Optimizing the embryo transfer technique
Abou-Setta, A.M.

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Chapter 3

Removal of cervical mucus prior to embryo transfer improves pregnancy rates in women undergoing assisted reproduction.

Mamdoh A. Eskandar, Ahmed M. Abou-Setta, Mohamed El-Amin, Mona A. Almushait, Adekunle A. Sobande

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Abstract
The removal of cervical mucus during embryo transfer has been postulated to increase the pregnancy and implantation rates by not interfering with embryo implantation. Even so, this is a time-consuming procedure that may increase the incidence of difficult transfers by removing the naturally lubricant mucus. In addition, any cervical manipulations at the time of embryo transfer may cause unwarranted uterine contractions. In this prospective, controlled study, 286 women undergoing embryo transfer between January and May 2006 were divided into two groups according to whether the cervical mucus was scheduled to be aspirated (group A) or not (group B). The two groups were similar with regards to the demographics, cause of infertility, characteristics of ovarian stimulation and embryos transferred. Even so, the clinical pregnancy rate was significantly higher in group (A) than group (B) (OR = 2.18, 95% CI = 1.32-3.58), although there were easier transfers in group (B) than group (A) (OR = 3.00, 95% CI = 1.05-8.55). This demonstrates that even though embryo transfers were easier to perform when the cervical mucus was left in place, aspiration resulted in an increased chance of clinical pregnancy.

Key words: embryo transfer, cervical mucus, aspiration, catheter, clinical trial
Introduction
The majority of patients undergoing assisted reproduction through 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). The pregnancy rate following embryo transfer is generally dependent upon multiple factors, including embryo quality, endometrial receptivity and the technique of embryo transfer itself (3).
Traditionally, unlike other aspects of assisted procreation which have been more thoroughly addressed by clinicians and researchers alike, the steps involved in the final stage of IVF, the transfer of embryos into the receptive uterus has mainly been left up to personal preferences. This fact is reflected by both the scarce volume of scientific publications regarding the embryo transfer technique as a whole, or its subunits in particular, compared with other aspects of IVF (e.g. ovulation induction). This is even more evident in the reluctance of physicians to modify their own personal habits to a more evidence-based approach.
Even so, in recent trends among clinicians, more stress is being placed on optimizing and standardizing the embryo transfer protocol. Factors such as the use of a dummy embryo transfer (e.g. trial transfer) (4), ease of the procedure (5), catheter choice (6, 7), and ultrasound guidance (8, 9) have proven to improve the clinical outcomes.
The influence of removing the cervical mucus prior to embryo transfer has been highly debated due to conflicting results in the medical literature. Some authors have demonstrated improved pregnancy rates, while others have shown no improvement (10-12). In addition, other studies supported the use of cervical mucus aspiration, but did not report the clinical pregnancy rates (13, 14).
In light of this controversy, and the need to clearly identify the potential value of removing the cervical mucus, it was decided to perform a prospective, controlled trial to investigate this individual step in the embryo transfer procedure.
Materials and methods
This prospective controlled trial was approved by the institutional review board. Two-hundred and eighty-six patients undergoing embryo transfer in the assisted reproduction unit between January and May 2006 were prospectively included. Patients were divided into two groups: group (A), which consisted of patients having the cervical mucus aspirated before the embryo transfer, and group (B), which consisted of patients not having the cervical mucus aspirated.

At the time of embryo transfer, patients in group (A) had the cervical mucus removed by a commercially available catheter designed for the removal of cervical mucus (9.3 Fr/25 cm) (Aspiracath, J-ASP-092500; Cook Women’s Health, USA). The cervix was not flushed, and the mucus was aspirated using the catheter introduced up to about 2 cm from the external cervical os. Patients in group (B) did not have the cervical mucus removed prior to embryo transfer. All other aspects of the ovarian stimulation, oocyte retrieval, embryo transfer and luteal phase support protocols were similar between the two groups. Moreover, all embryo transfers were performed on day 3 by a single physician using a standardized technique.

In brief, ovarian stimulation, oocyte retrieval and luteal phase support were performed in accordance with the standard protocol of the department. Women were down-regulated using a gonadotrophin-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 (Pergonyl; NV Organon) was given intramuscularly, and oocyte retrieval was performed 34–36 h later.

Embryo transfer was performed using a soft Edward-Wallace or Cook catheter connected to a tuberculin syringe. A complete column of fluid was used (e.g. no air bubbles) to avoid the possibility of artificially introducing air into the uterine cavity. In addition, the catheter was held with its tip slightly downwards to prevent embryos from travelling through the liquid column to the end connected to the syringe.

