Optimizing the embryo transfer technique
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Chapter 10

Ultrasound-guidance during embryo transfer: a prospective, single operator, randomized, controlled trial.

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Abstract

Objective: Ultrasound (US) assisted embryo transfer (ET) has been proposed to be more effective than the standard clinical touch (CT) method of catheter placement. Even so, heterogeneity between the published studies may be in part due to multiple operators.

Design: Prospective, single-operator, randomized, controlled trial comparing ultrasound to clinical touch methods of embryo catheter guidance.

Setting: Saudi Center for Assisted Reproduction.

Patient(s): 378 women.

Intervention(s): Transcervical, intrauterine embryo transfer with or without ultrasound guidance.

Main outcome measure(s): Primary outcomes were the live-birth/ongoing pregnancy and clinical pregnancy rates per randomized woman. Secondary outcomes were the incidences of difficult transfers, blood and/or mucus on the catheter tip, spontaneous miscarriages and ectopic pregnancies.

Result(s): Demographics and cycle characteristics were not different between the two groups. Live-birth/ongoing pregnancy rate was significantly higher in the US-ET group [68/183 (40.98%)] than the CT-ET group [50/190 (28.42%)] (O.R= 1.66, 95%CI= 1.07-2.57). In addition, there was a significantly higher number of clinical pregnancies in the US-ET group [75/183 (40.98%)] than the CT-ET group [54/190 (28.42%)] (O.R= 1.75, 95%CI= 1.14-2.69). Secondary outcomes were not significantly different between the two groups.

Conclusion(s): Ultrasound-guided embryo transfer significantly increases the chance of ongoing/live-birth and clinical pregnancy rates compared to the clinical touch method.

Key words: Ultrasound, clinical touch, embryo transfer, randomized controlled trial, in vitro fertilization
**Background**

The majority of patients undergoing assisted reproduction through in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) will reach the transfer stage, but a small proportion of them will achieve a clinical pregnancy, an ongoing pregnancy or a live-birth (1, 2). Although there have been miraculous improvements in respect to pituitary down-regulation and ovulation stimulation protocols, embryo culture medias, and laboratory techniques, embryo implantation remains the rate-limiting step in the success of IVF. Embryo implantation is considered by many to be the most important limiting factor to success in assisted reproduction. The main factors that are believed to affect embryo implantation are embryo quality, uterine receptivity, and more importantly the proper technique of embryo transfer (3).

Ever since the birth of the first in-vitro fertilization (IVF) baby in 1978 (4), the technique of uterine embryo transfer has remained largely unchanged since it was first described. Even so, recently a renewed interest in the embryo transfer technique has evolved among clinician and researchers, alike. Factors such as the use of soft embryo transfer catheters (5) and ease of the technique have been shown to affect the clinical outcomes.

A new innovation is the use of ultrasound-guidance during embryo transfer in order to improve the ease of transfer, and precision in determining the site of transfer when compared to the standard clinical touch method. Recent systematic reviews have reported that embryo transfer under ultrasound guidance improves clinical pregnancy, ongoing pregnancy and live birth rates (6 - 8). Even so, there was heterogeneity between the included studies. This heterogeneity may be a result of individual transfer techniques, role of multiple practitioners performing embryo transfer, or study quality.

As a result of the importance of the embryo transfer stage in determining the clinical outcomes for patients undergoing IVF treatment, it is vital for each program to properly evaluate their protocols and to adjust to new innovations in assisted reproduction treatment. Therefore we decided to perform a prospective, randomized, single operator, trial in order to determine if the implementation of ultrasound-guidance will improve the clinical outcomes in our patient population.
Materials and Methods
This prospective, randomized trial was approved by our institutional review board. Three-hundred and seventy-three patients undergoing embryo transfer in our assisted reproduction unit between January 2005 and November 2006 were prospectively included.

Patient population
The study objectives were explained thoroughly to all prospective patients and their partners entering our assisted reproduction program. Couples who agreed to enter the clinical trial provided both verbal and written consent. Inclusion criteria were broad so as to truly represent our center’s patient population. Women undergoing embryo transfer in a fresh cycle, and with good quality embryos on the day of transfer, were randomized to one of the two groups. Randomization was performed by the use of dark, sealed envelopes.

