Chapter 8

Air-fluid versus fluid-only models of embryo catheter loading: a systematic review and meta-analysis.

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Reproductive BioMedicine Online. 2007; 14:79-83.
Abstract
The objective of this systematic review was to determine the beneficial or detrimental effect of using air bubbles to bracket the embryo-containing medium during embryo transfer. To test this theory, a meta-analysis of randomized trials comparing air-fluid versus fluid-only methods was performed. The primary outcome measures were live birth, ongoing and clinical pregnancy rates. The secondary outcome measures were the rates of implantation, miscarriage, multiple and ectopic pregnancies and retained embryos. Electronic (e.g. PubMed, EMBASE, Cochrane Library) and hand searches of the literature revealed two included studies (298 women). Meta-analysis was conducted using the Mantel–Haenszel method (fixed-effect model). For the primary outcome measures, there were no significant differences between the two methods with regards to live birth (OR = 1.34; 95% CI = 0.59–3.07), ongoing pregnancy (OR = 1.34; 95% CI = 0.59–3.07) and clinical pregnancy (OR = 1.13; 95% CI = 0.70–1.83) rates. For the secondary outcomes, there were no significant differences between the two groups. In conclusion there is insufficient evidence to suggest that the fluid-only method is superior to the use of air brackets during embryo loading. There is a need for well-designed and powered randomized trials to determine any possible benefit to either method.

Key words: embryo transfer, IVF, loading of transfer catheters, meta-analysis
Introduction
Embryo transfer is the last stage of a series of complementary processes leading to success, or failure, of an IVF cycle. Over the years, it has been accepted as a non-decisive phase, as compared with other aspects of the IVF process. Even so, recently it has been shown that the pregnancy rates are strongly affected by the embryo transfer technique, and this has reflected in an increased interest and awareness among clinicians.
The pregnancy rate after embryo transfer is dependent upon multiple factors including embryo quality, endometrial receptivity and the technique of the embryo transfer itself (1). In recent years, more stress has been placed on optimizing and standardizing the embryo transfer protocol. Factors such as catheter choice (2, 3), ease of the procedure (4), ultrasound-guidance (5), and dummy embryo transfer (6) have proved to improve the clinical outcomes. Consequently, any modification in the standard protocol that will improve the outcomes has great value. The use of air brackets around the embryo-containing medium has, in theory, been debated to be beneficial to the success of the embryo transfer, by protecting the embryos from the cervical mucus and from accidental discharge before entering the endometrial cavity. On the other hand, supporters of the fluid-only method of catheter loading believe that the introduction of even a small amount of air in the uterus could be a non-physiological factor with a deleterious effect on the embryos and implantation (7).
In light of this controversy, and the need to clearly identify the relative efficacy of different embryo catheter-loading techniques, it was decided that there is a need for a systematic review to locate and analyse the best available evidence today in the medical literature.
Materials and methods

Criteria for considering studies for this review
All published, unpublished and ongoing randomized trials that had reported data comparing outcomes for women undergoing embryo transfer through the cervical route using air brackets (e.g. air–fluid) versus fluid-only methods of embryo catheter loading, following IVF or intracytoplasmic sperm injection (ICSI), were sought in all languages.

Types of outcome measures
The primary outcome measures were the live birth (LBR), ongoing pregnancy (OPR) and clinical pregnancy (CPR) rates. The secondary outcomes were the rates of embryo implantation, multiple and ectopic pregnancies and miscarriage rates. Lastly, the tips of the post-transfer catheters were evaluated for retained embryos.

Search strategy for identification of studies
Meticulous computerized searches (last performed July 2006) were conducted using Medline (PubMed) (1966 to present), EMBASE (1980 to present), the Cochrane Central Register of Controlled Trials (CENTRAL) on the Cochrane Library Issue 3, 2006, the National Research Register (NRR), and the trial register of controlled trials (www.controlled-trials.com). Furthermore, the reference lists of all known primary studies, review articles, citation lists of relevant publications, abstracts of major scientific meetings, e.g. European Society for Human Reproduction & Embryology (ESHRE) and American Society of Reproductive Medicine (ASRM) and included studies were examined to identify additional relevant citations. Finally, ongoing and unpublished trials were sought by contacting experts in the field and commercial entities.

