RESEARCH

The effect of embryo catheter loading technique on pregnancy rate

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Lay summary

Embryo transfer is the most emotional part for patients during *in vitro* fertilization treatment. Over the last decade, the embryo transfer procedure has undergone numerous changes in the guidelines in order to increase pregnancy rates. One such procedure is the loading of the embryo into the catheter, a thin tube that helps us transfer embryo into the uterine cavity. Very few research studies looked closely at embryo-loading technique per se. Furthermore, different infertility laboratories use various techniques to load embryo. The aim of our study was to compare the two most popular embryo-loading techniques. In 249 women, we transferred embryo aspirated into the catheter with small droplets of air, and in the group of 244 patients, we filled catheter only with fluid. Our main outcome measured was the clinical pregnancy rate. Based on our results, we did not find that embryo-loading technique affected patient's chances of achieving pregnancy.

Keywords: ▶ embryo transfer ▶ embryo-loading technique ▶ IVF ▶ implantation ▶ pregnancy

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Introduction

Embryo transfer is the final step in the complex process of *in vitro* fertilization (IVF) treatment and is considered to be the most emotional part for patients. A number of high-quality studies have been conducted over recent years to help optimize and standardize the embryo transfer process. Multiple variables affecting implantation and pregnancy rates have been identified (Schoolcraft 2016, Penzias *et al.* 2017*b*, Sigalos *et al.* 2017). The areas that gained most of the interest were the choice of embryo transfer catheter (Abou-Setta 2006, Buckett 2006), different embryo cultures (Zbořilová *et al.* 2018), oxygen tension parameters (Bagheri *et al.* 2018), volume of the transfer medium (Sigalos *et al.* 2018) and utilization of ultrasound guidance (Mirkin *et al.* 2003, Brown *et al.* 2016).

A number of different steps in embryo transfer preparation have been improved based on the outcomes of these studies, but loading of the catheter by an embryologist has not received much attention (Halvaei *et al.* 2013, Christianson *et al.* 2014).

According to a worldwide web-based survey conducted in 2014, there are several modifications to the embryo-loading technique, but the main difference is the usage of air bubbles in the transfer catheter (Christianson *et al.* 2014). Based on the survey, the most commonly utilized method is bracketing the embryo(s) with air bubbles, even though the presence of oxygen in transferring catheter has been considered controversial for decades (Abou-Setta 2007).

With advances in assisted reproductive technologies (ART) and tendencies to optimize and standardize treatment and lab protocols, we decided to conduct this retrospective study to compare air-fluid and medium-only loading methods as no data have been reported yet for frozen embryo cycles, and the debate concerning harmful

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effect of oxygen to the embryo(s) in transferring catheter is still ongoing.

Materials and methods

A total of 493 frozen embryo transfer (FET) cycles were used for our retrospective study. After getting approval from our institutional review board, we analyzed all embryo transfer cycles performed at our fertility center from November 2018 to July 2021. A total of 689 embryo transfers performed at our clinic during this period were assessed for eligibility. Fresh embryo transfer cycles, cycles involving gestational carriers and patients with incomplete embryo transfer record were excluded from the study (Fig. 1). Couples with the following diagnosis of infertility were included: male factor, unexplained, tubal factor, ovulatory dysfunction, endometriosis and diminished ovarian reserve. Eventually, 493 patients were enrolled in the study and their records were analyzed. Patients who had FET done from November 2018 to January 2020 were included in group A, and their embryo transfer was done using the air-fluid catheter loading method. In January 2020, a fluid-only catheter loading technique was implemented in our laboratory and all patients having embryo transfer after that time were included in group B.

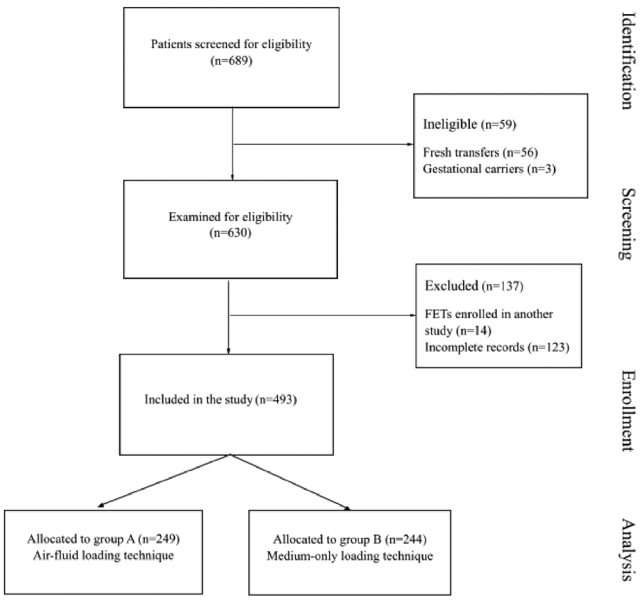

Figure 1 Flowchart showing the number of patients during each stage of the study.

