Implantation, clinical pregnancy and miscarriage rates after introduction of ultrasound-guided embryo transfer

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Abstract

The purpose of this study was to compare the reproductive outcome of ultrasound-guided (USG) embryo transfers versus clinical touch embryo transfers. A statistically powered retrospective analysis of women undergoing fresh and frozen embryo transfers in a National Health Service-based tertiary referral centre in the Department of Reproductive Medicine, St Mary’s Hospital, Manchester was carried out. A total of 1723 embryo transfers were included in the analysis. The implantation rate was significantly higher in the USG embryo transfer group compared with the non-USG embryo transfer group (fresh: 19.9 versus 9.5%, \(P < 0.0001\); frozen: 13.1 versus 7.3%, \(P < 0.0004\)). The clinical pregnancy rate was also significantly higher in the former group (fresh: 26.9 versus 12.5%, \(P < 0.0001\); frozen: 15.6 versus 8.9%, \(P < 0.0015\)). For the frozen embryos, the miscarriage rate was significantly elevated among the USG embryo transfer group [unadjusted rate ratio (RR) = 1.65, 95% CI: 1.04, 2.62], but this was of borderline significance when the model was adjusted for the potential confounders (adjusted RR = 1.56, 95% CI: 0.997, 2.45). There was no difference in the ectopic pregnancy rates between the two groups. The findings of this study show that the practice of USG embryo transfer is associated with statistically higher implantation and clinical pregnancy rates in IVF.

Keywords: clinical pregnancy, embryo transfer, implantation, IVF, miscarriage, ultrasound

Introduction

Embryo transfer is a crucial step in IVF cycles. Clinical pregnancy rates are influenced by several factors such as patient selection, ovarian stimulation protocol, embryo quality, uterine receptivity, embryo culture and embryo transfer techniques. In order to optimize the cycle outcome, all the aspects of IVF treatment need to be considered.

Embryo transfer techniques should be tuned to maximize the implantation potential of the embryos. Nearly 85% of all embryos transferred in the uterine cavity fail to implant (Edwards, 1995), and up to 30% of failed implantations can be due to poor transfer techniques (Li et al., 2005). An ideal embryo transfer method can be defined as one in which the embryos are deposited in the endometrial cavity without the catheter disturbing the endometrium and avoiding contact with the uterine fundus. The ideal site of embryo deposition at the time of transfer remains a matter of much debate.

The use of ultrasound guidance for proper catheter placement was first described over 20 years ago by Strickler and colleagues (Stuckler et al., 1985). With an increasing trend towards elective single embryo transfer, methods to improve embryo culture and embryo transfer become even more important to maintain the live birth rates. At St Mary’s Hospital, Manchester, UK,
ultrasound-guided (USG) embryo transfer has been part of standard practice since November 2004. This change in practice was implemented mainly because of an increasing body of evidence in support of USG transfers (Buckett, 2003; Sallam, 2003; NICE 2004; Abou-Setta et al., 2007). The objective of this large retrospective analysis was to compare the reproductive outcome of USG embryo transfers versus non-USG transfers (i.e. clinical touch method).

Materials and methods

All patients undergoing IVF, intracytoplasmic sperm injection (ICSI) and replacement of frozen–thawed embryos within a tertiary referral unit based at the Department of Reproductive Medicine, St Mary’s Hospital, Manchester were included in the analysis. The study was approved by the Institutional Review Board and Local Research Ethics Committee, Manchester (05/Q1402/70).

In the 12-month period between November 2003 and October 2004, 881 consecutive fresh and frozen embryo transfers were carried out. This was comparable with the number of fresh and frozen transfers (n = 842) performed between November 2004 and October 2005.

Apart from the introduction of ultrasound guidance for embryo transfer, no other changes were implemented between the two periods. The long-step-down ovarian stimulation protocol used in all cycles was as previously published (Gelbaya et al., 2006a,b). All the embryo transfers were performed 48 h after oocyte retrieval. Luteal phase support was either by 1500 IU human chorionic gonadotrophin (HCG, Pregnyl®; Organon Laboratories Ltd, Cambridge, UK) given intramuscularly 2 days after oocyte collection or by progesterone vaginal pessaries (Cyclogest®; Alpharma, Barnstaple, UK) 400 mg twice daily for 10 days, depending on the serum oestradiol concentration on the day of HCG administration (>10,000 pmol/l) and/or the total number of oocytes retrieved (>20).

