Clinical outcome analysis of sequential transplantation of frozen-thawed embryo transfer cycle

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Research Article

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Abstract

Research Question: What were the advantages or disadvantages of sequential transplantation compared with the other two strategies in freeze-thaw embryo transfer?

Design: A total of 767 patients undergoing frozen-thawed embryo transfer from July 1, 2020, to June 30, 2021, were enrolled in this study. All patients were divided into three groups based on the age of embryos: group A consisted of 60 cases of sequential transplantation, group B consisted of 576 cases of early embryo transfer, while group C was the blastocyst transplantation group consisting of 131 cases. The clinical data, embryo status, and pregnancy outcome in the three groups were compared and analyzed.

Results: The number of subsequent transplants and the number of embryos transferred in the sequential group were significantly higher than those in the other two groups ($p < 0.05$). The blastocyst group (group C) had the highest implantation rate, which was significantly different from the early embryo group (group B, 43.50% vs. 30.00%), but there was no significant difference in the other two groups (39.13% vs. 30.00%, 39.13 vs. 43.50%). The sequential transfer group had the highest rate of multiple births and spontaneous abortion among the three groups, but there was no statistical difference.

Conclusion: Sequential transplantation may achieve a higher clinical pregnancy rate than traditional single transplantation by increasing the number of transplants and embryos in a single cycle. Still, it increases embryo waste and may increase the rate of early abortion. The basic situation of the patient should be integrated to decide on the type of embryo transplant.

Background:

Transferring frozen-thawed embryos is an integral part of assisted reproductive technology. It can increase the cumulative pregnancy rate while preventing the occurrence of ovarian hyperstimulation syndrome (OHSS) [1, 2]. A single day-3 embryo or day-5/6 blastocysts is commonly used in the frozen-thawed embryo transfer cycle. In recent years, the concept of continuous embryo transfer has been put forward, a second embryo transfer method that combines the cleavage embryo transfer and blastocyst transfer. Day-3 embryos and day-5/6 blastocysts are sequentially transferred in one resuscitation transfer cycle to achieve better pregnancy outcomes [3]. This study analyzed the clinical outcomes of sequential transplantation, cleavage embryo transplantation, and blastocyst transplantation during the resuscitation cycle and discussed the advantages and disadvantages of sequential transplantation to provide some guidance to assist reproductive technology practitioners for the selection of frozen-thawed embryo transfer mode.

Materials And Methods:

Research materials:

A total of 767 frozen-thawed embryo transfer cycles from July 1, 2020, to June 30, 2021, were retrospectively analyzed. The cases of transplanted embryos were divided into three groups based on their ages: group A was the sequential transplantation group (60 cases); group B was the early embryo transfer group (576 cases), and group C was the blastocyst transplantation group (131 cases).

Research methods:

Endometrial preparation scheme

Hormone replacement therapy was used for all cycles. An ultrasound and serological examination were performed on the second day of menstruation. Under normal conditions (normal endometrial thickness, no uneven echo, no cyst, and no other abnormalities, and blood progesterone (P) < 1.5 nmol/mL), oral estradiol valerate tablet (Progynova®, Bayer, Germany) was given at a dose of 3 mg/twice a day for seven days and then increased to 4 mg/twice a day. On the 14th day of the menstrual cycle, ultrasound was used to examine the endometrium, and blood estradiol level (E2) and P levels were measured. The transplantation criteria were met when the endometrial thickness was ≥ 8 mm and E2 levels above 300 pg/mL. Those patients who did not meet the transplant requirements in one cycle opted out of the resuscitation embryo transfer and waited for the endometrium to be prepared for the next menstrual cycle.

Progesterone support program

Estradiol valerate tablet 6 mg/day and progesterone intramuscular injection 80 mg/day were given after the transplantation. Cleavage embryos were transplanted on day 4 of progesterone injection and blastocysts on day 6 of progesterone injection.

