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ASSISTED REPRODUCTION TECHNOLOGIES

Frozen-thawed embryo transfer cycles in China: clinical outcomes of two and three multicellular embryos transfers

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Abstract
Purpose To analyze the clinical outcomes of frozen embryo transfer (FET) cycles when two or three multicellular embryos were transferred in Chinese women.
Methods A retrospective study was conducted to analyze 980 FET cycles performed between January 2007 and October 2010. Two (785 cycles) or three (195 cycles) multicellular embryos were transferred.
Results Both in patients under 35 years (n=776) and those aged 35 to 39 years (n=169), the transfer of two versus three multicellular embryos results in similar clinical pregnancy rates (CPR), implantation rates (IR) and live birth rates (LBR). In both age groups, the multiple pregnancy rate (MPR) was significantly higher in the three-embryo groups. Among women over 40 years of age (n=35), there were no differences in the CPR, IR, MBR or LBR between the two groups
Conclusions Transferring two instead of three multicellular embryos in patients under 40 years old significantly decreases the risk of MPR without compromising PR, IR and LBR. In the age group above 40, transferring two instead of three multicellular embryos did not decrease PR, IR, MBR or LBR. Transferring more embryos when a patient had more unsuccessful cycles was not warranted in all patients.

Keywords Frozen embryo transfer · Number of multicellular embryos · Clinical outcome

Capsule Transferring two instead of three multicellular embryos in FET cycles improved clinical outcome regardless patients' age and the number of previous unsuccessful cycles.

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Introduction

In vitro fertilization and embryo transfer (IVF-ET) has developed rapidly during the past two decades, and frozen embryo transfer (FET) has become an essential component of IVF programs worldwide. FET allows practitioners to reduce the number of transferred embryos, thereby diminishing the risk of multiple pregnancy [1]. FET also helps to maximize the cumulative pregnancy rate per oocyte retrieval. Additionally, freezing all embryos in a fresh cycle can prevent ovarian hyperstimulation. However, FET is not free from the risk of multiple conception. Many countries have recommendations regarding the number of embryos that should be transferred in fresh embryo cycles. However, few such recommendations are in place for FET cycles. In China, ART practitioners are guided by a document published by the Ministry of Health. In October 2003, document No. 176, *The Revised Standard Document Regarding Assisted Reproduction Techniques for Humans*, stipulates that no more than two embryos should be transferred in payotients under the age of 35 in their first transfer cycle and that all other patients should have no more than three embryos transferred. Because of the family planning policy in China, which stipulates one child per family, most patients undergoing FET cycles are those who did not have a clinical pregnancy in their fresh cycles or previous FET cycles. There is a tendency to transfer three embryos in FET cycles, which results in a significant increase in the multiple pregnancy rate. This study was conducted to compare the outcomes of FET cycles when two or three multicellular embryos were transferred in women of different age groups, with a goal of developing recommendations regarding the number of embryos to be transferred in these populations.

Materials and methods

This retrospective study included patients undergoing FET treatment at the reproductive medical center of Ruijin Hospital of Shanghai Jiaotong University of Medicine in Shanghai between January 2007 and October 2010. The data analysis covered 785 two-embryo transfers and 195 three-embryo transfers with 1289 IVF- and 866 ICSI-derived embryos. Two embryos were transferred in the patients under the age of 35 in their first transfer cycle. The patients older than 35 years or when they have a prior failed cycle were informed of the adverse results of multiple pregnancy and two or three embryos were replaced with their consent. The FET cycles with embryos derived from donated oocytes and more than one preceding cycle were excluded. This study was reviewed and approved by our institutional review board.