It is important to note that although the vast majority of patients in each group were less than 35 years old, an average of about three
embryos were transferred since, as a referral centre, most of the patients had previous failed trials in different centres. Therefore, it was decided to transfer an average of three embryos for such patients according to the centre’s protocol.

It is also important to note that a trial transfer was not performed prior to the embryo transfer. This was because the use of a trial transfer is considered to be empirical, and there are limited clinical trials on the beneficial effect of using a trial, or dummy, transfer.

As a standard protocol in the unit, ultrasound-guided transcervical intrauterine embryo transfer is used exclusively. The embryos are deposited ~1 cm from the uterine fundus. Then the catheter is extracted and examined for retained embryos, blood and/or mucus by the embryologist under a stereomicroscope. Luteal phase support is provided in the form of daily progesterone vaginal suppositories t.i.d. (Cyclogest 400 mg; Hoechst Roussel Limited, UK).

The primary outcome measure for this trial was the clinical pregnancy rate per woman. Clinical pregnancy was defined by the presence of a positive β-HCG subunit measurement 2 weeks post-transfer and a clinically viable gestational sac with fetal heart pulsation on ultrasound 3 weeks later. In addition, embryo implantation rates, the incidences of difficult transfers and the presence of retained embryos, blood and/or mucus on the catheter tip was 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 min.

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 F-test. The results of the two groups were compared using the t-test or Mann-Whitney U-test for parametric and non-parametric 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). In addition, the two groups were similar with regard to other demographics and cycle characteristics.

There was no significant difference with regard to patient age, period of infertility, and day 3 FSH concentrations (Table 2). In addition, there were no significant differences in the numbers of gonadotrophin ampoules, days of stimulation, and numbers of oocytes retrieved, MII oocytes, injected oocytes, fertilized oocytes, embryos produced, embryo quality, and numbers of embryos transferred (Table 2). Finally, there were no significant differences in the catheter choice or the volume of injected culture media in the two groups (data not presented).

With regard to the primary outcome measure, clinical pregnancy rates, there was a significantly higher number of clinical pregnancies in group (A) (63/143) than group (B) (38/143) (P = 0.003; odds ratio = 2.18, 95% CI = 1.32-3.58). In contrast, there was a significantly higher incidence of easy transfers in favour of group (B) (Table 3, OR = 3.00, 95% CI = 1.05-8.55). The implantation rates were similar in both groups (13.80 versus 13.38%).

With regard 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 3), even though, ironically, there were more retained embryos in the mucus aspiration group than in the no-aspiration group (Table 3).

The results of this prospective clinical trial with 143 patients in each treatment arm demonstrated a statistically significant (P = 0.003) absolute difference of 17.48% between the two groups with regard to the clinical pregnancy rate. With this absolute difference, 129 women would be needed in each arm (with an alpha = 0.05) to have a power of 80%, therefore supporting the results. In addition, this difference relates to a number needed to treat of six (95% CI = 4-16).
**Discussion**

The embryo transfer is the final stage of the IVF cycle. It is also the area in which clinical manipulations can directly alter the outcomes of the IVF cycle, and has shown marked variability both among different IVF programmes and physicians in the same programme (15, 16).

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 (17). Physicians too often underestimate the importance of the embryo transfer technique, regarding it as an apparently simple manoeuvre. Most inexperienced clinicians do not consider inserting a catheter through the uterine cervix and ejecting embryo-contained fluid to be a difficult task, especially since many gynaecologists today perform intrauterine insemination (IUI) in a private clinic setting. Be that as it may, it has been shown recently that physician attitudes toward the embryo transfer technique are positively changing (18, 19).

Recently, the techniques and variables affecting the success of embryo transfer have attracted more attention. 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 (20).

In order to ascertain the importance of each step involved in the embryo transfer procedure, individual factors must be evaluated independently. The removal of cervical mucus prior to embryo transfer has been suggested to directly influence the embryo implantation rates. Nevertheless, this clinical query is not clearly answered in the literature. Cervical mucus has been postulated to interfere with proper embryo replacement. It has also been suggested that the presence of cervical mucous can prevent the embryos from leaving the catheter by acting as a 'plug' at the catheter tip (3). In addition, transferred embryos may stick to the cervical mucus around the catheter and be dragged from their original site of deposition during the withdrawal of the catheter.
Moreover, the mucus may also interfere with implantation if pushed or injected into the uterine cavity. This has even led some authors to test the efficacy of endometrial flushing to remove any excessive mucus that may prevent implantation (21). Although the theory of the cervical mucus acting as a ‘condom’ around the transfer catheter is possible, leading to retained embryos, this is unlikely to decrease the chances of treatment success since studies have shown that when the embryos are immediately transferred back into the endometrial cavity in a second attempt, the implantation and pregnancy rates are not reduced (14, 22). In addition, if the cervical mucus is dragged into the endometrial cavity with the transfer catheter, cervical canal mucous may entangle the embryos, interfere with implantation and increase the risk of cervical expulsion of the embryos post-transfer (13, 23).

