A randomized controlled clinical trial of 2295 ultrasound-guided embryo transfers

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BACKGROUND: We wanted to test the hypothesis that using abdominal ultrasound at the time of embryo transfer to guide replacement, improved pregnancy rates by at least 5%. METHODS: An RCT in a large assisted conception unit. A pilot study and power calculation suggested that at least 2000 embryo transfers were required to demonstrate a difference of 5%, for a test with 80% power and Type 1 error 0.05. Randomization, data entry and analysis were arranged independently. Randomization was stratified for age and fresh/frozen embryo transfer. Analysis was by intention to treat. RESULTS: There was no difference in clinical pregnancy or live birth rates between the two groups. The clinical pregnancy rate for ultrasound-guided embryo transfer was 22% and for non-ultrasound-guided embryo transfer was 23% (odds ratio: 0.96; 95% confidence interval: 0.79–1.18). CONCLUSIONS: We set out to determine whether ultrasound-guided embryo transfer improved clinical pregnancy rates and live birth rates in assisted conception. We used an appropriately powered RCT design. We did not demonstrate a difference. This outcome is at odds with the UKs National Institute of Clinical Excellence recommendations for fertility treatment (Fertility Assessment and Treatment for People with Fertility Problems. London, UK: RCOG Press, 2004, 112.) which used a meta-analysis of four smaller trials (range 362–800 patients, totalling 2051 embryo transfers) to conclude that ultrasound should be offered. We suggest that the current Cochrane review should be updated with data from our trial and recommend that consideration is given to accounting for heterogeneity between the included trials.


Keywords: assisted conception; embryo transfer; ultrasound; RCT

Background

Embryo transfer is a vitally important step in the assisted conception process. Clinicians are constantly testing and refining different ways to improve success. One such proposed innovation has been to use abdominal ultrasound to guide the positioning of embryo placement. There had been a few smaller randomized trials published in the years leading up to commencement of our trial, some of which demonstrated benefit (Tang et al., 2001; Garcia-Velasco et al., 2002), whereas others did not (Coroleu et al., 2000; Matorras et al., 2002). Some of these papers (Tang, Garcia-Velasco and Coroleu) describe a priori sample size calculations, based on 80 or 90% power. They set out to observe a significantly larger clinical difference than we aimed to observe (~15% or higher), hence why they needed such a smaller sample size. We felt that this was overly optimistic. Tang (800 patients) fell short of reaching his pre-specified difference but states in his conclusion, that he felt clinical significance was shown but not statistical significance and so a larger sample size was needed to confirm his clinical findings.

Nevertheless, when subjected to a systematic review and meta-analysis by Buckett (2003), collectively a benefit was shown. Around the same time, a further similar systematic review and meta-analysis was published (Sallam and Sadek, 2003) that included the same trials and arrived at the same conclusions. On the basis of these results, the UKs National Institute of Clinical Excellence (2004) made the recommendation that ultrasound-guided embryo transfer should be employed. This recommendation was given a Grade ‘A’ evidence rating based on randomized trial status. Clinical heterogeneity between the different trials was acknowledged by NICE. The guidance from NICE was published when recruitment to our trial was half completed. We felt that it was justified to complete our trial after the independent ‘data monitoring committee’ looked at trial data at the mid point.
A Cochrane review (Brown et al., 2007) of ultrasound versus ‘clinical touch’ for catheter guidance during embryo transfer has more recently been published and this again showed a benefit in favour of ultrasound guidance (USG). However, this review acknowledged that the included trials were limited by their quality and that none of the included trials had reported on live births, although data on this outcome were subsequently obtained from two authors. Clinical heterogeneity between the trials was again acknowledged. A further systematic review and meta-analysis was published in August 2007 (Abou-Setta et al., 2007) and again benefit in favour of USG was shown.

We wished to test the hypothesis that the use of abdominal ultrasound to guide embryo transfer improved clinical pregnancy and live birth rates in a large, appropriately powered randomized controlled clinical trial setting.

