of heparin with fresh versus frozen embryo transfer.

Factors such as length of follow-up and use of adjusted or unadjusted analysis will be considered in interpretation of any heterogeneity.

Sensitivity analysis

We will perform sensitivity analyses in order to explore the influence of the following factors on effect size:

1. Publication status of studies (published or unpublished)
2. Study quality, such as allocation concealment, blinding, and numbers lost to follow up (considered separately).

ACKNOWLEDGEMENTS

We thank Jane Clarke and Helen Nagels (Managing Editors), Marian Showell (Trials Search Coordinator), and the editorial board of the Cochrane Menstrual Disorders and Subfertility Group (MDSG) for their invaluable assistance in developing this protocol.

REFERENCES

Additional references

Arai et al 1994
Arai T, Parker A, Busby W Jr, Clemmons DR. Heparin, heparan sulfate, and dermatan sulfate regulate formation of the insulin-like growth factor-I and insulin-like growth factor-binding protein complexes. *The Journal of Biological Chemistry* 1994;**269**:20388–93.

Bick et al 2005
Bick RL, Frenkel EP, Walenga J, Fareed J, Hoppensteadt DA. Unfractionated heparin, low molecular weight heparins, and pentasaccharide: basic mechanism of actions, pharmacology and clinical use. *Hematology Oncology Clinics of North America* 2005;**19**:1–51.

Comper 1981
Comper WD. *Heparin (and related polysaccharides) structural and functional properties*. Gordon and Breach Publication group, 1981.

Corvinus et al 2003
Corvinus FM, Fitzgerald JS, Friedrich K, Markert UR. Evidence for a correlation between trophoblast invasiveness and STAT3 activity. *American Journal of Reproductive Immunology* 2003;**50**:316–21.

Di Simone et al 2007a
Di Simone N, Di Nicuolo F, Sanguinetti M, Ferrazzani S, D'Alessio MC, Castellani R, et al.Low-molecular weight heparin induces in vitro trophoblast invasiveness: role of matrix metalloproteinases and tissue inhibitors. *Placenta* 2007;**a 28**:298–304.

d'Souza et al 2007
D'Souza SS, Daikoku T, Farach-Carson MC, Carson DD. Heparanase expression and function during early pregnancy in mice. *Biology of Reproduction* 2007;**77**:433–41.

Egger et al 1997
Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**315 (7109)**:629–34.

Erden et al 2006
Erden O, Imir A, Guvenal T, Muslehiddinoglu A, Arici S, Cetin M, et al. Investigation of the effects of heparin and low molecular weight heparin on E-cadherin and laminin expression in rat pregnancy by immunohistochemistry. *Human Reproduction* 2006;**21**:3014–8.

Fluhr H et al 2010
Fluhr H, Spratte J, Ehrhardt J, Steinmüller F, Licht P, Zygmunt, M. Heparin and low-molecular-weight heparins modulate the decidualization of human endometrial stromal cells. *Fertility and Sterility* 2010;**93**:2581–7.

Germeyer et al 2007
Germeyer A, Klinkert MS, Huppertz A-G, Clausmeyer S, Popovici RM, Strowitzki T, et al.Expression of syndecans, cell-cell interaction regulating heparan sulfate proteoglycans, within the human endometrium and their regulation throughout the menstrual cycle. *Fertility and Sterility* 2007; **87**:657–63.

Higgins 2011
Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Intervention. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 . The Cochrane Collaboration 2011.

Kelly et al 1995
Kelly FD, Tawia SA, Rogers PAW. Physiology: immunohistochemical characterization of human endometrial microvascular basement membrane components during the normal menstrual cycle. *Human Reproduction* 1995;**10**:268–76.

Lai et al 2007
Lai T-H, King JA, Shih I-M, Vlahos NF, Zhao Y. Immunological localization of syndecan-1 in human endometrium throughout the menstrual cycle. *Fertility and Sterility* 2007;**87**:121–6.

Leach et al 2004
Leach RE, Kilburn B, Wang J, Liu Z, Romero R, Armant DR. Heparin-binding EGF-like growth factor regulates human extravillous cytotrophoblast development during conversion to the invasive phenotype. *Developmental Biology* 2004;**266**:223–37.

Moller et al 2006
Moller AV, Jorgensen SP, Chen JW, Larnkjaer A, Ledet T, Flyvbjerg A, et al. Glycosaminoglycans increase levels of free and bioactive IGF-I in vitro. *European Journal of Endocrinology* 2006;**155**:297–305.

Murray et al 1995
Murray WJ, Lindo VS, Kakkar VV, Melissari E. Long-term administration of heparin and heparin fractions and osteoporosis in experimental animals. *Blood Coagulation & Fibrinolysis* 1995;**6**:113–8.

Nelson and Greer et al 2008
Nelson SM, Greer IA. The potential role of heparin in assisted conception. *Human Reproduction Update* 2008;**14**: 623–45.

Poehlmann et al 2005
Poehlmann TG, Fitzgerald JS, Meissner A, Wengenmayer T, Schleussner E, FriedrichK, Markert UR. Trophoblast invasion: tuning through LIF, signalling via Stat3. *Placenta* 2005;**26 Suppl**:37–41.

Potter et al 1992
Potter SW, Morris JE. Changes in histochemical distribution of cell surface heparan sulfate proteoglycan in mouse uterus during the estrous cycle and early pregnancy. *The Anatomical Record* 1992;**234**:383–90.

Quenby et al 2004
Quenby S, Mountfield S, Cartwright JE, Whitley GS, Vince G. Effects of low-molecular-weight and unfractionated heparin on trophoblast function. *Obstetrics & Gynecology* 2004;**104**:354–61.

San Martin et al 2004
San Martin S, Soto-Suazo M, Zorn TMT. Perlecan and syndecan-4 in uterine tissues during the early pregnancy in mice. *American Journal of Reproductive Immunology* 2004; **52**:53–9.

Vail et al 2003
Vail A, Gardener E. Common statistical errors in the design and analysis of subfertility trials.. *Human Reproduction* 2003;**18**:1000–4.

Wang et al 2002
Wang L, Brown JR, Varki A, Esko JD. Heparin's anti-inflammatory effects require glucosamine 6-*O*-sulfation and are mediated by blockade of L- and P-selectins. *The Journal of Clinical Investigation* 2002;**110**:127–36.

Warkentin and Greinacher et al 2004
Warkentin TE, Greinacher A. Heparin-induced thrombocytopenia: recognition, treatment, and prevention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004;**126 Suppl**:311–37.

Warkentin et al 1995
Warkentin TE, Levine MN, Hirsh J, Horsewood P, Roberts RS, Gent M, Kelton JG. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *The New England Journal of Medicine* 1995;**332**:1330–5.

Weigert et al 2001
Weigert C, Brodbeck K, Haring HU, Gambaro G, Schleicher ED. Low-molecular-weight heparin prevents high glucose- and phorbol ester-induced TGF-beta 1 gene activation. *Kidney International* 2001;**60**:935–43.

Xu et al 2007
Xu X, Ding J, Rao G, Shen J, Prinz RA, Rana N, Dmowski WP. Estradiol induces heparanase-1 expression and heparan sulphate proteoglycan degradation in human endometrium. *Human Reproduction* 2007;**22**:927–37.

* *Indicates the major publication for the study*

APPENDICES

Appendix 1. CENTRAL search strategy

Menstrual Disorders and Subfertility Group Specialised Register (inception to present)
Ovid the Cochrane Central Register of Controlled Trials (CENTRAL) (inception to present)
There is no language restriction in these search.

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp insemination, artificial/ or exp reproductive techniques, assisted/
11 artificial$ inseminat$.tw.
12 iui.tw.

13 intrauterine insemination.tw.
14 nidation.tw.
15 reproductive technique$.tw.
16 reproduct$ technolog$.tw.
17 exp Embryo Implantation/
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 exp Infertility, Female/
21 ((Female$ or women) adj2 infertil$).tw.
22 ((Female$ or women) adj2 subfertil$).tw.
23 exp Abortion, Habitual/
24 recurrent miscarriage$.tw.
25 or/1-24 (8324)
26 exp heparin/ or exp heparin, low-molecular-weight/ or exp heparinoids/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38

Appendix 2. MEDLINE search strategy

Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) (1950 to present)
The MEDLINE search was combined with the Cochrane highly sensitive search strategy for identifying randomized trials which appears in the *Cochrane Handbook of Systematic Reviews of Interventions (version 5.0.2; chapter 6, 6.4.11)*
There is no language restriction in this search
1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp insemination, artificial/ or exp reproductive techniques, assisted/
11 artificial$ inseminat$.tw.
12 iui.tw.
13 intrauterine insemination.tw.
14 nidation.tw.
15 reproductive technique$.tw.
16 reproduct$ technolog$.tw.
17 exp Embryo Implantation/

18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 exp Infertility, Female/
21 ((Female$ or women) adj2 infertil$).tw.
22 ((Female$ or women) adj2 subfertil$).tw.
23 exp Abortion, Habitual/
24 recurrent miscarriage$.tw.
25 or/1-24
26 exp heparin/ or exp heparin, low-molecular-weight/ or exp heparinoids/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38
40 randomized controlled trial.pt.
41 controlled clinical trial.pt.
42 randomized.ab.
43 placebo.tw.
44 clinical trials as topic.sh.
45 randomly.ab.
46 trial.ti.
47 (crossover or cross-over or cross over).tw.
48 or/40-47
49 exp animals/ not humans.sh.
50 48 not 49
51 39 and 50

Appendix 3. EMBASE search strategy

Ovid EMBASE (01.01.10 to present)
EMBASE is only searched one year back as the UKCC has hand searched EMBASE to this point and these trials are already in CENTRAL.
The EMBASE search is combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) http://www.sign.ac.uk/mehodology/filters.html#random
There is no language restriction in this search
1 exp embryo transfer/ or exp female infertility/ or exp fertilization in vitro/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.

10 exp artificial insemination/
11 artificial$ inseminat$.tw.
12 reproductive technique$.tw.
13 reproduct$ technolog$.tw.
14 exp nidation/
15 (implant$ adj2 fail$).tw.
16 reproduct$ technique$.tw.
17 ((Female$ or women) adj2 infertil$).tw.
18 ((Female$ or women) adj2 subfertil$).tw.
19 exp recurrent abortion/
20 recurrent miscarriage.tw.
21 iui.tw.
22 intrauterine insemination.tw.
23 nidation.tw.
24 exp intracytoplasmic sperm injection/
25 or/1-24
26 exp HEPARIN/ or exp LOW MOLECULAR WEIGHT HEPARIN/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38
40 Clinical Trial/
41 Randomized Controlled Trial/
42 exp randomization/
43 Single Blind Procedure/
44 Double Blind Procedure/
45 Crossover Procedure/
46 Placebo/
47 Randomi?ed controlled trial$.tw.
48 Rct.tw.
49 random allocation.tw.
50 randomly allocated.tw.
51 allocated randomly.tw.
52 (allocated adj2 random).tw.
53 Single blind$.tw.
54 Double blind$.tw.
55 ((treble or triple) adj blind$).tw.
56 placebo$.tw.
57 prospective study/
58 or/40-57
59 case study/
60 case report.tw.
61 abstract report/ or letter/
62 or/59-61

63 58 not 62
64 39 and 63
65 (2010$ or 2011$).em.
66 64 and 65

Appendix 4. PsycINFO search strategy

Ovid PsycINFO (1806 to present)
There is no language restriction in this search

1 exp Reproductive Technology/
2 exp Infertility/
3 exp Embryo/
4 embryo transfer$.tw.
5 in vitro fertili?ation.tw.
6 ivf-et.tw.
7 (ivf or et).tw.
8 icsi.tw.
9 intracytoplasmic sperm injection$.tw.
10 (blastocyst adj2 transfer$).tw.
11 (assist$ adj2 reproducti$).tw.
12 artificial$ inseminat$.tw.
13 iui.tw.
14 intrauterine insemination.tw.
15 nidation.tw.
16 reproductive technique$.tw.
17 reproduct$ technolog$.tw.
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 ((Female$ or women) adj2 infertil$).tw.
21 ((Female$ or women) adj2 subfertil$).tw.
22 exp Spontaneous Abortion/
23 recurrent miscarriage$.tw.
24 or/1-23
25 exp Heparin/
26 heparin$.tw.
27 LMWH$.tw.
28 liquemin.tw.
29 enoxaparin.tw.
30 heparinic acid.tw.
31 dalteparin.tw.
32 tinzaparin.tw.
33 clexane.tw.
34 lovenox.tw.
35 indenox.tw.
36 xaparin.tw.
37 or/25-36
38 24 and 37

HISTORY

Protocol first published: Issue 11, 2011

CONTRIBUTIONS OF AUTHORS

Akhtar Muhammad A (Co-first author)

All correspondence with drafting of the protocol, develop a search strategy, search for trials, obtain copies of trials, select which trials to include, extract data from trials, enter data into RevMan, carry out the analysis, interpret the analysis, draft the final review and update the review.

Sur Shyamaly (Co-first author)

Drafting of the protocol, search for trials, obtain copies of trials, select which trials to include, extract data from trials, enter data into RevMan, carry out the analysis, interpret the analysis, draft the final review and update the review.