Controlled ovarian stimulation

Patients receiving either standard gonadotropin-releasing hormone (GnRH) agonist or standard GnRH antagonist protocol were both included in the study. Gonadotropin stimulation was initiated on day 2 or 3 of menstrual cycle and was conducted using human menopausal gonadotropins (Menopur, Ferring, Parsipanny, NJ, USA) and follicle-stimulating hormone (Gonal-F, Serono, MA, USA/Follistim Merck & Co, Whitehouse Station, NJ, USA). Dose of medications were modified according to the patient's ovarian reserve testing and her response to controlled ovarian stimulation. In 20 cases, in addition to standard gonadotropin injections, patients also received oral clomiphene citrate for the first 5 days of stimulation. We used flexible GnRH antagonist protocol.

When three or more follicles measuring >17 mm in average diameter were documented using transvaginal ultrasound, either leuprolide acetate (Sandoz Inc, Princeton, NJ, USA) or human chorionic gonadotropin (HCG) injection (Novarel, Ferring) was administered for the final maturation. Oocyte retrieval was performed 36 h after trigger injection.

Preparation for frozen embryo transfer

Endometrial preparation before FET was performed using the same protocol in both groups. Patients were started on transdermal estradiol (Sandoz Inc.) on day 2 or 3 of the menstrual cycle. Endometrial lining was measured on cycle day 15 and if thickness was 7 mm or more and hormone levels of estradiol and progesterone acceptable, i.m. progesterone in oil was administered for 5 days followed by embryo(s) transfer.

Embryo thawing procedures

Vitrified embryos were stored in liquid nitrogen loaded onto a Cryolock device (Irvine Scientific, Santa Ana, CA). Embryo warming was performed using a SAGE Warming kit (CooperSurgical, Trumbull, CT). The Cryolock was inserted into a MOPS (3-(N-morpholino)propanesulfonic acid)-based thawing solution with 1.0 M sucrose and 20% serum substitute supplement for 1 min at 37°C. After that, embryo(s) were transferred to the diluent solution with 0.5 M sucrose for 3 min, followed by incubation in 0.25 M sucrose for 5 min and sucrose-free MOPS medium for 5 min. Placing embryos in global total HP medium (CooperSurgical) completed the thawing process. We cultured embryos for 2 h before transfer.

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Embryo quality scores

All embryos were transferred at the blastocyte stage. Embryo grading was based on morphological features and took into account the degree of expansion of the blastocyst, development of the inner cell mass and tropchectoderm. The alphanumeric grading system was then translated into a simple scoring system and was reported as excellent (A), good (B) and poor (C) quality embryo.

Embryo catheter loading technique

The embryos selected for transfer were placed in a center-well ET dish (Thermofisher) containing 1–2 mL EmbryoGlue (Vitrolife, Sweden; May 2019 to November 2020) or global total HP (CooperSurgical; prior to May 2019). Transfer catheter was flushed with 1 mL of global total HP. After that, embryos were loaded according to one of the two methods (Fig. 2). In group A, loading was done by filling the catheter with 15–20 µL of the medium then aspirating 10 µL of air followed by 5–10 µL of medium containing embryo(s). At the tip of the catheter, additional 10 µL of air was added to complete loading and seal embryo(s) by air bubbles.

In group 2, the medium-only loading was performed by filling the catheter with 15–20 µL of medium followed by an additional 5–10 µL containing the embryo(s), without any air.

Embryo transfer technique and outcome measurements

The embryo transfer technique and protocol were the same for both groups.