Methods of the review
A standardized data extraction form was developed and piloted for consistency and completeness. Trials were considered for inclusion, and trial data extracted. Data management, statistical analyses and power calculations were conducted using the Review Manager (RevMan) 4.2 and Power and Sample Size Calculations (PS) 2.1.30 statistical software packages.
Individual outcome data were included in the analysis if they met the pre-stated criteria. Where possible, data was extracted to allow for an intention-to-treat analysis, defined as including all randomized cycles in the denominator. If data from the trial reports was insufficient or missing, the investigators of individual trials were contacted for additional information, in order to perform analyses on an intention-to-treat basis.

For the meta-analysis, the number of participants experiencing the event in each group of the trial was recorded. Heterogeneity of the included studies was determined by visual inspection of the outcome tables and by using the chi-squared test for heterogeneity. Meta-analysis of binary data was performed using the Mantel–Haenszel method utilizing a fixed effect model and the odds ratio (OR), and 95% confidence intervals (CI), evaluated.
Results

Search results

A total of three prospective randomized controlled trials were identified (7-9) (Figure 1). Of these studies, two were published as full-text articles in peer-reviewed journals and one was published as an abstract in a conference proceeding. One study was excluded because it compared the standard air-fluid method (e.g. two air bubbles used to bracket the embryo containing media) to a modified air-fluid method, using only one air bubble at the tip of the embryo transfer catheter (9). The methodological quality of the remaining trials was assessed and data extracted to allow for an intention-to-treat analysis.

Description of included studies

Krampl et al. (7) conducted a prospective, randomized controlled trial including 196 women undergoing 196 embryo transfer cycles. Patients were allocated at the time of embryo transfer into one of two groups by an unclear form of randomization, with an equal number in each group. None of the reported cycles were frozen embryo replacement or oocyte donation cycles.

In the air-fluid group, embryos were loaded as follows: 10 ml of air in the proximal part of the catheter, followed by 5–10 ml of medium containing the embryos to be transferred, and 10 ml of air at the tip of the catheter. In the fluid-only group, the syringe and the entire catheter were filled with medium and the embryo-containing medium (5–10 ml) was aspirated without being bracketed by air spaces.

Moreno et al. (8) conducted a prospective, randomized controlled trial including 102 women undergoing 102 embryo transfer cycles. Patients were allocated at the time of embryo transfer into one of two groups by a computer-generated randomization table, the results of which were placed in consecutively numbered and sealed opaque envelopes. The number of cycles in each arm was as follows: air-fluid group (52 women); fluid-only group (50 women). None of the reported cycles were frozen embryo replacement or oocyte donation cycles.

In the air-fluid group, embryos were loaded as follows: 200 ml of air in the syringe, 100–125 ml of air in the proximal part of the catheter, 20–25 ml of medium containing the embryos to be transferred, and 10 ml of air at the tip of the catheter. In the fluid-only group, the syringe and the entire catheter were filled with medium and the embryo-containing
medium (20–25 ml) was aspirated without being bracketed by air spaces. In both groups, the embryos were positioned about one-fourth of the way along the column from the catheter tip.

