Higher efficiency of frozen embryo transfer in male infertility cases in *in vitro* fertilization

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ABSTRACT

Objective: The aim of the present study was to analyze the success rates of frozen and fresh embryo transfer methods in different patient groups.

Material and methods: The study included 453 patients who underwent *in vitro* fertilization (IVF) treatment. The patients were further divided into three groups as male factor, tubal/ovarian/uterine factor, and other factors. IVF treatment was performed through either fresh or frozen embryo transfer (FET). Of the 453 patients, 298 had fresh embryo transfer, and 155 received FET. The implantation and live-birth rates of FET were compared with fresh transfer approach, focusing on the effects of male infertility.

Results: There was a significant difference between the pregnancy ratios of patients who underwent fresh embryo transfer versus patients who underwent FET. In patients who were receiving IVF treatment due to male factors, the pregnancy rate was 49.32% in the fresh embryo transfer group, whereas it was 69.70% in the FET group, revealing a significant difference between the two groups (p=0.0321). Although the live-birth ratios were higher in the FET group both among all patients who underwent IVF due to male factor, the differences between the groups were not statistically significant.

Conclusion: We observed higher pregnancy rates in FET patients compared with fresh embryo transfer in the study group. The differences in pregnancy rates and live-birth rates were especially evident in IVF cases where male factor was the reason for the treatment.

Keywords: Fresh embryo transfer; frozen embryo transfer; *in vitro* fertilization; male factor.

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Introduction

In vitro fertilization (IVF) has been an invaluable treatment method for infertility cases since its first use in 1978. As it has been used more and more over the following years, this process was also improved by many technical developments.[1,2] Advances in cryo-preservation methodologies enabled the use of IVF by frozen embryo transfer (FET) more common.[3-5] Studies suggest that FET technique can result in higher rates of implantation and pregnancy.[6,7] FET can also lead to improved live-birth rates.[8-10] FET may be recommended for patients at risk for ovarian hyperstimulation syndrome, patients whose embryos were tested after trophectoderm biopsy, patients with polycystic ovary syndrome (PCOS), and patients with premature elevation of serum progesterone.[6,10,11] The FET approach has been reported to result in better clinical pregnancy rates and lower miscarriage rates than the fresh transfer approach.[12,13] Pregnancy outcomes from FET were associated with lower risks of preterm birth, low birth weight, and perinatal death compared with fresh embryo transfer.[14-17] The implantation rates were higher in frozen embryos than in fresh embryos that are transferred in a retrospective study.[18]

Data do not suggest a total shift to the FET approach, but a personalized, selective approach.[6]

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This requires more information on the success rates of fresh embryo transfer versus FET in different patient groups to identify the patients who would benefit most from either approach.

The rate of male infertility in young men has been reported to increase worldwide.[19,20] In the case of male infertility, development of intracytoplasmic sperm injection technique improved the pregnancy rate in IVF patients with sperms from males with a low sperm count or who require harvesting.[21]

Although there is an increased focus on FET approach and its comparison with fresh transfer, the number of studies on this subject is limited. In addition, there is insufficient knowledge on the use of FET for the IVF treatment of male infertility cases. In the present study, we compared the implantation and live-birth rates of FET compared with fresh transfer approach, focusing on the effects of male infertility.

Material and methods

Study population

The study included 453 patients who underwent IVF treatment between 2014 and 2016. Of the 453 patients, 298 had fresh embryo transfer, and 155 received FET. Patients who underwent the semi-natural cycle or the ultra-long protocol, who were azoospermic and did not have sperm on microTESE, who had immature or low-quality oocytes, and who had mature but unfertilized oocytes were excluded from the study. All 453 patients in the study group were treated with either fresh embryo transfer or FET protocol. The study protocol was reviewed and approved by the Cukurova University Medical School Human Research Ethics Committee (approval no. 71/10). Written informed consent was obtained from each patient who participated in this study.

Fresh embryo transfer

For fresh embryo transfer, 90 mg progesterone gel was applied vaginally on the first day after oocyte pick up. On day 2, 2 mg estradiol, equivalent to 2 mg estradiol hemihydrate, which releases 25 μg in 24 h, was applied transdermally by using a 6.5 cm^2 patch. In addition, daily injections of 50 mg progesterone were performed intramuscularly, whereas 4 mg methyl prednisolone was applied per oral for 12 days after transfer.