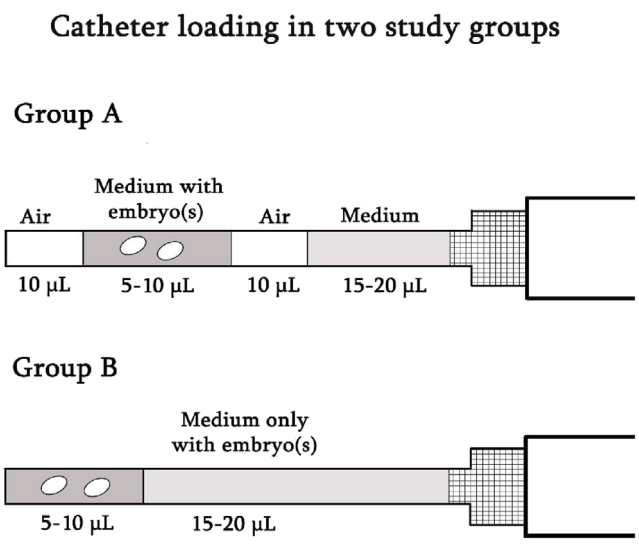

Figure 2 Embryo catheter loading techniques.

ET was standardized between clinicians and performed based on current recommendations provided by the American Society of Reproductive Medicine (ASRM) practice guideline (Penzias *et al.* 2017*a*). After the patient's ID was confirmed, she was taken to the procedure room and placed in dorsal lithotomy position. The vaginal speculum was inserted and cervix was cleaned with sterile cotton swabs soaked in 4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid (HEPES)-buffered Human Tubal Fluid (mHTF) medium (CooperSurgical). Cervical mucous was gently removed from external os. Soft-pass and Sydney catheters (CookMedical) were used in both groups for ET, and most cases were performed using the afterload technique. The clinician inserted a catheter handed off by the embryologist through the cervix and advanced it to 2 cm below the uterine fundus. Embryo unloading was performed under the ultrasound guidance, and the catheter was then carefully withdrawn with the plunger continuously compressed. Immediately after that, embryo transfer catheters were checked under the microscope for the retained embryo(s). Clinicians graded the transfer after the procedure and recorded if any difficulty occurred. ET was considered difficult if during the transfer the clinician had to use forceps or there was blood present on the catheter. The number of attempts was recorded as well. In 26 cases out of 493, a second attempt was needed to achieve a successful transfer.

Positive pregnancy test was defined as a positive beta hCG test 10 days after the embryo transfer. Clinical pregnancy was defined as a documented gestational sac with fetal pole and positive fetal heart rate at 6 weeks of gestation by transvaginal ultrasound. The positive pregnancy test and clinical pregnancy rates were calculated by dividing them by the number of women submitted to embryo transfers.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS, version 23.0, IBM Corp 2015) and was presented as mean ± S.E. Unpaired Student's *t*-test was used to compare quantitative variables, and the chi-square test was used to compare qualitative variables. *P*-values < .05 were considered statistically significant.

Considering our clinical pregnancy and positive pregnancy test rates, we also calculated our sample size to provide 80% statistical power of avoiding type II error and 5% chance of making a type I error. We would have been able to demonstrate 15% difference in pregnancy rates between the study groups by including at least 387 subjects in the study.

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Results

A total of 493 patients were included in the analysis. There was no statistically significant difference between the groups with regards to age, BMI, etiology of infertility, cryo cycle and ovarian stimulation characteristics, and quality of embryos transferred (Tables 1 and 2).

A total of 6459 oocytes were retrieved, of which 4204 were metaphase II oocytes. Six hundred and thirteen embryos were transferred in FET cycles, and there was a statistically significant difference between groups only in terms of the number of embryos transferred (Table 2).

Four hundred ninety-three FETs resulted in 279 positive pregnancy test results and 236 clinical pregnancies—defined as documented intrauterine gestational sac with fetal pole and positive fetal heartbeat on ultrasound. Positive pregnancy test (55.8% vs 57.3%, P = 0.4) and clinical pregnancy (47% vs 48.7% P = 0.36) rates showed no statistical difference between group A and group B, even though both percentages tended to be higher in the second group, where fluid-only embryo catheter loading method was utilized (Table 1).

Because our groups were different in terms of number of embryos transferred, we additionally calculated the outcomes using data only from the cases where the elective single embryo was transferred. The difference between the groups in regards to number of embryo transferred maybe partly explained by the fact that after the release of recommendations from ASRM on limiting the number of embryos to transfer in 2017 (Penzias *et al.* 2017*a*), our practice started to limit double embryo transfers. In total, a single embryo was transferred in 373 FETs and resulted in 164 clinical pregnancies and 194 positive pregnancy tests. Again, positive pregnancy test (49.7% vs 54.3%, P = 0.40) and clinical pregnancy (41.1% vs 46.7%, P = 0.3) rates were not significantly different between the two groups.

Difficult transfer was reported in 59 procedures and in 33 cases physician documented a bloody catheter after embryo transfer was complete. A second attempt was documented in only 26 procedures, and no retained embryos or catheter reloading cases were reported.

