The number of blastomeres in post-thawing embryos affects the rates of pregnancy and delivery in freeze-embryo-transfer cycles

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Abstract

Purpose To observe the differences in pregnancy rates (PRs), delivery rates, and abortion rates associated with frozen-embryo-transfer (FET)-based use of post-thawing embryos with different numbers of blastomeres.

Methods 959 FET cycles and 361 successful FET cycles performed between January 2007 and December 2007. Compare the PRs and abortion rates in post-thawing embryos with 8 blastomeres (8c), 7c, 6c, 5c, 4c, and 3c.

Results 1. The total PRs of post-thawing 8c, 7c, 6c, 5c, 4c, and 3c embryos were 44.1%, 41.0%, 34.4%, 23.8%, 12.5%, and 0%, respectively ($p < 0.05$). 2. The abortion rates for the transferred embryos of the 8c, 7c, 6c, 5c, and 4c groups were 17.92%, 19.35%, 27.69%, 24%, 20%, respectively ($p < 0.05$).

Conclusion The number of blastomeres in the post-thawing embryos is an important factor influencing the occurrence of pregnancy in FET procedures; however, the criterion that post-thawing embryos with 50% intact blastomeres will lead to pregnancy may not be valid.

Keywords Abortion rate · Blastomere survival · Frozen-embryo transfer · Pregnancy rate

Capsule PRs for FET cycles are correlated to the quality of post-thawing embryos, which is defined by the number of blastomeres in embryo.

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Since 1983, when the first frozen-embryo-transfer (FET)-based human pregnancy was successfully achieved [1], embryo cryopreservation has become one of the various widely used assisted reproductive techniques. However, the results appear to indicate that the rates of pregnancy and implantation in FET-based techniques are lower than those associated with the use of fresh embryos. One study has demonstrated that the pregnancy rate (PR) in FET cycles is <40% lower than that in IVF/ICSI cycles [2]. The rates of biochemical pregnancy and clinical abortion after FET have been shown to be 15–20% and 20–25%, respectively [3, 4].

Many factors influence the success rate of freeze-thaw cycles, including the age of the patient at time of cryopreservation, cause of infertility, grade of the embryos being transferred, the extent of embryo damage after thawing [5], level of estradiol, and the endometrial thickness at the time of transfer.

Although successful pregnancies can be achieved by transferring embryos that have 50% intact blastomeres after thawing [6], PRs are higher when all the blastomeres survive [5]. Indeed, if the embryos survive the freeze-thaw process with all their blastomeres intact, then the PR is comparable with that of fresh IVF cycles [5, 7].

The primary objective of the present study was to analyze the data of the FET cycles conducted in the years 2007. Among the frozen good-quality embryos (6–8 cells at day 3), embryos that showed at least 50% integration after thawing were transferred. We have compared the PRs, delivery rates, and abortion rates associated with the transfer of thawed embryos with different rates of blastomere survival.

Materials and methods

Materials

This retrospective study included data from 959 FET cycles and 361 successful FET cycles conducted between January 2007 and December 2007 at the assisted reproductive technologies center of Citic-xiangya reproductive and genetic hospital. This work was ratified by the Ethics Committee of Citic-xiangya reproductive and genetic hospital.

Methods

Controlled ovarian hyperstimulation, in vitro fertilization, and embryo culture

The patients underwent pituitary downregulation according to a long protocol or a short protocol, which was selected on the basis of the patient's age and ovarian reserve function. FSH treatment was initiated after satisfactory pituitary suppression. The FSH dose was adjusted on the basis of the results of ultrasound monitoring and the serum E2 levels. Oocyte maturation was induced by administering human chorionic gonadotropin (hCG; 5,000–10,000 IU) when at least 3 follicles had reached a mean diameter of 18 mm. Transvaginal follicular aspiration was carried out after 34–35 h. Regular IVF/ICSI was performed according to the patients' indications. After 16–18 h, the oocytes were observed under a microscope to determine evidence of fertilization. On day 3, the cleavage-stage embryos were graded on the basis of well-defined morphological and developmental criteria [8]. The embryos with more than 6 blastomeres and less than 20% fragments were considered as good-quality embryos.

