Optimizing the embryo transfer technique
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Difficult embryo transfers and the presence of blood on the embryo transfer catheter negatively affect clinical pregnancy rates.

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(Submitted)
Abstract

Introduction: The embryo transfer (ET) technique has become recognized as one of the most complex and significant variables in the success of assisted reproduction, with multiple factors associated with its success or failure.

Design: Retrospective cohort study.

Setting: Infertility centers.

Patient(s): 943 women, aged 18–39 years old, who underwent 1,122 ETs between January 1, 2005, and December 31, 2006.

Intervention(s): NA.

Main outcome measure(s): A semiquantitative system for grading and recording the difficulty of transfer, presence of retained embryos, mucus and blood found inside and outside the transfer catheter after ET was used to determine the correlation of each factor with the clinical pregnancy rate.

Result(s): Difficult transfer and presence of blood on the outside or inside of the transfer catheter after ET was associated with decreased clinical pregnancy rates. In addition, there was a correlation between difficult transfers and the presence of blood. In contrast, presence of retained embryos or mucus did not significantly affect the outcomes.

Conclusion(s): The relationship between difficult embryo transfer and the presence of blood on the outside or inside of the transfer catheter has been demonstrated to decrease the clinical pregnancy rates. Every effort should be made to minimalize difficulty, and hence bleeding, during embryo transfer.

Key words: Difficult embryo transfer, blood, embryo catheter, retained embryos, IVF
Background
Although most patients who undergo in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) will reach the embryo transfer stage and have good quality embryos available for transfer, embryo implantation remains the rate-limiting step in the success of this form of therapy. The main factors that affect embryo implantation can be related to uterine receptivity, embryo quality, and efficiency of the embryo transfer procedure (1). The aim must be to transfer the embryos with a high degree of reliability atraumatically.

In the early days of assisted reproduction, the embryo transfer technique was considered to be an unimportant factor in determining the outcome of the treatment cycle. This is mainly reflected by the relative lack of published studies or modification of the procedures constituting embryo transfer since it was first described. Even so, the importance of the transfer technique and each step within have recently become more appreciated by clinicians. In a series of communications with clinicians, factors such as lack of blood on the transfer catheter, mucus aspiration prior to transfer and lack of instrumental assistance during the transfer ranked high in importance (2, 3).

In addition, multiple factors have recently been shown to significantly affect the success of transcervical intra-uterine embryo transfer; including the softness of the catheter (4, 5), the use of ultrasound guidance (6 – 8), the ease of the procedure (9), the absence of blood on the catheter (10, 11), the use of cervical introducers or obturators (12), flushing of the cervical canal (13) or gentle aspiration (14) to remove cervical mucus, and retention of embryos in the catheter (15, 16).

Most importantly, difficulty in traversing the cervix has been considered to be one of the most important factors having a negative impact on the pregnancy rate. Furthermore, the presence of blood following embryo transfer has been associated with difficult embryo transfers and poor outcomes. The latter is believed to be as a result of endocervical and possibly endometrial damage, possibly resulting in uterine or junctional zone contractions (9, 17).

In this study, we aimed to determine whether the ease of the procedure, the presence or absence of blood, mucus or retained embryos on the tips of the post-transfer catheter were correlated with the probability of a clinical pregnancy in a large cohort of women.
undergoing ultrasound-guided embryo transfer with high quality embryos.
Materials and Methods

Patient Population
A detailed chart review of all ultrasound-guided embryo transfers performed in our centre from January 1, 2005 – December 31, 2006 was performed. Details on patient demographics, cycle characteristics and outcomes were extracted, with special emphasis on the presence of blood, mucus, retained embryos and occurrence of difficulty during embryo transfer. Inclusion criteria consisted of female partner age at the time of transfer between 18 – 39 years old, availability of high quality embryos (grade I or grade II) or blastocysts on the day of transfer, and undergoing a fresh embryo transfer. Patients aged ≥40 years, undergoing cryo-embryo transfer, or lacking high quality embryos on the day of transfer were excluded. The final cohort consisted of 943 women, undergoing 1,122 cycles. Of these, 787 women were having their first IVF-ET. This study was reviewed and approved by our local institutional review board (IRB).

Ovarian stimulation
In general, the majority of women were down-regulated using a long agonist protocol of pituitary down-regulation beginning on day 21 of the previous cycle. When down-regulation was considered adequate (e.g. serum E2 level of <35 pg/mL), ovarian hyperstimulation was begun with a standard initial daily dose of purified FSH, hMG and/or recombinant FSH. This was followed by individualization of the regimens according to the patient demographics (e.g. patient’s age, ovarian reserve, response to previous stimulation), follicular development and physician preference.
When at least two follicles ≥18 mm in diameter were seen on transvaginal ultrasound (US), human chorionic gonadotropin (10,000 IU IM) was administered, and transvaginal US-guided oocyte retrieval was performed ~36 hours later.