Ovulation induction and IVF protocols
All aspects of the IVF procedure were similar between the two groups, with the exception of the transfer technique. In brief, ovarian stimulation, oocyte retrieval and luteal phase support were performed in accordance with the standard protocol of our department. Women were down-regulated using a gonadotropin-releasing hormone agonist (GnRH-agonist) (Decapeptyl, Ferring NV, Belgium) protocol, followed by ovarian stimulation using recombinant FSH (rFSH, Puregon, NV Organon, Oss, The Netherlands) and/ or human menopausal gonadotrophin (Menogon, Ferring NV, Belgium) till the day of human chorionic gonadotrophin (HCG) administration. When the leading follicle reached ~18 mm in diameter, 10,000 IU of hCG (Pergnyl, NV Organon, The Netherlands) was given intra muscularly, and oocyte retrieval was performed 34 – 36 hours later. Luteal phase support is provided in the form of daily progesterone vaginal suppositories tid (Cycologest 400 mg; Hoechst Roussel Limited, UK).

Embryo transfer technique
All embryo transfers were performed on day 3 by a single physician (M.A.E.) using a standardized technique. In all cases patients were asked to maintain a full bladder prior to transfer. Embryo transfer was performed using a Sydney IVF Embryo Transfer Set (K-JETS- 7019-
SIVF) connected to a tuberculin syringe. A single air bubble was used to prevent the accidental expulsion of the embryo containing media. In addition, the catheter was held with its tip slightly downwards to prevent embryos from traveling through the liquid column to the end connected to the syringe.

In both groups, the target was to deposit the embryos ~2 cm from the uterine fundus. In the ultrasound group, this was assisted with the use of transabdominal ultrasound guidance (2101 Falcon B-K Medical, 3.5 MHz probe) by a trained ultrasonographer. In the clinical touch group, catheter placement was accomplished using clinical sense and judgment. Following the transfer the catheter was extracted slowly and examined for retained embryos, blood and/ or mucus by the embryologist under a stereomicroscope.

**Outcome measures**

The primary outcome measure for this trial was the live birth/ ongoing pregnancy and clinical pregnancy rates per randomized woman. Live birth was defined as a living fetus born ≥28 weeks gestation. Ongoing pregnancy was defined as an ongoing pregnancy ≥14 weeks gestation, and clinical pregnancy was defined by the presence of a positive β-hCG subunit measurement two weeks post-transfer and a clinically viable gestational sac with fetal heart pulsation on ultrasound three weeks later. In addition, the incidences of difficult transfers, blood and/ or mucus on the catheter tip, spontaneous miscarriages and ectopic pregnancies were evaluated. Difficult transfers were defined as difficulties in placing the catheter inside the uterine cavity due to position of the uterus in relation to the cervical canal, cervical stenosis or if embryo transfer took more than 5 minutes.