Embryo freezing, thawing, and embryo transfer

Freezing The surplus good-quality embryos were frozen after transfer. A gradual-freezing protocol with 1,2-propanediol and sucrose as the cryoprotectants (FREZEE-KIT; Vitrolife, Sweden) was used. The embryos were equilibrated in a 1.5 M propanediol solution for 10 min at room temperature, transferred to 1.5 M propanediol/0.1 M sucrose, and loaded into ministraws (3 M; Steri-Dual, KH-9991-5092-4), with 1–3 embryos loaded in each straw. Cooling, which was performed by using a programmable freezer (PLANER, UK), was initiated at 25°C at a rate of $-2^{\circ}\text{C}/\text{minute}$ and continued up to -7°C ; at this point, manual seeding was performed. Then, cooling was resumed at a rate of $-0.3^{\circ}\text{C}/\text{minute}$ and continued up to -30°C , then at a rate of $-50^{\circ}\text{C}/\text{minute}$ up to -140°C , after which the ministraws were immersed and stored in liquid nitrogen.

Thawing The embryos were rapidly thawed by removing them from liquid nitrogen, exposing them to air for 30 s, and immersing them in a water bath at 30°C for 30 s. The seal of the straw was snipped with disinfected scissors. The embryos were successively transferred to the following thawing solutions (THAW-KIT, Vitrolife, Sweden) to remove the cryoprotectants: ETs1, containing 1.0 M propanediol/0.2 M sucrose; ETs2, containing 0.5 M propanediol/0.2 M sucrose; and ETs3, containing 0.2 M sucrose. The thawed embryos were then microscopically assessed (magnification, $\times 200$) for blastomere survival and transferred into a culture medium at 37°C for 2–3 h before transfer. The embryos with more than 50% surviving blastomeres [embryos with 8 blastomeres (8c) before freezing, and more than 4c after thawing; 7c before freezing, and more than 4c after thawing; 6c before freezing and more than 3c after thawing] were considered suitable for transfer.

Confirmation of pregnancy Pregnancy was confirmed by a urine test for hCG, which was performed approximately 14 days after embryo transfer. Clinical pregnancy was defined by the presence of a gestation sac with fetal heartbeat in ultrasound scanning at 5 weeks after embryo transfer.

Groups

On the basis of the survival of blastomeres in the transferred embryos, we divided the data for the year 2007 into the following groups: embryos with 8c, 7c, and 6c before freezing. These groups were then subdivided into the following groups on the basis of the post-thawing blastomere survival: embryos with 8c, 7c, 6c, 5c, 4c, and 3c after thawing. We analyzed the differences in the PRs for these groups.

And we analyzed the differences in the delivery rates and abortion rates of the groups transferred with post-thawing 8c, 7c, 6c, 5c, 4c embryos.

Statistical analysis

The data were compared by chi-square analysis.

Results

Comparison of PRs

We assessed the data from 959 FET cycles conducted in 2007. Table 1 represents the PRs for frozen-thawed embryos with different survival rates. The PRs for the

Table 1 Clinical pregnancy rates classified according to the number of blastomeres in the prefreezing embryos and the number of blastomeres in post-thawing embryos in the data from FET cycles conducted in 2007

	Post-thaw 8c	Post-thaw 7c	Post-thaw 6c	Post-thaw 5c	Post-thaw 4c	Post-thaw 3c	P
Pre-freeze 8c	44.1% (173/392)	41.4% (36/87)	32.7% (16/49)	26.3% (5/19)	14.3% (1/7)		<0.05
Pre-freeze 7c		40.7% (57/140)	30.8% (12/39)	22.6% (7/31)	13.3% (2/15)		<0.05
Pre-freeze 6c			36.6% (37/101)	23.6% (13/55)	11.1% (2/18)	0% (0/6)	<0.05
Total pregnancy rates	44.1% (173/392)	41.0% (93/227)	34.4% (65/189)	23.8% (25/105)	12.5% (5/40)	0% (0/6)	<0.05

