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ORIGINAL ARTICLE

The effect of embryo transfer technique on pregnancy and live birth rates in infertile women undergoing IVF treatment with freeze-all strategy

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ABSTRACT

Objective: The aim of this observational retrospective cohort study was to define the effect of the embryo transfer (ET) technique on pregnancy and live birth rates.

Material and Methods: Infertile women with good prognostic factors undergoing *in vitro* fertilization treatment with freezing all the 5th day good quality embryos and consecutive frozen ET in 468 infertile good responders with first quality 5th day embryos were chosen for this study. Logistic regression analysis was performed to determine the effect of ET on the live birth rates.

Results: Of the six predictive values, four were statistically significant: The easy passage of the external catheter, ultrasonographic visualization of the bubble, leaving the embryo at the desired location, and bleeding found in the external catheter after the catheter were excluded from the cervical ostium. While the probability of pregnancy decreased by 98.1% in cases where the embryo could not be left at the expected location, it was observed that the probability of pregnancy decrease was 96.4% in cases where no bubble could be seen, and 59.2% in cases with bleeding in the external catheter. Similarly, a 74.4% decrease in the probability of pregnancy was observed in patients in whom the passage of the catheter was difficult. Even though the tip of the transfer catheter during the ET is easily seen, the blood in the external catheter decreases pregnancy chance.

Conclusion: Visualizing the internal catheter and the air bubble under the ultrasound guidance with the atraumatic placing of the embryo 1–1.5 cm away from the uterine fundus is crucial for the success of ET.

Keywords: Embryo transfer technique, freeze-all strategy, *in vitro* fertilization, transfer catheter, infertility.

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INTRODUCTION

Embryo transfer (ET) is a well-known rate-limiting factor on the way to pregnancy in assisted reproduction technology (ART). Usually, ET has been seen as an unimportant variable in the success of ART treatment. There is now a general acceptance in the *in vitro* fertilization (IVF) society that smooth, atraumatic ET is critical for achieving high success rates, and the choice of technique plays a very important role in the uterine embryo replacement cycle.[1] A study published by McDonald et al.[2] stated that the pregnancy rate is increased by 50% using a double-lumen soft catheter instead of a firm single lumen catheter. A meta-analysis in 2006 compared the different types of catheters used in ET and concluded that an increased chance of clinical pregnancy was achieved when soft ET catheters were used.[3] A recent Cochrane data systematic review on this topic concluded that ultrasound guidance improves the chance of live birth, ongoing and clinical pregnancies compared with clinical touch without increasing the chance of multiple pregnancy, ectopic pregnancy, or miscarriage.[4] Several investigators have debated this with the lack of consensus until a study including the retrospective analysis of 5055 ultrasound-guided ET belonging to 3930 infertile couples by Tiras et al.[5] concluded that pregnancy rates and ongoing pregnancy rates are higher if the embryos are replaced at a distance >10 mm from the fundal endometrial surface. As a result of studies investigating the effect of clearing cervical mucus at the beginning of the transfer process on pregnancy success, many guidelines recommended performing cervical cleaning and removing mucus before inserting the catheter carrying the embryos.[6] It also has been shown that detecting mucus at the tip of the transfer catheter immediately after ET reduces pregnancy success.[7] Studies investigating the effect of blood at the catheter tip after transfer on live birth rates have shown that implantation rates decrease, pregnancy and live birth rates are affected.[8] As it was noted in a fairly recent review that explored transfer techniques and the variables required to achieve a successful ET easy, atraumatic transfer without blood or mucus under ultrasound guidance using a soft catheter and proper placement in the uterine cavity injecting embryos slowly 1.5 cm from fundus optimize implantation and thus increases the success of the procedure.[9] Since we did not come across a study that simultaneously investigated the variables required to make ET more perfect, we planned this study and, for the 1st time, we kept stable the variables such as the person performing the ET, the catheter used and the transfer was done under ultrasound assistance for each patient. Making these variables unchanging, we investigated the effects of variables such as the degree of difficulty during ET, leaving the bubble carrying the embryo in the desired location, and monitoring of blood and/or mucus in the external catheter after the procedure on ET at the same time in each of the patient in this study.