Another benefit of cervical cleaning and mucous removal is to reduce the risk of bacterial contamination of the catheter and endometrial cavity. Contamination of the catheter tip with micro-organisms such as streptococci (groups B and D), E. coli, staphylococci, mycoplasma and ureaplasma has been shown to reduce implantation and pregnancy rates by 40-60% (24-28), even when prophylactic antibiotics have been used. The hypothetical, but ineffective, role of probiotics in this situation would be to positively support the presence of the natural vaginal flora (e.g. lactobacilli) (29). Cleaning the cervical canal can therefore play a beneficial role by modifying the cervical micro-environment and reducing the risk of introducing pathogens into the endometrial cavity.

As with any procedure, there are also negative aspects, and cervical mucus aspiration is no different. It is true that aspiration of the cervical mucus prior to embryo transfer only takes a few minutes, but in a busy IVF clinic it could accumulate to be time-consuming. This is due to the time taken to perform the procedure plus an additional period of time to allow the uterus to become quiescent again. It is generally preferable to have at least 5-10 min between the aspiration and the actual embryo transfer. Of course, this is an empirical approach, but since the extra wait does not do any harm to the parties involved, but could prove a beneficial factor, it has been decided to use it. Now in a busy IVF unit, when you add up the extra 5-10 min per embryo transfer, this could amount to a considerable amount of time invested in this procedure alone each day.
Mansour et al. (13) demonstrated that removing the cervical mucus before a methylene blue dummy transfer significantly reduced the extrusion of the dye. In addition, Nabi et al. (14) demonstrated that embryos were significantly more likely to be retained when the embryo catheter was contaminated with mucus (3.3 versus 17.8%, \( P = 0.000001 \)). Consequently, the removal of the cervical mucus prior to embryo transfer has been claimed to improve the pregnancy and implantation rates, but so far as is known, there have been only a limited number of randomized, controlled trials on the routine aspiration of the mucus prior to embryo transfer (11, 12). It is important to note also that they were all published as conference abstracts, and not in peer-reviewed journals.

McNamee et al. (10) performed a retrospective study to determine the effect of vigorous cervical irrigation prior to embryo transfer. The pregnancy rate following embryo transfer by five physicians (two who irrigated the cervix and three who did not) were compared. They demonstrated a significantly higher pregnancy (60 versus 32%) and ongoing pregnancy/delivery rates (44 versus 24%) in patients who had the cervical mucus removed.

Glass et al. (11) randomized 253 patients to mucus aspiration during a mock embryo transfer or a control group. Randomization was performed using sealed envelopes. Before the aspiration, the cervical canal was flushed intermittently with moderate force. If mucus or blood remained in the canal, a second irrigation with the trial catheter was performed. With regard to the clinical pregnancy rate, they noted that there was a trend towards significance in the control group.

In a double-blind randomized, controlled trial, performed on 424 patients, Visschers et al. (12) suggested that removal of cervical mucus prior to embryo transfer does not improve pregnancy rates. Even so, it is important to note that the patients in the cervical removal group underwent meticulous removal of cervical mucus prior to embryo transfer by means of a cervical brush. It is unclear if this brush increases endometrial contractility, as has been proven to occur with cervical and endometrial manipulations.

In this prospective, controlled trial, it was possible to demonstrate that the gentle removal of the cervical mucus has a beneficial effect on embryo implantation and clinical pregnancy rates. A significantly higher percentage of women undergoing cervical mucus removal prior to
embryo transfer have a clinical pregnancy ($P = 0.003$). However, there was also a greater number of patients in the cervical removal group with difficult transfers. This relationship points to the natural lubricant effect of the cervical mucus. It is important to note that no natural or synthetic lubricant is currently advised for use on the embryo transfer catheter in difficult cases, and this may be an issue for further research in the future. Moreover, logistic regression showed a trend towards significance ($P = 0.0606$) for the association between a difficult transfer and the presence of retained embryos. The actual reasons for the association between difficult embryo transfers and the presence of retained embryos is not clear, but may be due to more cervical bleeding and contamination of the catheter tip with blood in cases of difficult transfer. This may form a barrier to proper delivery of the embryos in the uterine cavity.