**Methods**

Local regional ethical committee approval was obtained. Couples undergoing assisted reproduction technology in the form of IVF or ICSI at the Hewitt Centre for Reproductive Medicine, Liverpool Women’s Hospital NHS Foundation Trust between February 2003 and May 2005 were recruited to a single centre RCT, with follow-up time allowed to collect live birth data.

A pilot study undertaken in 2002 of 30 patients having ultrasound-guided embryo transfers, suggested a 9% improvement in clinical pregnancy rates from 21 to 30% (or a 30% increase). The pilot study was performed by two clinicians involved in the larger trial. It was agreed that a change in practice would be indicated if a 5% increase in clinical pregnancy rates could be achieved using abdominal ultrasound. A power calculation using 80% power and Type I error of 0.05 indicated that at least 2000 embryo transfers would need to be randomized to demonstrate a 5% difference in pregnancy rates between the groups, assuming a control group clinical pregnancy rate of 21%.

The primary outcome of interest was to evaluate whether using ultrasound to guide embryo transfer led to an improvement in biochemical pregnancy or clinical pregnancy and importantly follow-up time was extended to include live birth rate. Biochemical pregnancy was defined as a positive urine β-HCG test; clinical pregnancy was defined as the observation of one or more fetal hearts on ultrasound screening at 7 weeks gestation. The secondary outcome concerned the safety of the procedure in terms of whether or not blood was observed on the catheter tip. Also of interest were the questions of whether ease of transfer, types of catheter and the observed airflow pattern (progression of the air bubble observed with ultrasound immediately after injecting the embryos) had an effect on clinical pregnancy outcome.

Block randomization was used, with random block size using computer randomization software (Randomization Generator Version 1.0, Jonathan Goddard, Medical Statistics, Health Care Research Unit, University of Southampton, UK). The randomization was stratified for age (older or younger than 38 years) and fresh or frozen transfers. The age of 38 was chosen as previously the UKs Human Fertilization and Embryology Authority (HFEA) data were presented in such a manner. The sizes of each group were estimated from data on patients receiving treatment in previous years, i.e. number of women being over 38 years of age and numbers having frozen embryo transfers. The randomization codes were prepared independently from the authors by the hospital’s research and development office and allocations sealed in four different coloured sequentially numbered envelopes. An independent data monitoring committee was established with the remit of examining the data after 1 year of the 2 year estimated recruitment process.

Couples were given patient information sheets during their IVF clinic appointments or when attending for follicular scans, thus allowing time to consider trial involvement. If couples agreed to participate, then on the day of embryo transfer an envelope was opened indicating ‘ultrasound’ or ‘clinical touch’ technique.

For both groups, a ‘comfortably full’ bladder was advised. The USG group underwent an abdominal scan using a Hitachi EUB-525 (Hitachi Medical Systems, Wellingborough, UK). A distance of 15 mm from the endometrial fundus in the sagittal plane was used as the mark to replace the embryos. Staff performing the ultrasound scans held fertility scanning certificates issued either by the British Fertility Society, Royal College of Obstetricians and Gynaecologists or UK Universities. The standard day to replace embryos in our centre is on Day 2 and was the same for both groups.

A Cook® Echotip Soft Pass™ embryo transfer catheter was used for women allocated to the USG group (Cook Ireland Ltd, Limerick, Ireland). For the ‘no ultrasound guidance’ (NUG) or ‘clinical touch’ group, a distance of 6 cm from the external cervical os was selected to be used (first graduated mark on the outer sheath of the embryo transfer catheter), as this has been previously estimated to be mid uterine cavity in the majority of women (Schoolcraft et al., 2001).

Either soft, non-echogenic Wallace® embryo replacement catheters (Smiths Medical International Ltd, Kent, UK) or Rocket Embryon® catheters (Rocket Medical Plc, Washington, UK) were used for this group as was current practice. The choice of the standard catheter used in the NUG group was left to the clinician performing the embryo transfer to decide on the day, without an additional randomization process. Although this aspect of the methods could be criticized, contractual obligation, higher cost of the echogenic catheters and previously published data concerning comparison of soft catheters used (Buckett, 2006) were the rationale behind the use of different catheters in the trial. Furthermore, we wanted to pragmatically test a proposed superior ‘package of care’, i.e. the use of ‘ultrasound plus echogenic soft catheter’ versus ‘clinical touch with older non-echogenic soft catheters’.