**Statistical analysis**

Statistical analysis was performed according to the intention to treat principle. All analyses of significance were two-sided and tested at the 5% level; values of \( P < 0.05 \) were considered to indicate significant differences. Continuous variables were tested if they presented normal distribution using the \( t \)-test. The results of the two groups were compared using the \( t \)-test or Mann-Whitney U test for parametric and nonparametric data, respectively. Qualitative variables were compared with the use of the chi-squared test with Yates correction or Fisher’s
exact test, when necessary, and the 95% confidence intervals (95% CI) using the Woolf (logit) approximation. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to examine the odds of improving clinical outcomes. Clinical and demographic data are also presented as mean (± SD) or as frequency distribution for simplicity. Statistical analysis was performed using the computer statistical package Stats Direct (Stats Direct, Ltd, UK).
Results
The cause of infertility was similar in both groups, mainly being male factor (Table 1). Also, the two groups were similar with regards other demographics, cycle characteristics.
There was no significant difference with regards to patient age, period of infertility, and day-3 FSH levels (Table 1). In addition, there were no significant differences in the days of stimulation, and numbers of oocytes retrieved, MII oocytes, injected oocytes, fertilized oocytes, embryos produced, embryo quality, and numbers of embryos transferred (Table 1). Finally, there were no significant differences in the volume of injected culture media in the two groups (data not presented).
With regards to the primary outcome measure, live-birth/ ongoing pregnancy rate there was a significantly higher number of live births/ ongoing pregnancies in the US-ET group [68/ 183 (40.98%)] than the CT-ET group [50/ 190 (28.42%)] (P = 0.0324; O.R = 1.66, 95% CI = 1.07 to 2.57). In addition, there was a significantly higher number of clinical pregnancies in the US-ET group [75/ 183 (40.98%)] than the CT-ET group [54/ 190 (28.42%)] (P = 0.0146; O.R = 1.75, 95% CI = 1.14 to 2.69).
With regards to the post-transfer examination of the catheter tips, there were no significant differences in the presence of blood (in and/or on the catheter tip), mucus or blood and mucus on the tip of the embryo transfer catheter (Table 2). Also, there was no significant differences in the incidence of easy transfers in the two groups. Finally, there were also no significant differences in the incidence of spontaneous miscarriages and ectopic pregnancies in the two groups.
Finally, a subgroup analysis was performed to determine if the presence of blood and/ or mucus on the catheter tip was a negative predictor of IVF outcome. In both the USG and CT groups, cycles with post-transfer evidence of blood or mucus had a less likely chance of a clinical pregnancy, but this did not reach statistical significance [(33.33% vs. 42.13%, Chi² = 0.35, P = 0.55) and (7.69% vs. 29.94%, Chi² = 1.96, P = 0.16), respectively].
Discussion
The embryo transfer is the final stage of the in vitro fertilization cycle. It is also the instance where clinical manipulations can directly alter the outcomes, and has shown marked variability among different IVF programs and physicians in the same program (9, 10).
During 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 (11). Recently, the techniques and variables affecting the success of embryo transfer have attracted more attention, and physician attitudes have been changing accordingly (12, 13). Today, in light of global trends such as single embryo transfer (SET), more stress has been placed on optimizing and standardizing the embryo transfer protocol than ever before.
Although most patients who undergo assisted procreation, via IVF or ICSI, will reach the embryo transfer stage with good quality embryos available for replacement, embryo implantation remains the rate-limiting step in the success of this form of therapy. The aim should be to meticulously and accurately place embryos within the uterus; in order to allow for proper implantation and fetal development (14).
In order to ascertain the importance of each step involved in the embryo transfer procedure, individual factors must be evaluated independently. This can only be properly performed in prospective, randomized trials in which all possible confounders are virtually the same in both the control and intervention groups. In addition, the two groups of patients should be similar with respect to the known clinical factors that influence pregnancy rate in fresh embryo transfer cycles (e.g. patient demographics, cause of infertility). Moreover, all transfers should be carried out by the same physician using the same transfer catheter, patient position, bladder preparation, and catheter loading techniques. In the current study, these factors were all similar with regards the two randomized groups.
Today, more than twenty-years since the first reports of the beneficial effect of ultrasound guidance during the ‘blind’ embryo transfer procedure were published (15, 16), the routine use of ultrasonography to guide the intrauterine embryo transfer catheter placement are still highly debated. This has been fueled by the conflicting results of published clinical trials, with some concluding that ultrasound guidance
improves the clinical pregnancy, and implantation rates, while others reporting no such improvement in their results.

In the literature there are numerous clinical trials that have tested the theory of improved success with ultrasound-guidance when compared to the standard clinical touch. Even so, the majority of published trials are flawed by study design (e.g. retrospective, quasi-randomization), including both fresh and frozen embryo replacement cycles and donor oocytes, and/or the inclusion of multiple cycles for the same patient in the trial. These issues may be a factor in the obvious heterogeneity in the presented results.

Recent systematic reviews have shown that ultrasound-guidance does in fact improve the clinical pregnancy, ongoing pregnancy and live-birth rates in women when compared to the standard clinical touch method (6 – 8, 17). Even so, the majority of studies did not provide the live-birth or ongoing pregnancy rates for the randomized groups.

