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Embryo flash migration in fresh and frozen embryo transfers for day 3 and day 5 embryos

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ABSTRACT

Objective: The aim of this study is to evaluate the embryo flash migration after 60 min from embryo transfer in fresh and frozen cycles.
Design: 80 fresh and 81 frozen embryo transfers implemented Ondokuz Mayıs University between December 2017 and May 2018 were included in this prospective study. The fresh transfers performed at day 3 embryos as the frozen transfers were day 5 embryos. The distance between the embryo and the fundus was measured in the sagittal plane within 1 min of the transfer. After 60 min of bed rest the distance between the air bubble and the fundus was measured. The transfers were divided into three groups based on the migration of the embryos after the transfer. Embryos were classified as static if they were within 15 mm of their initial position. If they moved more than 15 mm towards the cervix or more than 15 mm towards the fundus, it was classified as cervical and fundal, respectively.
Results: There was a statistically significant difference in embryo flash movements between frozen and fresh transfers ($p < 0.05$). In fresh transfers 48 patients (%60.0) were cervical, 14 patients (%17.5) were static and 18 patients (22.5%) were fundal. In frozen embryo transfers 31 patients (38.3%) were cervical, 31 patients (38.3%) were fundal and 19 (23.5%) patients were static.
Conclusion: We found that cervical migration is lower in frozen transfers than in fresh transfers. This result may be related with the day of embryo or the endometrium in fresh or frozen cycles. Because in this study the embryos transferred were day 3 in fresh cycle and day 5 in frozen cycle. In frozen transfers there was not any significant difference in embryo position between pregnant and non-pregnant group. But in fresh transfers the cervical migration was significantly high in non-pregnant patients ($p < 0.05$).

Introduction

Embryo transfer is the final and rate-limiting step of IVF therapy [1]. Therefore, in order to increase the probability of pregnancy, parameters that negatively affect transfer should be determined. Some factors that are known to affect transfer include type of catheter, location of the catheter tip, blood and mucus, clinician experience, presence of liquid in the cavity, and myometrial contractions [2].

Embryo transfers in the presence of ultrasonography often result in increased transfer success [3]. However, there are some questions regarding the use of ultrasonography for imaging embryo position. For example, it is not known how the initial position of the embryo during transfer affects the pregnancy outcome. Further, it has yet to be determined whether the embryo's position changes following transfer, and if so, how does this movement impacts pregnancy outcomes?

Some studies have shown that embryos close to the fundus had better pregnancy outcomes [4], while other studies have shown that embryos transferred ≥10 mm away from the fundus had better pregnancy outcomes [5]. Yet, other studies reported that embryo transfer positions did not affect pregnancy outcomes [6].

One study revealed that embryos can move following transfer, and that those moving towards the fundus had higher pregnancy rates, while those moving towards the cervix had lower [7].

The current study aimed to investigate the impact of embryo flash migration following transfer on pregnancy outcomes. In contrast to other studies, the current study evaluated both fresh and frozen embryo transfers, and to our knowledge, this study is the first to focus on frozen embryo transfers.

Our hypothesis is that the uterine contractility may expected to be more in fresh cycle than those of the frozen cycle due to the excess hormones. Current study will show the differences of air bubble migration between fresh and frozen transfers and the effect of this movement on pregnancy results.

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Materials and methods

In this prospective study there were 89 fresh and 90 frozen embryo transfers performed at Ondokuz Mayıs University between December 2017 and May 2018. Approval was obtained from the Ondokuz Mayıs University Ethics committee 2018/257. All of the patients provided informed consent were between 18 and 40 years of age, had FSH <15, underwent their first transfer and had regular cycles. Patients who had tubal factor or anovulatory infertility, myomas, uterine anomalies, adenomyosis and retroverted uteri were not included to study. Those having difficulty during transfer, a suspicion of contact with the fundus or experienced hemorrhage were excluded. As a result, 86 patients in fresh cycle and 85 patients in frozen cycle were included. However, in 6 patients from fresh cycle and 4 from frozen cycle air bubble was not seen and the remaining 80 fresh and 81 frozen cycle patients were involved the current study (Fig. 1).

Data collected from each patient included age, reason for infertility, FSH value on cycle day 2–3, number of collected oocytes, maximum E2 values, number of transferred embryos, and endometrium thickness on the day of transfer.