Raine-Fenning Nick R

Drafting of the protocol, select which trials to include, interpret the analysis, draft the final review and update the review.

Kannamannadiar Jayaprakasan:

Drafting of the protocol, select which trials to include, carry out the analysis, interpret the analysis, draft the final review and update the review.

Thornton Jim G

Drafting of the protocol, select which trials to include, help in carrying out the analysis, interpret the analysis, draft the final review and update the review.

Quenby Siobhan

Drafting of the protocol, select which trials to include, help in carrying out the analysis, interpret the analysis, draft the final review and update the review.

DECLARATIONS OF INTEREST

The authors have no commercial interest to disclose.

SOURCES OF SUPPORT

Internal sources

- Cochrane Menstrual Disorders and Subfertilty Group, New Zealand.
- Department of Obstetrics and Gynaecology, University of Auckland, New Zealand.
- Clinical Reproductive Medicine Unit, University Hospitals Coventry & Warwickshire NHS Trust, UK.
- Division of Obstetrics & Gynaecology, School of Clinical Sciences, University of Nottingham, UK.
- Clinical Sciences Research Institute, University of Warwick, UK.

External sources

- No sources of support supplied

Heparin for assisted reproduction (Review)

Akhtar MA, Sur S, Raine-Fenning N, Jayaprakasan K, Thornton JG, Quenby S

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2013, Issue 8

http://www.thecochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Review]

Heparin for assisted reproduction

Muhammad A Akhtar[1], Shyamaly Sur[2], Nick Raine-Fenning[2], Kannamannadiar Jayaprakasan[2], Jim G Thornton[3], Siobhan Quenby[4]

[1]Clinical Reproductive Medicine Unit, University Hospitals, Coventry & Warwickshire NHS Trust, Coventry, UK. [2]Division of Obstetrics and Gynaecology, School of Clinical Sciences, University of Nottingham, Nottingham, UK. [3]Department of Obstetrics and Gynaecology, University of Nottingham, Nottingham, UK. [4]Clinical Sciences Research Institute, University of Warwick, Coventry, UK

Contact address: Muhammad A Akhtar, Clinical Reproductive Medicine Unit, University Hospitals, Coventry & Warwickshire NHS Trust, Clifford Bridge Road, Coventry, UK. drmakh@hotmail.com.

Editorial group: Cochrane Menstrual Disorders and Subfertility Group.
Publication status and date: New, published in Issue 8, 2013.
Review content assessed as up-to-date: 6 May 2013.

Citation: Akhtar MA, Sur S, Raine-Fenning N, Jayaprakasan K, Thornton JG, Quenby S. Heparin for assisted reproduction. *Cochrane Database of Systematic Reviews* 2013, Issue 8. Art. No.: CD009452. DOI: 10.1002/14651858.CD009452.pub2.

ABSTRACT

Background

Heparin as an adjunct in assisted reproduction (peri-implantation heparin) is given at or after egg collection or at embryo transfer during assisted reproduction. Heparin has been advocated to improve embryo implantation and clinical outcomes. It has been proposed that heparin enhances the intra-uterine environment by improving decidualisation with an associated activation of growth factors and a cytokine expression profile in the endometrium that is favourable to pregnancy.

Objectives

To investigate whether the administration of heparin around the time of implantation (peri-implantation heparin) improves clinical outcomes in subfertile women undergoing assisted reproduction.

Search methods

A comprehensive and exhaustive search strategy was developed in consultation with the Trials Search Co-ordinator of the Cochrane Menstrual Disorders and Subfertility Group (MDSG). The strategy was used in an attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, and in progress). Relevant trials were identified from both electronic databases and other resources (last search 6 May 2013).

Selection criteria

All randomised controlled trials (RCTs) were included where peri-implantation heparin was given during assisted reproduction. Peri-implantation low molecular weight heparin (LMWH) during IVF/ICSI was given at or after egg collection or at embryo transfer in the included studies. Live birth rate was the primary outcome.

Data collection and analysis

Two review authors independently assessed the eligibility and quality of trials and extracted relevant data. The quality of the evidence was evaluated using GRADE methods.

Main results

Three RCTs (involving 386 women) were included in the review.

Peri-implantation LMWH administration during assisted reproduction was associated with a significant improvement in live birth rate compared with placebo or no LMWH (odds ratio (OR) 1.77, 95% confidence interval (CI) 1.07 to 2.90, three studies, 386 women, I^2 = 51%, very low quality evidence with high heterogeneity). There was also a significant improvement in the clinical pregnancy rate with use of LMWH (OR 1.61, 95% CI 1.03 to 2.53, three studies, 386 women, I^2 = 29%, very low quality evidence with low heterogeneity).

However these findings should be interpreted with extreme caution as they were dependent upon the choice of statistical method: they were no longer statistically significant when a random-effects model was used.

Adverse events were poorly reported in all included studies, with no comparative data available. However, LMWH did cause adverse effects including bruising, ecchymosis, bleeding, thrombocytopenia and allergic reactions. It appeared that these adverse effects were increased if heparin therapy was used over a longer duration.

Authors' conclusions

The results of this Cochrane review of three randomised controlled trials with a total of 386 women suggested that peri-implantation LMWH in assisted reproduction treatment (ART) cycles may improve the live birth rate in women undergoing assisted reproduction. However, these results were dependent on small low quality studies with substantial heterogeneity, and were sensitive to the choice of statistical model. There were side effects reported with use of heparin, including bruising and bleeding, and no reliable data on long-term effects. The results do not justify this use of heparin outside well-conducted research trials.

These findings need to be further investigated with well-designed, adequately powered, double-blind, randomised, placebo-controlled, multicentre trials. Further investigations could also focus on the effects of the local (uterine) and not systemic application of heparin during ART.

PLAIN LANGUAGE SUMMARY

Heparin for assisted reproduction

Review Question

Researchers in The Cochrane Collaboration reviewed the evidence about the effect of administration of heparin around the time of implantation, compared with placebo or no treatment, on clinical outcomes in subfertile women undergoing assisted reproduction.They found three randomised controlled trials.

Background

Heparin is a class of blood thinning drugs that are used in the prevention and treatment of blood clots. It had been suggested that heparin could improve the intrauterine environment by increasing growth factors to improve attachment of the embryo to the lining of the womb. The result could be an improvement in pregnancy rates during assisted reproduction.

Study Characteristics

Three studies with 386 participants were included in the review. All studies included subfertile women undergoing assisted reproduction. The characteristics of the participants differed across studies. One study included women having their first IVF cycle, with no blood clotting disorder. Another study included women with at least one blood clotting disorder. The third study included women with at least two previous unsuccessful assisted reproduction treatment cycles. Low molecular weight heparin (as daily injections) was given to women from the time of egg collection or embryo transfer during assisted reproduction in all three studies. There were no issues with source of funding in any of the studies. The evidence is current to May 2013.

Key Results

The results of this Cochrane review found evidence suggesting that heparin may increase live births and clinical pregnancies. However, these findings should be interpreted with extreme caution as the findings were no longer statistically significant when the review authors checked the effect of using an alternative method of analysis. Moreover, the quality of the trial evidence was poor, heparin had side

effects such as bruising and bleeding, and its long-term safety has not been established. The evidence does not justify heparin use outside well-designed clinical research trials. Such trials are a priority.

Quality of Evidence

The evidence was of very low quality due to small studies with different populations of subfertile women, and inconsistency when using different statistical tests and analyses. So we suggest that further well-designed randomised controlled studies are needed to clarify the possible role of heparin in assisted reproduction.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON *[Explanation]*

Heparin for assisted reproduction

Population: Subfertile women
Settings: Assisted reproduction treatment (ART)
Intervention: Heparin versus placebo or no heparin

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Heparin				
Live birth rate per woman	**173 per 1000**	**271 per 1000** (183 to 378)	**OR 1.77** (1.07 to 2.9)	386 (3 studies)	⊕◯◯◯ **very low**[1]	
Clinical pregnancy rate per woman	**250 per 1000**	**349 per 1000** (256 to 458)	**OR 1.61** (1.03 to 2.53)	386 (3 studies)	⊕◯◯◯ **very low**[1]	

*The basis for the **assumed risk** is the median control group risk across studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Selection Bias found in one study. High Heterogeneity. Results sensitive to choice of statistical model

BACKGROUND

Description of the condition

Infertility is the failure of a couple of reproductive age to conceive after having regular unprotected sexual intercourse for a period of 12 months or more. Primary infertility refers to couples who have never conceived, and secondary infertility refers to couples who have previously conceived but are unable to do so again after a year of trying.

Infertility affects 15% of couples and is becoming increasingly common. Of these couples, 70% will have primary and 30% secondary infertility. Assisted reproduction techniques (ART) have been employed to help some of these couples achieve a pregnancy. Assisted reproduction has significant physical, social, psychological and financial implications. The success of ART can be defined as the live birth of a child. Live birth rates with ART vary from 30% to 50%; hence various adjuncts have been employed during assisted reproduction to increase the likelihood of pregnancy and live birth. The effectiveness of these adjuncts remains to be determined in many cases. Heparin, given as an adjunct to women with or without a known thrombophilia, is one such therapy and has been suggested as being efficacious in improving implantation (attachment of the fertilised egg to the wall of the uterus) and achieving pregnancy.

Description of the intervention

Heparan sulphates have an important role in conception and early pregnancy events. However the role of heparin (a structural analogue of heparan) in assisted conception is not clear. Heparin is a linear polydisperse polysaccharide consisting of 1-4 linked pyranosyluronic acid and 2-amino-deoxyglucopyranose (glucosamine) residues (Comper 1981). Owing to their highly anionic nature, heparin and heparan sulphate have high binding affinity to antithrombin, growth factors, growth factor receptors, viral envelope proteins and extracellular matrix molecules.

Heparan sulphate proteoglycans (HSPGs) are expressed throughout the reproductive tract and are involved in the regulation of endometrial cycling (Potter 1992; Kelly 1995, San Martin 2004; Germeyer 2007; Lai 2007; Xu 2007).

Low molecular weight heparins (LMWH) are derived from heparin by enzymatic (for example tinzaparin) or chemical (for example dalteparin, nadroparin and enoxaparin) depolymerisation of unfractionated heparin (UFH), which in itself cannot be synthesised in vitro.

Unfractionated heparin and LMWH facilitate the anticoagulant effect of antithrombin (Bick 2005) but, compared with unfractionated heparin, LMWH has reduced antifactor IIa activity leading to inefficient inhibition of thrombin by antithrombin. However, the smaller weight LMWH inactivates factor Xa with equal efficacy. Low molecular weight heparin has a longer half-life, a more predictable antithrombotic response, and a substantially lower risk of heparin-induced thrombocytopenia (HIT) (Warkentin 1995; Warkentin 2004) and osteoporosis (Murray 1995), thus having obvious clinical benefits. So in practice, LMWH is used routinely with daily self-administered subcutaneous injections, not requiring close monitoring and with lower risk of side effects.

Low molecular weight heparins have a mean molecular weight of 4300 to 5000 kDa (range 1000 to 10,000 kDa), compared to 15,000 kDa for unfractionated heparin (Nelson 2008).

How the intervention might work

Implantation is a complex, dynamic process which involves coordination of various interactions at an intra- and intercellular level. The interaction between the developing embryo and the endometrium is still not fully understood; however heparin can potentially modulate many of the known mechanisms that underlie the successful implantation of the developing embryo.

Traditionally the role of heparin in early pregnancy was believed to be in the prevention of blood clotting during implantation and placentation in women with inherited and acquired thrombophilia. However, more recent work suggests a possible therapeutic role for heparin in other mechanisms fundamental to implantation. Unfractionated heparin as well as LMWH are able to modulate the process of decidualisation, whereby the cells in the lining of the womb prepare for pregnancy. This positive effect on decidualisation is a potential mechanism by which heparin improves implantation in ART (Corvinus 2003, Poehlmann 2005, Fluhr H 2010).

Heparin also has the ability to bind with and modulate a wide variety of proteins, which can influence a number of physiological processes involved in implantation and trophoblastic development. These processes include adhesion of the blastocyst to the endometrial surface (Wang 2002) and trophoblastic differentiation and invasion (Arai 1994; Weigert 2001; Leach 2004; Quenby 2004; Erden 2006; Moller 2006; Di Simone 2007; d'Souza 2007; Nelson 2008).

Why it is important to do this review

Heparin is often offered to couples as an adjunct in an attempt to improve live birth rates, its presumed effect being to improve implantation. Clinicians may be using heparin as an adjunct based on biological plausibility rather than evidence of efficacy. A systematic review is required to determine the efficacy of heparin to increase pregnancy and live birth rates and reduce adverse perinatal outcomes for all women undergoing assisted reproduction.

When heparin is used as an adjunct treatment during assisted reproduction, there has been no consensus regarding the optimum type of heparin (unfractionated heparin or LMWH) timing or the dose. This is an area which we considered in the review.