Frozen embryo transfer

For the endometrial preparation for FET, patients received 2 mg estradiol 3×1, 100 mg acetyl salicylic acid 1×1, and 400 µg folic acid 1×1 on days 2 or 3 of menstruation. In addition, daily vaginal applications of 90 mg progesterone were applied for 2 days for 2-day embryos, for 3 days for 3-day embryos, for 4 days for 4-day embryos, for 5 days for 5-day embryos, and for 6 days for 6-day embryos right before the transfer day. The endometrial thickness was measured, and the presence of endometrial pattern and ovarian follicular cyst was checked on day 11 of menstruation. Depending on the evaluation of the endometrium, 2-day embryos were transferred on days 15-16, 3-day embryos on days 16-17, 4-day embryos on days 16-18, and 5- and 6-day embryos on days 17-19 of menstruation. In addition, intramuscular daily injections of 50 mg progesterone and per oral application of 4 mg methyl prednisolone were performed for 12 days after transfer.

Statistical analysis

All statistical analyzes were performed by using GraphPad Prism program. Student's t-test or chi-square analyzes were performed for analyzing the difference between the groups. Chi-square test was performed and presented with 95% confidence interval (CI). A p-value of <0.05 was set as statistically significant. All data are presented as average±SD.

Results

The mean age of the patients was 33.39±5.45 (19-45) years. The medical reasons for undergoing IVF treatment were male infertility problems in 30.9%; tubal, ovarian, or uterine problems in 37.09%; and other factors including infertility due to unknown reasons or secondary infertility or IVF due to the presence of hereditary diseases in 32.01% of the patients.

Among patients who underwent fresh embryo transfer, 24.5% presented to the IVF clinic due to male factors, 40.6% presented for tubal, ovarian, or uterine factors, and 34.9% presented due to other factors. The ratio of patients who received FET because of male factor was 43.23%, whereas 30.32% received this treatment for ovarian, tubal, or uterine problems, and 26.45% received it due to other factors (Table 1).

The first pregnancy test was performed 12 days after embryo transfers. Patients who became pregnant were monitored until the

Table 1. The reason of IVF treatment for different patient groups

% diagnosis / Protocol	Male factor (%)	Tubal/ovarian/ uterine factor (%)	Other factors (%)
Fresh embryo transfer	24.50	40.60	34.90
Pregnant	26.09	32.61	41.30
Not pregnant	23.13	47.50	29.38
Frozen embryo transfer	43.23	30.32	26.45
Pregnant	52.87	22.99	24.14
Not pregnant	30.88	39.71	29.41

IVF: *in vitro* fertilization

end of pregnancy by the same clinic at 12 weeks of gestation and during birth. The mean endometrial thicknesses were 10.50±1.49 mm for the fresh embryo transfer group and 10.44±1.19 mm for the FET group, revealing no statistical significance between the two groups (p>0.05). The average numbers of transferred embryos were 1.50±0.50 in the fresh embryo transfer group and 1.47±0.50 in the FET group, revealing no statistically significant difference between the two groups (Table 2).

Pregnancy rate among all patients was 49.69% at 12 days after embryo transfer. When the pregnancy rates of the patient groups were evaluated, 46.31% of patients who received fresh embryo transfer were pregnant at 12 days, whereas 56.13% of the patients in FET were pregnant (Figure 1). The difference between the groups was found to be statistically significant (p=0.0473, odds ratio (OR) 0.6741, 95% CI 0.4562-0.9963).

Since IVF treatment due to male factors constitute a larger fraction of the patient group in the present study compared with other medical reasons, this group was further evaluated in detail. Of the 140 patients who were treated with IVF due to male factors, 73 received fresh embryo transfer, whereas 67 received FET. Among the male factor cases, the rate of pregnancy was 58.6% in total, which was higher than the pregnancy rate of the whole group. When the treatment groups were analyzed individually, the pregnancy rate was 49.32% in the fresh embryo transfer group, whereas it was 69.70% in the FET group, revealing a significant difference between the two groups (p=0.0321, OR 0.4953, 95% CI 0.2595-0.9454).

