Outcome Comparison Fresh versus Frozen Embryo Transfers in Bali Royal Hospital

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Abstract: ***Background**: The aim of this retrospective cohort study was to compare the outcome for the 'electively freeze-all' strategy and those destined for the 'fresh-transfer' strategy preceding subsequent FET. **Methods**: This retrospective single-center study analyzed the cumulative live birth rates (CLBRs) of 159 patients undergoing fresh or electively frozen blastocyst transfer cycles. In 81, cycles, the fresh embryo transfer (ET) strategy was applied for the 1st ET, whereas electively frozen ET (e-FET) was performed in other 78 cycles. The patients in each arm were further stratified into four subgroups according to the number of oocytes retrieved as follows: Group A: 1–5, group B: 6–10, group C: 11–15 and group D: 16–25 oocytes retrieved. The primary endpoint was the CLBR. The secondary endpoints were the live birth rates (LBRs), outcome in pregnancy such as IntraUterus Fetal Death, abortus, and hyperstimulation. **Results**: The CLBRs were similar between the fresh-transfer and e-FET arms in group 1–5 oocytes retrieved (2/7 [28.6%] vs 4/11 [36, 4%], p = 0.722), group 11–15 oocytes retrieved (6/12 [50%] vs 7/14 [50%], p=0, 440) and group 16–25 oocytes retrieved (8/13 [61, 5%] vs 6/9 [66, 7%], p=0, 362). However, in group 6–10 oocytes retrieved there is significant difference between two group (11/18 [61, 1%] vs 7/16 [43, 7%], p = 0.049). The perinatal outcome of Intra Uterine Fetal Death, abortus, and overstimulation were shown to be not significantly different between fresh ET and e-FET group. **Conclusion**: Compared to the fresh transfer strategy, the e-FET strategy resulted in higher CLBR among patients and the number of 6-10 oocytes collected during the cycle resulted in a better CLBR in the e-FET compared to fresh.***

Keywords: frozen embryo transfer, fresh embryo transfer, outcome

1. Introduction

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles coupled with frozen embryo transfer (FET) have been increasingly performed worldwide. Major factors contributing to this trend are improvements in extended culture conditions and the implementation of vitrification techniques with excellent survival rates. [1] Ovarian stimulation with the use of a gonadotropin realizing hormone (GnRH) antagonist protocol, which includes triggering with a GnRH agonist, elective cryopreservation of all embryos and FET in a subsequent cycle, namely, the 'freeze-all' concept with segmentation of IVF/ICSI treatment, has therefore been increasingly implemented in recent years. This concept originally emerged to eliminate the risk of ovarian hyperstimulation syndrome (OHSS).

A preliminary analysis of clinical studies demonstrated the strengths of the freeze-all concept, including increased maternal safety, improved pregnancy rates, decreased ectopic pregnancy rates and better obstetrical and perinatal outcomes. [2] However, emerging evidence supported an increased risk of hypertensive disorders of pregnancy in FET cycles compared with fresh embryo transfers. [3] Other adverse obstetrical and perinatal outcomes, including postpartum hemorrhage and macrosomia were reported to be increased in FET. The second reason for the evolution of the freeze-all strategy is the negative impact of controlled ovarian stimulation (COS), which leads to supraphysiological estradiol (E2) and progesterone (P) levels, on endometrial receptivity. [4] Many molecular and histological studies supported the detrimental effect of COS on endometrial receptivity. [5] However, clinical studies validating the positive pregnancy, obstetric and perinatal outcomes of freeze-all cycles in IVF/ICSI treatments are limited.

First randomized control trial (RCT) on freeze-all strategy indicated positive clinical pregnancy outcome in normal and hyper responders. [6] However, they had several methodological insufficiencies and the number of patients included in these studies were limited. In addition, in a retrospective cohort study it was reported that women with prior implantation failure, a freeze-all cycle had statistically significantly higher live birth rates than fresh cycle. [7] In an RCT of women with polycystic ovarian syndrome (PCOS), Chen et al. reported that the freeze-all strategy increases live birth rates (LBRs). [8] In an RCT of blastocyst transfer, Wei et al. also found the same result following blastocyst-rather than cleavage-stage transfers and advocated that blastocyst-stage transfer during an FET cycle mimics natural conception better than cleavage-stage transfer. [9] The other two RCTs did not demonstrate a significant difference between freeze-all and fresh transfer cycles. [10] Better embryo selection by extended culture up to the blastocyst stage, the better survival rates of blastocysts after thawing compared to cleavage-stage embryos and the different effects of the vitrification process on intracellular dynamics may explain the different results of the RCTs. [11] The connection between oocyte yield and the freeze-all strategy was reported in two population-based retrospective analyses. [12]