This Cochrane review aims to provide evidence about the efficacy of heparin given in the peri-implantation period (around the time of conception) to reduce implantation failure in women who have a history of infertility and are undergoing assisted reproduction treatments. In this review we do not assess the efficacy of heparin as an anti-thrombophilic agent (preventing blood clots) later in pregnancy or in women with a history of recurrent miscarriage.

OBJECTIVES

To investigate whether the administration of heparin around the time of implantation improves clinical outcomes in subfertile women undergoing assisted reproduction.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs).

Types of participants

We included trials of women undergoing assisted reproduction treatment (ART) with a history of infertility. Trials of women with a previously known thrombophilia were included.
Trials involving women undergoing stimulated or unstimulated intrauterine insemination (IUI) were not included.

Types of interventions

1. Heparin versus no treatment.
2. Heparin versus placebo.
3. Heparin versus aspirin.
4. Heparin versus heparin and aspirin.
5. Unfractionated heparin (UFH) versus low molecular weight heparin (LMWH).

Studies were included if heparin was administered in the peri-implantation period (from the day of egg collection or embryo transfer (ET) to 14 days later).

Types of outcome measures

Primary outcomes

1. Live birth rate per woman. Number of live births divided by the number of randomised women (live birth is defined as delivery of one or more live infants).
2. Adverse effects of heparin e.g. any bleeding, bruising, heparin-induced thrombocytopenia (HIT), anaphylaxis and any other unexpected side effects.

Secondary outcomes

1. Clinical pregnancy rate per randomised woman. The presence of at least one gestational sac with fetal heart beat on ultrasound scan defines a clinical pregnancy.
2. Multiple pregnancy rate per randomised woman. The demonstration of more than one sac with a fetal pole on ultrasound scan defines multiple pregnancies.
3. Maternal pregnancy complications including first trimester miscarriage, second trimester miscarriage, preterm delivery, pre-eclampsia, pregnancy-induced hypertension, any maternal bleeding.
4. Fetal complications during pregnancy including intrauterine growth restriction, placenta previa, placental abruption.

Additional outcomes not appropriate for statistical pooling

Data per cycle, per pregnancy or per ET are not appropriate for pooling because of what statisticians refer to as 'unit of analysis errors'. Simple group comparison tests for categorical data require that observations are statistically independent. The use of multiple observations per woman leads to unpredictable bias in the estimate of treatment difference Vail 2003. However, due to the frequency with which this form of data are reported in subfertility research, we planned to report the following outcomes in narrative form:

- implantation rate, the number of fetal sacs divided by the number of embryos transferred;
- incidence of miscarriage per total number of pregnancies;
- incidence of multiple pregnancies per total number of pregnancies.

Search methods for identification of studies

A comprehensive search strategy was developed in consultation with the Trials Search Co-ordinator of the Cochrane Menstrual Disorders and Subfertility Group (MDSG). The strategy was used in an attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, and in progress). Relevant trials were identified from both electronic databases and other resources.
This review will be updated every two years.

Electronic searches

We searched the following electronic databases, from inception to 6 May 2013 with the Cochrane highly sensitive search strategy for identifying randomised trials, which appears in the *Cochrane*

Handbook for Systematic Reviews of Interventions (version 5.1.0; chapter 6, 6.4.11) (Higgins 2011):

1. The Cochrane Central Register of Controlled Trials (CENTRAL) (*The Cochrane Library* latest issue) (see Appendix 1).
2. English language electronic databases: MEDLINE, EMBASE and PsycINFO (see Appendix 2, Appendix 3, Appendix 4).
3. *The Cochrane Library* (www.cochrane.org/index.htm) for DARE, the Database of Abstracts of Reviews of Effects (reference lists from non-Cochrane reviews on similar topics).
4. Current Controlled Trials (www.controlled-trials.com).
5. The World Health Organization International Trials Registry Platform search portal (www.who.int/trialsearch/Default.aspx).

Searching other resources

We searched the references lists of all included studies and relevant reviews to identify further relevant articles and when required, we contacted authors and experts in the relevant field for potential studies.

We performed a search for grey literature.

Data collection and analysis

We performed statistical analysis in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). Review Manager 5.1 was used to input data.

Selection of studies

The title, abstract, and keywords of every record retrieved were scrutinised independently by two review authors (MA, SS) to determine which studies required further assessment. The full texts were retrieved when the information given in the titles, abstracts, and keywords suggested that the randomised controlled study intervention was heparin as an adjunct to assisted reproduction therapy.

If there were any doubts regarding these criteria from scanning the titles and abstracts, the full articles were retrieved for clarification. Disagreements were resolved by discussion with a third review author (Professor S Quenby), if necessary. We contacted the authors of trials to provide missing data, if required.

Data extraction and management

The following information was extracted from the studies included in the review. It is presented in the table 'Characteristics of included studies'.

Trial characteristics

This includes the following items.

1. Method of generating randomisation sequence.
2. Allocation concealment.
3. Trial design.
4. Number of women screened for eligibility then randomised, excluded, and finally analysed.
5. Duration, timing, and location of the trial.
6. Source of funding.

Baseline characteristics of the studied groups

1. Age of the women.
2. Duration of infertility.
3. Type of ART.
4. Previous fertility treatments.

Intervention

1. Type of intervention and control group.
2. Dose regimen and timing.

Outcomes

1. Outcomes.
2. How outcomes were defined.
3. How outcomes were measured.
4. Timing of outcome measurement.

All data were extracted independently by two review authors (MA, SS) using forms designed according to Cochrane guidelines. Additional information was sought from the authors on trial methodology and trial data for trials that appeared to meet the eligibility criteria but had aspects of methodology that were unclear or where data were in an unsuitable form for meta-analysis. We planned to settle any differences of opinion by discussion between the review authors, but there were no disagreements.

Assessment of risk of bias in included studies

Assessment of risk of bias in the included studies was independently performed by two review authors (MA, SS). Disagreements were noted and resolved by a third review author (SQ).

The 'Risk of bias' table was included in the Characteristics of included studies

The following 'Risk of bias' domains were assessed according to the criteria specified by the *Cochrane Handbook for Systematic Reviews of Interventions* 5.1.0.

1. Selection bias: Random sequence generation method (e.g. computer-generated, random number tables, or drawing lots) and allocation concealment: adequate(e.g. third party, sealed envelopes); inadequate (e.g. open list of allocation codes); not clear (e.g. not stated).
2. Performance bias: Blinding of participants and personnel.

3. Detection bias: Blinding of outcome assessments.
4. Attrition bias: Incomplete outcome data and intention-to-treat analysis if used.
5. Reporting bias: selective outcome reporting.
6. Other bias: Any other potential sources of bias not included in this protocol.

Measures of treatment effect

All outcomes were dichotomous. We used the numbers of events in the control and intervention groups of each study to calculate odds ratios (OR) with 95% confidence intervals (CI).

Unit of analysis issues

The primary analysis was per woman randomised. Reported data that did not allow valid analysis (for example, 'per cycle' rather than 'per woman', where women contribute more than one cycle) were briefly summarised in an additional table and were not used in meta-analysis. Multiple live births (for example, twins or triplets) were counted as one live birth event.

Dealing with missing data

The data were analysed on an intention-to-treat basis as far as possible and attempts were made to obtain missing data from the original trialists. Where these were unobtainable, only the available data were analysed.

Assessment of heterogeneity

The review authors (MA, SS) considered whether the participants, interventions, and outcomes in the included studies were similar enough to consider pooling in a meta-analysis.
Tests for statistical heterogeneity in pooled data were carried out using the Chi^2 test, with significance set at $P < 0.1$. The I^2 statistic was used to estimate the total variation across studies that was due to heterogeneity, where < 25% was considered as low-level, 25% to 50% as moderate-level, and > 50% as high-level heterogeneity. If high levels of heterogeneity ($I^2 > 50\%$) were seen for primary outcomes, we explored possible sources of heterogeneity using sensitivity and subgroup analyses.

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases for primary outcomes, we performed a comprehensive search for eligible studies and were alert for duplication of data. We planned to use a funnel plot to explore the possibility of small study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies) if there were 10 or more studies in the primary analysis (Egger 1997).

Data synthesis

Meta-analyses were performed, as appropriate, where data were available from multiple studies investigating the same treatment and where the outcomes had been measured in a standard way. A fixed-effect model was used. We undertook this meta-analysis according to methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). An increase in the odds of a particular outcome, which may be beneficial (for example, live birth) or detrimental (for example, adverse effects), were displayed graphically in the meta-analyses to the right of the centre-line and a decrease in the odds of an outcome to the left of the centre-line.

Subgroup analysis and investigation of heterogeneity

If there were sufficient data, we planned to perform the following subgroup analyses.
1. Efficacy of heparin with different ART excluding IUI.
2. Efficacy of adjunct therapy of heparin with or without thrombophilia for women undergoing ART.
3. Duration, dose, timing and type of heparin therapy during ART.
4. Any other adjunct therapy used in addition with heparin during ART.
5. Efficacy of heparin during ART according to age.
6. Efficacy of heparin with fresh versus frozen ET.

Sensitivity analysis

We performed sensitivity analyses for the primary outcomes to determine whether the review conclusions would have differed if:
1. eligibility were restricted to studies without high risk of bias;
2. a random-effects model had been adopted;
3. the summary effect measure had been risk ratio rather than odds ratio.

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

A 'Summary of findings' table was generated using GRADEPRO software. This table evaluated the overall quality of the body of evidence for main review outcomes, using GRADE criteria (study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness and publication bias). Judgements about evidence quality (high, moderate or low) were justified, documented, and incorporated into reporting of results for main outcomes.

RESULTS

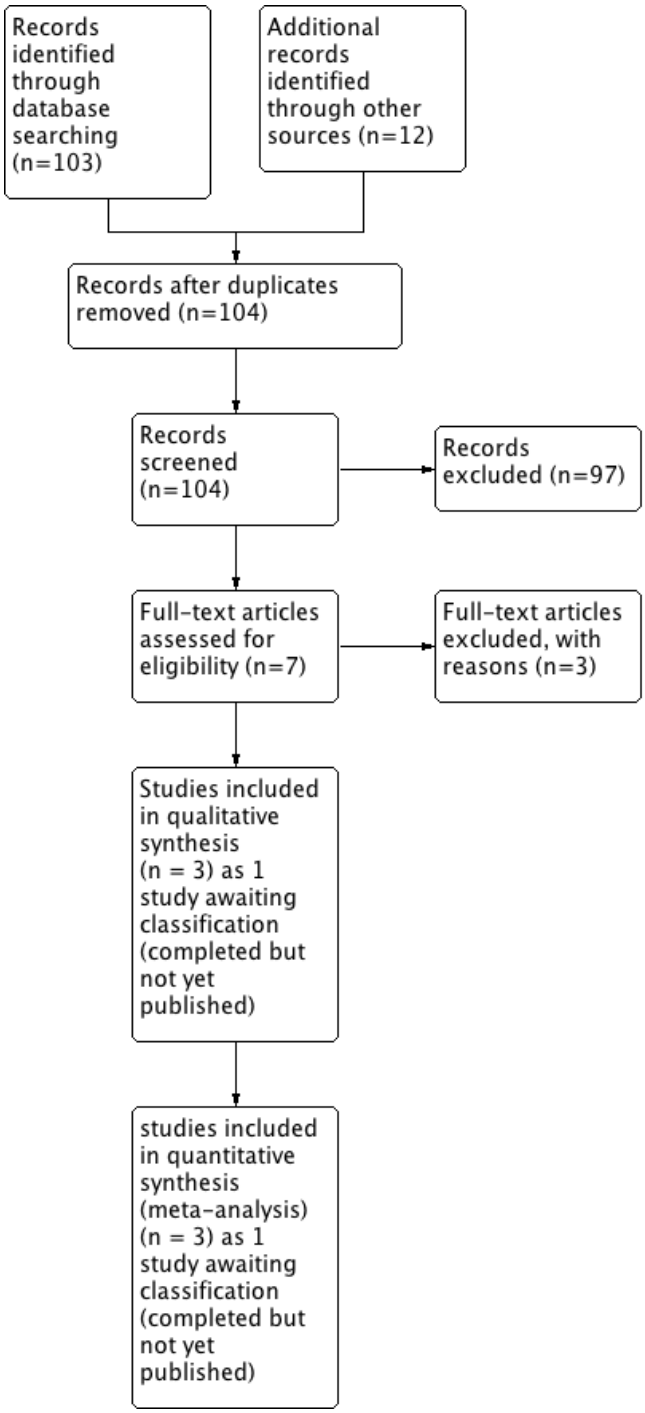
Description of studies

See: Characteristics of included studies; Characteristics of excluded studies; Characteristics of studies awaiting classification.

Results of the search

Seven studies were identified that assessed the use of peri-implantation heparin in assisted reproduction. Of these only three studies were eligible for the review. They compared heparin alone versus either no heparin or placebo. The results of one study were not published yet, however, the characteristics of that study (Mashayekhy 2011) are available in 'Characteristics of studies awaiting classification (completed but not yet published)'. Full agreement existed between the two researchers, concerning inclusion or exclusion of trials. Figure 1

Figure 1. Study Review flow diagram.