The transfer of cryopreserved embryos was carried out in down-regulated hormonally controlled cycles using a standard regime (Gelbaya et al., 2006a,b). The embryo freezing and thawing protocols used in the laboratory were also those previously published (Horne et al., 1997). Embryos were graded 1–4 according to the data reported by Steer and collaborators (Steer et al., 1992). The grading was as follows: grade 4: no fragmentation, clear embryos, and equal sized blastomeres; grade 3: 0–20% fragmentation or very slight irregularity; grade 2: 20–50% fragmentation, irregular dark blastomeres; grade 1: 50–100% fragmentation and extremely irregular and dark blastomeres. Grade 1 embryos were never transferred.

All embryo transfers were performed by five experienced operators, whose clinical pregnancy rate per embryo transfer rate did not differ significantly (ranges: 23–28% fresh and 17–21% frozen respectively). All operators showed an increase in success rate, and this was not proportional to their years of experience. Prior to the introduction of USG embryo transfer, all women had their uterine cavity length measured by sounding the uterus at the outpatient appointment preceding the treatment cycle. Both the USG and the non-USG embryo transfers were performed in theatre with the patient in the lithotomy position. All women undergoing USG transfer had a full bladder in order to obtain a clear image. Conversely, women were not required to have a full bladder if the clinical touch method was used. A sterile Cusco speculum was inserted to expose the cervix, which was then cleansed and the cervical mucous removed using sterile swabs or by simple aspiration with a 10 ml syringe. The embryo transfer was carried out using a soft embryo transfer catheter (Embryon® Thin Wall Soft Transfer Set; Rocket Medical, Newcastle upon Tyne, UK). Once the outer catheter had passed through the cervical os, the inner plastic stylet was removed and the catheter containing the embryos was introduced to a point equal to the length of the uterine cavity minus 1 cm. With the use of ultrasound, the inner catheter was introduced into the cavity until the tip could be seen at approximately 1 cm from the uterine fundus. Following the embryo transfer, patients had no restrictions to sit up, mobilize and empty their bladder.

The clinical pregnancy (i.e. a pregnancy where an ultrasound scan has shown at least one fetal heart beat) and ectopic pregnancy rates of USG and clinical touch embryo transfers were the primary outcomes. Implantation rates and miscarriage (i.e. a pregnancy that did not progress after a sac or fetal beat was seen on scan) rates were the secondary outcomes compared in this study.

Statistical analysis

Patients included in the two groups were demographically similar, using Student’s t-test. Data were analysed using the statistical package Stats Direct, Statistical Software Version 2.4.5 (Stats Direct Ltd, UK). The chi-squared test was used for comparisons as appropriate.

Log-linear binomial regression (Robbins et al., 2002) in Stata Software (Stata, 2003) was used to estimate the success rate ratio of pregnancy and miscarriages rate ratio. Rate ratios (RR) were adjusted for maternal age, parity, gravidity and serum FSH concentrations. When convergence was not achieved (a recognized problem with this model) log–linear Poisson regression with robust estimation variance was used (Zou, 2004). RR > 1 would indicate an increase in success rate or miscarriage rate among women who were treated with the USG embryo transfer compared with those treated with the clinical touch embryo transfer. A post-hoc analysis was performed. With the number of patients included in the analysis and a statistical power of 80%, this study was able to demonstrate a difference in pregnancy rate of 7.65 and 6.35% for fresh and frozen embryo transfers respectively. P < 0.05 was considered statistically significant.

Results

The patients included in the two study groups were comparable for demographics and clinical characteristics (Table 1). A total of 394 fresh and 448 frozen embryo transfers were carried out under ultrasound guidance, while 385 fresh and 496 frozen embryo transfers were performed using the clinical touch technique. All the operators performed the same proportion of embryo transfers. Both groups had a similar number of difficult transfers.

As shown in Table 2, the implantation rate (expressed as a percentage and calculated from the number of gestation sacs
with fetal heart visible on ultrasound scan divided by the total number of replaced embryos) was significantly higher in the USG embryo transfer group compared with the non-USG embryo transfer group (fresh: 19.9 versus 9.5%, \( P < 0.0001 \); frozen: 13.1 versus 7.3%, \( P < 0.0004 \)). The clinical pregnancy success rate for fresh embryos increased from 12.5% in the clinical touch group to 26.9% in the USG group. For frozen embryos, the pregnancy success rate increased from 8.9 to 15.6% (Table 3).