Table 1 Baseline characteristics of patients in both groups. Data are mean ± s.e. or *n* (%) unless otherwise specified.

Variable	Group A	Group B	*P*-value
Age	35.34 ± 4.7	35.35 ± 4.9	0.99
BMI (kg/m^2)	28.44 ± 6.8	28.11 ± 6.6	0.64
Diagnosis			0.94
Male factor	70	66	
Unexplained	23	32	
Tubal factor	43	45	
Ovulatory dysfunction	58	51	
Endometriosis	17	17	
Diminished ovarian reserve	50	51	
Other	15	14	
Endometrial thickness (mm)	10.5 ± 2.1	10.3 ± 1.9	0.40
Estradiol level on CD 15 (pg/mL)	925.7 ± 497.5	967.7 ± 528.5	0.46
Progesterone level on CD 16 (ng/mL)	24.7 ± 116.6	24.4 ± 85.3	0.98
Trigger medication			
HCG	178	160	0.18
Lupron	39	52	0.13
Positive pregnancy test rate (%)	139/249 (55.8)	140/244 (57.3)	0.40
Clinical pregnancy rate (%)	117/249 (47)	119/244 (48.7)	0.36

Table 2 Laboratory characteristics of patients in both groups. Data are mean ± s.e. or *n* (%) unless otherwise specified.

Variable	Group A	Group B	*P*-value
No of oocytes retrieved	14.68 ± 8.2	15.38 ± 8.4	0.37
No of metaphase II oocytes retrieved	9.81 ± 6.8	9.83 ± 6.6	0.97
Number of embryos transferred			0.04
1 embryo	175	198	
2 embryos	74	46	
Embryo quality scores			0.48
A	110	68	
B	99	147	
C	39	28	
Pre-implantation genetic testing			0.07
Yes	90	105	
No	159	138	
Difficult embryo transfer	27	32	0.49
Blood on catheter	14	19	0.37
Catheter used for embryo transfer			0.08
Soft pass	169	144	
Sydney	80	100	

Discussion

Few studies have looked closely at the embryo-loading process and how it may affect IVF outcomes. The aforementioned reports were published several years apart. None of the studies had a large enough sample size to demonstrate a statistical difference between the methods. Additionally, transfer guidelines and advances in ART have changed dramatically since these studies were published.

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In general, loading methods can be divided into two main models: the air-fluid and fluid-only loading techniques. In the air-fluid model, air is aspirated into the catheter alongside the medium containing embryo(s). Air may be used to bracket the medium containing the embryo(s) or this technique can be modified further by aspirating only one air pocket into the catheter to decrease the amount (Omidi *et al.* 2015) that we introduce into the uterine cavity during the transfer.

Krampl *et al.* were one of the first to demonstrate no difference in pregnancy rates between the air-fluid method and loading of the catheter with no air bubbles (Krampl *et al.* 1995). They conducted their prospective randomized trial in the early 90s and analyzed 196 fresh transfers, utilizing embryos at pronucleate or two to ten cell stage. Based on the outcomes, they proposed that air has no deleterious effect on implantation and it may be even advantageous to track the embryo transfer process on ultrasound. Numerous scientists have since argued that tracking the movement of air bubbles after the expulsion of embryos into the cavity may be used to predict implantation and success of the transfer (Friedman *et al.* 2011, Tiras *et al.* 2012).

It is noteworthy that in a study done by Krampl *et al.*, the amount of media (5–10 μL) and air (10 μL) used for catheter loading was quite small. That was not the case reported in the prospective randomized trial conducted by Moreno *et al.* (2004). In this study, they only reported fresh embryo transfers and included 102 participants. In the air-fluid group, they first loaded 200 μL of air into the syringe then 100–125 μL of air into the catheter, followed by 20–25 μL of medium containing the embryo(s) and then completed the process with an additional 10 μL of air at the tip. In the fluid-only method, the syringe and whole catheter were loaded with medium and at the distal end, 20–25 μL of medium with the embryo(s) was aspirated. Implantation and clinical pregnancy rate were higher using the air-fluid method but were not statistically significant, and the study was underpowered with a small sample size.