Assisted reproductive technology (ART) procedure

Ovarian stimulation was performed using an antagonist protocol with recombinant FSH (Gonal F, Serono, Spain) and GnRH antagonist (Cetrotide, 0.25 mg, Serono, Switzerland). Recombinant HCG (Ovitrelle, 250 mg, Serono, Switzerland) was applied when two or more follicles ≥17 mm were detected via serial ultrasonography measurements. Oocyte pick-up (OPU) was performed 36 h following HCG treatment and intracytoplasmic sperm injection (ICSI) was performed 4–6 h after OPU. Embryo transfer was performed at day 3 in fresh cycles, and day 5 in frozen cycles Starting from OPU, progesterone was given intramuscularly (Progestan 50 mg; Koçak, Turkey) and estrogen (Estrofem 2 mg; Novo Nordisk, Denmark) was given orally as luteal support in both cycles.

In frozen cycles, endometrium preparation was initiated using estrogen (Estrofem 2 mg; Novo Nordisk, Denmark) on cycle day 2–3 following transvaginal ultrasonography. The endometrium preparation protocol began with 4 mg/day of estrogen on days 1–4, 6 mg/day on days 5–8, and 8 mg/day from day 9 onwards. A second transvaginal ultrasonography was performed following 10 days of estrogen treatment. Embryo transfer was scheduled in cases where the endometrial thickness was at least 7 mm. Progesterone was administered intramuscularly (Progestan 50 mg; Koçak, Turkey) at a dose of 100 mg for 5 complete days prior to embryo transfer.

Embryo selection and embryo transfer (ET)

All transfers were performed without anesthesia using ultrasonography by the same reproductive endocrinologist (D. G). Considering patient age and embryo quality and number, either one or two embryos were transferred. Embryos were graded based on their degree of cleavage and amount of fragmentation. Patients were prepared in a lithotomy position with a full bladder. The cervix was visualized using a speculum, and cervical mucus was removed with a sterile sponge. The cervix region was washed using medium from a catheter (Cook Medical, IN, USA). Embryos were loaded into the catheter with the “three-drop technique.” First, an air bubble was loaded into the catheter. Then, 20 mcl of medium was drawn up into the catheter, followed by the embryo(s) in the smallest possible volume of medium. Then, a second air bubble was loaded into the catheter. Finally, enough medium was drawn into the catheter to bring the total volume to 30 mcl. The catheter was moved forward so that the outer part of the catheter was at the level of internal os, and the inner part of the catheter was 1.5–2 cm away from the fundus; then, the embryo was put into the endometrium, and the catheter was slowly removed. Then, the catheter was examined under the microscope by the same embryologist (B.A).

The distance between the embryo and the fundus was measured in the sagittal plane within 1 min of the ET. After 60 min of bed rest which is routine in our clinic, the patient stood up from her bed and lay down for the second ultrasonographic measurement. The distance between the air bubble and the fundus was measured at this time. The transfers were divided into three groups based on the migration of the embryos after ET. Embryos were classified as static if they were within 15 mm of their initial position, if they moved more than 15 mm towards the cervix or more than 15 mm towards the fundus it was classified as cervical and fundal, respectively (Fig. 2). Ultrasonographic measurements were performed by the same doctor (A.Z) by abdominal

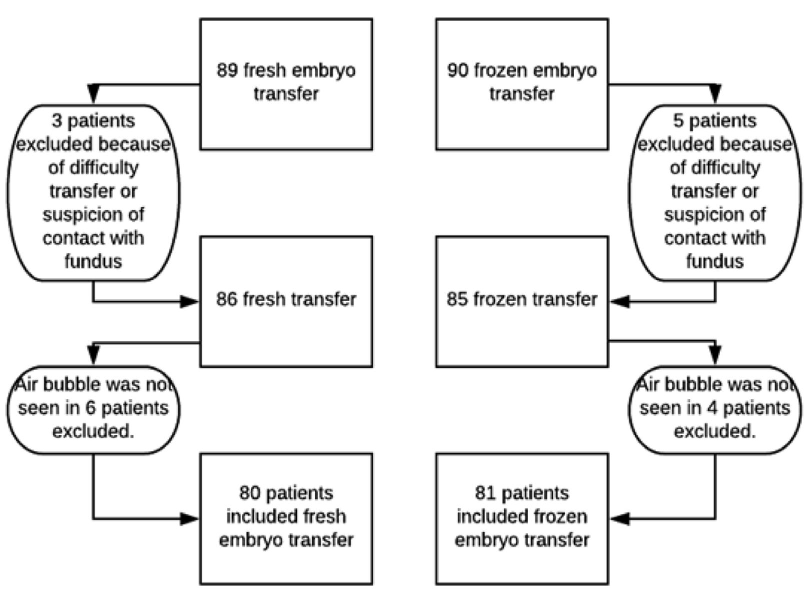

Fig. 1. Flow diagram for participants.