The results for the fresh embryos transfer showed that women who were treated with USG embryo transfer had more than twice the success rate compared with those who were treated with clinical touch embryo transfer (adjusted RR = 2.19, 95% CI: 1.73, 2.78). Success rate for frozen embryos among women who were treated with USG embryo transfer was 85% higher than those who were treated with clinical touch embryo transfer (adjusted RR = 1.85, 95% CI: 1.35, 2.53).

There was no difference in the ectopic pregnancy rates between the two groups (Table 3). Table 4 shows that the miscarriage rate for fresh embryos among women who were treated with USG embryo transfer was not significantly different from those who underwent clinical touch embryo transfer (adjusted RR = 1.10, 95% CI: 0.62, 1.92). For the frozen embryos, the miscarriage rate was significantly (unadjusted RR = 1.65, 95% CI: 1.04, 2.62) elevated among the USG embryo transfer group, but this was of borderline significance when the model was adjusted for the potential confounders (adjusted RR = 1.56, 95% CI: 0.997, 2.45).

The embryo grading, as well as the percentage of single embryo and double embryo transfers, was similar between the two study groups.

### Table 1. Characteristics and causes of infertility in the ultrasound-guided (USG) embryo transfer group and in the clinical touch embryo transfer group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fresh embryo transfer</th>
<th>Frozen embryo transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USG</td>
<td>Clinical touch</td>
</tr>
<tr>
<td>No. of patients</td>
<td>385</td>
<td>394</td>
</tr>
<tr>
<td>Age (years)</td>
<td>32.7</td>
<td>33.1</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>21.4</td>
<td>22.7</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.72</td>
<td>0.75</td>
</tr>
<tr>
<td>Parity</td>
<td>0.19</td>
<td>0.16</td>
</tr>
<tr>
<td>FSH concentration (IU/l)</td>
<td>4.88</td>
<td>6.06</td>
</tr>
<tr>
<td>Unexplained infertility %</td>
<td>32.3</td>
<td>32.0</td>
</tr>
<tr>
<td>Male factor %</td>
<td>18.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Tubal factor %</td>
<td>27.7</td>
<td>27.2</td>
</tr>
<tr>
<td>PCOS %</td>
<td>16.0</td>
<td>14.0</td>
</tr>
<tr>
<td>Endometriosis %</td>
<td>6.0</td>
<td>6.2</td>
</tr>
</tbody>
</table>

\( ^a \)t-test, \(^b \)chi-squared test. NS = not statistically significant; PCOS = polycystic ovarian syndrome.


### Table 2. Gestational sacs seen at ultrasound and implantation rate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Clinical touch embryo transfer</th>
<th>USG embryo transfer</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Frozen</td>
<td>Fresh</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>652</td>
<td>703</td>
<td>683</td>
</tr>
<tr>
<td>No. of gestational sacs(^a)</td>
<td>62</td>
<td>51</td>
<td>136</td>
</tr>
<tr>
<td>Implantation rate %</td>
<td>9.5</td>
<td>7.3</td>
<td>19.9</td>
</tr>
</tbody>
</table>

NS = not statistically significant; USG = ultrasound-guided.

\(^a\)With visible fetal heart on ultrasound.
Discussion

Following the initial reports (Stickler et al., 1985; Leong et al., 1986) on the use of ultrasound for embryo transfer, studies of various designs (Coroleu et al., 2000; Wood et al., 2000; Prapas et al., 2001; Tang et al., 2001; Matorras et al., 2002; Sallam et al., 2002), three meta analyses (Buckett, 2003; Sallam, 2003; NICE 2004; Abou-Setta 2007) and a Cochrane systematic review (Brown et al., 2007) have demonstrated the advantages of this approach over the clinical touch method. Conversely, other authors (Al-Shawaf et al., 1993; Kan et al., 1999; Garcia-Velasco et al., 2002) have shown no benefit when ultrasonography was used for embryo transfer. One study suggested that the difficulty of transfer can be overcome by the experience of the practitioner performing it (Garcia-Velasco et al., 2002). Ultrasound has not been shown to be of benefit in units with high success rates (Flisser et al., 2006). The lack of convergence of the data combined with inadequate quality of the studies so far published (Brown et al., 2007) makes it difficult to draw definite conclusions. The use of USG embryo transfer still requires further investigation.