Embryo transfer
The majority of cases received an embryo transfer (ET) 48 – 72 hours post-oocyte retrieval. In a minority of patients, a blastocyst transfer was performed. ET was performed with the patient in the dorso-lithotomy position. All transfers were performed by two experienced clinicians with similar pregnancy rates under ultrasound guidance. The Cook Sydney
IVF embryo catheter system was loaded with culture media till the tip (e.g. no air bubbles). The ultrasonography was performed by an experienced ultrasonographer, and patients were asked to maintain a full bladder during the transfer.

Following embryo transfer, the embryologist microscopically inspected the transfer catheter for retained embryos, and any found were immediately reloaded and transferred. This was followed by recording of all events that occurred during the transfer.

A semiquantitative scale was used to record the amount of mucus or blood (e.g., 0 = none, 1 = minimal, and 2 = significant). Scores were obtained separately for blood located inside and outside the catheter. In addition, the physician provided a score on the difficulty of the transfer (e.g. 0 = no resistance, 1 = minimal resistance, 2 = some resistance, 3 = marked resistance, 4 = dilator used, 5 = uterine sound used).

**Statistical analysis**

The primary outcome of this study was the clinical pregnancy rate per woman; defined as the visualization of a fetal sac 4 – 6 weeks post-transfer associated with an increasing maternal β-hCG titer. All analyses were performed on the overall cohort and on two selected populations: (1) patients undergoing their first cycle of IVF-ET, and (2) patients with only easy transfers.

Univariate analyses using the χ² test were performed to determine whether the occurrence of each variable was associated with the occurrence of a clinical pregnancy. Moreover, multivariate logistic regression analyses were performed to determine the influence of the independent variables on the occurrence of a clinical pregnancy.

For the purpose of the univariate analyses, in the blood or mucus categories, the cases with presence of minimal amounts of the respective variable were grouped with the absent group to form two distinctive groups: (1) absent or (2) present. In addition, difficulty during the embryo transfer was regrouped into two categories: (1) no to minimal resistance and (2) moderate to marked resistance, the latter including cases in which the use of instrumental assistance during transfer was required.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS). A P value <0.05 was considered to be statistically significant.
Results

Difficulty during the embryo transfer procedure was recorded during 45 embryo transfers (4.02%) in the overall cohort and in 29 transfers (3.68%) in the ‘1st cycle’ subgroup. Women with an easy transfer were significantly more likely to have a clinical pregnancy than women with a difficult transfer (45.40% vs. 15.56%; O.R = 4.52, 95% CI = 2.00 to 10.20; P < 0.0001). In addition, in the subgroup of patients with only their first transfer, the trend was also similar (46.31% vs. 24.14%; O.R = 2.71, 95% CI = 1.14 to 6.42; P = 0.03).

Mucus was noted post-transfer in 271 cases (24.15%) in the overall cohort and in 197 cases (25.03%) in the ‘1st cycle only’ subgroup. In the overall cohort, the presence of mucus did not seem to alter the chances for a positive outcome (44.65% vs. 42.80%; O.R = 1.08, 95% CI = 0.82 to 1.42; P = 0.64). In the ‘1st cycle’ subgroup, the results were also similar (45.42% vs. 45.69%; O.R = 0.99, 95% CI = 0.72 to 1.37; P = 1).

Blood was noted on the outside of the transfer catheter in 94 instances (8.38%) and on the inside of the catheter in 60 (5.35%) in the overall cohort. In the ‘1st cycle’ subgroup, the respective figures were 59 (7.50%) and 35 (4.45%). The presence of blood demonstrated a negative impact on the clinical pregnancy rates with pregnancy rates higher in cases in which there was no blood on the outside of the catheter (45.72% vs. 27.66%; O.R = 2.20, 95% CI = 1.38 to 3.52; P = 0.001), or on the inside of the catheter (44.82% vs. 33.33%; O.R = 1.63, 95% CI = 0.94 to 2.82; P = 0.11), but the latter did not reach statistical significance. In addition, the ‘1st cycle’ subgroup revealed similar results for the presence of blood on the outside of the catheter (46.57% vs. 32.20%; O.R = 1.84, 95% CI = 1.04 to 3.23; P = 0.046) and on the inside of the catheter (45.74% vs. 40.00%; O.R = 1.27, 95% CI = 0.63 to 2.5; P = 0.62).

Retained embryos were noted following 69 transfers (6.15%) and 44 transfers (5.59%) in the overall cohort and ‘1st cycle’ subgroup, respectively. The retention of embryos and subsequent retransfer did not seem to significantly affect the pregnancy rates in either group (44.44% vs. 40.58%; O.R = 1.17, 95% CI = 0.71 to 1.92; P = 0.62) and (45.49% vs. 45.45%; O.R = 1.00, 95% CI = 0.54 to 1.85), respectively.
Moreover, using a series of regression analyses to determine the association between the variable and the chance for a clinical pregnancy, in the original cohort, multiple linear regression analysis demonstrated that only the difficulty in embryo transfer significantly affected the clinical pregnancy rate \((P = 0.0017)\). The remaining variables (presence of mucus, retained embryos and blood) were considered to be non-significant.