MATERIAL AND METHODS

Our study was carried out using the data of 468 infertile women at Şişli Kolan Hospital Private IVF Center between 2016 and 2020. The indications for infertility treatment are explained in Table 1. Our study was designed as a retrospective cohort study to examine the effects of possible adverse events at every stage of the ET process on pregnancy and live birth rates. All patients underwent the ICSI procedure with a freeze-all strategy, a routine practice in patients with a good ovarian reserve in our center. Frozen ET (FET) was performed in these patients after 2 or 3 months following the freezing of all good quality embryos surviving to the 5th day after ovum pick-up. We aimed to ignore the embryo and endometrial factors. Hence, we chose 468 infertile women with good ovarian reserve (according to the antral follicle count), 38 years of age or younger, with good quality blastocyst embryos on the 5th day (5AA, 5AB, 4AA, and 4AB according to Gardner classification) and excluded patients with systemic disease, presence of endometriosis or endometrioma, severe male factor, uterine anomaly, or history of previous uterine surgery. Only one ET cycle for each of the patients was included in the study.

Table 1: Age of the patients and infertility indications for IVF treatment

	n	%
Age of the patients (years of age)	19–38 (median: 30)	
Patients with polycystic ovaries	120	25.6
Patients with unexplained infertility	158	33.8
Patients with mild male factor infertility	103	22.0
Patients with tubal factor infertility	87	18.6
Patients with double embryos transferred	260	55.6
Patients with single embryo transfer	208	44.4

IVF: *In vitro* fertilization.

The same specialist infertility doctor performed all transfer procedures under the guidance of transabdominal ultrasonography. Beginning from the intracytoplasmic sperm injection (ICSI), the same embryologist coordinated all stages of the embryo development and transfer procedure of 468 patients in each of the patients. All adverse event/s that occurred during the ET were recorded by the embryologist individually for each patient. The ET process was evaluated in three main sections; (1) insertion of the external catheter, (2) visualization of the internal catheter during insertion and transfer of embryos/embryos to the uterine cavity, and (3) after the procedure, macroscopic examination of the external catheter and microscopic examination of the internal catheter. In the first stage of the ET procedure, we recorded if it was difficult to insert the external catheter through the cervical area. The second stage recorded how the internal catheter was placed in the uterine cavity, and special attention was given to how the catheter was visualized under the transabdominal ultrasonography. The distance from the end of the inner catheter to the fundus at the time of transfer of the embryos was 1–1.5 cm, and it was accepted as the "optimum place." If embryos could not be transferred in the desired area, it was noted accordingly in the patient's file. It was also recorded if the air bubble coming out of the catheter could not be observed at the transfer time. After withdrawing the catheter from the cervix, the external catheter's macroscopic view of blood or mucus was carefully examined and noted in the file. It was also recorded if blood and/or mucus were observed under the microscopic examination in the internal catheter.

Table 2: The rates of performing (yes)/not performing (no) the embryo transfer steps as desired and the live birth pregnancy rates in percentages

	Yes		Live birth rate		No		Live birth rate		p
	n	%	n	%	n	%	n	%	
Easy passage of the catheter	440	94	255	58	28	6	9	32.1	0.008
Easily seen internal catheter	448	95.7	261	58.3	20	4.3	3	15	**<0.001**
Seeing the bubble	456	97.4	264	57.9	12	2.6	0	0	**<0.001**
Putting embryo/s in desired location	405	86.5	264	65.2	63	13.5	0	0	**<0.001**
Bleeding in external catheter	350	78.6	215	61.4	118	25.2	49	41.5	**<0.001**
Mucus on external catheter	360	76.9	208	57.8	108	23.1	56	51.9	0.276