Table 2. The pregnancy outcomes and clinical information of the study group

Protocol	No. of patients	Mean age±SD	No. of transferred embryos	Endometrial thickness
Fresh embryo transfer	298	33.91±5.69	1.50±0.50	10.50±1.49
Pregnant	138	32.73±5.49	1.47±0.50	10.47±1.36
Not pregnant	160	34.93±5.67	1.52±0.50	10.53±1.61
Frozen embryo transfer	155	32.37±4.82	1.47±0.50	10.44±1.19
Pregnant	87	31.26±4.31	1.46±0.51	10.48±1.53
Not pregnant	68	33.79±5.09	1.5±0.50	10.21±1.15

SD: standard deviation

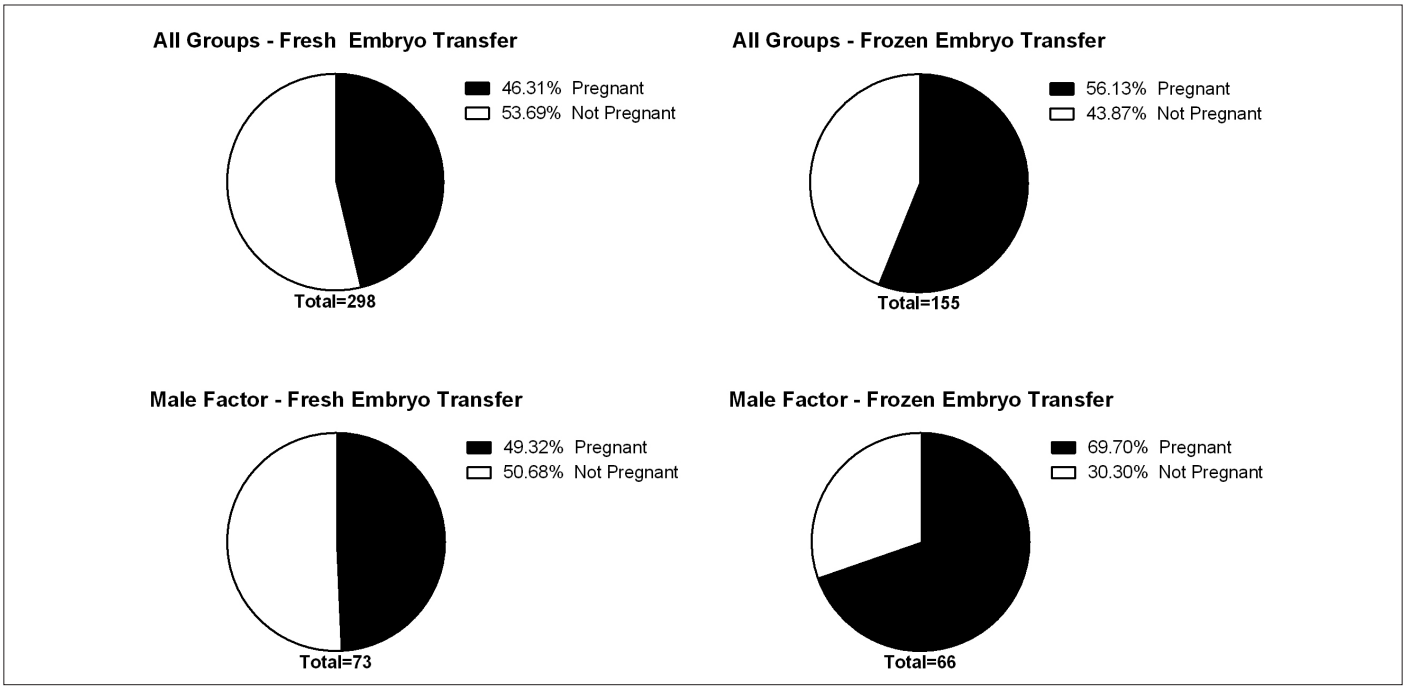

Figure 1. The distribution of pregnant versus not pregnant patients who underwent IVF through either fresh embryo transfer or FET protocols among all patients and among patients who were treated due to male factors

