Chapter I

Introduction
Assisted reproductive technology (ART) is a fast-paced and ever-changing field in medicine. All parts of the treatment cycle are evolving towards more evidence-based procedures, resulting in increasing success rates. Almost all aspects of the IVF procedure (e.g. ovarian stimulation protocols, laboratory techniques, techniques for embryo transfer and luteal phase support) have been evaluated and optimized through increased effectiveness and efficiency of each individual step.

Embryo transfer is the last phase of a complementing process involving ovarian stimulation, in vitro fertilization and finally intrauterine placement of the generated embryos. This step is considered to be an important one among all those involved in assisted fertilization procedures since any errors at this stage or poor technique negate all previous work.

Historically, little attention has been paid to the embryo transfer procedure. This fact is reflected by the dearth of scientific publications regarding embryo transfer compared with other aspects of IVF (e.g. ovarian stimulation), and also the reluctance of physicians to modify their own personal habits to encompass a more evidence-based approach. Physicians, too, often underestimate the importance of the embryo transfer technique, being an apparently simple manoeuvre. Most inexperienced clinicians do not consider inserting a catheter through the uterine cervix and ejecting embryo-containing fluid to be a difficult task. Be that as it may, multiple variables have been identified that might influence the success of the procedure (e.g. individual technical training and performance, catheter type and model, ultrasound-guidance and minimization of cervical/ endometrial trauma during transfer).

Over the years several modifications to the embryo transfer method have been introduced since it was first described by Steptoe and Edwards (1). Each has built on the available technical developments and previous experience in order to achieve an effective transfer. In general, the most used approach is through the natural vaginal-cervical-uterine orifice, the transcervical intrauterine transfer, with or without ultrasound-guidance. Other techniques, such as ultrasound-guided transmyometrial transfer, gamete intra-Fallopian transfer (GIFT), zygote intra-Fallopian transfer (ZIFT), and embryo intra-Fallopian transfer (EIFT) are more surgically invasive and have been described, but are nowadays hardly used.
However, until not long ago, most clinicians relied heavily on prior experience and personal preference when performing embryo transfer, even though the success rate after embryo transfer has been demonstrated to show marked variability among different IVF programmes, and also among physicians within the same programme (2-5). Recently it was shown that pregnancy rates are strongly affected by the embryo transfer technique (6). This has –fortunately– been reflected in an increased interest and awareness among clinicians and some evidence-based guidelines have been provided by medical organizations (5). Today, most patients who undergo IVF/ICSI will reach the embryo transfer stage with good quality embryos available for replacement. It is this development that emphasizes the clinical importance of an optimal embryo transfer.

There are several issues regarding the embryo technique that are still debated in the literature. They can easily be distinguished into the timing of the embryo transfer and aspects related to the procedure itself.

Re timing of the embryo transfer:
In the early days of IVF, Edwards et al. performed embryo transfer (ET) in the 8 – 16-cell stage (i.e. 3 – 4 days after egg collection) (1). Recently, variations to this timing have ranged from as little as 2, to as many as 7 days post-oocyte pick-up. In view of these discrepancies, we conducted a prospective, controlled trial to compare the IVF outcomes between days 2 and 3 transfer to determine if a change is policy is warranted.

Re the embryo transfer procedure:
In general, the transfer procedure may be arbitrarily divided into four distinct sections: (i) preparation prior to embryo transfer (e.g. dummy embryo transfer, cervical preparation, uterine position); (ii) technical aspects related to the embryo transfer catheter (e.g. catheter type and catheter loading); (iii) the embryo transfer procedure (e.g. the site of embryo deposition within the uterus and techniques to assist with the accurate placement of the embryo within the uterus); and (iv) post-transfer aspects (e.g. expulsion of fluid/embryos from the cervix after embryo transfer and bed rest following embryo transfer) (7).
Ad (i) preparation prior to embryo transfer. Data on preparation prior to the actual embryo transfer are conflicting. Techniques involved include: (i) performing a trial (e.g. dummy) embryo transfer prior to the actual transfer to determine the length and direction of the cervico-uterine axis, the cervico-uterine angle and identifying possible difficulty in traversing the cervix (8); (ii) aspiration of the cervical mucus prior to transfer to remove the natural cervical mucus, with or without irrigating the endometrial cavity to remove possible contaminants; (iii) passively widening the utero-cervical angle by filling the urinary bladder.

