

# Fresh versus frozen blastocyst transfer outcomes derived from the same ICSI cycle in male factor infertility

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DOI: [10.31083/j.ceog.2021.02.2317](https://doi.org/10.31083/j.ceog.2021.02.2317)

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Submitted: 07 October 2020 Revised: 10 December 2020 Accepted: 16 December 2020 Published: 15 April 2021

The aim of this study was to investigate the clinical outcomes of fresh and frozen-thawed blastocyst transfer derived from the same ICSI cycle because of male infertility. 2372 couples undergoing a total of 5075 ICSI cycles (2372 fresh + 2703 frozen embryo transfer) were enrolled in the study. Female factor infertility cases were excluded to prevent from the contribution of maternal factors that would influence the outcomes dramatically. Only the cycles for which day 5 blastocyst transfer was performed, had at least 1 surplus blastocyst available for freezing, and had at least one frozen embryo transfer (FET) cycle were included in the study. Clinical pregnancy rates and live birth rates were compared between fresh and FET cycles. No statistically significant difference was obtained in clinical pregnancy rates and live birth rates of fresh versus FET cycles of day 5 transfers, regardless of the number of embryos transferred. According to the results of the recent study, frozen embryo cycles appear to be as effective as fresh ICSI cycles provided that the patients' embryos can develop into blastocysts, which is proven by using the same patient as the control group regardless of the result of previous ICSI attempt but further research is required to test the efficacy in terms of cost-effectiveness and the duration for conception.

## Keywords

Frozen embryo; Blastocyst; ICSI; FET

## 1. Introduction

Since the introduction of freezing techniques in assisted reproduction technologies (ART), cryopreservation of human embryos has been a reliable tool that enables the use of surplus embryos for future use, thereby permitting fertility preservation over an extended period [1]. Freezing protocols have improved dramatically, from 'slow-freezing' with limited survival and success rates to the current 'vitrification', a rapid freezing method with very high survival and success rates. Improvement in freezing techniques, media and protocols used for preparation of frozen embryo transfers (FET) have increased the survival rates of frozen embryos significantly. As a result, FET cycles have become a promising option with success and live birth rates comparable to those of fresh cycles [2–4]. Improved success rates following FET have enabled 'elective single embryo transfer' (eSET) in a fresh ART cycle, primarily to prevent multiple

pregnancies [5–7], which were shown to be associated with numerous complications [8]. Single embryo transfers (SET) followed by one or more FET cycles have reportedly yielded higher cumulative pregnancy rates (per oocyte retrieval) [9–11]. Based on these research, a new legislation enabling elective single/double embryo transfer (eSET-eDET) that limits the number of transferred embryos up to two was introduced in 2010 in Turkey, in order to reduce the rate of multiple pregnancies. This protocol has enabled us to perform elective and limited embryo transfers, resulting in a significant increase in the number of embryos cryopreserved and FET cycles without a decrease in pregnancy rates [12–14]. Nevertheless, controversy continues regarding the benefits, efficacy and cost effectiveness of the technique as well as the suitable patient population. The aim of this study was to investigate the clinical outcomes of fresh and frozen-thawed blastocyst transfer derived from the same ICSI cycle because of male infertility.

## 2. Materials and methods

### 2.1 Study design

This retrospective observational study analyzed 2372 couples undergoing a total of 5075 ICSI cycles (2372 fresh + 2703 frozen embryo transfer) in the IVF center of Medicana Camlica Hospital between March 2010 and June 2018. Only the first ICSI attempts were included for fresh transfers. IVF cycles were excluded in order to prevent the possible contribution of natural selection mechanisms that are not bypassed as they are in ICSI cycles and that may dramatically influence the parameters in ART cycles.

Patients with male factor infertility (oligoasthenoteratozoospermia according to WHO, 2010 criteria) [15] who undertook fresh embryo transfer on day 5, had a minimum of 1 embryo left for freezing, and who had at least one frozen embryo transfer cycle in the study. Female factor infertility cases were excluded to eliminate the possible contribution of maternal factors on the outcome. ICSI cycles with preimplantation genetic diagnosis, assisted hatching, total fertilization failure, no embryo transfer (regardless of the reason), and coasting were also excluded from the study.

Patients were informed about the study and written informed consent forms were obtained from each couple. The study was approved by the Ethical Review Board of the Istanbul Medipol University ethics committee.

### 2.2 Ovarian stimulation and embryo manipulation

Ovarian stimulation was carried out as previously described. Then, gonadotrophin-releasing hormone (GnRH) analog suppression or antagonist protocols and human menopausal gonadotrophin or recombinant FSH were used for ovarian stimulation. When the leading follicle reached 17 or 18 mm in size, a human chorionic gonadotrophin (HCG) injection was administered. Oocytes were retrieved 36 h after the HCG injection under transvaginal ultrasound guidance. All the oocytes were performed via ICSI, Vitrolife (Sweden AB) culture media was used in one ART center, and Life-Global (IVFOnline, USA) was used in the remaining two centers for the culture and manipulation of oocytes and embryos.