The patients were given standard controlled ovarian stimulation with a long GnRH agonist or antagonist protocol. In the long GnRH agonist protocol, the patients underwent pituitary down-regulation with subcutaneous injections of 0.1 mg/day of a GnRH agonist (Decapeptyl 0.1 mg; Ferring, Kiel, Germany) for 7 to 10 days in the mid-luteal phase of the previous menstrual cycle. For the antagonist cycle, antagonist was administered from the sixth day of stimulation onward. Ovarian stimulation was achieved using recombinant FSH (Gonal-F; Serono, Italy) and human menopausal gonadotropin (HMG, Lizhu, China). Human chorionic gonadotropin (hCG, Lizhu, China) was administered to trigger egg maturation when two or more follicles reached the size of ≥20 mm in diameter, and transvaginal oocyte retrieval was performed 36 h later. Oocytes were incubated in fertilization medium (HTF medium, Irvine Scientific, USA) for 4 h and insemination or intracellular sperm injection (ICSI) was performed. Oocytes were checked for the presence of pronuclei and polar bodies 16–18 h after insemination or ICSI. Zygotes were transferred to culture medium (P-1 medium, Irvine Scientific, USA) for the following 48 h. The quality of embryos was evaluated 72 h after oocyte retrieval (D3) based on the number of blastomeres, the degree of fragmentation and the equality of blastomeres. (1) For number of blastomeres, a score of 4 was given for 8–9 blastomeres, 3 for 6–7 blastomeres or >=10 blastomeres, and 2 for 5 blastomeres. (2) For degree of fragmentation, a score of 4 was given for <5% fragments, 3 for 5–10% fragments, 2 for 11–25% fragments, and 1 for 25–50% fragments. (3) For equality of blastomeres, a score of 1 was given for equal blastomeres and 0 for unequal blastomeres. Embryo quality was scored as the sum of these three measures. Embryos with more than 5 blastomeres, <50% fragments and a total score more than 5 on D3 were considered suitable for transfer. The two best embryos were transferred in the fresh cycle and the rest were cryopreserved. All embryos were cryopreserved in the fresh cycle secondary to hyperstimulation or a poor endometrium.

Cryopreservation was performed using an automated Kryo 10 series II biological freezer (Planer Products Ltd., Sunbury-on-Thames, UK) following a two-step protocol utilizing 1,2-propanediol (PROH, Sigma, Saint Louis, MO, USA) and sucrose as cryoprotectants. The freeze solution was prepared in phosphate-buffered saline (PBS, Gibco, Life Technologies, UK) supplemented with 20% serum substitute supplement (SSS, Irvine Scientific, Santa Ana, California). Embryos were first incubated in 1.5 M PROH freezing solution at room temperature for 10 min, followed by incubation in 1.5 M PROH and 0.2 M sucrose for 5 min. During the second incubation, one to two embryos were loaded into plastic ministraws (0.25 mL, Pailette Souple, Industrie de la Medicine Veterinaire Mechelen, Belgium) and placed in the freezing machine at 22°C, cooled at a rate of −2°C/min until −7°C, held at −7°C for 10 min after manual seeding, cooled at a rate of −0.3°C/min to −30°C, and then cooled at a rate of −3°C/min to −150°C before being plunged into liquid nitrogen.

The thawing solution was prepared in PBS supplemented with 20% SSS and 0.2 M sucrose. The embryo thawing was performed as follows: the straws with frozen embryos were removed from liquid nitrogen, exposed to room temperature for 30 s and immersed in a water bath at 31°C for 35 s. Thawed embryos were then incubated in the series of thawing solutions with decreasing PROH concentrations (1 M for 5 min, 0.5 M for 5 min and thawing solution without PROH for 5 min). The embryos were then incubated in PBS supplemented with 20% SSS for 5 min and finally transferred to culture media. Embryos were accepted for transfer if more than half of the blastomeres remained intact after thawing.

Frozen embryo transfer was performed in a hormone replacement cycle. Estradiol (Progynova, Bayer Schering, Berlin, Germany) was given at a dose of 2–4 mg per day, and the dose was modified according to the thickness and morphology of the endometrium. Daily intramuscular progesterone injections were started when the endometrial thickness reached 7 mm and the serum estradiol reached 200 pg/mL on approximately cycle day 15. Embryos were transferred on the fourth day of progesterone injection. Confirmation of pregnancy was obtained by a positive serum hCG test (≥10 IU/L) 11 days after FET. Clinical pregnancy was determined by ultrasound scanning when gestational sac(s) with or without fetal heartbeat was detected.

Data are reported as mean±SD and were analyzed using SPSS 16.0. Chi-squared or Fisher's Exact test were used whenever there was a cell with a small number ($n<5$), and Student's t test analysis was also performed. A two-sided P value of <0.05 was considered statistically significant.

Results

During the study period, there were a total of 980 FET cycles involving the transfer of two or three multicellular embryos. A total of 2155 embryos were transferred in 785 two-embryo transfer cycles and 195 three-embryo transfer cycles. The mean age of women at the IVF/ICSI treatment was 31.27±4.53 years. All FET cycles resulted in 400 (40.8%) clinical pregnancies. The overall multiple pregnancy rate was 25.5% (102/400). One and two gestational sacs were observed in 298 (74.5%) and 100 (25%) clinical pregnancies, respectively. Three gestational sacs were observed in 2 (0.5%) clinical pregnancies. The total implantation rate was 23.4% (504/2155). The delivery rate was 35% (343/980).