**Outcome measures**

With regards to the primary outcome measures, there was no significant difference between the two methods in live birth rate (19/52 versus 15/50; OR = 1.34; 95% CI = 0.59–3.07) (Figure 2) and clinical pregnancy rate (54/150 versus 49/148; OR = 1.13; 95% CI = 0.70–1.83) (Figure 3). For the secondary outcomes, there were also no significant differences between the two methods in rate of embryo implantation (31/127 versus 24/128; OR = 1.40; 95% CI = 0.77–2.55), multiple pregnancy (1/22 versus 0/17; OR = 2.44; 95% CI = 0.09–63.75), ectopic pregnancy (0/150 versus 3/148; OR = 0.14; 95% CI = 0.01–2.72), miscarriage (3/22 versus 2/17; OR = 1.18, 95% CI = 0.17–8.02), and retained embryos (1/52 versus 1/50; OR = 0.96; 95% CI = 0.06–15.79).
Discussion

Embryo transfer is the final stage in the IVF cycle with manual clinical manipulation. Although most patients who undergo assisted reproduction with 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 of the embryo transfer procedure is to atraumatically and accurately place embryos within the uterus in order to allow for proper implantation and fetal development.

The embryo transfer procedure can be broken down into several parts, all of which are under the control of the clinician. In the past, the choice of catheters, ultrasound guidance, and other aspects (e.g. catheter-loading techniques) were left up to personal preferences. Today, in the era of evidence-based medicine, the performance of each step should be scrutinized in order to standardize and perfect this obviously inefficient technique.

In order to ascertain the importance of each step involved in the embryo transfer procedure, individual factors must be evaluated independently. In addition, since it would be difficult to accurately compare several factors at the same time, it was decided to concentrate on only one factor, the possible beneficial or detrimental role of using air brackets around the embryo-containing medium.

Embryo transfer protocols have shown that different combinations of air and fluid volumes may be introduced in the catheter during embryo loading. In general, there are two accepted catheter-loading techniques, the air-fluid and the fluid-only models. In the air-fluid model, the loading of the syringe–catheter complex with the transferred volume consists of the transfer media (which contains the embryos) separated by air spaces on both sides. In the fluid-only model, the embryos are placed in a complete column of fluid, without any air brackets or bubbles (10). In addition, a new modification of the standard air-fluid loading technique has been suggested which uses only one air bubble at the tip of the embryo transfer catheter instead of two air bubbles to bracket the embryo containing media (9), therefore introducing a lesser amount of air into the uterine cavity.

To this day, the use of air brackets is controversial, and the evidence to support its use or avoidance is lacking. Some clinicians prefer the use of air brackets so that the embryo-containing media is easily identified in
the catheter, and on ultrasound. In addition, it has a psychological benefit for both the clinician and patient. The physician is guaranteed that the embryos will not be released from the catheter prematurely before proper placement in the uterus (11), or move up towards the syringe, therefore increasing the risk of being retained. Also the added visibility on ultrasound helps to detect the embryo catheter tip, in order to allow proper distancing from the uterine fundus. As for the patients, they are given the added comfort of visualizing the embryo-containing droplet on ultrasound through the detection of the two air bubbles surrounding the media.

Even though some clinicians support the use of air columns to identify the positioning of embryos in the uterus, others suggest that the presence of air could increase the likelihood of embryo entrapment and increase reactive oxygen species, movement of embryos to other areas away from the uterus, or the occurrence of retained embryos within the catheter (7). It is important to note that these theories have yet to be proven, and the results of this systematic review provide evidence of clinical equivalence with regards to the two loading methods.

In general, systematic reviews and meta-analyses of randomized controlled trials have proven to be the highest level of evidence in the hierarchy of medical knowledge. Even so, publication and search biases may confound the results of any systemic review, as studies showing positive results are more likely to be published (12, 13). Therefore, every effort has been made to avoid bias by searching a wide variety of databases, including Medline, EMBASE, the Cochrane Library, with no language barriers, in addition to hand searching the abstract books of major conferences (e.g. ASRM, ESHRE), reference lists of review articles and included trials. Even with all these precautions, only a limited number of trials were retrieved from the literature. This is evidence for the need for more trials to be properly planned, implemented and published regarding the different steps of the embryo transfer technique.