Gonadotropin treatment was started in 468 patients diagnosed with infertility on the 2nd or 3rd day of the menstrual period for the controlled ovarian stimulation. The starting dose of gonadotropins was arranged according to each patient's age, body mass index, and ovarian reserve, calculated by counting the antral follicles. Medroxyprogesterone acetate with a daily dose of 10 mg was used to prevent premature luteinization started on the 6th day of stimulation or whenever the leading follicle reached 14 mm. After follicular monitoring, when the diameter of the leading follicle reached 17–18 mm, GnRH agonist (triptorelin acetate, Gonapeptyl 0.1 mg/mL, Ferring, Germany) was administered 1X0.2 mg/mL SC for final ovulation triggering. Ovum pick-up was scheduled 36 h after the final ovulation. ICSI, a standard procedure for all patients in this clinic, was applied to all cases after oocyte pick-up performed under general anesthesia. With the end of OPU procedure oocytes was collected for fertilization in a medium (Irvine Scientific) and placed in a Miri Benchtop incubator until the ICSI was performed approximately 3–4 h after the procedure.

Embryos were cultured in this sequential medium and observed for progress every day. The freeze-all procedure was applied to all cases at the blastocyst stage. Patients which embryos reached the blastocyst stage in more than 5 days were excluded from the study.

Good quality 5th day blastocyst embryos were transferred in all of the patients. Embryo classifications were described according to embryo evaluation criteria proposed by ESHRE/ALPHA consensus. The Grade A embryos described as expanded blastocyst with a tightly packed inner cell mass, and trophectoderm (TE) cells were with many cells forming a cohesive epithelium or TE cells were herniating through a breach in the thinned ZP. According to the local rules, two embryos were transferred only to patients over 35 years who had previously tried 2 or more times and failed. One embryo was transferred to patients outside of these parameters.

All patients included in the study were evaluated at D2 or D3 for FET cycles starting with 2 mg estradiol (Estrofem 2 mg tablet, estradiol valerate, Nova Nordisk, Denmark) and increasing the dose every 4 days. Progesterone treatment was started at D14 or D15 when the endometrial thickness exceeded 7 mm. After 6 days of progesterone (Crinone 8% gel progesterone 90 mg, Merck, UK) intravaginal twice-daily treatment, the transfer process was performed. All patients received luteal phase support using 8 mg of estradiol and twice-daily vaginal progesterone until the pregnancy test result on the 12th day after the transfer. This treatment was continued till the 10th week of pregnancy in patients with positive pregnancy results.

Statistical Analysis

Demographic data and frequency of successful/unsuccessful procedures in the steps of the ET procedure were presented together with frequency and descriptive statistics. Pearson Chi-square test and Fisher's exact tests were used to compare categorical variables and evaluate correlation. Logistic regression analysis was performed to determine the causality effect in cases with correlation. IBM SPSS version 25.0 was used for all statistical analyses.

RESULTS

In our study, 468 ET procedures and pregnancy results between 2016 and 2020 were examined. The median age of the patients participating in the study was 30 years, the age of patients was between 19 and 38. Two embryos were placed simultaneously in 260 (55.6%) of the ET procedures, while single ETs were done in 208 patients (44.4%) (Table 1). Since cervical cleaning was completed in all patients, it was not evaluated during statistical analysis considering the ET procedure steps. Data of the patients with bleeding during cleaning were also not calculated because this was observed in only three patients. Bleeding in the internal catheter was observed in only two patients, and mucus in the internal catheter was observed in only seven patients; this low number of occurrences also required that this information not be included in the calculations.