Observational studies and small randomized, controlled trials have shown higher pregnancy rates and better perinatal outcomes with frozen-embryo transfer than with fresh-embryo transfer. It has been hypothesized that frozen-embryo transfer may provide a more favorable intrauterine environment for embryo implantation and placentation by avoiding the supraphysiologic condition that occurs after ovarian stimulation. [5]The aim of this retrospective cohort study was to compare the outcome for the 'electively freeze-all' strategy and those destined for the 'fresh-transfer' strategy preceding subsequent FET.

2. Methods

Study population and design

This was a retrospective, single-center cohort study including all women who underwent ICSI at our center between January 2015 and December 2019. Electively frozen embryo transfer (e-FET) is a common policy in our clinic and it is offered to our patients as an option (e-FET arm). The patients who don't accept this opt undergo fresh embryo transfer (fresh ET arm). This retrospective study was approved by the *Institutional Review Board of BahceciFulya IVF Center* with a reference number of 38. The computer-based data was analyzed by the permission of ethical committee. A total of 300patient was included in this study. When the data set was initially filtered by the exclusion criteria 1) women aged >39 years (n = 37) 2) body mass index (BMI) >30 kg/m2 (n = 83) 3) missing data and loss of follow up (n = 20). At the end, a total of 159 patients, including 81 patients who underwent fresh ET and 78 patients who underwent e-FET were analyzed.

Main outcome measures

The primary outcome of this study was the cumulative live birth rate (CLBR). The CLBR for the fresh-transfer strategy was defined as the number of live births deriving from fresh and frozen/warmed embryos obtained during a single ovarian stimulation cycle following fresh and frozen cycles in the same woman (the percentage of deliveries with at least one baby born from conception within 2 years after the first ET). The CLBR for the freeze-all strategy was defined as the number of live births deriving from frozen/warmed ETs obtained during a single stimulation cycle in the same women (the percentage of deliveries with at least one baby born from conception within 2 years after the first ET). The secondary outcomes were the live birth rate (LBRs) (the percentage of patients with a live birth), and problem within pregnancy (including causes of unborn fetus and hypertension in pregnancy).

Statistical analysis

The women were grouped by cycle type based on whether they had undergone the freeze-all strategy, where no embryos were transferred in the stimulated cycle and all resulting embryos were cryopreserved for transfer in subsequent cycles, or the fresh-transfer strategy, where the morphologically best embryo were transferred in the stimulated cycle and the remaining embryos were cryopreserved for future use. The Kolmogorov-Smirnov test was performed on continuous parameters to test whether the continuous variables followed a normal distribution, which revealed that the continuous parameters did not follow a normal distribution. The independent mean test was used to test whether the median values of the continuous parameters were significantly different between the fresh ET and e-FET arms. The chi-squared test was used to compare patient and embryological characteristics and LBRs between the two arms in all subgroups. To determine which factors affected the cumulative pregnancy outcome in all subgroups, binary logistic regression models were evaluated and are reported. The outcome of the models was whether a patient achieved live birth. The independent factors imputed in the models at the initial step were patient age, BMI, menstrual cycle, reason for infertility and the causes of unborn fetus.

3. Result

A total of 159 patients, including 81 patients who underwent fresh ET (50, 9%) and 78 patients who underwent e-FET (49, 1%), were analyzed (Fig 1). The patients' baseline characteristics in the fresh ET and e-FET arms are presented in Table 1. Patient age, BMI, and type of infertility, time infertility, and number of oocytes were not significantly different between the fresh ET and e-FET. This indicates that the two groups have the same characteristics so that it does not have much effect on the results of the research analysis.

All data in the form of continuous data were tested for normality (data not shown) to assess whether all data were normally distributed or not. Based on the results of the normality test, all data were not normally distributed ($p < 0.05$) except for the maternal age data ($p = 0.448$). This resulted in a comparison of the averages carried out using non-parametric tests on the two groups. The results of p value based on comparison of the two groups are also shown in Table 1.

Based on the data obtained, more patients experienced primary infertility in the second group, namely 56.4% in the freeze group and 60.5% in the fresh group. There was no significant difference between the number of oocytes taken, the number of mature oocytes and the embryo yield obtained in the second group. But the results obtained were significant between the number of embryos that were successfully transferred between the 2 groups and the number of frozen groups was higher than the fresh group. In both groups, significant results were also found in the embryos born with a higher percentage in the frozen group than in the fresh group (84.6% vs.70.4%; p=0.038).