Diagnostic criteria for clinical pregnancy

Patients underwent blood human chorionic gonadotropin (HCG) level testing after 12 days of embryo transfer to determine pregnancy. Ultrasound examination was performed 28 days after embryo transfer to confirm pregnancy; pregnancy sac in ultrasonography assures clinical pregnancy. The clinical pregnancy, implantation, and early spontaneous abortion rate were then calculated using the following relation.

$$Clinical\ pregnacy\ rate = \frac{Number\ of\ clinical\ pregnancy\ cycles}{Number\ of\ transplant\ cycles} \times 100\%$$

$$Implantation\ rate = \frac{Number\ of\ intra\ and\ extrauterine\ pregnancy\ capsules}{Total\ number\ of\ embryos\ transferred} \times 100\%$$

$$Early\ spontaneous\ abortion\ rate = \frac{Number\ of\ spontaneous\ abortion\ cycles\ (within\ 12\ weeks)}{Number\ of\ clinical\ pregnancy\ cycles} \times 100\%$$

Statistical methods

SPSS 17.0 software was used for statistical analysis. Measurement data is expressed as mean ± standard deviation (X ± S). The count data is expressed by rate, and the chi-square test is used between groups. The $p < 0.05$ indicated statistically significant difference.

Results:

Comparison of general information:

The mean ages of patients in groups A, B, and C were calculated to be 33.37, 34.06, and 33.69 years, while the years of infertility were found to be 3.81 (primary infertility rate = 35.0%), 3.28 (primary infertility rate = 44.1%), and 3.28 (primary infertility rate = 35.88%) years for groups A, B, and C, respectively. There was no significant difference in age, years of infertility, and primary infertility rates among the three groups ($p < 0.05$). The average number of embryos transferred from high to low was 2.35 in group A, 1.88 in group B, and 1.53 in group C, with statistically significant differences ($p < 0.001$). The average number of transplantation cycles of groups A, B, and C in our center were 2.47, 1.5, and 2.11, respectively, showing statistical differences ($p < 0.001$). Pairwise comparison showed that the number of transplants in group A was significantly higher than in group B and C ($p < 0.001$, $p = 0.006$, respectively). The results are shown in Table 1.

Table 1
Basic clinical data of patients in the three groups.

	Cases (n)	Average age (y)	Infertility duration (y)	Primary infertility rate(%)	Transferred embryos (n)	Transplant number (n)
Group A	60	33.37 ± 4.59	3.81 ± 3.23	35.00(21/60)	2.35 ± 0.48	2.47 ± 0.11
Group B	576	34.06 ± 5.70	3.28 ± 2.73	44.10(254/576)	1.88 ± 0.34	1.55 ± 0.04
Group C	131	33.69 ± 5.17	3.28 ± 2.48	35.88(47/131)	1.53 ± 0.52	2.11 ± 0.07
F or χ^2	-	0.590	1.052	4.2632	97.92	50.13
p	-	0.555	0.350	0.1187	50.13	0.0

Comparison of IVF outcomes in three groups:
Clinical pregnancy rate and implantation rate

There were significant differences among the three groups in the clinical pregnancy rate and embryo implantation rate. The pairwise comparison revealed that the clinical pregnancy rate of group A was 65.0%, which was significantly higher than 45.66% of group B, indicating a statistical difference. There was no significant difference in clinical pregnancy rates between groups C and A (50.38% vs. 65.0%) and groups C and B (50.38% vs. 45.66%). In descending order, the embryo implantation rate was 43.50%, 39.13%, and 30.00% in groups C, A, and B, respectively. There was a statistically significant difference between groups A and B ($p = 0.029$) and no statistically significant difference between groups A and C ($p = 0.423$), while a statistically significant difference between groups B and C ($p < 0.001$). The results are shown in Table 2.

Table 2
Clinical outcomes of the three groups.