### 2.3 Embryo transfer

The number of embryos transferred was determined by legal regulations. According to the legislation in Turkey, regardless of the embryo quality and whether the cycle is fresh or frozen-thawed, one embryo transfer is permitted in the first and second ART treatment attempts of patients under thirty-five years; a maximum of two embryos can be transferred in the third or further attempts of this age group, and a maximum of two embryos can be transferred to patients over 35.

Blastocyst grading was done according to Gardner's classification [16] and top-quality blastocysts (5AA) were selected for transfer, if possible. Embryo transfers were performed on day 5 by the guidance of abdominal ultrasound with a Wallace 1816 N (Smiths Medical, USA) catheter. Serum  $\beta$ -hCG concentrations were measured 12 days following the embryo transfer and  $> 50$  mIU/mL was defined as a positive pregnancy. Observation of the gestational sac was defined as a clinically positive pregnancy.

### 2.4 Embryo cryopreservation and thawing

Vitrification was performed for the cryopreservation of embryos and rapid thawing was performed for warming by using Kitazato medium (Oocyte/EmbryoVitrification Kit - VT601-TOP; The VT602 Thawing media) according to the manufacture's recommendations. Embryos were stored individually in hand-made hemi-straws as cryo-tools. If an embryo is not viable then another embryo is thawed. If a patient do not have any viable embryos then the cycle is included in the non-pregnant group.

Clinical pregnancy rates (assessed by the presence of embryonic sac and fetal heartbeat) and live birth rates were compared between fresh and FET cycles regardless of the result of previous ICSI attempt, FET indication and number of embryos transferred.

### 2.5 Endometrial preparation regimen for frozen embryo transfer

The patients undergoing FET cycle were administered 100  $\mu$ g estrogen transdermal (Climara Forte patch; Bayer,

Turkey) on the first day of the cycle. The patch was renewed every other day and the were increased every 4 days. Patients underwent ultrasonographic examination on the 14th day; those with an endometrial thickness of  $> 8$  mm were administered vaginal progesterone (Crinone vaginal gel, Merck Serono) twice a day on day 15. Following 5-day progesterone use, the embryos were thawed and transferred. Subsequently, estrogen and progesterone were continued for 12 days until the  $\beta$ -hCG test and until the 10th–12th week of pregnancy in pregnant women.

### 2.6 Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences for Windows version 20.0 (SPSS, USA). Rates and proportions were evaluated by chi-squared test, and differences between groups were evaluated by *t*-test. The results were evaluated within a 95% confidence interval, and a *P*-value of  $< 0.05$  was defined as statistically significant.

## 3. Results

ICSI outcomes of 2372 couples with 5075 ICSI cycles are shown in Table 1. A total of 2372 fresh cycles and 2703 frozen embryo cycles were performed. Of the 2703 frozen cycles, 2372 were the first FET attempt, 213 were second, 99 were third and 19 were the fourth attempt.

Maternal age, number of embryos transferred, endometrium thickness, embryo qualities of transferred embryos and number of transferred embryos were found similar between the fresh and frozen groups (Table 1).

There was no statistically significant difference in clinical pregnancy rates and live birth rates in fresh and frozen cycles, although a slight decrease was detected for the overall frozen cycles (CPR: 68% and 62.52%; LBR: 48.05% and 45.05%, resp).

Analyzing the patients according to the number of embryos transferred, we found no statistically significant difference between the groups involving 1 and 2 embryo transfers, though there was a slight decrease in frozen cycles. When analyzing the patients according to results of the previous ICSI attempt, no statistically significant difference was obtained for the positive and negative pregnancy groups, although there was a slight increase in frozen cycles of previous positive pregnancy outcome.

## 4. Discussion

A successful frozen-thawed embryo transfer cycle was first reported in 1983 by Trounson and Mohr [17] and was followed shortly after by Zeilmaker in 1984 [18]. In a short timeframe, embryo cryopreservation has become an important method in fertility preservation, following a fresh ART program. A frozen-thawed embryo transfer cycle provides an infertile couple the chance of a less invasive, easier and cheaper method of conception [10]. Until the 2000s, success rates of FET cycles were shown to be not more than 20% [19, 20]. However, advances in cryopreservation techniques and the media used have provided significant improvements, and comparable success rates between fresh and

**Table 1. Comparison of day 5 fresh and frozen embryo transfer cycle parameters.**

	FRESH	FROZEN				P-value	
		Total	First FET	Second FET	Third FET		Fourth FET
Number of ICSI cycles	2372	2703 (3 no ET)	2372	213	99	19	0.12
Maternal Age (years)	31.3 ± 4.0	32.2 ± 6.2					0.24
Baseline BMI	25.2 ± 5.5	24.1 ± 4.0					0.32
E2 (pg/mL)	2261 ± 121	2169 ± 162					0.64
FSH (mIU/mL)	7.6 ± 0.6	7.7 ± 0.7					0.35
AMH (ng/mL)	2.4 ± 0.2	2.3 ± 0.3					0.18
Oocytes collected (n)	12.9 ± 6.2						
Number of Mature oocytes (n) (MII)	9.7 ± 4.2						
Endomet. Thickness (mm)	10.9	11.6	9.9	11.3	10.2	11.4	0.43
Top quality emb rate of trans. emb. (%)	40	34.7	23	42	34	40	0.09
Number of embryos transferred	1.3	1.4	1.6	1.1	1.7	1.2	0.31
Pregnancy rate (%)	68	62.5	58.6	64.1	62.1	65.3	0.24
Live birth rate (%)	48.05	45.05	42.3	42.6	47.2	48.1	0.46

Pregnancy rates and live birth rates were given as mean (%) and maternal age, and number of oocytes collected; number of mature oocytes (MII) were given as mean ± SD.