FET: frozen embryo transfer

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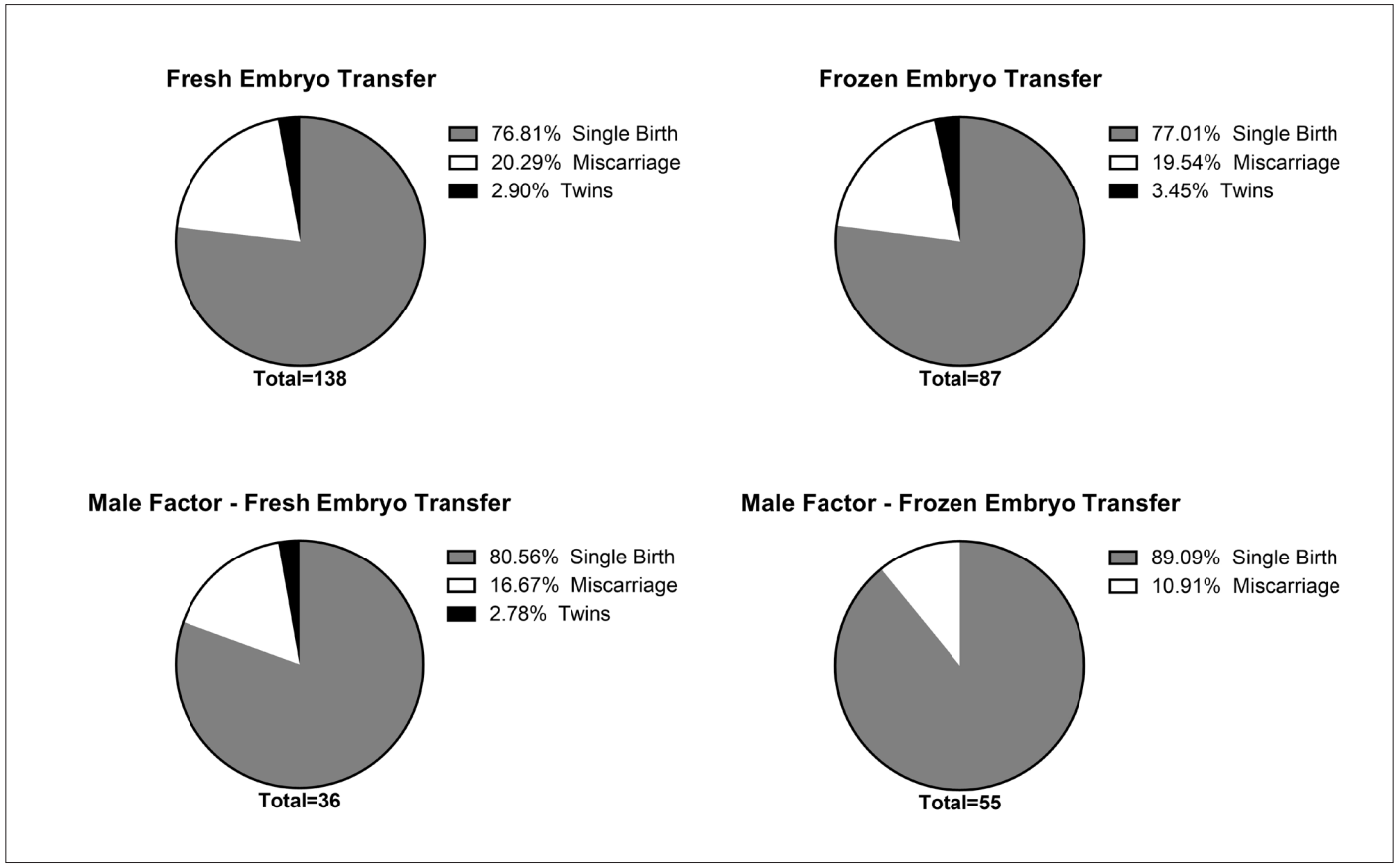

Figure 2. The distribution of single births, miscarriages, and twin births among pregnant women who underwent IVF through either fresh embryo transfer or FET protocols among all patients and among patients who were treated due to male factors
FET: frozen embryo transfer

When the live-birth versus miscarriage ratios were analyzed, there was no statistically significant difference between the fresh embryo transfer and FET groups (p=0.8911, OR 0.9541, 95% CI 0.4867-1.870). However, the percentage of miscarriage was slightly higher in the FET group with a value of 20.29% than in the fresh embryo transfer group with 19.54%. The ratio of twins was also slightly higher in the FET group with a percentage of 3.45% versus 2.9% in the fresh embryo transfer group (Figure 2).