Table 1: Demographic characteristic between freeze and fresh group

Characteristics	Freeze-all group (n=78)	Fresh transfer group (n=81)	p value
Age (mean, range)	32.90, 21-45	32.89, 24-42	0, 978
Body mass index (mean, range)	22.81, 17.3-33.7	22.72, 16.5-31.2	0, 914
Type infertility			0, 632
Primary	44/78 (56, 4%)	49/81 (60, 5%)	0, 052
Secondary	34/78 (43, 6%)	32/81 (39, 5%)	0, 062
Time infertility (years mean)	4, 32	5, 10	0, 179
Number of oocytes (mean)	11, 95	12.11	0, 932
1 – 5	4/78 (5, 1%)	6/81 (7, 4%)	0, 163
6 – 10	27/78 (34, 6%)	27/81 (33, 3%)	0, 069

11 – 15 16 – 25	28/78 (35, 9%) 19/78 (24, 4%)	30/81 (37, 0%) 18/81 (22, 2%)	0, 066 0, 083
Mature oocytes (mean)	9.71	9.63	0, 935
Embryo obtained (mean)	7, 78	7, 36	0, 625
Embryo transfer (mean)	2, 45	1, 81	<0, 001
Embryo born	66/78 (84, 6%)	57/81 (70, 4%)	0, 038

The CLBRs by the number of oocytes retrieved during the stimulated cycle are shown in Table 2. The CLBRs were similar between the fresh-transfer and e-FET arms in group 1–5 oocytes retrieved (5/6 [83.3%] vs 4/4 [100, 0%], p = 0.163), group 11–15 oocytes retrieved (19/30 [63, 3%] vs 24/28 [85, 7%], p=0, 066) and group 16–25 oocytes retrieved (16/18 [88, 9%] vs 15/19 [78, 9%], p=0, 083). However, in group6–10 oocytes retrieved there is significant difference between two group (17/27 [62, 9%] vs 23/27 [85, 2%], p = 0.049).

Table 2: CLBR by the number of oocytes retrieved

	Fresh ET			e-FET			p-value
No of oocytes retrieved	No of woman	No of live birth	CLBRs	No of woman	No of live birth	CLBRs	
1 – 5	6	5	83, 3%	4	4	100%	0, 163
6 – 10	27	17	62, 9%	27	23	85, 2%	0, 049
11 – 15	30	19	63, 3%	28	24	85, 7%	0, 066
16 – 25	18	16	88, 9%	19	15	78, 9%	0, 083
Total	81	57	70, 4%	78	66	84, 6%	0, 038

The outcome comparisons are presented in Table 3. In both groups, a significant result was found between abortion results and the fresh group was more likely to have an abortion than the freeze group (p=<0.001). The fresh group was also found to be more likely to experience non-viable embryos than the frozen group (p=0.045). Other results, namely infant mortality in the womb and the incidence of Blighted Ovum (BO) were not found to be significant differences between the two groups.

Table 3: Comparison of outcome in e-FET and fresh ET groups

Outcome	Freeze all group	Fresh transfer group	p value
LBRs	66 (84, 6%)	57 (70, 4%)	0, 038
IUFD	4 (5, 1%)	1 (1, 2%)	0, 200
Abortus	0 (0%)	6 (7, 4%)	<0, 001
Blighted ovum	6 (7, 7%)	5 (6, 2%)	0, 157
Not viable	2 (2, 6%)	12 (14, 8%)	0, 045

4. Discussion

The cumulative live birth rate (CLBR) is the preferred measure of success for IVF/ICSI procedures and allows us to better understand the true efficacy of the treatment. Based on this study, it was known that the frozen or frozen group had a higher cumulative probability of live births than the fresh group and was statistically significant (84.6% versus 70.6%; p value = 0.038). Based on a more specific analysis, it was found that the oocyte count group 6-10 had a significant CLBR ratio where the frozen group was better than the fresh group (85.2% versus 62.9%; p value = 0.049).

