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Frozen embryo replacement cycle: An analysis of factors influencing the outcome

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OBJECTIVE(S) : To assess factors that influence the outcomes in a frozen embryo replacement cycle (FERC)

METHOD(S) : An analysis of 205 fresh IVF/ICSI cycles and their first FERC was performed in a University hospital in UK.

RESULTS : The ongoing clinical pregnancy rate and implantation rate was 25.9% and 12.8% respectively in fresh and 16.1% and 7.5% respectively in frozen cycles. In FERC, the mean age (SD) of women with an ongoing pregnancy was comparable to those with a negative outcome 32.85 ± 3.57 vs 33.53 ± 4.31 years. Also, women with a non-tubal cause of subfertility appeared to have a better outcome. The ongoing pregnancy rates were not influenced by the laboratory technique of fertilisation ICSI 20.39% vs IVF 11.76% (P=0.093) nor by the number of embryos transferred. The risk of multiple gestation was comparable in fresh and frozen cycles.

CONCLUSION(S) : FERC increases the cumulative pregnancy rate by nearly 16%. An ongoing pregnancy in the fresh cycle, age, laboratory technique, length of cryostorage and number of embryos transferred do not appear to influence conception rates in FERC. A nontubal etiology of subfertility has a better pregnancy outcome.

Key words : assisted reproductive technology (ART), frozen embryo replacement cycle (FERC), cryopreservation.

Introduction

A frozen embryo replacement cycle is a bonus to couples that have a good harvest of oocytes and embryos in their fresh in-vitro fertilization and intracytoplasmic sperm injection (ICSI) cycles. Trounson carried out the first successful embryo cryopreservation in 1983[1]. The contribution of cryopreservation has been reported to increase the take home baby rate from 5.2% to 19%[2,3].

The frozen cycles has its advantages in being a cheaper cycle as compared with the complete fresh cycle and is also relatively stress free for couples due to the absence of daily gonadotropin injections. The technology of cryopreservation has now enabled couples to have multiple embryo transfers from one fresh cycle thereby reducing the risk of ovarian hyperstimulation syndrome.

We assessed whether the outcome of the fresh cycles influenced the outcome in a frozen cycle. We also looked at other factors that might be associated with the same outcome like the women's age at the time of the frozen cycle, cause of infertility, interval between the fresh and frozen cycle, and the number of embryos transferred.

Methods:

In this observational study, the available information has been derived from 205 infertile women. All of them had undergone a fresh IVF/ICSI cycle and its first frozen embryo replacement cycle between 1999 and 2004. Only cycles using early cleavages embryos in the fresh and frozen cycles were

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considered. All freeze cycles and those with embryos frozen in the pronuclear stage were omitted from analysis.

Briefly, ovarian downregulation in the fresh cycle was achieved with the help of GnRH analogue followed by ovarian stimulation with gonadotropins for between 10 and 12 days (long protocol). Human chorionic gonadotropin (hCG; 10,000 IU) was administered approximately 36 hours prior to oocyte retrieval. The number of embryos transferred (ET) was generally two. Three embryos were transferred in cases with more than two previous failed attempts at fresh IVF/ ICSI or in those with embryo grading of 2.5-4. ET was performed 48 hours after oocyte retrieval and the luteal phase was supported with vaginal progesterone pessaries.

Early cleavage embryos were stored frozen in LN_2 (liquid nitrogen) for future use after a fresh embryo transfer (day 2) provided there were at least two embryos of good quality (less than 20% fragmentation with at least two cells). The criteria for grading are as shown in Table 1. Embryos that were not suitable for freezing were either donated to research or allowed to perish humanely as per couple's consent.

Freezing and thawing of embryos was carried out by the standard technique previously described by Testart[4] using Medicult freezing and thawing solutions containing 1,2 propanediol and sucros (Medicult Laboratories, Denmark)[45]. One or two embryos were frozen in each straw based on the number of embryos that required freezing per person and all straws were carefully labelled.

The frozen early cleavage embryos were thawed on the morning of the transfer. Survival was defined as at least 50% of blastomeres being intact. The embryos were regarded (as per Table 1) with degenerated cells being considered the equivalent of the fragmented one.

Table 1. Embryo Grading.

