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Original Article

The factors affecting the outcome of frozen–thawed embryo transfer cycle

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Abstract

Objective: To determine the impact of the clinical and embryological factors on the pregnancy outcome of frozen–thawed embryo transfer.
Materials and Methods: The data of 247 frozen–thawed embryo transfer cycles were assessed at Royan Institute from March 2006 to March 2008. Appropriate statistical analysis was performed using Student *t* test and Chi-square or Fisher exact test. Forward logistic regression was done to predict the individual impact of factors on the success of frozen embryo transfer.
Results: According to our results, 1,523 frozen embryos were thawed with a survival rate of 79.8%. The overall chemical and clinical pregnancy rates per embryo transfer cycle were 28.1% and 26.3%, respectively. A total of 71 gestational sacs were implanted (7.9%). The pregnancy outcome was higher in women who were stimulated with the gonadotrophin releasing hormone agonist long protocol, treated by a combination of follicle stimulating hormone and luteinizing hormone, who had endometrial thickness greater than or equal to 8 mm on the embryo transfer day, and who had positive fresh-cycle pregnancy test.
Conclusion: Protocol type, gonadotrophin preparations, fresh-cycle outcome, endometrial thickness and the numbers of obtained oocytes, embryos, and high-quality thawed embryos transferred are the factors affecting pregnancy outcome of frozen–thawed embryo transfer.

Keywords: Clinical factors; Embryological factors; Frozen–thawed embryo transfer; Outcome

Introduction

Cryopreserved embryo transfer cycle is an advantage for women who produce large numbers of oocytes and embryos in their fresh *in vitro* fertilization (IVF) cycle [1,2]. Lowering the risk of multiple pregnancy [3] and ovarian hyperstimulation syndrome [4–7]; providing cost effectiveness [7] and almost stress-free procedure for couples [1,8]; giving the chance of donating embryos to other couples with a genetic disease or to women with premature ovarian failure; arranging the opportunity for couples to have an additional child [7]; increasing the take-home baby [9,10] and cumulative pregnancy rates [11,12] are all important advantages of embryo cryopreservation that persuade IVF centers to use this service for couples. On the other hand, several investigators have detected lower implantation and pregnancy rates in frozen–thawed embryo replacement cycles compared with fresh IVF cycles [13–15], and two important etiologies of this lowered rate are (1) adverse effect of the freeze–thawing process and ice crystal damage and (2) transferring higher-quality embryos in the fresh cycle and freezing the lower-grade ones [16]. The question that comes to mind is whether there is any way to improve the

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pregnancy outcome of a cryopreserved embryo transfer cycle. A few studies have assessed the clinical and embryological factors affecting pregnancy outcome [1,13,17,18], and there is controversy about the parameters among the performed studies. This retrospective study has been scheduled in our center for a better understanding about some of the clinical and embryological factors that may influence pregnancy outcome and to apply more measurements for promoting the pregnancy outcome of frozen−thawed embryo transfer cycles.

Materials and methods

All infertility cases who underwent IVF or intracytoplasmic sperm injection (ICSI) treatment at Royan Institute (Infertility and Reproductive Medicine Research Centre) and had cryopreserved embryo transfer were reviewed retrospectively. From March 2006 to March 2008, 297 cycles were identified. The cycles were excluded if (1) the cause of infertility was uterine factor; (2) the embryos were acquired from donated oocytes; (3) endometrial preparation was performed in a natural cycle; and (4) the couples had more than three ART cycles. Women with pituitary downregulation and "long or antagonist protocols," who agreed on cryopreservation of the additional retrieved embryos, were included in our study. Finally, 247 cycles were included in this study according to the mentioned inclusion and exclusion criteria.

After ovarian stimulation and oocyte retrieval, which was described previously [19], embryos were produced by IVF or ICSI procedures. The generated embryos were assessed and graded as A, B, and C, according to their morphology, cleavage stage, and fragmentation rate [20]. The remaining embryos were cryopreserved on Day 2 or 3 after IVF or ICSI. The procedure used for embryo cryopreservation in patients with surplus excellent- or good-quality embryos was slow freezing [21]. At the time of frozen embryo transfer, the embryos were thawed, and those with at least 50% of the blastomeres intact were selected for intrauterine transfer without any interference from the embryologists. The selected embryos were transferred into 50-μL embryo Glue (Vitrolife, Sweden) for 2 hours before intrauterine transfer.

For the endometrial preparation, estradiol valerate (Aburaihan, Tehran, Iran) 4 mg/d was given and continued until the optimal endometrial thickness was obtained. Intramuscular progesterone 100 mg (Aburaihan, Tehran, Iran) was given, and embryo transfer was performed 3 days later. Between one and six embryos were transferred according to the age, number of unsuccessful outcomes of the IVF or ICSI cycles, and quality of embryos. The patients were tested using serum β-human chorionic gonadotropin (β-hCG) assay 15 days after embryo transfer, and if the pregnancy test was positive, a prescription of estradiol valerate (6 mg/d) and progesterone (800 mg/d) was continued until 12 weeks of gestation.

