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Comparison of Pregnancy and Neonatal Outcomes in Frozen and Fresh Embryo Transfer in ART Cycles: A Retrospective Cohort Study

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ABSTRACT

Background & aim: In addition to the benefits of frozen embryo transfer (FET), the key question is whether freezing or melting embryos can cause fetal harm and prenatal complications. This study aimed to assess pregnancy and neonatal outcomes after FET and fresh embryo transfer (ET).

Methods: This retrospective cohort study investigated the pregnancy outcomes of infertile women undergoing intracytoplasmic sperm injection with FET and ET in Kowsar Infertility Center of Motahary hospital in Urmia, Iran between March 2014 and March 2016. A questionnaire was completed based on the hospital records of pregnancy or neonatal outcomes. To assess the continuation of pregnancy and delivery, the questionnaires were completed through phone call. The pregnancy and neonatal outcomes were compared between FET (n=96) and fresh ET (n=93) using Student's t-test, Chi-square, Fisher's exact tests and multiple logistic regression using SPSS software (version 21.0).

Results: The rate of ongoing pregnancy were reported as 60.2% and 76% in the FET and fresh ET groups, respectively. The rate of multiple pregnancy and ongoing pregnancy were lower in FET, compared to those reported for fresh ET (OR=0.38, 95% CI: 0.18-0.79, P=0.01; OR=0.48, 95% CI: 0.25-0.85, P=0.02). The frequency of spontaneous abortion was not different between two groups (P=0.07). The FET and fresh ET increased the odds of lower neonatal weight; however, it was not statistically significant.

Conclusion: In this study, a lower rate of ongoing pregnancy was reported in the FET group. It seems that FET can cause damage to the embryo during freezing and melting.

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Introduction

The first live birth after frozen embryo transfer (FET) was reported in 1984, and this method has been gradually used in assisted reproductive technology (ART) (1). One of the most important in vitro fertilization (IVF) programs is embryo cryopreservation; however, there are limited studies on the evaluation of the obstetric and neonatal outcomes of neonates born after the FET cycle (2–7). The incidence rates of prematurity, low birth weight (LBW), and neonatal mortality were matched in two FET and fresh embryo transfer (ET) groups (5). Decreasing the risk of multiple pregnancy and hyperstimulation syndrome and increasing the use of single embryo transfer in the FET cycle and pregnancy rates were reported in multiple

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studies (8). In addition, the rate of major abnormalities in the neonates born after FET is similar to those of fresh ET and in children after spontaneous conception (10–12).

The prevalence of abortion in FET is higher than that reported for fresh ET (13) which is in line with the results of a study carried out by Liu et al. (14). Furthermore, the FET method was reported with higher safety and cost-effectiveness and better outcomes for neonates than the ET method; nevertheless, the reasons for better outcomes are not clear (15). In the FET cycle, because ovarian stimulation is not used, the estrogen levels remain according to the physiology of the body; therefore, high estrogenic effects on endometrial and implantation will not be observed (16). Moreover, the endometrial arrangement may be achieved using estradiol and progestrone, and the development of the endometrium can be controlled more accurately than the controlled ovarian hyperstimulation (COH) cycles with gonadotropins (17, 18).

In addition to the benefits of FET, the key question is whether freezing or melting these embryos can cause fetal harm and prenatal complications in pregnancy. In addition, reducing the rate of cooling injury can increase the rate of embryo survival (19, 20). Some studies have shown the increased risk of ovarian hyperstimulation syndrome in the ET cycle (21–23). Therefore, according to different results of studies on FET and ET (10-13), the present study aimed to compare the pregnancy and neonatal outcomes of the two FET and ET groups.

Materials and Methods

This retrospective cohort study was carried out on 250 women after the transfer of embryo (i.e., fresh or frozen) in Kowsar Infertility Center of Motahary hospital in Urmia, Iran, within March 2014 to March 2016. The subjects with the age of over 40 years, history of abortion, premature labor, uterine anomalies, underlying medical conditions (e.g., chronic blood pressure, diabetes, and lupus) were excluded from the study. Then, all of the included participants were followed up to the end of pregnancy outcomes. The participants were divided into two groups of FET (n=96) and ET (n=93).

In all patients, ovarian stimulation was performed using recombinant or urinary follicle-stimulating hormone or human menopausal gonadotrophin combination with the gonadotropin-releasing hormone agonist. Two or four embryos with the best morphology were selected and transferred based on (19) the criteria of excellent or good quality. The vitrification method was used in the 4-8 cell stage for freezing the embryo.