Another important issue in clinical trials and systematic reviews is sample size. Since none of the included studies performed power calculations a priori, and due to their small sample sizes, it is postulated that they did not have sufficient power to detect minor differences between the study groups. Moreno et al. (8) determined from the
results of their implantation rates that a sample size of more than 800 patients in each arm would be necessary. Moreover, since clinical pregnancy rates are a more accepted outcome measure than implantation rates, it was decided to determine the properly necessary sample size. In order to detect a 5% difference between the air-fluid and fluid-only methods, it was determined that 1,307 women would be needed in each arm to provide an 80% statistical power of avoiding a type II error and a 5% chance of making a type I error in a two-tailed analysis (assuming a clinical pregnancy rate of 30% with the air-fluid method and a significance level of 0.05). The final outcome of this systematic review demonstrates that until today, even though there is no proven beneficial role to the use of air brackets when compared with the fluid-only method of embryo loading, there is also no evident detrimental role. In addition, it once again indirectly supports the notion of both clinical and publication bias against the all-so-important embryo transfer procedure in the literature. This is, of course, owing to the small number of studies planned, executed and published available to date. It is hoped that this trend will change in the near future, and the importance of each step in the embryo transfer technique will be further realized and scrutinized in order to create a truly evidence-based protocol for the transfer of human embryos following assisted reproduction. In conclusion, there is insufficient evidence to suggest the superiority of the air-fluid or fluid-only methods during embryo loading. There is also a strong need for more well-designed and powered randomized controlled trials in order to truly determine any possible beneficial, or detrimental, effect to either method.
Acknowledgements
The author would like to thank all the corresponding authors that were contacted for more information and provided us with assistance. A special thanks is in order to Dr. Juan Balasch, whose direct assistance has helped to increase the accuracy of this systematic review by providing missing information.
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Potentially relevant RCTs identified and screened for retrieval \((n = 3)\)

RCTs included in meta-analysis \((n = 2)\)

Potentially relevant RCTs identified and screened for retrieval \((n = 3)\)

RCTs excluded, \((n = 0)\)

RCTs retrieved for more detailed evaluation \((n = 3)\)

RCTs excluded, (compared standard air-fluid method to modified method using only one air bracket at tip of catheter) \((n = 1)\)

Potentially appropriate RCTs to be included in the meta-analysis \((n = 2)\)

RCTs withdrawn, \((n = 0)\)

RCTs included in meta-analysis \((n = 2)\)

RCTs excluded, \((n = 0)\)

RCTs with usable information, by outcome \((n = 2)\)

RCT = randomized controlled trial.
**Figure 2.** Meta-analysis forest plot showing live birth rates.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Air-fluid n/N</th>
<th>Fluid-only n/N</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allahbadia 2005</td>
<td>9/20</td>
<td>17/16</td>
<td>0.91 [0.31, 2.74]</td>
</tr>
<tr>
<td>Krampl 1995</td>
<td>32/98</td>
<td>32/98</td>
<td>1.00 [0.55, 1.82]</td>
</tr>
<tr>
<td>Moreno 2004</td>
<td>22/52</td>
<td>17/50</td>
<td>1.42 [0.64, 3.10]</td>
</tr>
</tbody>
</table>

Total (95% CI) 170 184 1.10 [0.71, 1.70]

Total events: 63 (Air-fluid), 66 (Fluid-only)
Test for heterogeneity: Chi² = 0.60, df = 2 (P = 0.74), I² = 0%
Test for overall effect: Z = 0.41 (P = 0.68)

**Figure 3.** Meta-analysis forest plot showing clinical pregnancy rates.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Air-fluid n/N</th>
<th>Fluid-only n/N</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
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<td>Moreno 2004</td>
<td>22/52</td>
<td>17/50</td>
<td>1.42 [0.64, 3.18]</td>
</tr>
</tbody>
</table>

Total (95% CI) 150 148 1.13 [0.70, 1.83]

Total events: 54 (Air-fluid), 49 (Fluid-only)
Test for heterogeneity: Chi² = 0.48, df = 1 (P = 0.49), I² = 0%
Test for overall effect: Z = 0.52 (P = 0.61)