Information was recorded on data collection sheets pertaining to patient details, randomization number, treatment allocated, treatment received, catheter type, ease of transfer, catheter distance (for USG group), airflow pattern, clinician name, observation of blood on catheter tip and biochemical and clinical pregnancy outcome. Patients were also followed-up to observe whether a live birth was achieved. Data were independently double entered onto a database and analysed by a statistician.

**Statistical analysis**

Analysis was by intention to treat, i.e. patients were analysed in the groups to which they were randomized regardless of which treatment they actually received. To calculate the odds of achieving pregnancy when USG is given relative to when no USG is given, a logistic regression was undertaken. A regression approach was chosen so that any imbalances in pertinent characteristics between the two treatment arms could be adjusted for, and also since this approach lends itself readily to the necessary sensitivity analyses described below. The binary outcome of interest was whether or not pregnancy was achieved and patient level covariates found to have a statistically significant effect on outcome and/or on the effect of treatment, were included in the logistic regression. The patient covariates believed to be of interest a priori and hence investigated were age being greater than 38 years, use of fresh embryos as opposed to frozen, catheter type used and the mean number of embryos transferred. A stepwise selection approach was adopted to select which of these patient...
level covariates had a statistically significant effect on outcome and hence should be retained in the model.

Some patients were entered into the trial for more than one attempt at embryo transfer and outcomes recorded for these patients were not deemed independent of one another. To account for this issue of non-independence, sensitivity analyses were undertaken. The first sensitivity analysis involved again fitting a logistic regression but this time limiting the data to that for the first attempt of each patient only. The second included all the data but involved including an additional covariate in the regression to represent attempt number. The final sensitivity analysis again made use of all the data but adopted a two-level logistic regression model with the added assumption that treatment effect varied randomly depending on the number of the attempt.

Live birth and the secondary outcome of observing blood on the catheter tip were again binary outcomes and so a similar logistic regression approach was adopted for these outcomes also.

For the purpose of investigating the effect of ‘airflow pattern’ (progression of the air bubble observed with ultrasound immediately after injecting the embryos) on pregnancy outcome, the pattern of airflow was classified into the three categories: ‘laminar’, ‘nil’ and ‘reverse’ and a Chi-squared test was undertaken to assess whether airflow pattern was associated with the outcome of clinical pregnancy. This analysis was limited to patients in the USG group only since airflow pattern is not observable without ultrasound. Airflow pattern was only recorded from the study midpoint onwards.

‘Ease of transfer’ was again a categorical measure but this time there were four possible categories: ‘straightforward’, ‘outer sheath’, ‘stylet’ and ‘tenaculum’. These categories can be considered as ordinal, as they denote the degree of difficulty in ascending order, with ‘straightforward’ being the easiest. A Chi-squared test for trend was therefore undertaken to assess for any association with the outcome of clinical pregnancy.

For the purpose of investigating these three secondary issues (i.e. blood on the catheter tip, airflow pattern, ease of transfer), in addition to an analysis including all data, a sensitivity analysis was undertaken including data from the patients’ first attempt only.

Results

A total of 2295 embryo transfers were entered into the trial and allocation was confirmed from completed data sheets cross-checked with the randomization code. Data are available for 2294 embryo transfers as the randomization group for one patient was not recorded. An additional 295 embryo transfers were randomized as two of the four groupings were exhausted before the largest group (fresh and <38 years) was completely entered. The additional envelopes were supplied in the same manner as the first 2000. A total of 1144 were randomized to the USG group (834 of these women were on their first randomization) and 1150 were randomized to the NUG group (816 of these women were on their first randomization). The total number of women involved in the trial was 1650. The Consort flow diagram is shown in Fig. 1. Compliance with treatment allocation was 99%. The patient groups were comparable in terms of age (median 34 years, range 28–43), whether fresh/frozen embryos were used, mean number of oocytes received and mean number of embryos transferred (Table I). With regard to the type of catheter used, the Cook echotip catheter, designed to be seen clearly on ultrasound was used most frequently (97%) in the USG group with the remaining transfers in this group undertaken using Wallace (1%) and Rocket (2%) catheters. For this 3% of women, transfers started with Cook echotip, but due to technical difficulty