The first meta-analysis comparing fresh ET and e-FET was published in 2013 and included3 RCTs analyzing normal responders and hyper-responders, and the RCTs reported higher clinical pregnancy rates in favor of e-FET. [13] In 2017, the Cochrane Library reported a meta-analysis including 1892 patients. [14]Unfortunately, this analysis did not include any trial with a poor response. The LBR after the first ET for all embryo stages (cleavage-stage embryos and blastocysts) was higher in the freeze-all group than that in the fresh ET group. However, no difference was observed between the freeze-all and fresh ET cycles regarding the CLBR per patient as the primary outcome (OR = 1.09, 95% CI: 0.91–1.31). This meta-analysis included four parallel-design RCTs. In three of these studies, cumulative results were not reported, but the authors of this study may be able to obtain these data through personal communication with the authors of the original articles.

Theoretically, the freeze-all strategy is likely superior to the fresh-transfer strategy for the first ET. Using the 'best embryo' in a more receptive endometrium may have an effect on the CLBR outcome. In previous study, the LBR after the first single blastocyst transfer was comparable in patients from whom fewer than11 oocytes were collected for both strategies. Conversely, the LBR after the first single blastocyst transfer showed significantly lower results in the fresh ET arm based on the increased number of oocytes retrieved (>11), which was considered to reflect the first transfer effect because in the second and third ET cycles, no statistically significant differences were found between the fresh strategy and e-FET strategy. [15]

Although some observational studies have compared fresh ET and freeze-all strategies, no RCTs have evaluated the effects of the e-FET strategy on poor responders. [15] Contrary to this finding, in 2017, Berkkannoglu et al. compared fresh ET and freeze-all strategies in poor responders (≤4 oocytes) with both cleavage-and blastocyst-stage embryos and reported improved pregnancy outcomes from blastocyst transfers, thus favoring the freeze-all strategy. [16]

In a different point of view, Acharya et al. evaluated US national data by dividing patients into cohorts based upon the number of oocytes retrieved: high (>15), intermediate (6–14), and low responders (1–5). [12] In low responders, the LBR was higher for the fresh transfers (25.9%) than that for FETs (11.5%) (p<0.001). These findings relate with our study. The authors declared that they were unableto distinguish which embryos were electively frozen due to patient and clinical preferences and which embryos were frozen for premature luteinization, which is associated with a poorprognosis.

Although the number of patients is under power, in the poor ovarian response group (<6 oocytes retrieved) we concluded that the LBRs for the first ET and CLBRs were not significantly different for fresh ET and e-FET. The main rationale for e-FET is the altered endometrial receptivity, which is more commonly observed in hyper-responders whose uterine environment is exposed to supraphysiological hormonal levels. [15] The hormonal levels after gonadotropin stimulation in poor responders are closer to physiological levels, which may explain the comparable pregnancy outcomes.

The studies evaluating the LBRs for fresh ET reported that the LBR fails to increase when more than 10–11 oocytes are retrieved. [16] Moreover, the LBR decreases when >15 oocytes are retrieved, which is an indirect indicator of impaired endometrial receptivity. [16] The available RCTs comparing fresh ET versus freeze-all policy included patients with both a goodprognosis and patients with a good ovarian response (normal and high responders). Chen et al. included cleavage stage embryo transfers of PCOS patients in their study and reported significantly higher LBR in FET arm compared to fresh ET arm but failed to show any statistical significance with respect to CLBRs.

Shi et al. conducted their study on normo-responder patients and compared both transfer strategies following cleavage stage embryo transfers. [9] Pregnancy outcomes after the first transfer attempts were comparable between groups. The data did not report CLBR calculation. In a similar fashion, Vuong et al. declared comparable pregnancy outcomes following cleavage stage transfers in fresh ET and e-FET groups. [10]In addition, CLBRs were also not significant at the end of the 12months follow-up period.

Similarly, a US national study evaluated the results of intermediate responders with6–15 oocytes retrieved. However, in a retrospective analysis, Roque et al. (2017) divided normal responders into two subgroups: with 4–9 oocytes retrieved and with 10–15 oocytes retrieved. [17] They concluded that patients with 4–9 oocytes retrieved had a similar ongoing pregnancy rate in the freeze-all group. In our study, we found that the e-FET strategy is beneficial for LBRs in the first transfer as well as CLBRs in patients with >10 oocytes retrieved whereas the LBR and CLBRs in patients with 6–10 oocytes retrieved were similar in fresh transfer and e-FET group. In a parallel with the poor responders, in this group uterine environment is suggested to be less effected.

5. Conclusion

Compared with a fresh-transfer strategy, the e-FET strategy results in a higher CLBR among patients and the number of6-10 oocytes collected during the cycle resulted in a better CLBR in the e-FET compared to fresh. In both groups, there were significant results between abortion results and the fresh group was more likely to have an abortion than the freeze group (p=<0.001).

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