Included studies

Three studies Qublan 2008; Urman 2009; Noci 2011 met the criteria for inclusion in this review. For details see Characteristics of included studies

Participants

The total number of trial participants was 386. The upper age limit was < 40 years in all participants in the included studies.

Interventions

All women were included for a single IVF/ICSI (in vitro fertilisation/intracytoplasmic sperm injection) cycle only. Low molecular weight heparin (LMWH) was administered from either oocyte retrieval or embryo transfer (ET), so the treatment protocol varied across studies.
In Qublan 2008, LMWH therapy treatment was started from the day of ET until results of Beta-hCG were available two weeks after ET. If Beta-hCG was 425 IU/mL, LMWH was continued either until delivery or foetal demise was diagnosed. In Noci 2011 LMWH treatment was started on the day of oocyte retrieval until nine weeks of pregnancy with positive pregnancy results. In Urman 2009 LMWH treatment was started a day after oocyte retrieval until 12 weeks of pregnancy with positive pregnancy test results.
Control groups in these studies received placebo (Qublan 2008) or no heparin (Urman 2009; Noci 2011)

Outcomes

All three included studies reported live birth rate per woman as the primary outcome, adverse effects, clinical pregnancy rate per woman, multiple pregnancy rate per woman, implantation rate per woman and miscarriage rate per woman.

Additional outcomes not appropriate for statistical pooling

Data per cycle, per pregnancy or per ET were not appropriate for pooling. We have reported the following in additional tables:

- implantation rate, the number of fetal sacs divided by the number of embryos transferred; Table 1
- incidence of miscarriage per total number of pregnancies; Table 2
- incidence of multiple pregnancies per total number of pregnancies; Table 3

Excluded studies

Three studies failed to meet the inclusion criteria. Colicchia 2011 was excluded because LMWH was used in conjunction with prednisolone. Stern 2003 was excluded because unfractionated heparin (UFH) was used in conjunction with low-dose aspirin. Berker 2011 was excluded because it was a quasi-randomised study. Details are provided in Characteristics of excluded studies.

Risk of bias in included studies

The methodological quality of included studies was documented in the 'Risk of bias' table for each individual study. The 'Risk of bias' summary and 'Risk of bias' graph are presented as Figure 2 and Figure 3.

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

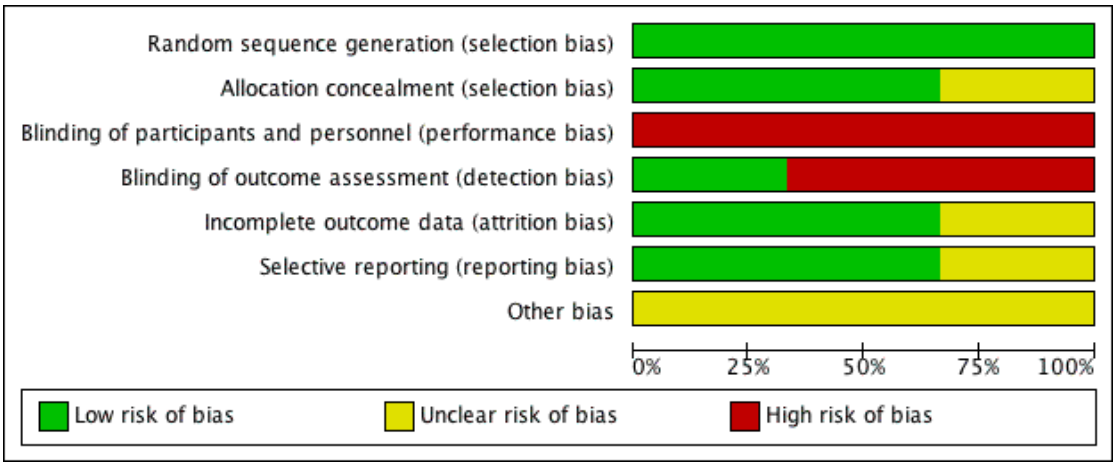
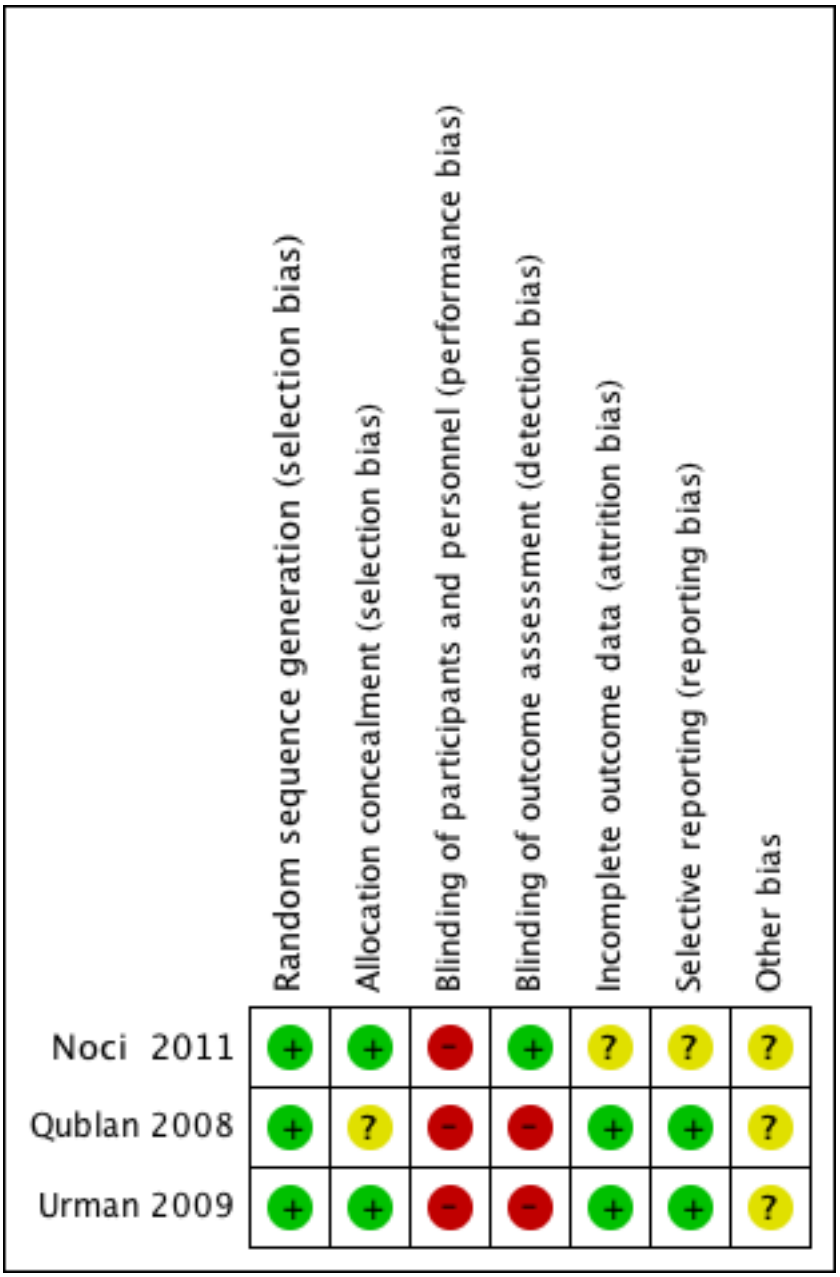


Figure 3. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Sequence generation

All three studies were rated as at low risk of this bias.

Allocation concealment

Two studies were rated as at low risk of this bias (Noci 2011; Urman 2009). The third study was rated as at unclear risk, as concealment of allocation was not described Qublan 2008.

Blinding

All three studies were rated as at high risk of performance bias. Two studies were rated as at high risk of detection bias (Qublan 2008; Urman 2009); the other as low risk of detection bias (Noci 2011).

Incomplete outcome data

No dropouts of participants were reported in one study (Qublan 2008).

In Urman 2009, 153 women were recruited to the trial. Three women in the treatment and control groups were lost to follow-up before completion of initial follow-up (completion of the 20th gestational week for the latest recruited participant who achieved an ongoing pregnancy), and another two women in the LMWH group were lost to follow-up after completion of the 20th gestational week but before delivery or expected completion of the 40th gestational week. Women lost to follow-up during the first period were considered not to have an ongoing pregnancy, and women lost to follow-up in the second period were considered not to have a live birth in the intention-to-treat analysis. The dropout rate was 5.22%. In the final analysis, 75 women in each group were considered. The study was rated as at low risk of attrition bias because trialists compensated for dropouts by imputing a negative outcome to losses to follow-up.

Noci 2011 enrolled 210 patients presenting all the necessary requirements and subjected to ovarian stimulation for IVF/ICSI. On the day of oocyte retrieval, 38 patients were excluded: 30 for the absence of retrieved oocytes or cancelled cycles and eight who decided to decline their participation. One hundred and seventy-two women were allocated to intervention and divided into two groups: 86 women in the control group and 86 women in the treatment group. The final series for analysis contained 153 women because 13 women belonging to the treatment group and six women belonging to the control group had no embryos to transfer, thus they were immediately excluded from the study. Thus in the final analysis, 73 women were in treatment group and 80 women were in the control group. The dropout rate was 8.72% after allocation to the intervention. The study was rated as at unclear risk of attrition bias.

Selective reporting

Two studies were at low risk of bias related to selective reporting (Qublan 2008; Urman 2009). There was no evidence to suggest that the decision by authors of included studies to either publish or not publish any specific outcomes was based on perceived statistical significance. One study (Noci 2011) did not report adverse events and was rated as at unclear risk of selective reporting bias.

Other potential sources of bias

No other potential sources of bias were found in any of the included studies. There were no significant differences noted in the baseline characteristics of intervention and control groups in any of the included studies.

Effects of interventions

See: **Summary of findings for the main comparison** Heparin for Assisted Reproduction - live birth rate

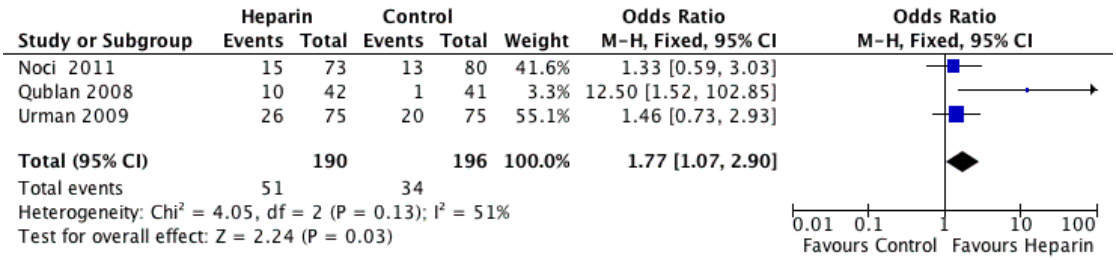
Primary Outcomes

1. Live birth rate per woman

All three included studies assessed the primary outcome, namely 'live birth rate per woman'.

Results pooled in meta-analysis (fixed-effect model) showed that there was a significant improvement in live birth rate with the use of LMWH (odds ratio (OR)1.77, 95% confidence interval (CI) 1.07, 2.90 P = 0.03, I^2 = 51%, three studies, 386 women) in comparison to placebo or no LMWH (Figure 4). Sensitivity analysis performed with a random-effects model showed that there was a non significant improvement in live birth rate with the use of LMWH compared to no LMWH (OR1.85, 95% CI 0.80, 4.24 P,=,0.15, $I^{2,}$ =,51%, three studies, 386 women)

Figure 4. Forest plot of comparison: 1 Heparin versus control, outcome: 1.1 Live Birth Rate per woman.



This finding should be viewed with extreme caution due to high heterogeneity and sensitivity to choice of statistical model.

The evidence was of very low quality as shown in Summary of findings for the main comparison.

2. Adverse effects

Direct adverse effects of heparin including bleeding, bruising, thrombocytopenia or any other side effects were described in all the included studies.

Qublan 2008 reported that the most frequent complications encountered in the heparin-treated group were bleeding (3/42, 7.1%) followed by thrombocytopenia (2/42, 4.8%) and allergic reactions (1/42, 2.4%).

Urman 2009 revealed that platelet counts did not change significantly in the LMWH group during the study period. None of the participants experienced any adverse effects other than small ecchymosis around the LMWH injection sites. None of the participants in the LMWH group discontinued treatment due to pain or ecchymosis around the injection site.

Noci 2011 reported no other adverse effects in the study except minimal bruising at injection site of heparin.

It appeared from the studies that longer duration of heparin therapy increased the number of side effects; however this interpretation must be looked with caution as there was no available controlled comparative data for duration of therapy.

In Qublan 2008 LMWH therapy was started from the day of ET until results of Beta-hCG were available two weeks after ET. If Beta-hCG was 425 IU/mL, LMWH was continued either until delivery or foetal demise was diagnosed. In Noci 2011, LMWH treatment was started on the day of oocyte retrieval until nine weeks of pregnancy with positive pregnancy results. In Urman 2009 LMWH treatment was started a day after oocyte retrieval until 12 weeks of pregnancy with positive pregnancy test results.