In 2015, Omidi *et al.* for the first time included FETs in a study comparing different embryo-loading techniques (Omidi *et al.* 2015). Out of 401 cases included in the analysis, 194 were frozen-thawed embryo transfers. All embryos in both arms were transferred on day 2. The loading techniques compared in this study both utilized air bubbles, and the amount of medium and air loaded was similar to our study. In group A, only one air bracket was loaded into the catheter before aspirating medium containing the embryo(s), and in the second group, catheter loading was done by sealing embryo(s) between two 5 μL air pockets. The analysis did not reveal any statistically significant difference between the groups in terms of implantation, clinical pregnancy, or live birth rates, although the pregnancy rates and deliveries were higher in group B, where the double air pocket technique of loading was utilized. There was also no benefit or improvement of outcomes demonstrated by the technique of loading with only one air pocket at the tip of the catheter (Allahbadia *et al.* 2005).

In contrast to the above studies, showing no effect of air in a catheter on a positive pregnancy test or clinical pregnancy rates, Ebner *et al.* reported that a small volume of medium and the use of air bubbles in the catheter may negatively affect IVF outcomes (Ebner *et al.* 2001). This is the only study so far that has described a potentially harmful effect of air on embryo transfer outcomes.

We conducted this study to gain more knowledge and insight into this issue. Only two prospective trials comparing air-fluid to medium-only catheter loading methods have been published, both more than 20 years ago. A more recent study chose not to compare these two techniques but rather modifications of various air-fluid models to each other (Omidi *et al.* 2015). Studies by Moreno *et al.* and Krampl *et al.* were conducted by analyzing data only from fresh cycles using day 2 embryos. Even though no significant difference with regard to pregnancy rates was demonstrated between methods at that time, routine use of air brackets is still controversial. The presence of air in the catheter may offer some psychological comfort to the doctor and to the patient by making it easier to appreciate the echogenic air bubble in the uterus on ultrasound image (Zinger *et al.* 2004, Lambers *et al.* 2007).

Some clinicians also share the opinion that the presence of air bubbles may play a role in preventing accidental preterm spillage of embryo(s) from the catheter (Poindexter *et al.* 1986, Woolcott & Stanger 1998) and possibly protect them from cervical mucus (Krampl *et al.* 1995). In his randomized clinical trial, Madani *et al.* even argued the fact that injecting air immediately after the embryo transfer into the endometrial cavity may not only prevent embryo expulsion but also can improve implantation and clinical pregnancy rates (Madani *et al.* 2010). This fact, though, does not eliminate the theoretical assumption of damage that reactive oxygen species may have on an embryo, given the fact that the introduction of air is not physiologic.

None of the studies so far have had large enough sample sizes to demonstrate a significant difference between methods. Additionally, transfer guidelines and advances in ART have changed dramatically since their publication. Our study is the first to analyze day 5 embryo transfers using

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data exclusively from FET cycles. We have also calculated our sample size to demonstrate a 15% difference in pregnancy rates between the groups. We collected our data during two different chronological timeframes, before and after the implementation of a new catheter loading technique. This can be considered as one of the limitations of the study, but our doctors and embryologist that were performing transfers were the same for the two groups. Also, during the same period, our practice started to limit double embryo transfers according to the newly released ASRM recommendations. Those changes most likely resulted in difference we observed between the groups in regards to the number of embryo transferred. In order to overcome this limitation, we analyzed cases with single embryo transfers separately and did not find any difference between the groups in terms of clinical pregnancy or positive pregnancy rates. Another possible limitation is the fact that same participant could have been enrolled in the study more than once. We acknowledge that this could have introduced potential bias in the study, but we had to make this permission in order to achieve our calculated sample size.

In conclusion, no significant difference was observed between the two loading techniques concerning positive pregnancy test and clinical pregnancy rates. Our results do not support the hypothesis that air aspirated into the transfer catheter might have any negative effect on the embryo implantation. There is insufficient evidence at this time to recommend one method of catheter loading over the other in FETs. More prospective studies, which should include live birth rates, are needed to draw a final conclusion.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Availability of data and material
The data sets used and analyzed during the current study are available from corresponding author on reasonable request.

Ethics approval
Institutional Review Board at Eastern Virginia Medical School waived ethical approval in view of retrospective nature of the study.

Informed consent
Institutional Review Board at Eastern Virginia Medical School waived ethical approval in view of retrospective nature of the study.

Consent to participate
This retrospective chart review study involving human participants was in accordance with ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Institutional Review Board at Eastern Virginia Medical School approved this study (IRB # 20-05-XX-0131).

Author contribution statement
Tamar Matitashvili contributed to the design and implementation of the study. Performed data collection, analysis of the results and drafted the manuscript. Seifeldin Sadek contributed to the implementation of the study and analysis of the results. Gerard Celia contributed to the main conceptual idea of the study. Contributed to the design and to the final version of the manuscript.

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