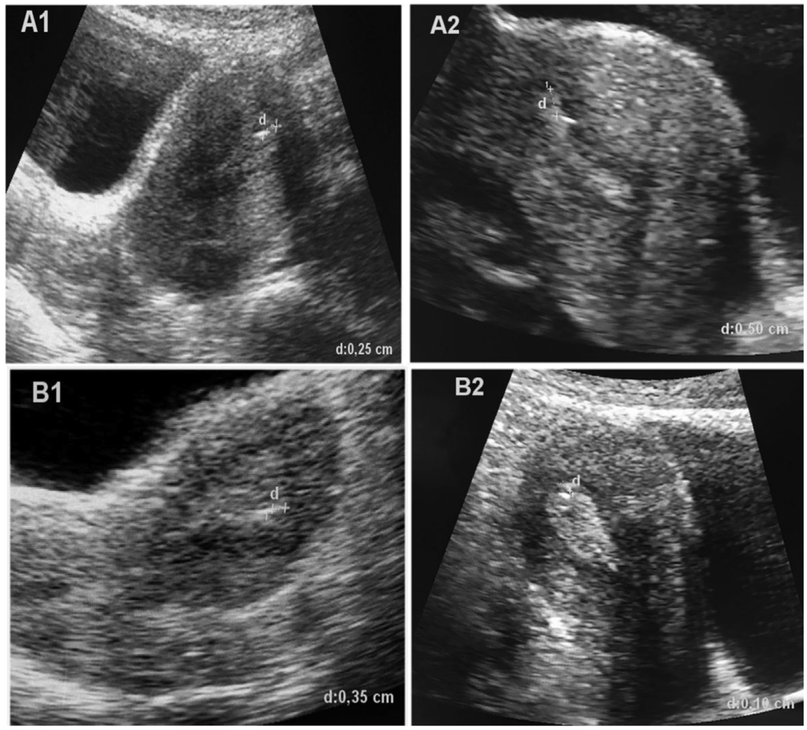

Fig. 2. Embryo flash migration A: Cervical B: Fundal.

ultrasonography (GE LOGIQ P5 Ultrasound Machine). If there was more than one air bubble or the air bubbles dispersed, the air bubble that was closer to the fundus was measured. In some cases embryo flash was not visualized and these patients were excluded from the study.

Visualization of the sac via vaginal ultrasonography was defined as a clinical pregnancy. The follow up period was three weeks after the latest ET, so we did not mention the abortion rates. And there was not any ectopic pregnancy during follow up period.

Statistical analysis

In this study, definitive statistics were expressed as averages, standard deviations, and 95% confidence intervals for continuous

Table 1
Characteristics of patients in pregnant and non-pregnant groups.

		Non-pregnant (n:93)		Pregnant (n:68)		
		Mean	Std. Dev.	Mean	Std. Dev.	*p.
Age		31,33	4,70	30,10	4,47	0,09
Day 2-3 FSH of cycle		8,88	3,37	7,40	2,98	0,004
Maximum E2 level		1896,88	1212,69	2105,35	1033,04	0,25
Number of collected oocytes		11,15	6,29	12,38	5,97	0,21
Endometrial thickness		8,69	1,33	9,06	1,12	0,06
		Non-pregnant (n:93)		Pregnant (n:68)		
		N	N%	N	N%	p
Reason of infertility	Unexplained infertility	61	65,6	53	77,9	0,22
	Poor ovarian reserve	19	20,4	8	11,8	
	Male factor	13	14,0	7	10,3	
Embryo Grade	Grade1	56	60,2	61	89,7	<,001
	Grade2	30	32,3	5	7,4	
	Grade3	7	7,5	2	2,9	
Number of transferred embryo(s)	1	58	62,4	50	73,5	0,13
	2	35	37,6	18	26,5	
Position of embryo	Static	18	19,4	15	22,1	<,001
	Fundal	16	17,2	33	48,5	
	Cervical	59	63,4	20	29,4	
Embryo transfer cycle	Fresh	60	64,5	20	29,4	<,001
	Frozen	33	35,5	48	70,6	

*Fresh transfer was Day 3 embryos, frozen transfer was Day 5 embryos.
$P < 0.05$ significant difference.