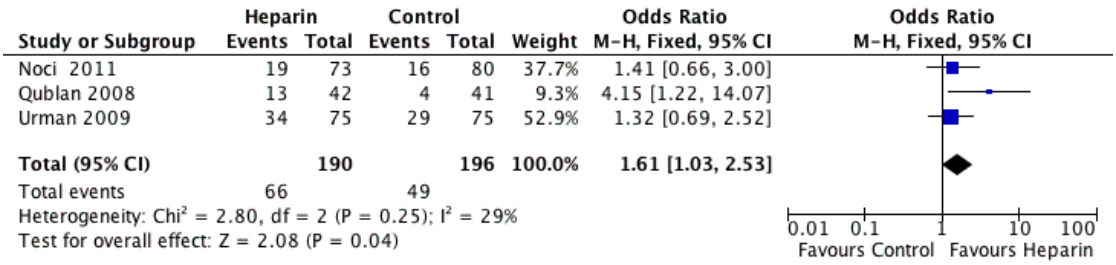
Secondary Outcomes

1. Clinical pregnancy rates per woman

'Clinical pregnancy rate per woman' was described in all included studies.

Results pooled in meta-analysis (fixed-effect model) showed a significant improvement in clinical pregnancy rate with the use of LMWH compared with placebo or no LMWH (OR 1.61 95% CI 1.03, 2.53 P = 0.04, I^2 = 29%, three studies, 368 women) Figure 5. Sensitivity analysis performed with a random-effects model showed no significant improvement in clinical pregnancy rate with the use of LMWH compared to no LMWH (OR 1.66, 95% CI 0.94 to 2.90, I^2 = 29%, three studies, 368 women).

Figure 5. Forest plot of comparison: 1 Heparin versus control, outcome: 1.2 Clinical Pregnancy Rate per woman.



These results should be viewed with caution due to high heterogeneity and sensitivity to choice of statistical model.
The evidence is of very low quality, as shown in Summary of findings for the main comparison.

2. Multiple pregnancy rates per woman

'Multiple pregnancy rates per woman' were not reported in any of the included studies. "Multiple pregnancy rates per total number of pregnancies" was reported in all studies but cannot be pooled for meta-analysis due to unit of analysis errors. Please see Table 3

3. Maternal pregnancy complications

Qublan 2008 reported placental abruption (1/42, 2.4%) in LMWH group. Two (4.9%) women in the placebo group developed pre-eclampsia.
Urman 2009 reported that total numbers of preterm deliveries were nine (34.6%) in LMWH and six (30.0%) in control groups (P = 0.74). Three women delivered in the 32nd week (one set of quadruplets, one set of twins and a singleton, all in LMWH group), one woman (singleton in control group) delivered in the 33rd week, four women delivered in the 34th week (two sets of twins in LMWH group and two sets of twins in the control group), four women delivered in the 35th week (all twins, three and one in LMWH and control groups, respectively) and three women delivered in the 36th week (one singleton in LMWH group and two sets of twins in the control group).
Noci 2011 did not describe any maternal pregnancy complications.

4. Fetal complications during pregnancy

Qublan 2008 reported two intrauterine foetal deaths in the heparin-treated group compared to none in the control group. No further details were provided.
Urman 2009 reported that none of the infants delivered in the study had any congenital malformations. One boy (from the LMWH group) had a unilateral undescended testis, and another infant delivered at the 32nd week (from the LMWH group) underwent surgery due to necrotising enterocolitis.
Noci 2011 did not describe any fetal complications during pregnancy.

Other analyses

There were insufficient studies to conduct the planned subgroup analyses or to construct a funnel plot to assess publication bias.
We considered clinical and methodological differences between the studies that might account for the high heterogeneity in the analysis of live birth. Exclusion of the study that was clearly restricted to women with at least one thrombophilic defect (Qublan 2008) eliminated the heterogeneity (I^2 = 0%). However, with so few studies available for analysis it is unclear whether the effects of the intervention may differ in this population.

DISCUSSION

Summary of main results

The aim of this review was to investigate whether the administration of heparin during the peri-implantation period improves clinical outcomes in subfertile women undergoing assisted reproduction. We found evidence suggesting that administration of peri-implantation low molecular weight heparin (LMWH) may improve live birth and pregnancy rates during assisted reproduction, however the studies were few and small (three studies, total 386 participating women) with high heterogeneity and sensitivity to choice of statistical model. Therefore all results must be interpreted with extreme caution.
Low molecular weight heparin was associated with adverse events, including bruising, ecchymosis, bleeding, thrombocytopenia and allergic reactions. It appeared that adverse effects increased if heparin therapy was used over a longer duration. There were no reliable data on long-term side effects of heparin at this stage of pregnancy.
Overall, this evidence does not justify the present widespread use of LMWH in this population subgroup (previous failed IVF), outside well-conducted randomised trials. Such trials should be a priority.

Overall completeness and applicability of evidence

There were only three studies that could be included in the review and the total sample size was small (386 women) so the findings have to be viewed with caution. Moreover, study characteristics varied: one was a multicentre study Noci 2011 while the two others were conducted at a single centre (Qublan 2008; Urman 2009). There was no uniformity of dose, timing or duration of the intervention. Only one study Qublan 2008 used sodium chloride as placebo control, the other two included studies had no placebo, hence the patients were not blinded. Furthermore, none of the studies described blinding of clinicians.
We were unable to adequately assess the effect of heparin in women with or without thrombophilia undergoing assisted reproduction as only one study (Qublan 2008) included women with thrombophilia, Noci 2011 included women without thrombophilia, the other remaining study(Urman 2009), did not report about the presence or absence of thrombophilia in including participants.
The small numbers of underpowered trials means that there was insufficient evidence to change clinical practice until results of large high quality randomised controlled trials (RCTs) are available.

Quality of the evidence

The studies were small, low quality, and had high heterogeneity reflecting different inclusion criteria. Allocation concealment was not adequately described in one of the three studies, none were double blinded and placebo was used only in one study, creating a risk of performance bias. Risk of detection bias (due to failure to blind of outcome assessment) was also noted in two studies. There was significant heterogeneity noted in the analyses. The quality of the evidence for the main findings was rated as very low, using GRADE criteria (Summary of findings for the main comparison).

Potential biases in the review process

The findings were sensitive to methodological decisions made in the review process, and are therefore to be regarded very cautiously.

Agreements and disagreements with other studies or reviews

It has been suggested that heparin could potentially modulate many of the known mechanisms that underlie successful apposition, adhesion and penetration of the developing embryo. Heparin could improve the endometrial environment for implantation of embryo. Confirmation of the outlined potential of heparin to alter the molecular processes underpinning successful implantation was urgently required given the potential for clinical translation to increased pregnancy and live birth rate and a reduction in adverse perinatal outcomes for all women undergoing assisted reproduction (Nelson 2008).The following studies showed no efficacy of heparin in improving outcome.

- In one small non-randomised study, heparin with low-dose aspirin was given to women with antiphospholipid positive antibodies undergoing assisted reproduction. There were no statistically significant differences detected in implantation, pregnancy and ongoing pregnancy rates between both groups (Kutteh 1997).
- A double-blind, randomised cross-over trial was conducted to investigate whether heparin and low-dose aspirin increase the pregnancy rate in antiphospholipid antibody or antinuclear antibody-seropositive women with IVF implantation failure. Unfractionated heparin and low-dose aspirin were given from day of embryo transfer. It found that there was no significant difference in pregnancy rates or implantation rates between treated and placebo cycles. However, a cross-over design is not appropriate for a pregnancy trial (Stern 2003).
- Heparin was given to women with thrombophilia and repeated implantation failure undergoing assisted reproduction in this prospective cohort study. Authors suggested that it showed improvement in biochemical and clinical pregnancy rates. However, no precise data were published. This study also looked at other factors of implantation failure, therefore it cannot be inferred that this intervention of heparin only improved the success rate of assisted reproduction (Sharif 2010).

The American Society for Reproductive Medicine (Practice Committee of ASRM 2008) assessed available data in 2008 and suggested that assessment of antiphospholipid antibodies was not indicated among couples undergoing IVF, and heparin therapy was not justifiable on the basis of existing data to improve pregnancy and live birth rates.
In agreement with our review, Ricci 2010 suggested that heparin should not be used in women undergoing IVF until its efficacy is demonstrated in carefully designed RCTs.
Three published studies suggested that heparin did improve clinical outcome:

- One single centre non-randomised study found that heparin with low-dose aspirin given to women undergoing assisted reproduction with positive antiphospholipid antibodies showed improvement in live birth rate and clinical pregnancy rate Sher 1994.
- The same results were shown by a single centre case control study by the same author Sher 1998. However, these studies are non-randomised and significant bias was found.
- Lodigiani 2011 presented observational retrospective analysis of women with previous implantation failure and screened for thrombophilia undergoing assisted reproduction who were given LMWH showed significantly higher pregnancy rates. The results also showed that there was no relation between inherited thrombophilia and pregnancy rate in patients with previous IVF implantation failures. This was an observational retrospective study, which could be influenced by various other factors.

We found two reviews on this topic which also agree with our conclusions:

- Nardo 2009 suggested that clinicians should inform patients of factors including: our current lack of knowledge; potential adverse effects; and available weak evidence regarding adjuvant therapy during assisted reproduction. There was need for good clinical trials in many of the areas surrounding medical adjuncts in IVF to resolve the empirical/evidence divide.
- Bohlmann 2011 suggested that the available studies on heparin in assisted reproduction were characterised by heterogeneous inclusion criteria and a lack of proven effectiveness in special constellations. In conclusion, the application of heparin to improve assisted reproduction treatment (ART) outcome rates was not justified. A large RCT should be undertaken to answer this.

AUTHORS' CONCLUSIONS

Implications for practice

There was insufficient evidence to determine whether routine ad-

ministration of peri-implantation heparin improved the clinical outcome in subfertile women undergoing assisted reproduction. In addition, heparin caused a number of adverse events.

Implications for research

Well-designed RCTs with sufficient power are warranted to assess the efficacy of peri-implantation heparin in improving assisted reproduction outcomes. These should be large parallel-group RCTs with populations of subfertile women with unexplained infertility, recurrent failure of embryo implantation or a positive thrombophilia screen. No additional adjunct therapies should be used. Cross-over designs should always be avoided in trials where pregnancy is an intended outcome.

Studies should be done where local (uterine) rather than systemic heparin is used to see the effects of heparin on decidualisation, implantation and pregnancy rates in an attempt to avoid adverse effects.

ACKNOWLEDGEMENTS

We especially thank Helen Nagels and Jane Clarke (Managing Editors), Jane Marjoribanks, Marian Showell (Trials Search Co-ordinator), and the editorial board of the Cochrane Menstrual Disorders and Subfertility Group (MDSG) particularly Prof C Farquhar, for their invaluable assistance, support and advice for the review.

REFERENCES

References to studies included in this review

Noci 2011 *{published data only}*

Noci I, Milanini MN, Ruggiero M, Papini F, Fuzzi B, Artini PG. Effect of dalteparin sodium administration on IVF outcome in non-thrombophilic young women: a pilot study. *Reproductive BioMedicine Online* June 2011;**22**(6): 615–20.

Qublan 2008 *{published data only}*

* Qublan H, Amarin Z, Dabbas M, Farraj AE, Beni-Merei Z, Al-Akash H, et al.Low-molecular-weight heparin in the treatment of recurrent IVF-ET failure and thrombophilia: A prospective randomized placebo-controlled trial. *Human Fertility* December 2008;**11**(4):246–53.

Urman 2009 *{published data only}*

Urman B, Ata B, Yakin K, Alatas C, Aksoy S, Mercan R, et al.Luteal phase empirical low molecular weight heparin administration in patients with failed ICSI embryo transfer cycles: a randomized open-labeled pilot trial. *Human Reproduction* April 2009;**24**(7):1640–7.

References to studies excluded from this review

Berker 2011 *{published data only}*

Berker B, Taşkin S, Kahraman K, Taşkin EA, Atabekoğlu C, Sönmezer M. The role of low-molecular-weight heparin in recurrent implantation failure: a prospective, quasi-randomized, controlled study. *Fertility and Sterility* June 30, 2011;**95**(8):2499-502.

Colicchia 2011 *{published data only (unpublished sought but not used)}*

Colicchia, A Pergolini I, Gilio B, Rampini MR, Alfano P, Marconi D, et al.Role of heparin in embryo implantation in women without thrombophilia: A pilot study. 27th Annual Meeting of the European Society of Human Reproduction and Embryology, ESHRE 2011 Stockholm Sweden. Conference Publication. July 2011; Vol. 26:pp i157.

Stern 2003 *{published data only}*

Stern C, Chamley L, Norris H, Hale L, Baker HWG. A randomized, double-blind, placebo controlled trial of heparin and aspirin for women with in vitro fertilization implantation failure and antiphospholipid or antinuclear antibodies. *Fertility and Sterility* August 2003;**80**(2): 376–83.

References to studies awaiting assessment

Mashayekhy 2011 *{published data only (unpublished sought but not used)}*

Mashayekhy M, Dehghani Firouzabadi R Ghasemi N. The effect of heparin in treatment of recurrent IVF-ET failure. *Iranian Journal of Reproductive Medicine* Spring 2011;**9 (Suppl 2)**:30.