Grade 1:	Regular cleavage, no fragmentation
Grade 2:	Regular / slightly irregular cleavage, < 20% fragmentation, cells dark
Grade 2.5:	Regular / slightly irregular cleavage, > 20% fragmentation, cells dark
Grade 3:	Irregular cleavage, extensive fragmentation, > 1 cell intact
Grade 4:	One cell intact, extensive fragmentation
Grade 5:	No viable cells

The frozen thawed embryos were replaced in a programed cycle. Ovarian suppression was achieved with 3.75mg of a subcutaneous Leuprorelid acetate injection. After downregulation with an endometrial thickness of < 5mm, estradiol valerate tablets were commenced for ten days (6mg for 7 days followed by 8mg for 3 days). The dose was increased if suitable endometrial thickness was not achieved in 10 days. If the endometrium measured $\geq$ 8mm, 800 mg of progresterone vaginal pressaires were commenced for 3 nights prior to the transfer. Following the embryo transfer (ET), all patients were advised to continue with the same dose of medications until their first pregnancy test. Serum hCG estimation was performed 12 days after ET and if the value was $\geq$ 50 IU patients were booked for an ultrasound scan 2-3 weeks later and continued on the medication until the 12th week of gestation. Patients with evidence of an ongoing clinical pregnancy (positive outcome) on untrasound were discharged from the IVF clinic at 12 weeks gestation. The serum hCG estimation was repeated after 48 hours if the values were between 2-50 IU. If the repeat values were low, patients were warned of possible poor outcome. In cases with values < 2 IV (negative outcome) patients were advised to stop all medications and await withdrawal bleeding.

Statistical analysis : The continuous and normally distributed variables are presented as means and standard deviations (SD), whereas the skewed ones as medians and ranges, or interquartile ranges (IQR). Categorical variables are described with number and percentages. The tests implemented in this analysis are: McNemar, chi-square, Fisher's exact and unpaird t-test: A P value $\leq$ 0.05 was considered statistically significant. The SPSS software (SPSS 1.2.0.1. SPSS Inc., USA) was used for analysis.

Results

A total of 3296 oocytes were retrieved and 2025 embryos were formed. Nearly 955 were frozen and subsequently 765 were thawed with 695 embryos surviving the freeze-thaw process. The number of embryos transferred in the fresh and frozen cycles was 431 and 480 respectively. The embryos characteristics are shown in Table 2. The survival rates of IVF and ICSI embryos were comparable. (IVF: overall freeze-thaw rate 68.9% and thaw-survival rate 91.5%; ICSI: overall freeze-thaw rate 77.5% and thaw-survival rate 90.1%).

Pregnancy rates in fresh and frozen cycles : The clinical pregnancy rate per ET and implantation rate were 25.9% (53/205) and 12.8% in the fresh cycle and 16.1% (33/205) and 7.5% in FERC respectively. The cumulative pregnancy rate after one fresh and the following frozen cycle was 41.9%. There were three sets of twins in the fresh cycles (5.66%) as compared with 4 sets in the frozen cycles (12.12%). The multiple pregnancy rates were comparable in the two cycle types. In the fresh cycles, one of the three

cases with multiple pregnancies had three embryos transferred as compared with two of the four cases in the frozen cycles.

Table 2. Embryo Characteristics.

	Fresh Cycle Median (Range)	Frozen Cycle Median (Range)
Number of embryos transferred	2 (2-3)	2 (1-3)
Grade of embryo 1	1 (1-2)	2 (1-5)
Number of cells in embryo 1	4 (2-8)	4 (1-8)
Grade of embryo 2	1 (1-2)	2 (1-4)
Number of cells in embryo 2	4 (2-7)	3 (1-6)
Grade of embryo 3	2 (2-4)	2 (2-4)
Number of cells in embryo 3	4 (1-5)	2 (1-6)

Table 3 shows the cross tabulation of outcomes in the fresh and frozen cycles. To assess the probability of having a higher chance of an ongoing pregnancy in a frozen cycle if a woman had had a clinical pregnancy in the fresh cycle we used the McNemar test which showed that women with a positive outcome in the fresh cycle were more likely to have a negative outcome in the frozen cycle, than the other way around ($c^2 = 5.014$; P=0.025).

Table 3. Outcome of frozen cycle for women with ongoing clinical pregnancy (positive), and negative outcome during the fresh cycle. Data presented as numbers (percentages).

	Frozen cycle		
Fresh cycle	Positive	Negative	Total
Positive	7 (3.42%)	46 (24.44%)	53
Negative	26 (12.68%)	126 (61.46%)	152
Total	33	172	205

Figures in brackets represent percentages. Positive outcome indicates ongoing pregnancy.

Age : The mean ± SD age of patients undergoing a fresh cycle and the subsequent FERC was 33 ± 4.2 vs 33.4 ± 4.2 years.

The mean age ± SD of women with an ongoing clinical pregnancy in the FERC was comparable to that with a negative outcome in the frozen cycle 32.85 ± 3.57 vs 33.53 ± 4.31 years; P=0.391.

Causes of infertility : Eleven different categories of infertility were identified, with most of them having very small numbers. We merged them into five groups based on the most predominant cause of infertility to help draw inferences i.e. (i) Male factor: varying degrees of oligoasthenoteratozoospermia (73 cases), male factor polycystic ovary syndrome (PCOs) (13 cases), and male factor mild tubal factor (6 cases); (ii) PCOS consisted of PCOS (6 cases), PCOS/ minimal endometriosis (1 cases) and PCOS/mild tubal factor (4 cases); (iii) Tubal factor, in which only women with unilateral, bilateral blocked tubes and those with a previous history of salpingectomy were included (51 cases); (iv) Endometriosis; consisted of women with moderate to severe endometriosis (6 cases), endometriosis / mild male factor (6 cases), endometriosis/ mild tubal factor (2 cases) and (v) unexplained infertility (37 cases). The number and percentage of ongoing clinical pregnancies in each group is shown in Table 4. The chi square test confirmed that the clinical pregnancy rates were not significantly different in five groups ($(?^2_4 = 4.19$; P=0.258).