Chemical pregnancy was determined by the number of positive β-hCG results. The clinical pregnancy was clarified by the number of women with gestational sacs on transvaginal sonography at 4−6 weeks of gestation. In the calculation of implantation, the number of gestational sacs was considered. This study was approved by the Royan Ethics Committee, and all couples gave an informed consent for using their information for analysis before starting their treatment.

Data analysis was performed by means of the SPSS version 13.0 software program (SPSS Inc., Chicago, IL, USA). Appropriate statistical analysis was performed using Student *t* test, Chi-square test, and Fisher exact test. A p value less than 0.05 was considered statistically significant. Forward logistic regression was performed to predict the individual impact of factors on the success of frozen embryo transfer. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Results

A total of 222 patients who underwent 247 frozen embryo transfer cycles were included in this study. The patient's mean age was 30.6 ± 5.3 years (range, 17−45 years). The total number of obtained embryos was 2,687, of which 2,010 were frozen. One thousand five hundred twenty-three out of 2,010 frozen embryos were thawed, with a survival rate of 79.8% (1,215 embryos survived), and 899 embryos were transferred (74% transfer rate). The mean number of embryos transferred was 3.6 per cycle (range, 1−6 per cycle), and the median storage time was 170 days (range, 53−1,671 days).

According to our analysis, positive β-hCG results were seen in 61 (Vitrolife, Sweden) cycles. The overall chemical and

Table 1
Impact of clinical factors (age, gonadotrophin, protocol, infertility etiology, endometrial thickness, and fresh-cycle β-hCG result) on the embryo cryopreservation outcome

Clinical factors	Implantation rate % (*n*)	Clinical pregnancy % (*n*)
Age (yr)		
≤40	7.9 (67/847)	26.3 (54/205)
>40	7.7 (4/52)	25 (3/12)
Gonadotrophin		
FSH	5.3 (22/413)*	18 (18/100)
FSH + LH	16.9 (11/65)*	37.5 (6/16)
FSH + LH + HCG	8.4 (21/249)*	30 (18/60)
NA	17/172	17/41
Protocol		
Long	8.5 (69/813)*	27.8 (55/198)
Antagonist	2.3 (2/86)*	10.5 (2/19)
Infertility etiology		
PCO	7.4 (23/312)	25.3 (19/75)
Non-PCO	8.2 (48/587)	26.8 (38/142)
Endometrial thickness** (mm)		
≥8	8.6 (67/779)	28.2 (53/188)
<8	0 (0/36)	0 (0/9)
NA	4/84	4/20
Fresh-cycle β-hCG		
Positive	14 (19/136)*	42.9 (15/35)*
Negative	6.5 (44/680)*	22.2 (36/162)*
NA	8/83	6/20

*A p value ≤ 0.05.
**$p = 0.065$.
β-hCG = β-Human Chorionic Gonadotropin; FSH = follicle stimulating hormone; LH = luteinizing hormone; NA = not available (unregistered information in the patients' files); PCO = polycystic ovary syndrome.

clinical pregnancy rates per embryo transfer cycle were 28.1% (61 of 217) and 26.3% (57 of 217), respectively. A total of 71 gestational sacs were observed by ultrasonography (implantation rate of 7.9%). The number of singletons was 45 (78.9%), and multiple pregnancies were observed in 21.1% (17.6% twins and 3.5% triplets).

Table 1 indicates the main pregnancy outcomes of the cryopreserved embryo transfer cycles separately stratified by age, type of gonadotrophin, protocol type, infertility etiology, endometrial thickness, and the result of fresh-cycle β-hCG test. Statistical analysis indicated that there were no differences in implantation and clinical pregnancy rates between the two maternal age groups (≤40 years and >40 years).

The implantation rate in women stimulated with gonadotrophin releasing hormone agonist long protocol was higher than that in the patients stimulated with antagonist protocol ($p < 0.05$), and in comparison with the different gonadotrophin preparations, women previously treated by follicle stimulating hormone (FSH) + luteinizing hormone had a better implantation rate ($p < 0.05$). There were no significant differences in pregnancy outcomes between women with polycystic ovary syndrome and those without polycystic ovary syndrome.

Our results indicated that women with an endometrial thickness greater than or equal to 8 mm on the embryo transfer day had higher rates of clinical pregnancy and implantation as compared with those with a lesser endometrial thickness (<8 mm), but this difference was not statistically significant ($p = 0.06$). The results also showed that endometrial thickness negatively correlated with the maximal dosages of estradiol (E2) ($r = -0.321$, $p < 0.0001$).