Additional references

Arai 1994

Arai T, Parker A, Busby W Jr, Clemmons DR. Heparin, heparan sulfate, and dermatan sulfate regulate formation of the insulin-like growth factor-I and insulin-like growth factor-binding protein complexes. *Journal of Biological Chemistry* 1994;**269**:20388–93.

Bick 2005

Bick RL, Frenkel EP, Walenga J, Fareed J, Hoppensteadt DA. Unfractionated heparin, low molecular weight heparins, and pentasaccharide: basic mechanism of actions, pharmacology and clinical use. *Hematology Oncology Clinics of North America* 2005;**19**:1–51.

Bohlmann 2011

Bohlmann MK . Effects and effectiveness of heparin in assisted reproduction. *Journal of Reproductiv Immunology* 2011 Jun;**90**(1):82–90.

Comper 1981
Comper WD. *Heparin (and Related Polysaccharides) Structural and Functional Properties*. Gordon and Breach Publication Group, 1981.

Corvinus 2003
Corvinus FM, Fitzgerald JS, Friedrich K, Markert UR. Evidence for a correlation between trophoblast invasiveness and STAT3 activity. *American Journal of Reproductive Immunology* 2003;**50**:316–21.

Di Simone 2007
Di Simone N, Di Nicuolo F, Sanguinetti M, Ferrazzani S, D'Alessio MC, Castellani R, et al.Low-molecular weight heparin induces in vitro trophoblast invasiveness: role of matrix metalloproteinases and tissue inhibitors. *Placenta* 2007;**28**(4):298–304.

d'Souza 2007
D'Souza SS, Daikoku T, Farach-Carson MC, Carson DD. Heparanase expression and function during early pregnancy in mice. *Biology of Reproduction* 2007;**77**:433–41.

Egger 1997
Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;**31**(7109):629–34.

Erden 2006
Erden O, Imir A, Guvenal T, Muslehiddinoglu A, Arici S, Cetin M, et al. Investigation of the effects of heparin and low molecular weight heparin on E-cadherin and laminin expression in rat pregnancy by immunohistochemistry. *Human Reproduction* 2006;**21**:3014–8.

Fluhr H 2010
Fluhr H, Spratte J, Ehrhardt J, Steinmüller F, Licht P, Zygmunt, M. Heparin and low-molecular-weight heparins modulate the decidualization of human endometrial stromal cells. *Fertility and Sterility* 2010;**93**:2581–7.

Germeyer 2007
Germeyer A, Klinkert MS, Huppertz AG, Clausmeyer S, Popovici RM, Strowitzki T, et al.Expression of syndecans, cell-cell interaction regulating heparan sulfate proteoglycans, within the human endometrium and their regulation throughout the menstrual cycle. *Fertility and Sterility* 2007; **87**(3):657–63.

Higgins 2011
Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Intervention Version 5.1.0. The Cochrane Collaboration. Available from www.cochrane-handbook.org 2011.

Kelly 1995
Kelly FD, Tawia SA, Rogers PAW. Physiology: immunohistochemical characterization of human endometrial microvascular basement membrane components during the normal menstrual cycle. *Human Reproduction* 1995;**10**(2):268–76.

Kutteh 1997
Kutteh WH, Yetman DL, Chantilis SJ, Crain J. Effect of antiphospholipid antibodies in women undergoing in-vitro fertilization: role of heparin and aspirin. *Human Reproduction* 1997;**12**(6):1171–5.

Lai 2007
Lai TH, King JA, Shih IeM, Vlahos NF, Zhao Y. Immunological localization of syndecan-1 in human endometrium throughout the menstrual cycle. *Fertility and Sterility* 2007;**87**(1):121–6.

Leach 2004
Leach RE, Kilburn B, Wang J, Liu Z, Romero R, Armant DR. Heparin-binding EGF-like growth factor regulates human extravillous cytotrophoblast development during conversion to the invasive phenotype. *Developmental Biology* 2004;**266**:223–37.

Lodigiani 2011
Lodigiani C, Di Micco P, Ferrazzi P, Librè L, Arfuso V, Polatti F, et al. Low-molecular-weight heparin in women with repeated implantation failure. *Womens Health (Lond Engl)* 2011 Jul;**7**(4):425–31.

Moller 2006
Moller AV, Jorgensen SP, Chen JW, Larnkjaer A, Ledet T, Flyvbjerg A, et al. Glycosaminoglycans increase levels of free and bioactive IGF-I in vitro. *European Journal of Endocrinology* 2006;**155**:297–305.

Murray 1995
Murray WJ, Lindo VS, Kakkar VV, Melissari E. Long-term administration of heparin and heparin fractions and osteoporosis in experimental animals. *Blood Coagulation & Fibrinolysis* 1995;**6**:113–8.

Nardo 2009
Nardo LG, Granne I, Stewart J. Policy & Practice Committee of the British Fertility Society. Medical adjuncts in IVF: evidence for clinical practice. *Human Fertility* 2009 Mar;**12**(1):1–13.

Nelson 2008
Nelson SM, Greer IA. The potential role of heparin in assisted conception. *Human Reproduction Update* 2008;**14**: 623–45.

Poehlmann 2005
Poehlmann TG, Fitzgerald JS, Meissner A, Wengenmayer T, Schleussner E, FriedrichK, et al. Trophoblast invasion: tuning through LIF, signalling via Stat3. *Placenta* 2005;**26 Suppl**:37–41.

Potter 1992
Potter SW, Morris JE. Changes in histochemical distribution of cell surface heparan sulfate proteoglycan in mouse uterus during the estrous cycle and early pregnancy. *Anatomical Record* 1992;**234**:383–90.

Practice Committee of ASRM 2008
The Practice Committee of the American Society for Reproductive Medicine. Anti-phospholipid antibodies do not affect IVF success. *Fertility and Sterility* 2008;**90**(3): S172–S173.

Quenby 2004
Quenby S, Mountfield S, Cartwright JE, Whitley GS, Vince G. Effects of low-molecular-weight and

unfractionated heparin on trophoblast function. *Obstetrics & Gynecology* 2004;**104**:354–61.

Ricci 2010
Ricci G, Giolo E, Simeone R. 'Heparin's potential to improve pregnancy rates and outcomes' is not evidence based. *Human Reproduction Update* 2010;**16**:225–7.

San Martin 2004
San Martin S, Soto-Suazo M, Zorn TMT. Perlecan and syndecan-4 in uterine tissues during the early pregnancy in mice. *American Journal of Reproductive Immunology* 2004; **52**:53–9.

Sharif 2010
Sharif KW, Ghunaim S. Management of 273 cases of recurrent implantation failure: results of a combined evidence-based protocol. *Reproductive BioMedicine Online* 2010;**21**:373–80.

Sher 1994
Sher G, Feinman M, Zouves C, Kuttner G, Maassarani G, Salem R, et al.High fecundity rates following in-vitro fertilization and embryo transfer in antiphospholipid antibody seropositive women treated with heparin and aspirin. *Human Reproduction* 1994;**9**:2278–83.

Sher 1998
Sher G, Matzner W, Feinman M, Maassarani G, Zouves C, Chong, P, et al.The selective use of heparin/aspirin therapy, alone or in combination with intravenous immunoglobulin G, in the management of antiphospholipid antibody-positive women undergoing in vitro fertilization. *American Journal Reproductive Immunology* 1998;**40**:74–82.

Vail 2003
Vail A, Gardener E. Common statistical errors in the design and analysis of subfertility trials. *Human Reproduction* 2003;**18**:1000–4.

Wang 2002
Wang L, Brown JR, Varki A, Esko JD. Heparin's anti-inflammatory effects require glucosamine 6-*O*-sulfation and are mediated by blockade of L- and P-selectins. *Journal of Clinical Investigation* 2002;**110**:127–36.

Warkentin 1995
Warkentin TE, Levine MN, Hirsh J, Horsewood P, Roberts RS, Gent M, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *New England Journal of Medicine* 1995;**332**:1330–5.

Warkentin 2004
Warkentin TE, Greinacher A. Heparin-induced thrombocytopenia: recognition, treatment, and prevention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004;**126 Suppl**:311–37.

Weigert 2001
Weigert C, Brodbeck K, Haring HU, Gambaro G, Schleicher ED. Low-molecular-weight heparin prevents high glucose- and phorbol ester-induced TGF-beta 1 gene activation. *Kidney International* 2001;**60**:935–43.

Xu 2007
Xu X, Ding J, Rao G, Shen J, Prinz RA, Rana N, et al. Estradiol induces heparanase-1 expression and heparan sulphate proteoglycan degradation in human endometrium. *Human Reproduction* 2007;**22**:927–37.

* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

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

Noci 2011

Methods	Multicentre Prospective randomised control pilot study
Participants	172 patients were allocated to intervention and divided into two groups: 86 women in the control group and 86 women in the treatment group. The final series for analysis contained 153 patients because 13 women belonging to treatment group and 6 women belonging to the control group had no embryos to transfer, thus they were immediately excluded from the study So in the final analysis 73 women were in treatment group (A) and 80 women were in the control group (B). Both groups were matched. Every woman was recruited for only one cycle. Cause infertility: variety of causes
Interventions	IVF or ICSI. The treatment group (A) received both luteal phase support with vaginal progesterone (Prometrium 200 mg twice per day) and a prophylactic dose of dalteparin sodium (Fragmin, 2500 IU s.c. daily; Pfizer Italia, Latina, Italy) from the afternoon of the day of oocyte retrieval until the day of pregnancy test. The control group (B) received luteal phase support with progesterone only until pregnancy test. Platelet count was performed on days7-8 of dalteparin treatment to evaluate possible adverse effects of the therapy. If platelet values dropped to below 50% of basal levels or <100,000/ µ L, dalteparin administration was immediately stopped because of the risk of heparin induced thrombocytopenia COH: FSH, GNRH analogue. HCG 250 mcg. Luteal support: progesterone 200 mg pessaries vaginally twice daily until a pregnancy test was performed. If the test was positive, progesterone treatment was continued up to 12 gestational weeks
Outcomes	Live birth rate per woman: LMWH group (A): 21%, Control group (B): 16 % Adverse effect: Thrombocytopenia was not observed in any of the 73 patients treated with dalteparin and only a few patients reported the presence of minimal bruising at the injection point of the drug Clinical pregnancy rate per woman: LMWH group (A): 26%, Control group (B): 20% Multiple pregnancy rate per woman: LMWH group (A): 31.57%, Control group (B): 12.5% Implantation rate/ embryo transferred LMWH group (A): 15% Control group (B): 12% Spontaneous Miscarriage rate per woman: LMWH group (A): 21%, Control group (B) : 19%
Notes	Study population consisted of women aged < 40years, without congenital or acquired thrombophilia and undergoing their first IVF cycle

Risk of bias

Bias	Authors' judgement	Support for judgement

Noci 2011 (*Continued*)

Random sequence generation (selection bias)	Low risk	Computerised random sequence generation method was used
Allocation concealment (selection bias)	Low risk	Described clearly with sealed and numbered envelopes containing the allocation information
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not described
Blinding of outcome assessment (detection bias) All outcomes	Low risk	The ultrasonography was performed by a gynaecologist unaware of the allocation of the patients
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	The study had a follow-up rate of 89%
Selective reporting (reporting bias)	Unclear risk	No adverse effects were reported in the study
Other bias	Unclear risk	Not described

Qublan 2008

Methods	Single centre Prospective randomised placebo controlled
Participants	Of the 137 women with a history of three or more previous IVF failures and who had at least one thrombophilic defect, 39 did not meet the inclusion criteria and 15 refused participation. The remaining 83 women were randomly allocated to each arm of the study. Randomisation was started on the day of ET
Interventions	The treatment group (A) (n = 42) had enoxaparin 40 mg/day subcutaneous injections. Control Group (B) (n = 41) received placebo (equivalent volume of NaCl 0.9% subcutaneous; Pharmaceutical Solutions Industry Ltd., Jeddah, SA). Treatment was started from the day of ET until results of Beta-hCG were available 2 weeks after ET. If Beta-hCG was 425 IU/mL, LMWH was continued either until delivery or foetal demise was diagnosed COH: HMG, GNRH antagonist. HCG 10,000 IU. Luteal support: Progesterone pessaries (Cyclogest: Alpharma, Barnstaple, UK) were used for luteal phase support in the two study groups
Outcomes	Live birth rate per woman: LMWH group (A): 23.8%, Control group (B): 2.4% Adverse effect: The frequency of complications did not differ between the two study groups. The most frequent complications encountered in the heparin-treated were bleeding (7.1%) followed by thrombocytopenia (4.8%), allergic reactions (2.4%) and placental abruption (2.4%)

Qublan 2008 (*Continued*)