![Figure 1: Consort flow diagram](image-url)

Table I. Baseline characteristics of patients undergoing embryo transfer in the RCT.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>First attempts only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USG (=1144)</td>
<td>NUG (=1150)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &gt; 38</td>
<td>249 (22%)</td>
<td>267 (23%)</td>
</tr>
<tr>
<td>Age &lt; 38</td>
<td>894 (78%)</td>
<td>883 (77%)</td>
</tr>
<tr>
<td>Total</td>
<td>1143¹</td>
<td>1150</td>
</tr>
<tr>
<td>Fresh or frozen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>901 (88%)</td>
<td>914 (88%)</td>
</tr>
<tr>
<td>Frozen</td>
<td>242 (12%)</td>
<td>236 (12%)</td>
</tr>
<tr>
<td>Total</td>
<td>1143²</td>
<td>1150</td>
</tr>
<tr>
<td>Mean number oocytes (SD)</td>
<td>10.87 (6.3)³</td>
<td>10.61 (5.9)²</td>
</tr>
<tr>
<td>Mean number embryos transferred (SD)</td>
<td>1.87 (0.38)⁴</td>
<td>1.89 (0.36)³</td>
</tr>
<tr>
<td>Catheter type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wallace</td>
<td>18 (1%)</td>
<td>519 (51%)</td>
</tr>
<tr>
<td>Cook echotip</td>
<td>1098 (97%)</td>
<td>18 (2%)</td>
</tr>
<tr>
<td>Rocket</td>
<td>26 (2%)</td>
<td>539 (47%)</td>
</tr>
<tr>
<td>Trans myometrial</td>
<td>0</td>
<td>1 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>1143³</td>
<td>1149³</td>
</tr>
<tr>
<td>Echo tip visualization¹</td>
<td>963 (90%)</td>
<td>695 (89%)</td>
</tr>
<tr>
<td>Echo tip visual: yes</td>
<td>109 (10%)</td>
<td>85 (11%)</td>
</tr>
<tr>
<td>Echo tip visual: no</td>
<td>1072 (90%)</td>
<td>780</td>
</tr>
</tbody>
</table>

USG, ultrasound guidance; NUG, no ultrasound guidance. ¹Age and fresh/frozen status was not recorded for one woman in the USG group. ²Number of oocytes taken not recorded for nine patients in USG group and 11 patients in NUG group. ³Based on 1142 women in USG group and 1149 in NUG group since embryo transfer did not take place for two women in USG group and one woman in NUG group. ⁴Based on 1072 women only as not recorded for 70 women out of those receiving embryo transfers.
introducing the catheter into the uterus, switched to other brands. We found the supplied stylet to be quite flimsy compared to Wallace or Rocket. In the NUG group, the Wallace and Rocket type catheters, not designed for ultrasound use, were used most frequently (51 and 47%, respectively) with the remaining transfers in this group undertaken using a Cook catheter (2%) necessitated on a rare occasion by stock supply. The possible impact of this imbalance on the outcomes is accounted for by considering catheter type as a potential covariate in the logistic regressions.

Results for the outcomes of biochemical pregnancy, clinical pregnancy, live birth and blood on catheter tip are given in Table II. It can be seen that there was no significant difference between the groups in terms of any of these outcomes, both when including data from all patients and when including data from patients’ first attempt only.