The results were analyzed separately for the following three age groups: patients under 35 years old at the time of the fresh cycle, patients aged 35 to 39 years and patients aged more than 40 years at the time of the fresh cycle. In patients under 35 ($n=776$), the two- and three-embryo transfer groups were not significantly different with respect to their fresh cycle characteristics, average endometrial thickness and score of transferred embryos. Average age and ET cycle number was significantly higher in the three-embryo group than in the two-embryo group (Table 1). The PR, IR and LBR were similar between the two groups (Figs. 1, 2, 4). The MBR was significantly higher in the three-embryo group than in the two-embryo group (Fig. 3). The multiple pregnancies in the two-embryo group were all twin gestations, but there were 27 twin gestations and 2 triplet gestations in the three-embryo group.

In patients aged 35 to 39 years at the time of the fresh cycle ($n=169$), the average age, ET cycle number, and fresh and frozen cycle characteristics were all similar in the two- and three-embryo groups (Table 1). Similarly, the PR, IR and LBR were similar in the two groups (Figs. 1, 2, 4). The MBR in the three-embryo group was significantly higher than in the two-embryo group (Fig. 3). The multiple pregnancies in the two groups were all twin gestations.

In patients over 40 years of age, the number of cycles was relatively small ($n=35$). All data between the two- and three-embryo groups were similar (Table 1, Figs. 1, 2, 3, 4).

Discussion

Since the first report of pregnancy from FET, embryo cryopreservation has been an essential component of IVF programs worldwide. The factors predicting the pregnancy outcome of FET cycles include patient's age [2], type of infertility [3], the developmental stage of the embryo at freezing [4], the quality of the embryos at freezing and the cleavage capacity [5]. A successful conception in fresh cycles is also shown to be associated with the successful outcome of a subsequent FET cycle [6]. The pregnancy rate in FET cycles was reported lower than fresh IVF/ICSI cycles [7, 8]. Therefore, practitioners and patients are motivated to transfer more embryos in FET cycles, thus increasing the risk of multiple pregnancies. The Practice Committee of the American Society for Reproductive Medicine recommended that the number of good-quality thawed embryos transferred in frozen embryo transfer cycles should not exceed the recommended limit on the number of fresh embryos transferred for each age group [9]. In recent years, efforts have been made to decrease the number of embryos transferred during frozen cycles in order to lower the risks of multiple pregnancies [10–12]. To our

Table 1 Analysis of the baseline and cycle characteristics of the two- versus three-embryo transfer groups by patient age

	Age <35 years			Age 35–39 years			Age >40 years		
	2 embryos transferred	3 embryos transferred	P value	2 embryos transferred	3 embryos transferred	P value	2 embryos transferred	3 embryos transferred	P value
Number of cycles	631	145	–	129	40	–	25	10	–
Age	29.50±2.94	30.37±2.63	0.001	36.43±1.39	36.68±1.52	NS	41.82±1.6	41.56±2.1	NS
Number of previous ET cycles	0.62±0.65	1.14±0.53	0.005	0.85±0.81	1.27±1.19	NS	1.3±1.5	1.5±1.4	NS
Basal FSH (mIU/mL)	5.4±1.9	5.3±1.9	NS	6.2±1.6	6.3±1.8	NS	8.1±1.9	7.7±2.0	NS
Peak E2 (in fresh cycles)	3875±1343	3842±1288	NS	3682±1232	3594±1198	NS	2837±1423	3392±1996	NS
ICSI performed	251	61	NS	50	18	NS	9	3	NS
Average endometrial thickness (mm) in FET cycles	8.9±0.1	9.0±0.2	NS	9.1±1.4	9.0±1.5	NS	8.9±1.6	9.0±1.1	NS

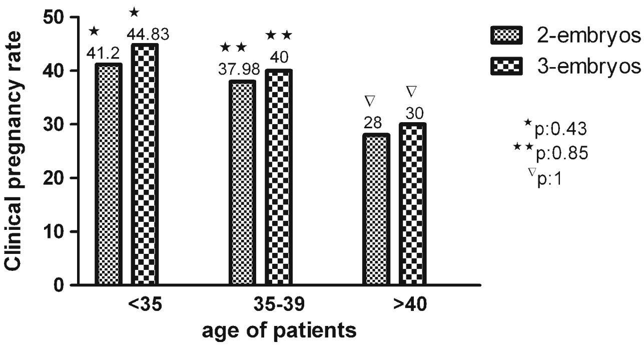

Fig. 1 Clinical pregnancy rate in two-versus three-embryo groups

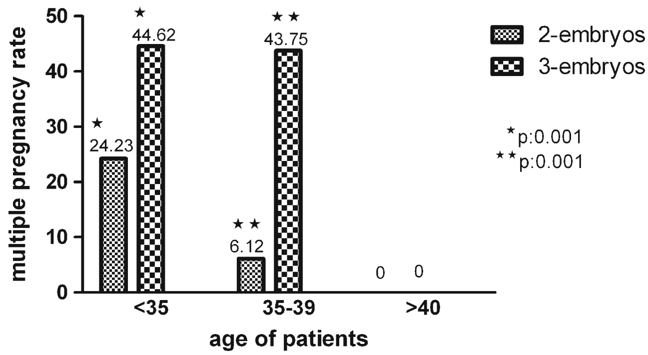

Fig. 3 Multiple pregnancy rate in two-versus three-embryo groups

knowledge, there is no study that has examined outcomes of the number of multicellular embryos transferred in FET cycles.