	Pregnancy rate per woman: LMWH group (A): 31%, Control group (B): 9.6% Multiple pregnancy rate per woman: LMWH group (A): 23.1%, Control group (B): 25% Implantation rate/ embryo transferred LMWH group (A): 19.8% Control group (B): 6. 1% Spontaneous Miscarriage rate per woman: LMWH group (A): 7.7%, Control group (B) : 50% Intrauterine Fetal death rate: LMWH group (A) 15.4%, control group 0%
Notes	Study population consisted of women aged 19-35 years with a history of three or more previous IVF failures, and who had at least one thrombophilic defect

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Allocation was done by selection from table of random numbers
Allocation concealment (selection bias)	Unclear risk	Not described
Blinding of participants and personnel (performance bias) All outcomes	High risk	Participants were blinded but not clinicians
Blinding of outcome assessment (detection bias) All outcomes	High risk	Outcome assessors were not blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	It appears that the data are complete
Selective reporting (reporting bias)	Low risk	It appears that the data are complete
Other bias	Unclear risk	Not described

Urman 2009

Methods	Single centre Open labelled randomised controlled pilot trial
Participants	150 consecutive couples who met the inclusion criteria and gave informed consent were recruited to the trial. Each woman was included for one cycle only. 3 women in the LMWH and control group each were lost to follow-up before completion of the initially planned follow-up period (completion of the 20th gestational week for the latest recruited participant that achieved an ongoing pregnancy), and another 2 women in the LMWH group were lost to follow-up after completion of the 20th gestational week but before delivery or expected completion of the 40th gestational week. 75 women in each arm of the study

Interventions	ICSI. The study group was administered LMWH group (A) (Enoxaparin Sodium, Clexane, Aventis Pharma) at a dose of 1 mg/kg/day starting on the day after oocyte retrieval. Patients' weights were rounded to the closest multiple of 10 kg, and 0.1 mL/10 kg/day Clexane was self-administered subcutaneously by the participants. LMWH was discontinued if the pregnancy test 12 days after ET was negative, but continued up to the 12th week of pregnancy if the test was positive. The control group (B) received no medication besides progesterone gel. In the study group the platelet count was done on the day of oocyte retrieval and 1 week after commencement of LMWH treatment COH: FSH, GNRH agonist. HCG 10,000 IU. Luteal support: Progesterone pessaries 90 mg vaginal progesterone gel (Crinone 8%, Serono, Serono, Bedfordshire, UK) starting from the day of oocyte collection. LPS was continued until the pregnancy test performed 12 days after ET. Women with a positive pregnancy test continued the vaginal progesterone gel until the 12th week of gestation
Outcomes	Live birth rate per woman: LMWH group (A): 34.7%, Control group (B): 26.7% Adverse effect: Platelet counts did not change significantly in the LMWH group during the study period. Small ecchymoses around the LMWH injection sites were noted Clinical Pregnancy rate per woman: LMWH group (A): 45.3%, Control group (B): 38.7% Ongoing Pregnancy rate per woman: LMWH group (A): 37.3%, Control group (B): 26.7% Multiple pregnancy rate per woman: LMWH group (A): 35.3%, Control group (B): 34.5% Implantation rate/ embryo transferred LMWH group (A): 24.5% Control group (B): 19.8% Numbers of preterm deliveries were (34.6%) in LMWH and (30.0%) in control groups
Notes	Study population consisted of women aged < 38 years with a history of two or more previous IVF failures. Women lost to follow-up during the first period were considered not to have an ongoing pregnancy, and women lost to follow-up in the second period were considered not to have a live birth in the intention-to-treat analysis

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Women were randomised according to a computer-generated randomisation list. Study subjects were randomised in blocks of 10; i.e. of every 10 women randomised, five were allocated to the LMWH arm, and five were allocated to the control arm, in a random manner
Allocation concealment (selection bias)	Low risk	Opaque envelopes that were numbered and sealed containing the allocation information were given to the ART centre nurse coordinator who assigned patients to study

Urman 2009 (*Continued*)

		arms following recruitment by attending physicians on the morning of oocyte retrieval procedure
Blinding of participants and personnel (performance bias) All outcomes	High risk	Not described
Blinding of outcome assessment (detection bias) All outcomes	High risk	Not described
Incomplete outcome data (attrition bias) All outcomes	Low risk	This study compensated for dropouts by imputing a negative outcome to losses to follow-up
Selective reporting (reporting bias)	Low risk	It appears that the data are complete
Other bias	Unclear risk	Not described

COH: controlled ovarian hyperstimulation
ET: embryo transfer
FSH: follicle-stimulating hormone
GNRH: gonadotropin-releasing hormone
HCG: human chorionic gonadotropin
ICSI: intracytoplasmic sperm injection
IVF: in vitro fertilisation
IU: international units
LMWH: low molecular weight heparin
LPS: lipopolysaccharide,
NaCl: sodium chloride
s.c.: subcutaneous

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

Study	Reason for exclusion
Berker 2011	Not a True RCT as quasi randomisation was performed for the purposes of this study
Colicchia 2011	Low molecular weight heparin (LMWH) was used in conjunction with prednisolone
Stern 2003	Unfractionated heparin (UFH) was used in conjunction with low-dose aspirin. Cross-over design study

RCT: randomised controlled trial

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

Mashayekhy 2011

Methods	Single centre Prospective randomised controlled trial
Participants	86 patients with recurrent IVF-ET failure.
Interventions	Ovarian stimulation was performed with long protocol. The patients were randomly divided into two groups after embryo transfer, and one group received unfractionated heparin 5000 IU twice a day plus 100 mg progesterone and another group only received progesterone
Outcomes	There were no significant differences between individual characteristics of two groups. However, implantation rate and clinical pregnancy were significantly higher in patients who received unfractionated heparin. Thirty-six women had at least one thrombophilic mutation
Notes	Only the abstract has been published in The Iranian Journal of Reproductive Medicine spring 2011;9 (Suppl 2):30-30 The authors were contacted regarding the details of study results. The study is presently not able to be included in the review as it has been completed and submitted for publication. The authors were unable to provide me with the details of results till publication

ET: embryo transfer
IU: international units
IVF: iv vitro fertilisation

DATA AND ANALYSES

Comparison 1. Heparin versus control

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live Birth Rate per woman	3	386	Odds Ratio (M-H, Fixed, 95% CI)	1.77 [1.07, 2.90]
2 Clinical Pregnancy Rate per woman	3	386	Odds Ratio (M-H, Fixed, 95% CI)	1.61 [1.03, 2.53]

Analysis 1.1. Comparison 1 Heparin versus control, Outcome 1 Live Birth Rate per woman.

Review: Heparin for assisted reproduction

Comparison: 1 Heparin versus control

Outcome: 1 Live Birth Rate per woman

Study or subgroup	Heparin n/N	Control n/N	Odds Ratio M-H,Fixed,95% CI	Weight	Odds Ratio M-H,Fixed,95% CI
Noci 2011	15/73	13/80		41.6 %	1.33 [0.59, 3.03]
Qublan 2008	10/42	1/41		3.3 %	12.50 [1.52, 102.85]
Urman 2009	26/75	20/75		55.1 %	1.46 [0.73, 2.93]
Total (95% CI)	**190**	**196**		**100.0 %**	**1.77 [1.07, 2.90]**

Total events: 51 (Heparin), 34 (Control)

Heterogeneity: $Chi^2 = 4.05$, df = 2 (P = 0.13); $I^2 = 51\%$

Test for overall effect: Z = 2.24 (P = 0.025)

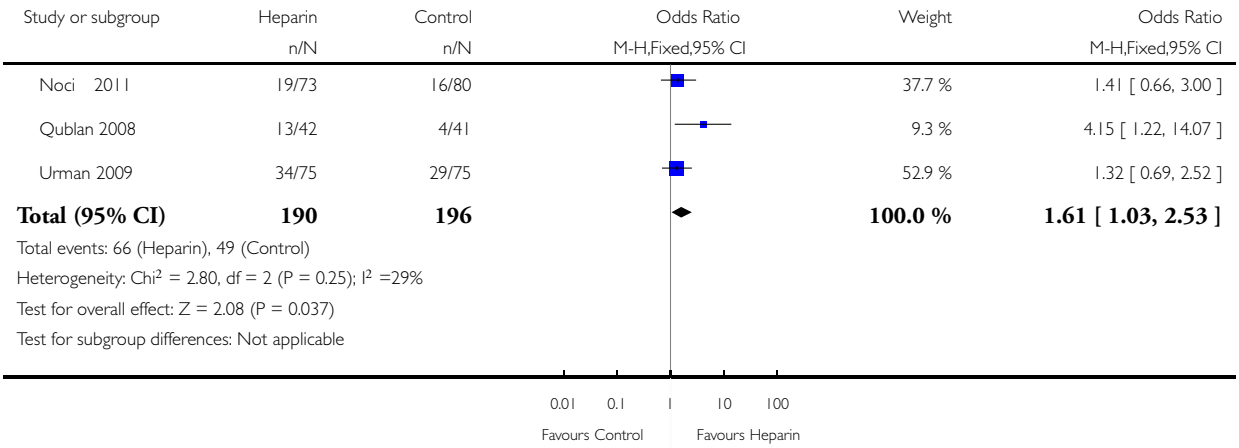
Test for subgroup differences: Not applicable

Analysis 1.2. Comparison 1 Heparin versus control, Outcome 2 Clinical Pregnancy Rate per woman.

Review: Heparin for assisted reproduction

Comparison: 1 Heparin versus control

Outcome: 2 Clinical Pregnancy Rate per woman



ADDITIONAL TABLES

Table 1. Table of Comparisons: Implantation rate per embryos transferred

Study ID	Heparin group	Control group
Noci 2011	15%	12%
Urman 2009	24.5%	19.8%
Qublan 2008	19.8%	6.1%

Table 2. Table of Comparisons: Incidence of miscarriage per total number of pregnancies and per woman

Study ID	Heparin group per pregnancy	Control group per pregnancy	Heparin group per woman	Control group per woman
Noci 2011	4/19	3/16	4/73	3/80
Urman 2009	n/a	n/a	n/a	n/a

Table 2. Table of Comparisons: Incidence of miscarriage per total number of pregnancies and per woman (*Continued*)

Qublan 2008	1/13 *IUFD 2/13	2/4 *IUFD 0/4	1/42 *IUFD 2/42	2/41 *IUFD 0/41

IUFD: Intraunterine fetal death

Table 3. Table of Comparisons: Incidence of multiple pregnancies per total number of pregnancies

Study ID	Heparin group	Control group
Noci 2011	(6/19) 31.5%	(2/16) 12.5%
Urman 2009	(12/34) 35.3%	(10/29) 34.5%
Qublan 2008	(3/13) 23.1%	(1/4) 25%

APPENDICES

Appendix 1. CENTRAL search strategy

Menstrual Disorders and Subfertility Group Specialised Register (inception to 2 July 2012)
Ovid the Cochrane Central Register of Controlled Trials (CENTRAL) (inception to 2 July 2012)
There is no language restriction in these search.

1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp insemination, artificial/ or exp reproductive techniques, assisted/
11 artificial$ inseminat$.tw.
12 iui.tw.
13 intrauterine insemination.tw.
14 nidation.tw.
15 reproductive technique$.tw.
16 reproduct$ technolog$.tw.
17 exp Embryo Implantation/
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 exp Infertility, Female/
21 ((Female$ or women) adj2 infertil$).tw.

22 ((Female$ or women) adj2 subfertil$).tw.
23 exp Abortion, Habitual/
24 recurrent miscarriage$.tw.
25 or/1-24 (8324)
26 exp heparin/ or exp heparin, low-molecular-weight/ or exp heparinoids/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38

Appendix 2. MEDLINE search strategy

Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) (1950 to 2 July 2012)
The MEDLINE search was combined with the Cochrane highly sensitive search strategy for identifying randomized trials which appears in the *Cochrane Handbook of Systematic Reviews of Interventions (version 5.0.2; chapter 6, 6.4.11)*
There is no language restriction in this search
1 exp embryo transfer/ or exp fertilization in vitro/ or exp sperm injections, intracytoplasmic/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp insemination, artificial/ or exp reproductive techniques, assisted/
11 artificial$ inseminat$.tw.
12 iui.tw.
13 intrauterine insemination.tw.
14 nidation.tw.
15 reproductive technique$.tw.
16 reproduct$ technolog$.tw.
17 exp Embryo Implantation/
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 exp Infertility, Female/
21 ((Female$ or women) adj2 infertil$).tw.
22 ((Female$ or women) adj2 subfertil$).tw.
23 exp Abortion, Habitual/
24 recurrent miscarriage$.tw.
25 or/1-24
26 exp heparin/ or exp heparin, low-molecular-weight/ or exp heparinoids/

27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38
40 randomized controlled trial.pt.
41 controlled clinical trial.pt.
42 randomized.ab.
43 placebo.tw.
44 clinical trials as topic.sh.
45 randomly.ab.
46 trial.ti.
47 (crossover or cross-over or cross over).tw.
48 or/40-47
49 exp animals/ not humans.sh.
50 48 not 49
51 39 and 50

Appendix 3. EMBASE search strategy

Ovid EMBASE (01.01.10 to 2 July 2012)
EMBASE is only searched one year back as the UKCC has hand searched EMBASE to this point and these trials are already in CENTRAL.
The EMBASE search is combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) http://www.sign.ac.uk/mehodology/filters.html#random
There is no language restriction in this search
1 exp embryo transfer/ or exp female infertility/ or exp fertilization in vitro/
2 embryo transfer$.tw.
3 in vitro fertilisation.tw.
4 ivf-et.tw.
5 (ivf or et).tw.
6 icsi.tw.
7 intracytoplasmic sperm injection$.tw.
8 (blastocyst adj2 transfer$).tw.
9 (assist$ adj2 reproducti$).tw.
10 exp artificial insemination/
11 artificial$ inseminat$.tw.
12 reproductive technique$.tw.
13 reproduct$ technolog$.tw.
14 exp nidation/
15 (implant$ adj2 fail$).tw.
16 reproduct$ technique$.tw.
17 ((Female$ or women) adj2 infertil$).tw.
18 ((Female$ or women) adj2 subfertil$).tw.