(quantitative) variables, or as numbers (N) and percentages (%) for categorical variables. The Kolmogorov-Smirnov test was used to determine whether the averages of continuous variables were normally distributed, and parametric tests were used in cases where the variables had a normal distribution. Student's *t*-test was used to compare continuous variables in fresh and frozen cycles. Logistic regression analysis was used to determine parameters that were thought to be effective on pregnancy outcomes. The Chi-square test was used to determine the variations in categorical data in fresh and frozen cycles. Values of $P < 0.05$ were considered statistically significant, and SPSS (IBM SPSS for Windows, Ver.24) statistics software was used for all calculations. The power calculation is 80 for 95% confidence and 99,77 test power [2].

Results

There were significant differences in embryo position between pregnant and non-pregnant patients ($p < 0.01$). 33 of the pregnant patients (%48.5) were fundal, 20 patients (%29.4) were cervical and 15 patients (%22.1) were static. In non-pregnant patients 59 patients (%63.4) were cervical, 16 patients (%17.2) were fundal and 18 patients (%19.4) were static. There were significant differences in embryo grade between pregnant and non-pregnant patients ($p < 0.01$). There were significant differences in Day 2–3 FSH levels between pregnant and non-pregnant patients (p = 0,004).The differences in the other parameters were not significant ($p > 0.05$) (Table 1).

In fresh embryo transfer group, 20 patients were pregnant and 60 patients were not pregnant. On the other hand in frozen embryo transfers cycle, 48 patients were pregnant and 33 patients were not pregnant. There was a significant difference in pregnancy results between fresh and frozen embryo transfers ($p < 0.01$).

There was a statistically significant difference in embryo flash movements between frozen and fresh transfers ($p < 0.05$). In fresh transfers 48 patients (%60.0) were cervical, 14 patients (%17.5) were static and 18 patients (22.5%) were fundal. In frozen embryo transfers 31 patients (38.3%) were cervical, 31 patients (38.3%) were fundal and 19 (23.5%) patients were static (Table 2).

In frozen transfers there was not any significant difference in embryo position between pregnant and non-pregnant groups. But in fresh transfers the cervical migration was significantly more in non-pregnant patients ($p < 0.05$).

Considering the logistic regression analysis, cervical position were related to the pregnancy results ($p < 0.05$). The other parameters were not related to the pregnancy results ($p > 0.05$) (Table 3).

Table 2
Embryo flash movements within 60 min of embryo transfer.

			Cycle		Total
			Fresh	Frozen	
Position	Static	N	14	19	33
		%	17.5%	23.5%	20.5%
	Fundal	N	18	31	49
		%	22.5%	38.3%	30.4%
	Cervical	N	48	31	79
		%	60.0%	38.3%	49.1%
Total		N	80	81	161
		%	100.0%	100.0%	100.0%

Chi-square = 7.859.
p = 0.02.
* Fresh transfer was Day 3 embryos, frozen transfer was Day 5 embryos.
$P < 0.05$ significant difference.

Discussion

To our knowledge, there are no studies comparing embryo flash movement in frozen and fresh cycles. Although all of the transfers in our study were performed by the same reproductive endocrinologist, there was less frequent cervical migration of embryos in frozen cycles than in fresh cycles. Fundal migration and static embryos were more frequently observed in frozen cycles (Table 4).

There are several published studies on the movement of embryos following embryo transfer. Using 3D ultrasonography, Baba et al. demonstrated that the implantation region of gestation was the initial area of embryo transfer in 81% of patients [8]. However, the study by Confino et al. revealed that only 11% of embryos were static following embryo transfer [9].

Previously, it was thought that embryo movement was due to physiological uterine contractility, rather than gravity [9]. It was shown that this contractility negatively affected pregnancy outcomes [10,11].