In conclusion, cervical aspiration prior to embryo transfer should be performed routinely in all patients undergoing embryo transfer through the cervical route. Even so, randomized controlled studies with adequate sample sizes should be performed to confirm these findings, and to determine if there is a true relationship between the presence of a difficult embryo transfer and the presence of retained embryos.
References

12. Visschers BAJT, Bots RSGM, Mol BW, Van Dessel HJHM 2006 Removal of cervical mucus prior to embryo transfer does not improve pregnancy rates in IVF/ICSI. Human Reproduction 21 (Suppl. 1), i84
Table 1. Frequencies of indications leading to assisted reproduction.

<table>
<thead>
<tr>
<th></th>
<th>Mucus aspiration</th>
<th>No aspiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male factor</td>
<td>65 (45.5)</td>
<td>61 (42.7)</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>38 (26.6)</td>
<td>50 (35.0)</td>
</tr>
<tr>
<td>Anovulation</td>
<td>28 (19.6)</td>
<td>16 (11.2)</td>
</tr>
<tr>
<td>Unexplained infertility</td>
<td>9 (6.3)</td>
<td>15 (10.5)</td>
</tr>
<tr>
<td>Mixed male and female factors</td>
<td>3 (2.1)</td>
<td>1 (0.7)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.
There were no significant differences between groups.

Table 2. Patient demographics and cycle characteristics between the two groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mucus aspiration</th>
<th>No aspiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (≤30 years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66 (46.15)</td>
<td>71 (49.65)</td>
</tr>
<tr>
<td>Age (range 31–35 years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41 (28.67)</td>
<td>40 (27.97)</td>
</tr>
<tr>
<td>Age (range 36–40 years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26 (18.18)</td>
<td>22 (15.38)</td>
</tr>
<tr>
<td>Age (range 41–45 years)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 (6.99)</td>
<td>10 (6.99)</td>
</tr>
<tr>
<td>Period of infertility (years)</td>
<td>6.51 ± 3.16</td>
<td>6.43 ± 3.25</td>
</tr>
<tr>
<td>Day 3 FSH concentration</td>
<td>5.19 ± 2.27</td>
<td>5.06 ± 2.21</td>
</tr>
<tr>
<td>No. of ampoules</td>
<td>32.55 ± 11.24</td>
<td>33.50 ± 8.93</td>
</tr>
<tr>
<td>Days of stimulation</td>
<td>10.20 ± 2.07</td>
<td>10.46 ± 2.34</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>11.69 ± 6.29</td>
<td>11.03 ± 7.36</td>
</tr>
<tr>
<td>No. at metaphase II</td>
<td>9.59 ± 5.51</td>
<td>8.97 ± 6.43</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Mucus aspiration</td>
<td>No aspiration</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Clinical pregnancy rate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63 (44.1)</td>
<td>38 (26.6)</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>69/500 (13.8)</td>
<td>63/471 (13.4)</td>
</tr>
<tr>
<td>Rate of difficult embryo transfer&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 (9.8)</td>
<td>5 (3.5)</td>
</tr>
</tbody>
</table>

**Embryo transfer catheter tip**

<table>
<thead>
<tr>
<th></th>
<th>Mucus aspiration</th>
<th>No aspiration</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>No blood or mucus present</td>
<td>119 (83.2)</td>
<td>109 (76.2)</td>
<td>1.55</td>
<td>0.86–2.77</td>
</tr>
<tr>
<td>Presence of blood only</td>
<td>5 (3.5)</td>
<td>13 (9.1)</td>
<td>0.36</td>
<td>0.10–1.12</td>
</tr>
<tr>
<td>Presence of mucus only</td>
<td>14 (9.8)</td>
<td>19 (13.3)</td>
<td>0.71</td>
<td>0.34–1.47</td>
</tr>
<tr>
<td>Presence of both blood and mucus</td>
<td>5 (3.5)</td>
<td>2 (1.4)</td>
<td>2.55</td>
<td>0.41–27.17</td>
</tr>
<tr>
<td>Retained embryos&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15 (10.5)</td>
<td>2 (1.4)</td>
<td>8.26</td>
<td>1.85–36.8</td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise indicated.
<sup>a</sup>Mean (%).
<sup>b</sup>Values are numbers with percentages in parentheses.
<sup>b</sup>Differences between groups are statistically significant.