With regard to our findings, pregnancy in the fresh embryo transfer cycle had a statistically significant effect on pregnancy outcome in the freeze cycle, as the rates of implantation and clinical pregnancy were higher in women with a positive previous fresh-cycle β-hCG test ($p < 0.05$). The method of fertilization, storage time, number of embryos transferred, and assisted hatching were factors that we assessed, and they were found not to be associated with pregnancy outcomes of cryopreserved embryo transfer (Table 2).

Table 2
Impact of embryological factors (number of embryos transferred, storage time, method of fertilization, and assisted hatching) on the embryo cryopreservation outcome

Embryological factors	Implantation rate % (*n*)	Clinical pregnancy % (*n*)
Number of embryos transferred		
1–3	10.9 (13/119)	20 (9/45)
≥4	7.4 (58/780)	27.9 (48/172)
Storage duration (d)		
≤180	6.5 (33/506)	22.7 (27/119)
>180	9.7 (38/393)	30.6 (30/98)
Method of fertilization		
ICSI	8.3 (49/592)	27.4 (40/146)
IVF	8.3 (5/60)	28.6 (4/14)
IVF/ICSI	6.9 (17/247)	22.8 (13/57)
Assisted hatching		
Yes	7.8 (65/829)	25.5 (51/200)
No	8.6 (6/70)	35.3 (6/17)

ICSI = intracytoplasmic sperm injection; IVF = *in vitro* fertilization.

Table 3
The forward logistic regression analysis with odds ratios and 95% confidence intervals on the factors affecting the β-hCG result [oocyte retrieval, embryo retrieval, and quality of embryo (Grade A)] after frozen–thawed embryo transfer

Variables	β	SE	*p*	OR	95% CI for OR	
					Lower	Upper
Oocyte retrival	−0.086	0.032	0.007	0.918	0.863	0.976
Retrieval embryo	0.107	0.049	0.028	1.113	1.011	1.226
Quality of embryo transferred (Grade A)	0.234	0.100	0.020	1.263	1.038	1.538

Adapted from Refs. [4–7,40]
CI = confidence interval; OR = odds ratio; SE = standard error.

We also performed forward logistic regression for all clinical and embryological factors that we assumed to have an influence on β-hCG result. According to the results, the number of retrieval oocytes, the number of retrieval embryos, and high quality of embryos transferred (Grade A) were important factors predicting a positive β-hCG test in cryopreserved embryo cycles (Table 3). The OR calculated for a positive hCG result for oocyte retrieval was 0.918 (95% CI, 0.863–0.976). Women with a lower number of retrieved oocytes had more chance to have a positive β-hCG result. Our analyses showed that the mean numbers of retrieved oocytes in women with positive β-hCG and women with negative β-hCG results were 16.4 ± 6.6 and 18.7 ± 7.7, respectively ($p < 0.05$). In the evaluation of embryo quality, transferred embryos with a higher quality (Grade A) was also another factor that predicted a positive β-hCG result (OR, 1.263; 95% CI, 1.038–1.538). The computed OR for the positive β-hCG for embryo retrieval was 1.113 (95% CI, 1.011–1.226).

Discussion

In this retrospective study, we assessed the outcome of cycles after frozen–thawed embryo transfers and also the clinical and embryological factors affecting the pregnancy outcome of embryo cryopreservation.

Our data showed that the overall clinical pregnancy rate was 26.3%, which is comparable to those in some other studies [12,22–24]. However, our implantation rate per transfer was lower than those in these studies (7.9%). The reason for this decrease is the high number of embryo transfers in our center during this time (range, 1–6).

Some large studies have shown that the female's age has a converse effect on the pregnancy and implantation rates [10]. However, we did not find such significant differences in pregnancy outcome in the two maternal age groups (≤40 years and >40 years). This finding shows that if we have enough good-quality eggs and embryos, the age does not have an effect on pregnancy outcome.

As anticipated, our results indicated better implantation rate in women stimulated with the GnRH agonist long protocol

than those stimulated with the antagonist protocol. Generally, after stimulation with the GnRH agonist long protocol, a large amount of oocytes are obtained in women with normal ovaries, and large numbers of embryos are provided for embryo transfer or freezing. Consequently, significantly lower cancellation and higher pregnancy rates are observed in the long protocol in comparison with those of other stimulation protocols [25–27]. Oehninger et al [17] reported higher pregnancy and implantation rates in women stimulated with the long protocol than those in women with GnRH agonist stop protocol in freezing–thawing embryo transfer cycles. Other researchers have reported no significant differences in implantation rates between GnRH agonist and GnRH antagonist protocols after cryopreserved embryo transfer [28,29].