It has been hypothesized that uterine contractility can be affected by many parameters during transfer. Some of these parameters include injection speed, amount of liquid in the catheter, unrecognized endometrial trauma, catheter located close to the fundus, increased process time, and fast removal of catheter. In our current study, it is important to note that all transfers were performed by the same reproductive endocrinologist in order to minimize the variability of these parameters.

In 2001, Fanchin et al. evaluated uterine contractility on the day when hCG was given, at day 4 and day 7 of embryo transfer in 43 patients undergoing IVF treatment due to unexplained infertility [12]. Results of that study indicated that uterine contractility was only moderately lower in patients who had non-cavitary embryo transfer at day 4 compared with the day hCG was given. On the other hand, uterine contractility was significantly lower in patients who had blastocyst transfer at day 7 compared with the day hCG was given. The authors hypothesized that low uterine contractility was one of the reasons for high pregnancy rates in blastocyst transfer. Results of our current study support these findings, in that our study revealed that there were a greater number of embryos with cervical migration in fresh transfers performed 3 days after hCG. We found that cervical migration is lower in frozen transfers than in fresh transfers. This result may be related with the day of

Table 3
Embryo flash movements in pregnant and non-pregnant groups of Fresh and Frozen cycles.

		Fresh Embryo Transfers					Frozen Embryo Transfers				
		Non-pregnant		Pregnant			Non-pregnant		Pregnant		
		Mean	Std. Dev.	Mean	Std. Dev.	p	Mean	Std. Dev.	Mean	Std. Dev.	P*
Position of the embryo	Static	10	16.7	4	20.0		8	24.2	11	22.9	
	Fundal	8	13.3	10	50.0	0,001	8	24.2	23	47.9	0.06
	Cervical	42	70.0	6	30.0		17	51.5	14	29.2	

* $P < 0.05$ significant difference.

Table 4
Impact of categorical variables on pregnancy rates.

	B	*p.	OR	95% CI	
				Lower	Upper
Fresh or Frozen Transfers	−,91	0,33	0,40	,06	2,54
Grade 1	,62	0,49	1,86	,30	11,38
Grade 2	−,82	0,40	0,44	,06	3,01
Number of transferred embryo(s)	,05	0,90	1,05	,43	2,54
Fundal migration embryos	,85	0,07	2,35	,93	5,96
Cervical migration embryos	1,61	0,001	5,02	2,08	12,12
Constant	−1,30	0,23	0,27		

Logistic Regression; OR: Odds Ratio; CI: Confidence Interval*.
* $P < 0.05$ significant difference.

embryo or the preparation of endometrium in fresh or frozen cycles. So we routinely cryopreserved day 5 embryos and transferred day 3 embryos.

We believe that the decreased cervical movement of embryos seen in frozen cycles compared with fresh cycles is most likely due to lower estrogen levels and lower contractility in frozen cycles. However, previous studies on this topic have conflicting results. Some studies have shown that supraphysiological estrogen levels in stimulated cycles increased uterine contractility [13], while on the other hand, in 2000, Fanchin et al. [14] showed that uterine contractions measured at the day hCG were not different compared than those measured in normal cycles.

There are some limitations in our study; as the Day 3 embryos were transferred in the fresh cycle, the frozen transferred embryos were Day 5. The cervical movement was more in fresh transfers than frozen transfers. Is that result due to the day of embryo transfer or the type of cycle? So we will design a study to evaluate the effect of cycle for day 5 embryos prospectively. And a big study for a long following period is designed by us to compare the abortion or ectopic pregnancy rates.

Previous studies have shown that pregnancy rates were low for embryos with cervical migration [2,4,7]. However, all of these studies were performed in fresh cycles. In our current study, we also observed that embryo flash movement was effective in pregnancy outcomes in fresh transfers, which we performed as described in the literature. According to our results in fresh cycles the pregnancy is lower in the cervical migration group however more embryos transferred. So we think that in fresh cycles embryo flash movement is more important than number of transferred embryos. However, we observed that embryo flash movement did not significantly affect pregnancy outcomes in frozen cycles. This difference in results may be due the low number of our patient population.

Conclusions

Our current study revealed a significant difference between embryo flash migrations in frozen embryo transfer compared with fresh embryo transfer. There is a need for randomized, controlled studies with a higher number of patients in order to more fully compare embryo flash migrations in fresh and frozen cycles.

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