We conducted a prospective, controlled trial to determine whether removal of cervical mucus was beneficial. In addition, we systematically reviewed the available evidence on the use of passive uterine straightening during embryo transfer.

Ad (ii) technical aspects related to the embryo transfer catheter. Several embryo transfer catheters are commercially available. All are mainly composed of non-toxic plastics and/or metal, but vary in length, calibre, location of the distal port (end- or side-loading), and degree of stiffness and malleability. They can be subdivided by the material they are made off (i.e. metal, hard or soft plastics) and whether they are equipped with, or without, an introducing cannula that facilitates the transfer procedure. In general, soft catheters have been associated with better pregnancy rates, but also with a higher incidence of failed and difficult transfers.

To determine the most suitable catheter for embryo transfer in a clinical setting, we conducted a multicenter, randomized controlled trial comparing two commercially available soft transfer catheters. In addition, we performed a retrospective comparison of soft versus firm catheters under ultrasound guidance. Finally we systematically reviewed the current evidence comparing clinical pregnancy rates achieved with soft versus firm embryo transfer catheters.

Catheter loading, i.e. the use of air brackets around the embryo-containing medium has been suggested to be beneficial to the success of the embryo transfer. By these air brackets the embryos would be protected from the cervical mucus and from accidental discharge before entering the endometrial cavity. On the other hand, it has also been suggested that even a small amount of air in the uterus could be a non
physiological factor with a deleterious effect on the embryos and implantation (9). Accordingly, we systematically reviewed the literature to summarize the evidence on the use of air brackets during embryo transfer.

(iii) the embryo transfer procedure.
With regards the embryo transfer procedure itself, the influence of the depth of embryo replacement into the uterine cavity has been postulated as being one of the most important factors to the success of an IVF treatment cycle (10). Traditionally, most IVF programmes have relied on the clinician’s ‘clinical touch’ for the placing of the transfer catheter within the uterine cavity at a point ‘near’ the fundus (3, 11). At present, the best site of embryo deposition is still not clear and remains highly debated. Therefore, we performed a systematic review of the literature to determine the best site of embryo deposition.

Another important aspect of the embryo transfer technique is whether ultrasound guidance is able to assist with accurate placement of the embryo within the uterus. More than twenty-years after the first reports on ultrasound guidance during the ‘blind’ embryo transfer procedure, the routine use of ultrasonography to guide the intrauterine embryo transfer catheter placement are still highly debated (12, 13). This has been fuelled by the conflicting results of published clinical trials, with some concluding that ultrasound guidance improves clinical pregnancy and implantation rates, while others reporting no such improvement in their results.

We therefore compared the use of ultrasound guided embryo transfer to the standard clinical touch embryo transfer performed by a single operator, and at the same time performed a systematic review of the literature to summarize the evidence on the use of ultrasound guidance during embryo transfer.

Lastly, during the embryo transfer, the aim is to manipulate the catheter atraumatically through the cervix into the uterine cavity; without touching the fundus and minimizing trauma to the endometrium. Factors related to tissue trauma such as the presence of blood and/or mucus on the transfer catheter has been shown to decrease implantation and pregnancy rate (14, 15).
This led us to investigate the relationship between difficulties during transfer and presence of blood on the catheter tips following embryo transfer and the outcomes of IVF. In addition any clinical manoeuvre that increases the ease of transfer is highly welcomed. Therefore we investigated whether the use of Propofol anaesthesia improves the clinical outcomes of difficult embryo transfers. Propofol acts as both a hypnotic agent and exerts smooth muscle relaxation. It has been shown to be safe and useful in alleviating anxiety in women undergoing embryo transfer (16).
**Aim of this thesis**
The aim of this thesis was to answer the following questions:-

1- Is the pregnancy rate improved by delaying embryo transfer from ‘day two’ to ‘day three’?

2- Does removal of cervical mucus prior to embryo transfer improve the results?

3- What is the effect of passive uterine straightening during embryo transfer on pregnancy rate?