Even so, the influence of each individual variable on the occurrence of a clinical pregnancy using simple linear regression revealed differing results. The analyses not only confirmed that difficult transfers were correlated with the probability of a clinical pregnancy, but so were the presence of blood either on the outside or inside of the transfer catheter (Table 1). In addition, the presence of mucus and the occurrence of retained embryos were not associated with the primary outcome.

Subgroup analyses of only patients undergoing their first embryo transfer (e.g. 1\textsuperscript{st} cycle subgroup) revealed similar results as the multiple linear regression analysis of the original cohort. Moreover, there was more homogeneity with the results of the individual simple linear regression analyses, with the difficulty of the embryo transfer being the only significant variable \([(r) = -0.08, (r^2= 0.007); 95\% \text{ CI for } r = -0.15 \text{ to } -0.02; P = 0.02]\].

Finally, in a subgroup analysis of only patients with an easy transfer revealed an association with the presence of blood, especially on the outside of the catheter with the occurrence of a clinical pregnancy. Other variables were considered to be non-significant.
Discussion
The current study emphasizes the correlation between difficulty in performing the embryo transfer, presence of blood on the catheter and poor outcomes. Since difficult transfers have been associated with a poorer outcome than easy transfer, it would be useful to directly examine the uterine cavity for any lesions post-transfer. Since this is only possible in mock transfers, indirect measures of the degree of difficulty were utilized. These include physician assessment of difficulty, the need for instrumental assistance (e.g. tenaculum), and the presence of blood on the catheter post-transfer.

Different approaches have been described in cases of difficult embryo transfers with varying success rates (18 – 21). A commonly used initial approach is to negotiate the cervix using the outer sheath of the catheter, with its inner noodle withdrawn (22). Once the uterine cavity is entered, the inner noodle is used to deposit the embryos, taking care to avoid the fundus. Even so, the pregnancy rates with this method have not been acceptable when the catheter guide enters the endometrial cavity (23).

Moreover, more invasive and potentially traumatic events are sometimes undertaken by clinicians to overcome the problematic cervix. These include the use of a tenaculum, stylette, sounding and/or cervical dilatation. Overall these events have been associated with increased uterine junctional zone contractions and a decreased pregnancy rate (9, 20, 24, 25). Alternatively, the cervical route may be bypassed and the embryos may be transferred transmyometrially into the uterine cavity using the ‘Towako method’ (19).

Another tell-tale sign of a difficult transfer is the post-transfer presence of blood on the transfer catheter. Amongst clinicians, the absence of blood on the catheter or cannula is ranked high as an important factor towards success (2, 3). This opinion is supported by literature reports in which the presence of blood on the transfer catheter has been associated with lower pregnancy rates (10, 24). In addition, Perin et al. (26) found that contamination of the catheter with blood and mucus accounted for significantly lower implantation and clinical pregnancy rates.

In the current study blood was negatively correlated with the clinical pregnancy rate. This was more evident for blood on the outside of the catheter than on the inside of the catheter. Moreover, even in easy
transfers, blood on the outside of the catheter was found to be a negative predictor of success. Finally, the role of retained embryos and mucus in decreasing the pregnancy rate is controversial with some studies claiming a negative effect (24) and other claiming no such effect (10, 27). In our study, we could not find an association between the retransfer of retained embryos and a drop in the pregnancy rate. In conclusion, the relationship between difficult embryo transfer, the presence of blood on the transfer catheter has been demonstrated to decrease the clinical pregnancy rates. Therefore, every effort should be made to minimalize difficulty, and hence bleeding, during embryo transfer.
References


23. Abdelmassih VG, Neme RM, Dozortsev D, Abdelmassih S, Diamond MP, Abdelmassih R. Location of the embryo-transfer catheter guide before the internal uterine os improves the outcome of in vitro fertilization. Fertil Steril. 2007 May 15; [Epub ahead of print].


Table 1: Simple linear regression of independent variable and clinical pregnancy rate in the overall cohort.

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient</th>
<th>95% CI for r</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td><strong>Difficulty in transfer</strong></td>
<td>$r = -0.11$</td>
<td>$-0.17$ to $-0.05$</td>
<td>$P = 0.0002$</td>
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<td>$r^2 = 0.01$</td>
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<tr>
<td><strong>Blood on the outside of the catheter</strong></td>
<td>$r = -0.09$</td>
<td>$-0.15$ to $-0.03$</td>
<td>$P = 0.002$</td>
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<td>$r^2 = 0.008$</td>
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<tr>
<td><strong>Blood on the inside of the catheter</strong></td>
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<td>$-0.15$ to $-0.03$</td>
<td>$P = 0.004$</td>
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<td>$r^2 = 0.008$</td>
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<tr>
<td><strong>Mucus</strong></td>
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<td>$-0.09$ to $0.03$</td>
<td>$P = 0.31$</td>
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<td></td>
<td>$r^2 = 0.0009$</td>
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<tr>
<td><strong>Retained embryos</strong></td>
<td>$r = -0.02$</td>
<td>$-0.08$ to $0.04$</td>
<td>$P = 0.53$</td>
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<td></td>
<td>$r^2 = 0.0004$</td>
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