ET, embryo transfer; FET, frozen embryo transfer.

frozen-thawed cycles [2–4, 11, 21]. One of the most important steps taken towards higher success rates was the introduction of the vitrification procedure which has been correlated with higher survival and pregnancy rates [2–4, 22]. In addition to this technique, the design of more suitable cryopreservation devices has provided easier and safer storage of embryos as well [23]. Improvements in the culture media have further provided better outcomes in FET cycles.

Adoption of the SET policy worldwide accounts for another potential cause of increased success rates following FET cycles due to the increased number of good-quality embryos frozen. In March 2010, legislation limiting the number of transferred embryos to 2 was introduced in Turkey, a landmark for the shift to eSET or eDET strategy. Clinical outcomes of this new policy have been evaluated by several groups including ours, and have revealed unchanged clinical pregnancy rates and significantly reduced multiple pregnancy rates [22–24].

However, debate remains due to the limited number of RCTs comparing fresh versus FET cycles. Most studies report the outcomes of different IVF cycles, ignoring the dynamics of different IVF cycles including drug dosage and type, duration and type of stimulation, over-response and psychological factors. While some authors have reported better pregnancy outcomes in FET cycles compared to fresh cycles [25–27], others have reported better outcomes in normal and high-responders only, in favor of FET in one meta-analysis [28].

In our study, we retrospectively investigated clinical outcomes of a total of 5075 patients who undertook ICSI treatment because of male factor infertility. This is the first study that analyzes the outcomes of fresh and frozen blastocyst transfer cycles that originate from the same ICSI cycle and that exclude numerous drawbacks concerning the effect of in-

dividual variables among different IVF cycles. We found no significant difference in the clinical pregnancy rates between overall fresh and frozen-thawed cycles derived from the same ICSI, although there was a slight decrease in frozen cycles.

When we analyzed the patients according to the number of embryos transferred, no statistically significant difference were found between groups transferring 1 or 2 embryos, although again there was a slight decrease in frozen cycles. This may be due to the difference in mean maternal ages and mature oocyte numbers in two groups that were in the favor of 1 ET patients depicting these patients as having a better prognosis. The new embryo transfer policy restricting the number of transferred embryos to 2 did not appear to affect the outcome in either fresh or frozen cycles.

Data observed in this study will help to analyze the efficacy of fresh or frozen cycles according to the new legislation obtained from the same ICSI cycle in cases of blastocyst transfer. The primary and most important feature of this study is that it compares the outcomes of both fresh and frozen cycles deriving from the same ICSI cycle while excluding interfering parameters of different IVF cycles that may affect the results.

The main limitation of our study was its retrospective nature. We adjusted the statistical analysis for the best interfering factors, however, there were others that could not be eliminated. Further detailed observational studies should be conducted in order to investigate the outcomes of cleavage status transfer and freezing.

Limiting the number of embryos transferred did not affect pregnancy chance of the couples from the same ICSI cycle and the frozen embryo transfers success rates were found to be as effective as fresh transfers. Considering the deleterious effects of multiple pregnancies, transferring fewer embryos while freezing the others and having another chance in subsequent FET cycles with no significant decrease in preg-

nancy and live birth rates appears to be a beneficial strategy. However, FET cycles should be evaluated cautiously for cost-effectiveness, especially in countries whose patients are partially funded or not funded by the government.

## 5. Conclusions

According to the results of the present study, the success of the FET cycle was found to be comparable to fresh cycles when blastocyst transfer could be performed. Further study is necessary to shed light on questions raised by the freezing strategy and to counsel patients facing the issue, including suitability for FET, or fresh transfer; the number and timing of embryo transfer; the number of FET cycles. Besides, cost-effectiveness and time lost should be considered thoroughly by the experts before cementing policy and counselling patients.

## Author contributions

SK and PK developed the theory. SK, PK and İK contributed substantially to the conception of this essay. All authors performed the literature review. İK contributed to the conception, design of the study and interpretation of the data. OK contributed to analysis of the data. All authors contributed to drafting the article and provided critical revision. SK provided final approval of the version to publish.

## Ethics approval and consent to participate

The institutional review board of the İstanbul Medipol University ethics committee approved the study, code 10840098-604.01.01.-E22292.

## Acknowledgment

We thank two anonymous reviewers for excellent criticism of the article.

## Funding

This research received no external funding.

## Conflict of interest

The authors declare no conflict of interest.

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