There are several factors associated with our study. We study the outcome of multicellular embryos and pay more attention to the patients with failed cycles. No more than three embryos were transferred in all age groups. Although extended embryo culture has allowed patients the advantage of selecting the best embryo, it also increases the risk of cycle cancellation. Patients with fewer embryos are not suitable for blastocyst transfer and embryos are more precious for them. Because infertile patients must pay out-of-pocket for their medical care in China, elective single embryo transfer is also not feasible to many patients. According to the recommendation of document No. 176 from the Ministry of Health of China, three embryos can be transferred in FET cycles when patients are older than 35 years or when they have a prior failed cycle. The average number of embryos transferred is generally much greater in FET cycles than in fresh cycles. In this study, we analyzed the relationship of the number of thawed embryos transferred in the context of women's age and the number of previous transfer cycles and the chances of clinical pregnancy and multiple pregnancy in the FET cycles. The results demonstrate that both in patients under 35 years and those aged 35 to 39 years, the transfer of two instead of three frozen embryos can result in a significant decrease in the multiple pregnancy rate without compromising the clinical pregnancy rate. The occurrence of triplet gestations can be reduced greatly. The baseline characteristics of the two- and three-embryo groups were similar, except for the increased age and number of embryo transfers in the three-embryo group among patients under 35 years of age. Both physicians and patients preferred to transfer three embryos when patients were older or there were more prior failed cycles. However, our study found that prior failed ET cycles were not an indication that three embryos should be transferred. In patients aged 35 to 39 years of age, a statistically significant increase in MPR was observed in the three-embryo group compared with the two-embryo group. This result was different with the conclusion of the prior study, which reported that transferring two instead of three embryos did not decrease the PR and the LBR [10]. Increasing the number of embryos transferred did not improve the chance of pregnancy in patients over 40 years of age. We regret that the number of cycles in this group is relatively small and we need more cycles to evaluate.

The results presented in our study suggest that transferring three multicellular embryos is unnecessary in a patient with prior failed cycles regardless of patient age. Two-embryo transfer will decrease the risk of multiple pregnancy and maximize the cumulative pregnancy rate of one controlled ovarian hyperstimulation cycle.

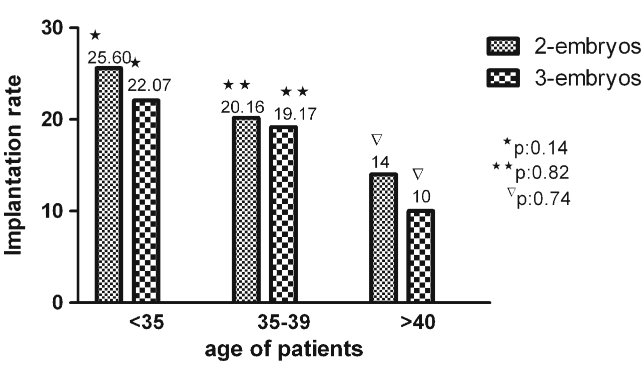

Fig. 2 Implantation rate in two-versus three-embryo groups

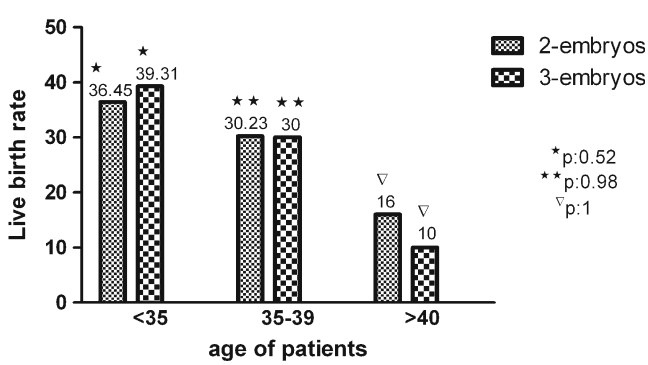

Fig. 4 Live birth rate in two-versus three-embryo groups

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Conflict of interest No conflict of interest exits in the submission of this manuscript.

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