19 exp recurrent abortion/
20 recurrent miscarriage.tw.
21 iui.tw.
22 intrauterine insemination.tw.
23 nidation.tw.
24 exp intracytoplasmic sperm injection/
25 or/1-24
26 exp HEPARIN/ or exp LOW MOLECULAR WEIGHT HEPARIN/
27 heparin$.tw.
28 LMWH$.tw.
29 liquemin.tw.
30 enoxaparin.tw.
31 heparinic acid.tw.
32 dalteparin.tw.
33 tinzaparin.tw.
34 clexane.tw.
35 lovenox.tw.
36 indenox.tw.
37 xaparin.tw.
38 or/26-37
39 25 and 38
40 Clinical Trial/
41 Randomized Controlled Trial/
42 exp randomization/
43 Single Blind Procedure/
44 Double Blind Procedure/
45 Crossover Procedure/
46 Placebo/
47 Randomi?ed controlled trial$.tw.
48 Rct.tw.
49 random allocation.tw.
50 randomly allocated.tw.
51 allocated randomly.tw.
52 (allocated adj2 random).tw.
53 Single blind$.tw.
54 Double blind$.tw.
55 ((treble or triple) adj blind$).tw.
56 placebo$.tw.
57 prospective study/
58 or/40-57
59 case study/
60 case report.tw.
61 abstract report/ or letter/
62 or/59-61
63 58 not 62
64 39 and 63
65 (2010$ or 2011$).em.
66 64 and 65

Appendix 4. PsycINFO search strategy

Ovid PsycINFO (1806 to 2 July 2012)
There is no language restriction in this search

1 exp Reproductive Technology/
2 exp Infertility/
3 exp Embryo/
4 embryo transfer$.tw.
5 in vitro fertili?ation.tw.
6 ivf-et.tw.
7 (ivf or et).tw.
8 icsi.tw.
9 intracytoplasmic sperm injection$.tw.
10 (blastocyst adj2 transfer$).tw.
11 (assist$ adj2 reproducti$).tw.
12 artificial$ inseminat$.tw.
13 iui.tw.
14 intrauterine insemination.tw.
15 nidation.tw.
16 reproductive technique$.tw.
17 reproduct$ technolog$.tw.
18 (implant$ adj2 fail$).tw.
19 reproduct$ technique$.tw.
20 ((Female$ or women) adj2 infertil$).tw.
21 ((Female$ or women) adj2 subfertil$).tw.
22 exp Spontaneous Abortion/
23 recurrent miscarriage$.tw.
24 or/1-23
25 exp Heparin/
26 heparin$.tw.
27 LMWH$.tw.
28 liquemin.tw.
29 enoxaparin.tw.
30 heparinic acid.tw.
31 dalteparin.tw.
32 tinzaparin.tw.
33 clexane.tw.
34 lovenox.tw.
35 indenox.tw.
36 xaparin.tw.
37 or/25-36
38 24 and 37

CONTRIBUTIONS OF AUTHORS

Akhtar Muhammad A (Co-first author)

All correspondence with drafting of the protocol, develop a search strategy, search for trials, obtain copies of trials, select which trials to include, extract data from trials, enter data into RevMan, carry out the analysis, interpret the analysis, draft the final review and update the review.

Sur Shyamaly (Co-first author)

Drafting of the protocol, search for trials, obtain copies of trials, select which trials to include, extract data from trials, enter data into RevMan, carry out the analysis, interpret the analysis, draft the final review and update the review.

Raine-Fenning Nick

Drafting of the protocol, select which trials to include, interpret the analysis, draft the final review and update the review.

Kannamannadiar Jayaprakasan:

Drafting of the protocol, select which trials to include, carry out the analysis, interpret the analysis, draft the final review and update the review.

Thornton Jim G

Drafting of the protocol, select which trials to include, help in carrying out the analysis, interpret the analysis, draft the final review and update the review.

Quenby Siobhan

Drafting of the protocol, select which trials to include, help in carrying out the analysis, interpret the analysis, draft the final review and update the review.

DECLARATIONS OF INTEREST

The review authors have no commercial interest to disclose.

SOURCES OF SUPPORT

Internal sources

- Cochrane Menstrual Disorders and Subfertilty Group, New Zealand.
- Department of Obstetrics and Gynaecology, University of Auckland, New Zealand.
- Clinical Reproductive Medicine Unit, University Hospitals Coventry & Warwickshire NHS Trust, UK.
- Division of Obstetrics & Gynaecology, School of Clinical Sciences, University of Nottingham, UK.
- Clinical Sciences Research Institute, University of Warwick, UK.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

Biological pregnancy rates and ongoing pregnancy rates per woman were included in the protocol but not in the review, as these outcome measures are not as important from a patient perspective as live birth rates and clinical pregnancy rates. We made these changes on the advice of the MDSG Co-ordinating Editor.

Heparin for assisted reproduction: summary of a Cochrane review

Muhammad Ahsan Akhtar, M.B.B.S.,[a] Shyamaly Sur, Ph.D.,[b] Nick Raine-Fenning, Ph.D.,[c] Kannamannadiar Jayaprakasan, Ph.D.,[d] Jim Thornton, M.D.,[c] Siobhan Quenby, M.D.,[e] and Jane Marjoribanks, M.P.H.[f]

[a] Reproductive Medicine, St. Mary's Hospital, Manchester; [b] Queen Charlotte's and Chelsea Hospital, Imperial College Healthcare Trust, London; [c] Division of Child Health, Obstetrics and Gynaecology, School of Medicine, University of Nottingham, Nottingham; [d] Fertility Unit, Royal Derby Hospital, Derby; and [e] Clinical Sciences Research Institute, University of Warwick, Coventry, United Kingdom; and [f] Cochrane Office, University of Auckland, Auckland, New Zealand

It is suggested that heparin given in the peri-implantation period may improve clinical outcomes in women undergoing assisted reproduction techniques (ART). This systematic review evaluates the use of heparin in subfertile women undergoing ART. (Fertil Steril® 2014;■: ■–■. ©2014 by American Society for Reproductive Medicine.)

Key Words: Anticoagulants, heparin, live birth, pregnancy, assisted reproductive techniques

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/akhtarm-heparin-assisted-reproduction-cochrane-review/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

BACKGROUND

It is suggested that heparin may improve clinical outcomes in women undergoing assisted reproduction techniques (ART) by enhancing decidualization and thus improving the intrauterine environment. It is given in the peri-implantation period (at or after egg collection or at ET). This systematic review evaluates the use of heparin in subfertile women undergoing ART.

METHODS

We included all randomized controlled trials (RCTs) of the use of peri-implantation heparin in subfertile women undergoing ART. Study selection, quality assessment, and data extraction were conducted independently by two review authors. The literature search was conducted in May 2013 and included Cochrane databases, MEDLINE, EMBASE, PsycINFO, trial registries, and other sources. Primary review outcomes were live birth and adverse effects.

We calculated odds ratios (ORs) and 95% confidence intervals (CIs) and pooled data using a fixed-effects model. A sensitivity analysis was conducted using random effects. Statistical heterogeneity was assessed using the I^2 statistic. The overall quality of the evidence was evaluated using GRADE methods.

Institutional Review Board approval was not required for this work, as it is secondary research.

RESULTS

We included three RCTs (386 women) in which low molecular weight heparin given at ET (one RCT) or egg collection (two RCTs) was compared with placebo or no treatment. Participant characteristics varied across studies. One study included women having their first IVF cycle, with no blood clotting disorder; one included women with at least one blood clotting disorder; and the third included women who had undergone at least two previous unsuccessful ART cycles.

Our findings require very cautious interpretation as they differed according to choice of statistical model. Use of a fixed-effects analysis suggested that peri-implantation heparin may be associated with an improvement in live-birth and pregnancy rates compared with placebo or no heparin, but there was high heterogeneity for the outcome of live birth ($I^2 = 51\%$). When a random-effects model was used there was no longer a difference between the groups for either live birth (OR, 1.85; 95% CI, 0.80–4.24, three studies, 386 women, $I^2 = 51\%$, very low quality evidence) or clinical pregnancy (OR, 1.66; 95% CI, 0.94–2.90, three studies 386 women, $I^2 = 29\%$, low-quality evidence; see Fig. 1 and Table 1).

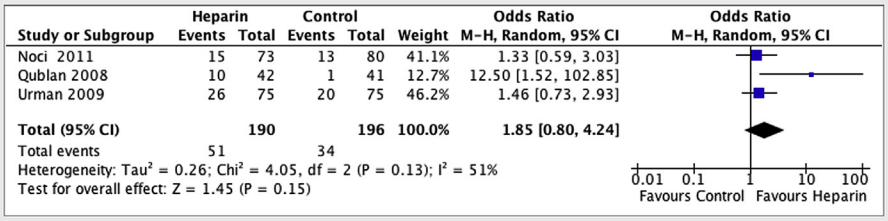
Adverse events were poorly reported in all the included studies. Events such as bleeding and thrombocytopenia

Received June 23, 2014; accepted September 4, 2014.
M.A.A. has nothing to disclose. S.S. has nothing to disclose. N.R.-F. has nothing to disclose. K.J. has nothing to disclose. J.T. has nothing to disclose. S.Q. has nothing to disclose. J.M. has nothing to disclose.
This is a summary of a systematic review published on the Cochrane Library: *Heparin for Assisted Reproduction*. Cochrane Database of Systematic Reviews 2013, Issue 8. Art. No.: CD009452. http://dx.doi.org/10.1002/14651858.CD009452.pub2.
Correspondence: Jane Marjoribanks, M.P.H., 260 Hot Springs Road, Katikati RD2, New Zealand (E-mail: j.marjoribanks@auckland.ac.nz).

Fertility and Sterility® Vol. ■, No. ■, ■ 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.09.005

FIGURE 1



Forest plot for live birth: heparin versus no heparin.

Akhtar. Heparin for assisted reproduction. Fertil Steril 2014.

TABLE 1

Summary of findings table: heparin versus no heparin.

Outcome	Illustrative comparative risks[a] (95% CI)		Relative effect (95% CI) using a random-effects model	No. of Participants (no. of studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk control	Corresponding risk heparin				
Live-birth rate per woman	173 per 1,000	280 per 1,000 (144–471)	OR, 1.85 (0.8–4.24)	386 (3)	⊕○○○ very low[b,c]	Estimate using a fixed effects model: OR, 1.77; 95% CI, 1.07–2.9
Clinical pregnancy rate per woman	250 per 1,000	356 per 1,000 (239–492)	OR, 1.66 (0.94–2.9)	386 (3)	⊕⊕○○ low[c]	Estimate using a fixed-effects model: OR, 1.61; 95% CI, 1.03–2.53
Adverse effects	No comparative data available so no conclusions could be drawn. Adverse effects such as bleeding and thrombocytopenia were reported in the heparin groups and affected 5%–7% of women in one study.					

Note: The population was subfertile women. The setting was an ART laboratory. The interventions were heparin versus placebo or no heparin. GRADE Working Group grades of evidence: high quality means further research is very unlikely to change our confidence in the estimate of effect. Moderate quality means further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality means further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality means we are very uncertain about the estimate.

[a] The basis for the assumed risk is the median control group risk across studies. The corresponding risk (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

[b] Inconsistency (high heterogeneity: $I^2 = 51\%$).

[c] Imprecision: low overall event rate, confidence intervals compatible with substantial benefit or no appreciable benefit, findings sensitive to choice of statistical model.

Akhtar. Heparin for assisted reproduction. Fertil Steril 2014.

were reported in women receiving heparin and affected 5%–7% of women in the heparin group in one study. However, no studies reported data suitable for analysis and so no firm conclusions could be drawn regarding the safety of heparin.

The evidence was seriously limited by inconsistency, imprecision, and inadequate reporting of adverse events.

CONCLUSIONS

It is unclear whether peri-implantation heparin improves live-birth and pregnancy rates in subfertile women undergoing ART, as the evidence is seriously limited by inconsistency and imprecision. No benefit is apparent when a random-effects model is used.

Adverse events were inadequately reported, and no firm conclusions could be drawn regarding the safety of heparin.

Our findings do not justify the use of heparin in this context except in well-conducted research trials, and further such studies are recommended. Further investigations could also focus on the effects of local (uterine) heparin during ART.