Table 4. Numbers and percentages of women with an ongoing clinical pregnancy (positive) inthe 5 infertility subgroups.

Cause of infertility	No. of women in the group	No. of women with ongoing clinical pregnancy	Percentage ongoing clinical pregnancy
Male factor	92	17	18.48%
PCOS	11	1	9.09%
TUBAL	51	4	7.84%
Endometriosis	14	4	28.57%
Unexplained	37	7	18.92%
Total	205	33	16.09%

Treatment received : Of the 205 women that participated in this study 103 underwent ICSI and 102 the IVF treatment in the fresh cycle. The numbers and percentages of women with positive and negative outcome during the frozen cycle can be seen in Table 5.

Table 5. Outcome of frozen cycles.

Outcome of frozen cycles	Treatment	
	ICSI	IVF
Positive	21 (20.39)	12 (11.76)
Negative	82 (79.61)	90 (88.24)
Total	103	102

Figures in brackets represent percentages. Positive outcome indicates pregnancy.

As association between the given treatment and the outcome of the frozen cycle was tested with the chi-square test. No significant association was found to be present (($?^2_1$ 2,822, P=0.093).

Interval between fresh and frozen cycles : The median (IQR) interval for both the ongoing clinical pregnancies and negative outcome women in the frozen cycles was comparable at 5 (4-9) months.

Number of frozen embryos transferred : We found that 16/ 205 had a single embryo transferred, 96/205 had 2 embryos transferred and 93/205 had 3 embryos transferred during the frozen cycles the women with an ongoing clinical pregnancy in each of these three groups was 1(6.3%), 16 (16.7%) and 16(17.2%). In order to test for a linear trend in the proportions of positive results we implemented in Mantel-Henszel test. The result showed that no statistically significantly linear trend was present between the outcome of the frozen cycle and the number of embryos transferred ($?^2_{MH}$ =0.624; P=0.429).

Discussion

The proportion of children born through ART in the UK is nearly 2% of the total number of live birth. Embryo cryopreservation allows for storage and later transfer of surplus good quality embryos obtained through fresh IVF and ICSI cycles [3]. A good quality embryo cleaves better with a low fragmentation rate. Current, scoring of embryos is based on morphological criteria and polarity of the pronuclei [8]. Cryopreservation damages mammalian embryos by inducing intracelluar ice formation, solution effects and osmotic effects. In view of loss of some embryos during the freeze-thawing process, a fresh transfer is inherently preferable in the first instance [9]. Embryo survival rate has been found to be comparable between IVF (70.5%) and ICSI patients (73.2%) and between embryos frozen at the early cleavage and pronuclear stage [10]. This is comparable to our findings. We also found that the laboratory technique of fertilization neither influenced the embryo survival rate nor the clinical pregnancy rate in FERC. The length of cryopreservation was comparable between cycles with a positive and negative outcome. Also a nontubal etiology for subfertility has a better outcome through the frozen embryo replacement cycle. Our pregnancy rates in tubal infertility are in keeping with previous similar studies [5].

It has been suggested that the chances of implantation and pregnancy following a frozen cycle is higher if conception occurred in the fresh cycle [5]. A positive outcome in the fresh cycle does not seem to increase the chances of pregnancy in the frozen cycle. However, follow up of subsequent frozen cycles undertaken by couples in this study might have added valuable information to our study. The risks of multiple births have increased progressively with advent of assisted, conception treatments, with ensuing effects on perinatal mortality and morbidity. The twin pregnancy rate in our study was comparable between the fresh and frozen cycles. Also, the ongoing pregnancy rates were comparable between cases having two or three embryos transferred. This once again highlights the fact that there is no advantage in transferring three embryos [11,12]. Efforts should be made to adhere to the national UK guidelines that allow a maximum transfer of two embryos.

Conclusion

Frozen embryo cycles increase the cumulative pregnancy rates by nearly 16% Achieving an ongoing clinical pregnancy in the fresh cycle does not increase the chance of an ongoing pregnancy in the frozen cycle. Patients with positive and negative outcome in the frozen cycle were not found to different in terms of age and cyostorage length. The laboratory technique of fertilization and the number of embryos transferred in the frozen cycle do not appear to influence the outcome. Finally, women with a nontubal cause of subfertility seem to have a better outcome in the frozen cycle. However, no statistically significant difference was found to be present between their pregnancy rate and those of the tubal infertility women.

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