A logistic regression was undertaken to assess the effect of USG on the pregnancy and live birth outcomes. Treatment effect was not found to have a significant effect on any of these outcomes [odds ratio (OR) (95% confidence interval (CI))—clinical pregnancy: 0.96 (0.79, 1.18); biochemical pregnancy: 0.93 (0.78, 1.12); live birth: 0.97 (0.79, 1.20)] (Table II). Sensitivity analyses supported these conclusions, as is illustrated in the results from including data from the patients’ first attempt only (Table II). The patient level covariates found to have a significant effect on the outcomes and hence adjusted for in the logistic regressions were age greater than 38 years, use of fresh embryos and mean number of embryos transferred (P-value < 0.001 for each). Type of catheter used was not found to have a significant effect on any of the outcomes.

A further logistic regression was undertaken to assess the effect of USG on the odds of observing blood on the catheter tip. This was not found to have a statistically significant effect on outcome (OR (95% CI): 0.99 (0.43, 2.25)) (Table II). Again, sensitivity analyses supported this result. Catheter type was the only patient level covariate found to have a significant effect on observing blood on the catheter tip, however, this is unlikely to have real clinical significance if pregnancy outcome is unaffected (P-value = 0.001).

To assess whether either airflow pattern or ease of transfer had an impact on the outcome of clinical pregnancy, Chi-squared tests for association were undertaken (Table II). Owing to the lack of observations in the ‘reverse’ airflow category, this and the ‘nil’ airflow category were combined. The Chi-squared test undertaken on all data gave a P-value of 0.10 and that on the data from first attempts only gave a P-value of 0.19, and so there was no evidence to support association between airflow pattern and the outcome of clinical pregnancy.

To investigate whether ease of transfer had an impact on the outcome of clinical pregnancy, a Chi-squared test for trend was undertaken, and an association with outcome was detected. When including all data in the analysis (Table II) a P-value of 0.025 was achieved and when limiting the analysis to data on first attempts only, a P-value of 0.03 was achieved. Hence, we had evidence in favour of ease of transfer being predictive of clinical pregnancy success.

**Discussion**

We wanted to test the hypothesis that the use of abdominal ultrasound at the time of embryo transfer improved pregnancy and especially live birth rates, an outcome that has been largely ignored in previous trials in assisted conception. The study design used to test this hypothesis was a RCT. We did not find that USG helped improve pregnancy or live birth rates. Indeed outcome rates for the two groups were remarkably similar. The pregnancy rates achieved are typical of those found in the UK based on HFEA figures (www.hfea.gov.uk).

We used abdominal ultrasound to locate a position 15 mm from the uterine fundus to deposit the embryos. For the standard ‘clinical touch technique’, we used the assumption that mid uterine cavity was ~6 cm from the external os. We
based these parameters on previously published work (Schoolcraft et al., 2001). We did not routinely measure the uterine dimensions in the ‘clinical touch’ group, nor perform dummy transfers in either arm of the study prior to trial entry.

The ease of the embryo transfer, however, appeared to have a statistically significant effect on clinical pregnancy outcome, with an easier transfer being more likely to lead to a clinical pregnancy. We found that using ultrasound, at least abdominally, very rarely helps the clinician negotiate a tight or deviated cervical canal. In the obese woman, visualization of even the echogenic catheter can be difficult. Ultrasound does, however, enable the clinician to demonstrate to the couple, that the catheter is located in the uterus prior to releasing the embryos. Neither type of catheter used nor the observed airflow pattern was found to be associated with pregnancy outcome. This does not support earlier promising work from smaller studies (Cruikshank et al., 2003).

We acknowledge that the use of different brands of catheters is a methodological weakness of the trial in the evaluation of ultrasound per se, however, the results of a recent meta-analysis comparing different catheter types used in embryo transfer provide reassurance in this regard (Buckett, 2006). The aforementioned meta-analysis concluded that there was no significant difference in clinical pregnancy rates when comparing soft catheters. In our trial, we considered all the catheters used for both groups as being ‘soft’. Thus, we feel our conclusions remain valid. A pertinent conclusion to draw from this study is whether an assisted conception unit, at considerable expense in terms of time, personnel and resource, should insist on the routine use of USG for all embryo transfers. The pragmatic question is therefore whether to abandon the clinical touch technique using non-echogenic and less expensive catheters altogether, in favour of using ultrasound plus more costly echogenic catheters. We believe that this study addresses that issue.