4- Is there a difference in clinical outcomes when two soft embryo transfer catheters are compared for embryo transfer?

5- Is there a difference in clinical outcomes between firm and soft embryo transfer catheters when embryo transfer is performed under ultrasound guidance?

6- Are soft embryo transfer catheters more favourable than firm catheters during embryo transfer?

7- What is the effect of using air to bracket embryos in the transfer catheter?

8- What is the best site in the uterine cavity for embryo replacement?

9- Does ultrasound-guidance during embryo transfer improve the outcomes when performed by a single operator?

10- Is the probability of pregnancy and live birth improved with ultrasound-guidance than with clinical touch alone?

11- What is the effect of blood found on the tips of the post-transfers on clinical outcomes?

12- Does the use of Propofol anaesthesia improve the clinical outcomes of difficult embryo transfers?
Outline of this thesis

Chapter 2 provides results of a prospective, controlled, clinical trial comparing the clinical outcomes following embryo transfer on ‘day two’ and ‘day three’. The primary outcome was the clinical pregnancy rate per woman.

Chapter 3 provides results of a prospective, controlled, clinical trial comparing the clinical outcomes following cervical mucus aspiration compared with no aspiration prior to embryo transfer. The primary outcome was the clinical pregnancy rate per woman.

Chapter 4 presents a systematic review and meta-analysis of randomized controlled trials that compare between the uses of passive uterine straightening during embryo transfer compared with no intervention. The studies were included and assessed according to the principles of the Cochrane Collaboration. Primary outcomes were live-birth, ongoing and clinical pregnancy rates.

Chapter 5 presents the results of a randomized controlled trial that investigated the clinical outcomes following the use of two soft embryo transfer catheters during embryo transfer. Primary outcomes were the live-birth and clinical pregnancy rates per woman randomized.

Chapter 6 presents the results of a retrospective analysis of using firm embryo transfer catheters compared with soft catheters under ultrasound-guidance. Primary outcome was the clinical pregnancy rate.

Chapter 7 reports the results of a systematic review and meta-analysis of randomized controlled trials that compared soft embryo transfer catheters with firm catheters during embryo transfer. The studies were included and assessed according to the principles of the Cochrane Collaboration. The primary outcome measures used for this systematic review were implantation rate, clinical pregnancy rate and ongoing/take-home baby rate.

Chapter 8 presents a systematic review and a meta-analysis of randomized controlled trials that compare between the air-fluid and the fluid-only models of embryo catheter loading. The studies were included
and assessed according to the principles of the Cochrane Collaboration. Primary outcomes were live-birth, ongoing and clinical pregnancy rates.

**Chapter 9** presents a systematic review and meta-analysis of randomized controlled trials that compare different sites of embryo placement in the uterine cavity. The studies were included and assessed according to the principles of the Cochrane Collaboration. Primary outcomes were live-birth, ongoing and clinical pregnancy rates.

**Chapter 10** presents the results of a randomized controlled trial that compared ultrasound with clinical touch methods of embryo catheter guidance performed by a single operator. Primary outcomes were the live-birth/ongoing pregnancy and clinical pregnancy rates per randomized woman.

**Chapter 11** reports on results of a systematic review and meta-analysis of randomized controlled trials that compared ultrasound with clinical touch methods of embryo catheter guidance. The studies were included and assessed according to the principles of the Cochrane Collaboration. Primary outcome measures were the live-birth, ongoing pregnancy, and clinical pregnancy rates.

**Chapter 12** reports on results of a retrospective analysis on a large cohort of women who underwent embryo transfer to determine whether the presence of difficulty during embryo transfer, the presence of blood and mucus on the post-transfer catheter and retained embryos affected the clinical outcomes. Primary outcome was clinical pregnancy rate.

**Chapter 13** presents a retrospective analysis to determine whether the use of Propofol anaesthesia improves the clinical outcomes when used in women presenting with difficulty during embryo transfer. The primary outcome measures for this study were the odds of a clinical pregnancy and embryo implantation following Propofol anesthesia compared to no anesthesia.

**Chapters 14 and 15** present a general discussion and the summary and conclusions of the preceding chapters.
References