We also found a significantly higher implantation rate in women treated with LH–FSH than those treated with only FSH or a combination of luteinizing hormone, FSH, and hCG. However, other studies noted similar implantation rate among different preparations of gonadotrophin [17,28].

Endometrial thickness is a major factor in identifying the time of progesterone supplementation and frozen–thawed embryo transfer [24,30]. More studies have evaluated the relationship between endometrial thickness and treatment outcome after fresh IVF cycles and reported significantly higher pregnancy rate in women with endometrial thickness greater than or equal to 9 mm on hCG or embryo transfer day in comparison with that in women with an endometrial thickness of 7–8 mm [24,31,32]. In another study, no differences were found in pregnancy outcome in women with endometrial thickness less than or equal to 8 mm and those with endometrial thickness of 9–14 mm [33]. In our study, none of the women with endometrial thickness less than 8 mm became pregnant, although this finding was not statistically significant ($p = 0.06$).

Similar to the study by Check et al [33], in the present study, no differences were found in pregnancy outcomes according to the maximal daily E2 dosages. However, there was a significantly poor converse correlation between endometrial thickness and total dosages of E2, showing the resistance of women consuming higher dosages of E2 to endometrial thickness, which is comparable to that in the study by Check et al.

Our observation demonstrated that patients with a positive β-hCG result in the fresh cycles were more likely to have a positive outcome in the cryopreserved embryo transfer cycles than patients with a negative β-hCG result. Wang et al [10] retrospectively analyzed 3,570 frozen embryo cycles and discovered higher implantation rate among younger women with a previous positive pregnancy test. Conversely, another study has reported impaired implantation rate for patients who became pregnant in their fresh treatment cycle [1].

Simon et al [34], in a retrospective study, indicated impaired clinical pregnancy rates for patients with frozen embryo transfer who used ICSI in their fresh cycle. With regard to our study, no differences were found in pregnancy outcomes according to the method of fertilization. Similarly, several investigators have failed to detect any significant differences in implantation or clinical pregnancy rates in cycles in which IVF or ICSI was used for fertilization [1,15,16,35]. This finding shows that the pregnancy outcome is independent from fertilization method.

Storage time was another factor that was assessed. On the basis of our data, there was no significant difference in pregnancy outcome according to the duration of cryopreservation. Wang et al [10] reported a nonsignificant improved implantation rate in patients with longer storage time (more than 2 years), which, from their point of view, was because of the confounding effect of women who became pregnant in their fresh cycle. In another study performed by Singh et al [1], the storage time was comparable between patients with positive and negative pregnancy results.

Analysis of our data also indicated that the number of retrieval oocytes has a converse impact on the β-hCG result. It means that with increasing number of oocytes, the chance of pregnancy decreases. This suggests that with increasing number of oocytes, the quality may reduce.

Another factor that had a direct impact on the β-hCG result was the number of higher-quality transferred embryos (Grade A). Salumets et al [12] have, in fact, reported significantly higher implantation rates in those who had higher quality of embryo transfer (Grade A or B) than in those with poor-quality embryo transfer. The adverse effect of cryopreservation on pregnancy outcome by crystal damage has been demonstrated by several investigators [3,16]. Selick et al [3] evaluated the effect of cryopreservation on embryo quality and pregnancy outcome of embryos in fresh and frozen ovum recipient from the same donor and discovered that cryopreservation has a detrimental effect on embryo quality, but the rates of implantation or pregnancy are not impaired after transfer of high quality of embryos.

According to the results of other investigators, there is a controversy among studies in the relationship between the number of embryos transferred and pregnancy outcome. Some researchers have reported a higher pregnancy rate with greater number of embryos transferred [12,23], and other studies fail this relationship [36]. In the present study, the rate of pregnancy outcome was calculated for a one- to three-embryo transfer because of the small number of one- and two-embryo transfers. Our data showed no differences in pregnancy outcomes between women with a one- to three-embryo transfer and those with an embryo transfer greater than or equal to four.

The analysis of our study showed that laser-assisted hatching has no impact on the rates of pregnancy outcome. However, in the present study, the number of women for whom laser-assisted hatching had not been performed was small. Some studies reported higher clinical pregnancy and implantation rates for women who had assisted hatching protocol than those in nonhatched women [37,38]. In another study by Valojerdi et al [21], the impact of assisted hatching on pregnancy outcome in women 37 years or older was discovered. Conversely, Ng et al [39], in a randomized double-blind controlled study, observed that laser-assisted hatching did not improve the implantation rate after cryopreserved embryo transfer.

In conclusion, protocol type, gonadotrophin preparations, outcome of the previous fresh cycle, endometrial thickness, number of obtained oocytes, retrieval embryo, and the number of high-quality thawed embryos transferred (Grade A) are the factors that have an effect on pregnancy outcome of frozen–thawed embryo transfer.

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