Table 3. Pregnancy, miscarriage and ectopic pregnancy rates in the two study groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Clinical touch embryo transfer</th>
<th>USG embryo transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
<td>Frozen</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>385</td>
<td>496</td>
</tr>
<tr>
<td>Positive pregnancy test (%)</td>
<td>65 (16.9)</td>
<td>66 (13.3)</td>
</tr>
<tr>
<td>Clinical pregnancy (%)</td>
<td>48 (12.5)</td>
<td>44 (8.9)</td>
</tr>
<tr>
<td>Miscarriage before fetal heart (%)</td>
<td>15 (3.9)</td>
<td>21 (4.2)</td>
</tr>
<tr>
<td>Miscarriage after fetal heart (%)</td>
<td>4 (1.0)</td>
<td>7 (1.4)</td>
</tr>
<tr>
<td>Total miscarriage (%)</td>
<td>19 (4.9)</td>
<td>28 (5.6)</td>
</tr>
<tr>
<td>Ectopic pregnancy (%)</td>
<td>2 (0.5)</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

NS = not statistically significant; USG = ultrasound-guided.

Table 4. Relative risk of successful pregnancy and miscarriage among women receiving USG and clinical touch embryo transfer.

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Fresh embryos</th>
<th>Frozen embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Success rate</td>
<td>2.14 (1.57, 2.92)</td>
<td>2.19 (1.73, 2.78)</td>
</tr>
<tr>
<td>Total miscarriage rate</td>
<td>1.23 (0.69, 2.22)</td>
<td>1.10 (0.62, 1.92)</td>
</tr>
<tr>
<td>Miscarriage rate (no fetal heart)</td>
<td>1.30 (0.68, 2.51)</td>
<td>1.11 (0.58, 2.10)</td>
</tr>
</tbody>
</table>

Values are rate ratio (95% confidence interval).
<sup>a</sup>Relative risk adjusted for maternal age, parity, gravidity and FSH concentrations.

Without doubt, ultrasound guidance enables assessment of the uterus and utero–cervical angle prior to the transfer. It ensures accurate placement of the catheter tip without touching the fundus and allows visualization of the bubble containing the embryos. Besides being reassuring to the anxious couple, fertility physicians believe that not touching the fundus prevents uterine junctional zone contractions and may, therefore, increase the success of embryo transfer. The presence of uterine contractions at
the time of transfer has been shown to be associated with reduced pregnancy and implantation rates (Lesny et al., 1999).

The type of catheter used for the transfer is still debated. A recent meta-analysis by Buckett (2006) has demonstrated that there is an increased chance of clinical pregnancy when soft embryo transfer catheters are used. All embryo transfers included in the present analysis were performed using the same type of soft catheter and the plastic stylet was always removed after passing through the internal cervical os.

Many would agree that ultrasound guidance for embryo transfer plays a crucial role in the process of training, and its routine use in teaching centres should be advocated. It enables the trainee to be confident that the catheter has been correctly placed (Papageorgiou et al., 2001). As the practice of USG embryo transfer requires additional resources in terms of equipment and trained staff, it has to be acknowledged that it may not be very cost-effective for some small units.

Previous reports (Coroleu et al., 2000; Matorras et al., 2002; Sallam, 2003) and the present study have shown that ultrasound guidance for embryo transfer does not affect the ectopic pregnancy rate. Of note, this is in contrast with the data reported in a meta-analysis (Buckett, 2003). Studies with adequate reporting of randomization and power calculations are, therefore, required to address this issue further.

Coroleu and collaborators (2000) found an increased miscarriage rate in the clinical touch embryo transfer group. The present study and the elegant meta-analysis by Sallam (2003) have noted no difference in the miscarriage rate after the introduction of ultrasonography for embryo transfer. Interestingly, in the present study a higher miscarriage rate was found in women undergoing USG transfer of frozen embryos. It is plausible to speculate that factors other than the ultrasound technique, including quality of cryo-preserved embryos and the precise embryo–endometrium cross-talk, could be responsible for this finding.

It is noteworthy that the recent detailed review by Flisser and Grifo (2007) concluded that routine use of ultrasound can be justified, as no trial has shown an adverse effect and situations where use of ultrasound would be advantageous cannot be reliably predicted.

In conclusion, the present study adds to the current body of evidence confirming that USG embryo transfer is associated with increased implantation and clinical pregnancy rates in women undergoing assisted reproductive technology. Although limited by its retrospective design, this study is adequately powered to make a strong case in favour of USG embryo transfer. The potential confounding variables are reduced by the inclusion of matched groups of patients and clinicians performing the transfers. The reassurance that visualization of an accurate embryo transfer provides the couple should not be discounted.

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