The exact mechanism whereby ultrasound-guidance improves the outcomes following embryo transfer remains unclear. Several theories have been proposed to identify the mechanisms including confirming the position of the catheter tip within the uterine cavity, properly determining the site of embryo deposition, and decreasing the frequency of “difficult” embryo transfers (7). Nevertheless, some clinicians argue that the real benefit of ultrasound guidance lies is the ability of increasing the clinical appreciation of the pelvic anatomy, and to notice early signs of ovarian hyperstimulation prior to transfer. Whatever the underlying mechanism, the overall conclusion from this randomized controlled trial is that ultrasound-guided embryo transfer using 2D-transabdominal ultrasound is significantly more effective than embryo transfer by clinical touch alone.
References
Table 1: Review table of demographic and cycle outcomes:

<table>
<thead>
<tr>
<th></th>
<th>US-ET Mean (SD)</th>
<th>CT-ET Mean (SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>32.41 (5.47)</td>
<td>31.69 (5.46)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>FSH</strong></td>
<td>5.19 (2.09)</td>
<td>5.17 (2.32)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Days of stimulation</strong></td>
<td>9.81 (1.87)</td>
<td>9.78 (1.98)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>No. of Oocytes Retrieved</strong></td>
<td>10.81 (7.37)</td>
<td>11.56 (7.90)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>No. of MII Injected</strong></td>
<td>8.79 (6.12)</td>
<td>9.07 (6.41)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fertilized</strong></td>
<td>8.78 (6.10)</td>
<td>9.05 (6.38)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Good Embryo G1</strong></td>
<td>6.16 (4.28)</td>
<td>6.48 (4.66)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fair Embryo G2</strong></td>
<td>3.20 (3.10)</td>
<td>2.98 (2.94)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Poor Embryo G3, G4</strong></td>
<td>1.90 (2.01)</td>
<td>2.16 (2.01)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>No. of ET</strong></td>
<td>1.05 (1.63)</td>
<td>1.36 (1.90)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Clinical Pregnancy Rate (%)</strong></td>
<td>3.36 (1.07)</td>
<td>3.32 (1.04)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Clinical Pregnancy Rate (%)</strong></td>
<td>75/183 (40.98)</td>
<td>54/190 (28.42)</td>
<td>P = 0.0146; OR = 1.75, 95% CI = 1.14 to 2.69</td>
</tr>
<tr>
<td><strong>Multiple Pregnancy Rate</strong></td>
<td>3</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Ectopic Pregnancy</strong></td>
<td>1</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>
### Miscarriages

<table>
<thead>
<tr>
<th></th>
<th>US-ET Mean (SD)</th>
<th>CT-ET Mean (SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miscarriages</td>
<td>5</td>
<td>4</td>
<td>NS</td>
</tr>
</tbody>
</table>

### Live-birth/ Ongoing Pregnancy Rate (%)

<table>
<thead>
<tr>
<th></th>
<th>US-ET Frequency (%)</th>
<th>CT-ET Frequency (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live-birth/ Ongoing Pregnancy Rate (%)</td>
<td>68/ 183 (37.16)</td>
<td>50/ 190 (26.32)</td>
<td>P = 0.0324; O.R = 1.66, 95% CI = 1.07 to 2.57</td>
</tr>
</tbody>
</table>

Table 2: ET Catheter tip:

<table>
<thead>
<tr>
<th></th>
<th>US-ET Frequency (%)</th>
<th>CT-ET Frequency (%)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blood or mucus</td>
<td>159 (86.89)</td>
<td>177 (93.16)</td>
<td>NS</td>
</tr>
<tr>
<td>Blood</td>
<td>5 (2.73)</td>
<td>3 (1.58)</td>
<td>NS</td>
</tr>
<tr>
<td>Mucus</td>
<td>15 (8.20)</td>
<td>7 (3.68)</td>
<td>NS</td>
</tr>
<tr>
<td>Blood + Mucus</td>
<td>4 (2.19)</td>
<td>3 (1.58)</td>
<td>NS</td>
</tr>
<tr>
<td>Retained embryos</td>
<td>3 (1.64)</td>
<td>5 (2.63)</td>
<td>NS</td>
</tr>
</tbody>
</table>