The rates of performing/not performing the ET steps as expected and the percentages of getting pregnant are given in Table 2, and the percentages of live births are given in Table 3 accordingly. Three hundred and fifty-five of the total 468 patients in the study had positive pregnancy results (75.8%), and 264 of these pregnancies ended with at least one healthy child. The correlation of the ET steps and the abortion or biochemical pregnancies were not the study's primary endpoint; thus, it was not included in the statistical analysis. When the correlation between the procedural steps was evaluated, it was observed that bleeding was less common in the external catheter in patients with easy access to the endometrial cavity during

Table 3: Correlation between the procedural steps performing (YES)/not performing (NO) the embryo transfer as desired

	Yes		Bleeding		No		Bleeding		p
	n	%	n	%	n	%	n	%	
Easy passage of the catheter	440	94	104	23.6	28	6	14	50	**0.002**
	Yes		**Putting embryo/s in desired location**		**No**		**Putting embryo/s in desired location**		**p**
	n	%	n	%	n	%	n	%	
Easily seen internal catheter	448	95.7	394	87.9	20	4.3	11	55	**<0.001**
Seeing the bubble	456	97.4	400	87.7	12	2.6	5	41.7	**<0.001**

Number of patients and percentages among all patients.

the ET. Similarly, it was observed that the rate of leaving the embryo at the desired location inside the endometrial cavity under the ultrasonographic guidance was higher in cases where the internal catheter was clear, and the bubble was easily seen (Table 3). Logistic regression analysis was performed to determine the effect of procedural steps on the live birth rates. The regression model was statistically significant, χ^2 (6)=181.2, p<0.001. The model explained 48% (Nagelkerke R2) of the variance in pregnancy development and accurately predicted 87.6% of cases. Sensitivity was 97.5%, specificity was 56.6%, positive predictive value was 87.5%, and negative predictive value was 87.6%. Of the six predictive values, four were statistically significant: The easy passage of the external catheter, ultrasonographic visualization of the bubble, leaving the embryo at the desired location, and bleeding found in the external catheter after the catheter was excluded from the cervical ostium. While the probability of pregnancy decreased by 98.1% in cases where the embryo could not be left at the desired location, it was observed that the probability of pregnancy decrease was 96.4% in cases where no bubble could be seen and 59.2% in cases with bleeding in the external catheter. Similarly, a 74.4% decrease in the probability of pregnancy was observed in patients in whom the passage of the catheter was difficult. Age of the patient and good ultrasonographic visualization of the internal catheter were not affecting the possibility of a pregnancy negatively as well as there was no increase in the probability of pregnancy.

DISCUSSION

All patients in this study were selected from young infertile patients aged 19–38 years with a good prognosis. The intent of choosing the patients with a good prognosis for IVF treatment was to focus our study on the effects of the ET technique and the possible stages that can go wrong during the ET procedure. The physician doing the transfer is also an important factor in the success of the ET. There is a study stating that the efficacy of the ET catheter in IVF and ICSI is operator dependent.[10] The fact that a single physician in our study transferred all patients also caused the bias on the results of possible technique and experience of the physician difference to be removed.

In studies carried out so far, ET has been classified according to whether it is easy or difficult, and its effect on pregnancy outcomes has been investigated. Many studies have stated that difficult ET adversely affects pregnancy outcomes, and the negativities experienced in which stages did not give clear information about what caused us to reach this conclusion.[11–15] However, contrary to this, studies have been done that suggest no correlation between a difficult transfer and poor pregnancy rates.[16]

There is not an accepted consensus on describing the optimal ET technique, it is usually recommended that the touching of the endometrium and the induction of uterine contractions be avoided during ET. We should decrease the exposure of embryos to unwanted conditions, and the embryo(s) should be placed at an optimal (desired) position within the fundal region of the uterine cavity.[17] The presence of blood on the catheter once removed at the time of ET and its possible implications have been studied in many studies, suggesting an increased interest and concern about blood and ET techniques. Considering the different results of many studies, it is difficult to conclude that blood seen on the catheter once it was withdrawn is related to the implantation or pregnancy results.[18] When there is difficulty in passing an external catheter, the pregnancy rate decreases. However, sometimes, even if the passage is easy, blood can be observed in the external catheter. Maybe a situation such as pushing the catheter too far and contacting the endometrial cavity may cause macroscopic blood to be seen in the external catheter. In our study, blood in the external catheter reduces the pregnancy rate in the regression analysis. Although the external catheter is easily passed, the sight of blood should be considered. Many studies support the effectiveness of ultrasound guidance during ET.[19,20] The transfer catheter is usually loaded using a "three-drop technique," in which the drop of medium containing the embryo(s) is/are separated from a preceding and a following drop of the medium by an air bubble. Surprisingly enough, only a few studies analyze the relationship between the air bubble position and pregnancy rates. The internal catheter may not always be well visualized. The inexperience of the nurse, assistant, or obesity may complicate optimal imaging. In our study, visualization of the internal catheter significantly changes the pregnancy rate, but in the regression analysis, only visualizing the