A questionnaire was completed based on hospital records about pregnancy or neonatal outcomes. To determine the continuation of pregnancy and delivery, the questionnaires were completed through phone calls. This study was approved by the Ethics Committee of Urmia University of Medical Sciences (IR.UMSU.rec.1395.207). The outcome variables included ongoing pregnancy, ectopic pregnancy, abortion, preterm labor (<34 weeks), neonatal birth weight, intrauterine fetal demise (IUFD), multiple pregnancy, congenital malformation, and live birth.

Differences between fresh FET and ET variables were analyzed using the Student's t-test for normally distributed continuous variables and Chi-square and Fisher's exact tests for categorical variables. Multiple logistic regression was used for binary outcomes. A p-value of less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS software (version 21.0; SPSS Inc, Chicago, IL, USA).

Results

Table 1 shows that maternal age distribution is similar between the two groups.

The mean values of the number of ETs were 3.75±1.35 and 2.76±0.88 in the fresh ET and FET groups, respectively. Moreover, out of 95 pregnancies in the fresh ET group, 73 pregnancies (76%) were continued. In addition, out of 87 pregnancy (60.2%) in the FET group, 56 pregnancies were continued. In the fresh ET and FET groups, the numbers of live births were reported as 102 (30.45%) and 67 (27.45%), respectively, with no significant difference (P=0.43).

According to Table 2, 1 ectopic pregnancy (1%) and 22 abortions (23%) were observed in the fresh ET group. Moreover, out of 93 pregnancies, 6 ectopic pregnancies (6.5%) and

31 abortions (35.6%) were reported in the FET group.

Table 1. Characteristics of fresh and frozen embryo transfer groups

	Fresh group n=96	Frozen group n=93	P-value
***Mother's age (year)**	29.62±5.07	28.5±4.10	0.09
***Number of transferred embryos**	3.75±1.35	2.76±0.88	0.001
Total transferred embryo (n)	335	244	
****Live birth**	102 (30.45%)	67 (27.45%)	0.43
Pregnancy continuation n	73/95 (76%)	57/87 (60.2%)	0.09
Live birth	102 (30.45%)	67 (27.45%)	0.43

* Data presented as mean±standard deviation; Student's t-Test

** Data presented as n (%); Chi-square test

Table 2. Pregnancy outcomes in fresh and frozen embryo transfer groups

	Fresh group	Frozen group	Odds ratio (95 % CI) δ	P-value
***Ectopic pregnancy**				
Yes	1 (1%)	6 (6.5%)		0.06
No	95 (99%)	87 (93.5%)		
Abortion				
Yes	22 (23.2%)	31 (35.6%)	1.84 (0.96-3.51)	0.07
No	73 (76.8%)	56 (64.4%)		
Ongoing pregnancy				
Yes	73 (76 %)	56 (60.2%)	0.48 (0.25-0.85)	0.02
No	23 (24%)	37 (39.8%)		
Multiple pregnancy				
Singletons	65 (61.4%)	74 (85.1%)	1	
Twins	27 (28.4%)	12 (13.8%)	0.38 (0.18-0.79)■	0.01
Triplets	3 (3.2%)	1 (1.1%)		
Preterm labor <34 weeks				
Yes	6 (8.2%)	2 (3.6%)	0.41 (0.8-2.13)	0.29
No	67 (91.8%)	54 (96.4%)		
Third trimester bleeding				
Yes	22 (30.1%)	15 (26.8%)	0.84 (0.39-1.84)	0.67
No	51 (69.9%)	41 (73.2%)		
***Intrauterine fetal demise**				
Yes	1 (1.4%)	2 (3.6%)		0.57
No	72 (98.6%)	54 (96.4%)		
Neonatal anomalies				
Yes	2 (2.7%)	2 (3.6%)	1.31 (0.18-9.63)	0.78
No	71 (97.3%)	54 (96.4%)		
Maternal hypertension				
Yes	7 (9.6%)	9 (16.1%)	1.8 (0.63-5.19)	0.27
No	66 (90.4%)	47 (839%)		
Neonatal weight (g)				
2500≤	58 (56.9)	40 (59.7)	1	-
1500-2500	32 (31.4)	22 (32.8)	0.97 (0.51-1.95)	0.99
<1500	12 (11.8)	5 (7.5)	0.6 (0.19-1.85)	0.37

δ: Logistic regression■ Odds ratio of twins, triplets, and singletons* Due to the observed low frequency, the estimation of the odds ratio is not accurate.