	Clinical pregnancy rate (%)	Implantation rate (%)	Early miscarriages rate (%)	Multiple pregnancies (%)
Group A	65.00(39/60)	39.13(54/138)	20.51(8/39)	30.77(12/39)
Group B	45.66(263/576)	30.00(324/1080)	14.83(39/263)	22.42(59/263)
Group C	50.38(66/131)	43.50(87/200)	18.18(12/66)	31.82(21/66)
$\chi 2$	8.5092	16.742	1.0912	3.2532
p	0.0142	0.0002	0.5795	0.1967

Discussion:

As the conditions for embryo culture in assisted reproductive technology continue to improve, embryo transfer strategies also evolve, beginning with the first early embryo transfer and progressing to blastocyst transfer, and then the sequential transfer strategy was derived, which is presented in this work. The sequential transplantation was originally intended to increase embryo implantation rates in patients who had previously failed repeated implantation attempts. Synchronization of embryo implantation and endometrial receptivity is the key to improving the implantation pregnancy rate. The 'implant window period' of the endometrium of different patients is not entirely consistent, and it may be opened in advance or delayed. Studies[4] have shown that the 'implantation window' opening time may vary from cycle to cycle in the same patient. Approximately one-third of infertility patients experience changes in the endometrial implantation window, which is manifested as the shortening, advance, or delay [5]. Sequential transplantation allows the embryo to be placed in the uterine cavity twice in one implantation process, increasing the chances of the embryo and endometrial "implantation window" synchronization and thus improving the patient's pregnancy rate[]. The mechanism involves the transplantation stimulation enhances the sensitivity of the endometrium as first step, which causes endometrial cells to secrete cytokines such as IL-1, transforming growth factor, epidermal growth factor, and granulocyte-macrophage stimulation factor, which seeds the endometrium to enter the "planting window" state and improves the transplantation outcomes[5, 7].

This study showed that sequential transfer had a higher clinical pregnancy and embryo implantation rate than early embryo transfer cycle but had no advantage over the blastocyst transfer cycle. The results were consistent with the findings of Wang Miaomiao's et al. research[8, 9]. Sequential transplantation required at least two embryos per cycle, significantly higher than the blastocyst transfer cycle, but it did not improve clinical outcomes. Due to the low utilization rate of embryos in the sequential transplantation cycle, the sequential transplantation strategy should be carefully used for patients undergoing the first transplant to avoid embryo waste [10]. Meanwhile, sequential migration is still controversial. Machtinger

et al. [11] hypothesized that two-step transplantation could improve embryo implantation and clinical pregnancy rates in patients with repeated implantation failure [12]. Fang et al. [13]. pointed out in their study that sequential transplantation could significantly improve clinical pregnancy and embryo implantation rates in patients with repeated implantation failure compared to embryo transfer at the cleavage stage. However, Jacob Ashkenazi et al. [14] believed that when compared to traditional embryo transfer at the cleavage stage, two-step transplantation is not conducive to improving embryo implantation and clinical pregnancy rate, which could be due to repeated intrauterine operations that may lead to infection and endometrial damage, increasing the risk of abortion. However, Tur-Kaspa et al. [15] showed that sequential transplantation did not increase the risk of infection. Some researchers compared the rate of early abortion after blastocyst and cleavage embryo transplantation. It concluded that blastocyst transfer has a lower risk of early abortion than embryo transfer in both the egg and freeze-thaw cycle stages, which did not appeared in our data. There remains a scarcity of multi-center, large-sample data that can be used to confirm the feasibility of sequential transplantation. The existing controversies also suggest that close attention should be paid to the application indications of sequential transplantation strategies to achieve greater social benefits.

In conclusion, sequential transplantation is an emerging strategy for embryo transfer. By increasing the number of transplants and the number of embryos in the cycle, the ideal pregnancy rate, and the number of endometrial transplant preparations for infertile patients can be reduced, as well as the economic and time costs of patients. Simultaneously, more clinical evidence is needed to support the study of birth outcomes and the subsequent development of the sequential transplantation cycle. In future studies, it is necessary to comprehensively evaluate various factors of patients, and try to combine with immune indicators to classify and stratified detection of patients, so as to further clarify the mechanism of sequential transplantation, so as to accurately target populations and give full play to the advantages of sequential transplantation.

Declarations:

Ethics approval and consent to participate:

The research protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Hangzhou Women's Hospital. Written informed consent was obtained from the study individual.

Consent for publication:

Not applicable.

Availability of data and material:

All data generated or analysed during this study are included in this published article

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

Zhou Wenjing conducted the research design. Cheng Zhaojun is responsible for data analysis, review and editing. Wang Chong is responsible for data supervision and audit. Feng Ying is responsible for data collection and management. All authors reviewed the manuscript.

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