bubble and leaving it at the desired location seem to give statistically significant results. However, good visualization of the catheter in the relationship analysis is associated with seeing the bubble and placing it at the desired location. For this reason, assistant support is vital for good imaging under ultrasound. Although the image quality is important, it turns out that it is essential for a good visualization also to be able to position the uterus well so that the catheter can pass easily. The positioning of the uterus requires experience of the physician and optimal fullness of the bladder. Seeing bubble is very meaningful and directly related to the regression analysis. The bubble does not show the embryo directly, but still, when the bubble is detected in the right place, the probability of pregnancy and live birth increases. This was shown in a study done in 2007 by Lambers et al.,[21] the position of the air bubbles after ET is related to pregnancy rate; the highest pregnancy rates are found when the air bubbles end up closer to the fundus. Of all embryos that implant successfully, 81% do so in the area where the air bubbles were initially seen at ET.[22] Therefore, the air bubbles can be regarded as an indication of the position of the embryos.[23] We also found that one of the most critical points in the results was to leave the embryo in the desired place under ultrasound imaging during the transfer. In other words, the phenomenon that is wanted to be explained under the name of leaving it in the desired place is that the tip of the catheter is 1-1.5 cm away from the uterine fundus at the exact moment of transfer. The American Society of Reproductive Medicine guidelines also recommend as described.[18] It means that it is necessary to follow the tip of the catheter well and leave the embryo in the right area. In our study, single or double ET does not affect pregnancy and live birth outcomes. This means that performing a single ET under the age of 39 in the group with a good prognosis gives successful results. The live birth rate was 54.3% in two embryo transferred patients, while we had a 58.1% live birth rate in single embryo transferred patients. According to our study, even though when the tip of the catheter is easily seen, the blood in the external catheter decreases pregnancy chance. It seems that visualizing the internal catheter and the air bubble under the ultrasound guidance with the atraumatic placing of the embryo 1–1.5 cm away from the uterine fundus is crucial for the success of ET in patients with good quality embryos.

Limitations and Strengths of the Study

Data of the patients with bleeding during cleaning were also not calculated because this was observed in only three patients. Bleeding in the internal catheter was observed in only two patients, and mucus in the internal catheter was observed in only seven patients; this low number of occurrences made us to discard this information from the calculations. The strengths of this study are large sample size and very detailed documentation of ET technique.

CONCLUSION

Our study divides the ET process into stages. It is valuable in terms of seeking an answer to the question of what the effect of the problems is experienced at which stage of ET on pregnancy and live birth. Thus, this study examined all the so-called difficult transfer problems holistically. According to our study, the presence of blood in the external catheter and the distance from the point where the embryo is left to the fundus are outside the area of 1–1.5 cm are the most important factors that negatively affect pregnancy success.

Statement

Ethics Committee Approval: The Haliç University Non-Interventional Clinical Research Ethics Committee granted approval for this study (date: 24.06.2021, number: 142).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – SO, AŞ, YKA, SÇ, NY; Design – SO, AŞ, YKA, SÇ, NY; Supervision – SO, AŞ, YKA, SÇ, NY; Resource – SO, YKA, AŞ; Materials – SO, YKA, AŞ; Data Collection and/or Processing – SO, AŞ; Analysis and/or Interpretation – SO, AŞ, NY; Literature Search – SO, AŞ, YKA, SÇ, NY; Writing – SO, AŞ, YKA, SÇ, NY; Critical Reviews – SO, AŞ, YKA, SÇ, NY.

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