transferred embryos that were 8c before freezing, and 8c, 7c, 6c, 5c, and 4c after thawing were 44.1% (173/392), 41.4% (36/87), 32.7% (16/49), 26.3% (5/19), and 14.3% (1/7), respectively. There were significant differences among these groups ($p<0.05$). The PRs for the transferred embryos that were 7c before freezing and 7c, 6c, 5c, and 4c after thawing were 40.7% (57/140), 30.8% (12/39), 22.6% (7/31), and 13.3% (2/15), respectively. There were significant differences among these groups ($p<0.05$). The PRs for the transferred embryos groups that were 6c before freezing, and 6c, 5c, and 4c after thawing were 36.6% (37/101), 23.6% (13/55), and 11.1% (2/18), respectively. There were significant differences among these groups. ($p<0.05$). In case of embryos with the same number of blastomeres before freezing, a greater number of surviving blastomeres after thawing corresponded to higher PRs.

The PRs associated with the transfer of 7c post-thawing embryos that had 8 or 7 blastomeres before freezing were 41.4% (36/87) and 40.7% (57/140), respectively. There were no significant differences between these 2 groups. The PRs associated with transferring 6c post-thawing embryos that were 8c, 7c, or 6c before freezing were 32.7% (16/49), 30.8% (12/39), and 36.6% (37/101), respectively. The PRs associated with transferring 5c post-thawing embryos that were 8c, 7c, or 6c before freezing were 26.3% (5/19), 22.6% (7/31), and 23.6% (13/55), respectively. The PRs associated with transferring 4c post-thawing embryos that were 8c, 7c, or 6c before freezing were 14.3% (1/7), 13.3% (2/15), and 11.1% (2/18), respectively (refer to Table 1).

In Table 1, it can be seen that the total PRs associated with the transfer of 8c, 7c, 6c, 5c, 4c, and 3c post-thawing embryos are 44.1%, 41.0%, 34.4%, 23.8%, 12.5%, and 0%, respectively. There were significant differences between these values.

Comparison of delivery rates and abortion rates

We compared the delivery rates and abortion rates of 361 subjects who had successfully undergone FET cycles between January 2007 and December 2007. The abortion rates of 8c, 7c, 6c, 5c, and 4c transferred embryos were 17.92% (31/173), 19.35% (18/93), 27.69% (18/65), 24% (6/25), 20% (1/5), respectively, and there were significant differences between these values (refer to Table 2).

Discussion

The processes of freezing and thawing involves the formation of ice crystals [9]. Ice crystal damage the cell membranes and lead to blastomere lysis [10], or osmotic shock, which may cause degeneration of lipoproteins due to prolonged cell exposure to the hyperosmotic environment [11, 12].

The selection of embryos for cryopreservation has been suggested to be a vital factor in predicting the occurrence of pregnancy in FET cycles. Traditionally, embryo freezing is performed at the pronucleus stage or at the cleavage stage, i.e., at the 4-cell- (day 2) or the 8-cell-blastomere (day 3) stages [13, 14]. The best implantation rate was observed on transferring embryos with 4 cells and less than 10% fragmentation at day 2 [15, 16]. Edgar et al [17] concluded that the prefreezing growth rate, rather than the prefreezing blastomere number, correlates with the developmental potential of stored embryos. In their study, the ratio of the implantation rate of thawed intact 4c (day 2) to that of thawed intact 2c (day 2) embryos was 2.35:1, and the ratio of the implantation rate of frozen 4c (day 2) to that of frozen 4c (day 3) embryos was 2.33:1.

Table 2 Comparison of the delivery rates and abortion rates in different qualities of thawed embryos in 2007

	Post-thaw 8c	Post-thaw 7c	Post-thaw 6c	Post-thaw 5c	Post-thaw 4c
Abortion rate	17.92% (31/173)	19.35% (18/93)	27.69% (18/65)	24% (6/25)	20% (1/5)
Delivery rate	82.08% (142/173)	80.65% (75/93)	72.31% (47/65)	76% (19/25)	80% (4/5)
Sum total	173	93	65	25	5

In our study, day-3 embryos with more than 6 blastomeres and less than 20% fragmentation were considered as good-quality embryos that could be used for transfer; this criterion was finalized on the basis of the results of a previous study [8]. Thus, the surplus good-quality embryos were frozen after transferring.