A question of interest in terms of interpreting the results was whether there was evidence of a ‘learning curve’ associated with the use of abdominal ultrasound. We investigated this issue by imagining a ‘sliding window’ moving across the trial with time. The first window included the first 200 patients recruited to the ultrasound arm of the trial, the second window excluded the first 100 patients but included the next 200 and so forth. Clinical pregnancy success rate in the USG group was calculated for each window. A learning curve was not directly apparent (results not shown). Furthermore, different clinicians were involved in undertaking the embryo transfers, with the number of embryo transfers undertaken by a single clinician ranging from 4 to 563. Since it is possible that these clinicians will vary in terms of their embryo-transferring skills, it was important for us to investigate whether this variation was sufficient to affect our results. Covariates to represent clinicians, as well as clinician-treatment group interaction terms, were therefore incorporated into our final regression model for the outcome of clinical pregnancy, however, their inclusion did not reach statistical significance (P-value, 0.57).

The results for clinical pregnancy and live birth outcome are at odds with the current fertility NICE guidelines published in 2004. The NICE recommendation was based on accumulated evidence from four studies, all smaller than ours, with varying patient characteristics (Coroleu et al., 2000; Tang et al., 2001; Garcia-Velasco et al., 2002; Matorras et al., 2002). In total, NICE based its recommendation on pooled data involving 2051 women, whereas our study included 2295. Our results are also at odds with the conclusions of the Cochrane review (Brown et al., 2007) published more recently, although the authors did acknowledge that the quality of some of the trials included in the review was questionable.

Our trial is by far the largest trial to compare USG with the clinical touch procedure and it also reports on live birth rate, distinguishing it from other studies on the same subject. We would therefore recommend that the Cochrane review is updated to include the results from our trial as well as those from any further trials undertaken since the review’s publication. Further, it is evident from the forest plots prepared in the Cochrane review that there is some degree of heterogeneity in effect size between studies (ongoing pregnancy outcome, $I^2$ statistic: 45.8%; clinical pregnancy outcome, $I^2$ statistic: 21.6%) and this will only increase with the inclusion of our trial. Although some heterogeneity is to be expected in any systematic review, we would suggest that the possible sources of heterogeneity are investigated with consideration given as to how some potentially key characteristics differ between trials. We suggest that the characteristics considered should include: requirements regarding fullness of bladder in the two treatment groups; whether patients received bed rest following transfer; whether a dummy transfer was undertaken prior to real transfer; type of catheter used and where the embryos were placed in relation to the uterine fundus. It is evident from Tables I and III of the Cochrane review that these characteristics vary between trials and these differences could be adjusted for by way of meta-regression.

It is important to subject new interventions to adequately powered randomized trials before they become entrenched in routine clinical practice. By insisting on abdominal ultrasound-guided embryo transfers, assisted conception clinics would have to purchase abdominal probes and have a machine available, plus a practitioner experienced in abdominal ultrasound present for the embryo transfer, allowing additional time for the ultrasound component of the procedure. The manufacturers of embryo transfer catheters have responded to requests from the specialty to make echogenic catheters, which usually attract a higher purchase price than the traditional catheters. This all adds to the cost of the treatment cycle and is usually passed onto the patient or funding body.

We present arguably the largest randomized trial conducted within the field of reproductive medicine, certainly in the area of ultrasound-guided embryo transfer, and have the added benefit of having followed-up our patients to record live birth status, an outcome seldom reported in previous trials of this intervention. We have found that in this trial, the use of ultrasound did not have either a positive or negative effect on live birth rates in assisted conception cycles. There have been several recently published meta-analyses, all concluding a benefit of USG, but we feel it would be worthwhile exploring the heterogeneity between the included smaller trials and repeating the meta-analyses, including our data.
We do believe however, from clinical experience, that ultrasound does have a role in the difficult embryo transfer, especially when doubt exists whether the catheter is correctly located in the uterus. This study was not designed with enough power to demonstrate a difference in pregnancy rates in this fortunately rare subset.

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**References**


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