Patients who underwent IVF treatment due to male factor also showed a similar pattern in the analysis of live-birth ratios. The ratio of miscarriage in the fresh embryo transfer group was 16.67%, whereas it was 10.91% in the FET group; however, the difference between the two groups was not statistically significant (p=0.4273, OR 0.6122, 95% CI 0.1808-2.073).

Discussion

In IVF treatments, both fresh embryo transfer and FET are routinely used with varying success rates. The studies that show that FET is more efficient propose that the differences observed between fresh embryo transfer and FET result from the differences in the uterine environments.[3] The medications that are used for ovarian stimulation may alter endometrial receptivity.[22-24] In the frozen transfers, the uterine environment has time to heal after the ovarian stimulation; however, in the fresh transfers, the uterine environment is not completely healed at the time of the transfer.

Strikingly, we observed even larger difference between pregnancy rates of the fresh transfer versus frozen transfer groups among patients who underwent IVF treatment due to male factor. Although pregnancy rates in both the fresh transfer and frozen groups were higher in the male factor cases than in other factors, we observed an even higher increase in the frozen transfer-male factor group. To the best of our knowledge, the difference between fresh embryo transfer and FET in male factor IVF cases has not been studied previously, and our study shows the importance of preference of IVF method for these cases. Similar to previously published studies, our data suggest that a personalized and selective approach is important for selecting the right methodology for IVF treatment for individual cases.[6]

Another reason for the difference between success rates of fresh embryo transfer and FET could be the extra selection step during freezing and thawing of embryos. When the frozen embryos are thawed, even if they survive, they may not resume development. It was observed that even though 100% of the blastocysts that were frozen by vitrification survived the thawing procedure, only 45% resumed development.[25] In the case of slow frozen bipronuclear oocytes, 85.5% of them were viable, whereas only 53.5% formed blastocysts.[26] In our cohort, we also observed higher pregnancy rates in FET cases than those in fresh embryo transfers, and the difference between the groups was statistically significant. It is possible that during these freeze-thaw cycles, the embryos that are stronger survive better than the weaker ones. In addition to the selection of less damaged embryos during freezing, the weaker ones are eliminated, which would explain the increased pregnancy rates in the IVF by freezing.

It was also previously suggested that the freezing process has a therapeutic effect on the embryos.[27] During the thawing process, physical stress may cause membrane and organelle damage, protein denaturation, and change in the physiological environment, including pH. The embryos that are exposed to this stress may change the regulation of genes and adapt to the environment. These may result in reduced reactive oxygen species levels, detoxification of the cell, and reduced mutated mtDNA, which results in more healthy embryos and increases the implantation rate.[27] The results of our study also fit this hypothesis. In the male infertility cases, the benefit of the embryos from the freeze/thaw process may be higher, because the eggs are relatively healthier and can adapt more easily.

Very few studies have compared the effects of the fresh versus frozen approach in the live-birth rate. Chen et al. reported higher live-birth rates in FET even though they showed similar pregnancy rates between the fresh embryo transfer and FET groups in patients with PCOS.[10] They showed a 7.3% increase in live-birth rate in the FET group compared with the fresh embryo transfer group. In our study, although the live-birth rates in the FET group were slightly higher than those in the fresh embryo transfer group with a 0.7% difference, the difference between the groups was not statistically significant. In the male factor cases, the difference between live-birth ratios of the fresh transfer and frozen transfer groups was much larger, approximately 5.8%, while still not statistically significant. This difference might become statistically significant if larger numbers of patients are analyzed.

In conclusion, in a cohort of 453 IVF patients, we observed that FET resulted in higher pregnancy rates than fresh embryo transfer. The difference in pregnancy rates and live-birth rates was especially evident in IVF cases where male factor was the reason for the treatment. Our results suggest that the frozen treatment approach is a better option for male factor patients. More studies with larger cohorts are needed, especially to validate the live-birth results.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Cukurova University (approval no. 71/10).

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

Peer-review: Externally peer-reviewed.

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