Usually, post-thaw embryos with 50% intact blastomeres are considered to have survived the freezing procedure. These embryos can be transferred to achieve successful pregnancies. Toukhy et al [18] analyzed embryos with no blastomere damage and those with at least 1 damaged blastomere, and they found that transferring these embryos resulted in clinical PRs of 22.8% and 13.5%, respectively, and implantation rates of 17.3%, and 8.1%, respectively. Tang et al [19] showed that there was no significant difference between the implantation rates associated with transferring post-thawing embryos with less than 25% blastomere lysis and embryos with no blastomere damage.

According to a recent report, the blastomeres that are closer to the breach in the zona are more prone to lysis after thawing [20]. The transfer of partially damaged cleaved embryos is associated with decreased PRs, when compared with the PRs associated with the transfer of fully intact embryos [21]. Researchers have suggested that the decrease in viability is due to the release of necrotic cytoplasmic material by the degenerated blastomeres, which impairs the embryo's viability. Degenerated or necrotic cells can disrupt cell signaling [22, 23], and they may also disrupt morphological restructuring of embryonic cells. Consequently, the development to blastocyst stage may be impaired. In this respect, necrotic cells in a frozen–thawed embryo may exhibit a similar effect as the cytoplasmic fragments in a fresh, cleavage-stage embryo. However, as described by Alikani et al [22], the degenerated cells in frozen–thawed embryos will not or cannot be reabsorbed by the remaining living cells of the embryo. Recent studies [24, 25] have shown that the removal of necrotic blastomeres from human embryos significantly increased the rate of implantation of partially damaged frozen–thawed embryos.

In our study, the total PRs of post-thawing embryos showed a gradual degradation, corresponding to a decrease in the number of surviving blastomeres ($p < 0.05$). In accordance with the previous studies, our findings reconfirmed the vital role of the post-thawing embryos in the success of FET procedures. In case of pre-freezing embryos with the same number of blastomeres, a greater number of blastomeres in the post-thawing embryos corresponded to higher PRs ($p < 0.05$). However, there were no significant differences between the PRs associated with the transfer of post-thawing embryos with the same number of blastomeres, regardless of differences in the number of blastomeres in the corresponding prefreezing embryos. Thus, it is evident that

the number of blastomeres in the post-thawing embryos is a vital factor influencing the occurrence of pregnancy in FET procedures, but the criterion that embryos with 50% intact blastomeres are capable of survival may not be as valid. In other words, the transfer of post-thawing embryos with 4 or 5 blastomeres would result in low PRs, regardless of the number of blastomeres before freezing or the percentage of blastomeres that were intact after freezing.

In a previous study, it was reported that there was no relationship between the abortion rate and the embryo quality, which was defined according to the number of embryo cells, the percentage of fragmentation, and the evenness of the cleavage [26]. This study showed that there were significant differences in the number of biochemical pregnancies (defined only by the elevation in hCG levels without any assessments of the gestational sac) and in the number of early pregnancy losses, and there were low chances of a pregnancy when transferring embryos with >10% fragmentation or with 5 or fewer blastomeres at day 3 [26, 27]. Studies focusing on the obstetric outcome of conceptions achieved by FET have reported 20–25% spontaneous abortions from all clinically ascertained gestations [4].

We demonstrated that the abortion rate associated with transferring 6-cell embryos was 27.69%, and the abortion rate associated with transferring 5-cell embryos was 24%; both these values were higher than the corresponding values for other groups. Moreover, the abortion rate associated with transferring 4-cell embryos was 20%. Because of the limited data, we could not analyze these findings thoroughly.

Conclusion

The present study confirms that PRs for FET cycles are strongly correlated to the quality of the post-thawing embryos, which is defined by the number of the blastomeres in embryo. Furthermore, our findings show that embryos with less than 6 blastomeres show low potential for pregnancy, despite the fact that they are considered to have survived. We also studied the influence of the embryo quality on the delivery outcomes in FET cycle.

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