Discussion

In this study, the pregnancy and neonatal outcomes were compared between the two groups of FET and ET. The results of the present study showed that the rates of multiple pregnancy, ongoing pregnancy, and neonatal birth weight were lower in the FET group in comparison to those reported for the fresh ET group. In addition, abortion, maternal hypertension, and neonatal anomalies were higher in the FET group, compared to those of the fresh ET group. However, abortion and maternal hypertension had borderline statistical significance.

A systematic review showed a higher rate of ongoing pregnancy in fresh ET, compared to that reported for FET (25) that is consistent with the results of the present study. These results may be achieved by the improvement of endometrial synchrony instead of the COH cycles in fresh cycles (25). The results of the current study demonstrated a higher rate of spontaneous abortion in the FET group, compared to that reported for the ET group, with borderline statistical significance. This finding is consistent with the results of studies conducted by Van Steirteghem et al., Liu et al., and Aflatoonian et al. (13, 14, 24). However, in a study carried out by Belva et al., the abortion rate was similar between the two groups (5). It can be concluded that FET can cause damage to the embryo during the freezing and melting.

The rate of preterm labor was lower in the FET group than that of the fresh ET group, but not statistically significant. By increasing the sample size, this difference may be statistically significant. Additionally, in some studies, the lower rates of preterm birth and LBW were reported in FET compared to those of fresh ET in a singleton pregnancy (6, 7, 15, 26, 27). The findings of the aforementioned studies are consistent with the results of the present study. Regarding neonatal outcomes, the present study showed that neonatal birth weight was lower in the FET in comparison to that of fresh ET, but not statistically significant (P>0.05)

In another study, FET has similar neonatal outcomes, including LBW, stillbirth, neonatal prematurity, neonatal mortality, and congenital malformation, compared to fresh ET (13). A study carried out by Zhang et al. and other studies reported a higher neonatal birth weight and higher rates of macrosomia and large for gestational age in the FET cycle than those reported for fresh ET in normal pregnancy (28-32). However, the findings of the present study are not consistent with the results of multiple clinical studies in this regard (33-36).

The difference in the results probably may be due to the higher odds of maternal hypertension in the present study in FET, compared to those of fresh ET; therefore, pregnancy complications would have had a significant effect on neonatal outcomes. Accordingly, in other studies consistent with the current study (33-36), the two methods were compared in high-risk patients, such as the subjects with chronic hypertension and preeclampsia. In the present study, it was also observed that FET was associated with higher odds of maternal hypertension than fresh ET which may be related to the type of study and those at risk. In the current study, the birth weight of newborns in the FET group was lower due to the increased maternal hypertension; however, it is not statistically significant.

The present study showed that the rate of neonatal anomalies was higher in the FET group, compared to that reported for the fresh ET group; however, it was not statistically significant (P>0.05). Nevertheless, several studies showed that FET seems similar to fresh ET in this regard (6, 13, 24). A recent study reported that the rate of major abnormalities in FET was higher than that of fresh ET. The reason for this difference could be due to differences in the day of freezing, type of cryopreservation protocols, number or quality of frozen transferred embryos (5). However, Wada et al. reported that the rate of major malformation in the FET group was significantly lower than that reported for the standard IVF group (2).

A large study conducted on frozen embryos concluded that freezing does not cause genetic changes and an increase in fetal abnormalities (37). In addition, a study carried out by AbdelHafez reported a higher uterine receptivity in the FET cycle than fresh ET (38). Consequently, it is required to perform further

thorough studies to evaluate the effect of freezing and thawing methods and clinical factors on implantation and fetal growth due to different results.

The limitations of the present study included the retrospective design, inadequate sample size, and collection of data from a single public IVF center in Urmia, and no control of confounding variables (e.g., LBW and neonatal gender). Accordingly, it is recommended to consider the limitations of the current study and perform further randomized control trials and follow-up studies on the long-term outcomes of children born in two groups of FET and ET in order to determine the effectiveness of each method.

Conclusion

The rates of pregnancy outcomes, such as multiple pregnancy, were lower in the FET group, compared to those reported for the fresh ET group. The rates of abortion and maternal hypertension were higher in the FET cycle, but with borderline statistical significance. The obtained results of the present study showed that FET had a lower rate of ongoing pregnancy. The FET can cause damage to the embryo during freezing and melting. Therefore, it is required to carry out further randomized clinical trials for the assessment of endometrial receptivity and ART outcomes.

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Conflicts of interest

Authors declared no conflicts of interest.

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