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Outcome Analysis of Day 4 Embryo Transfer During Fresh Cycle in Assisted Reproductive Technology: A Retrospective Analysis

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Abstract

Objective: Clinical outcomes of Day 4 transfer cycles in fresh cycles were followed up to explore the feasibility of Day 4 embryo transfer in fresh cycles.

Methods: This study retrospectively counted infertile patients who underwent fresh cycle transfer at Hangzhou Women's Hospital from January 1st, 2019 to December 31st, 2021. The embryos were grouped according to the embryonic age and divided into Day 3 and Day 4 groups. Analyzed differences in general, of infertility factors, the average number of eggs obtained, the average number of embryos transferred, clinical pregnancy outcomes and birth weight of newborns.

Results: There was a no difference in clinical data of average age, average years of infertility, average blood AMH value, percentage of primary infertility, endometrial thickness on the day of transplantation, and infertility factors in both groups ($p>0.05$). Differences in the average number of eggs obtained between groups D3 and D4 ($p>0.044$). However, there was no statistical differences in the average number of embryos transferred, clinical pregnancy rate, implantation rate, multiple birth rate, early miscarriage rate, live birth rate, birth weight etc. ($p>0.05$).

Conclusion: Transferring Day 4 embryos in a fresh cycle is safe and feasible, and it have similar clinical pregnancy outcomes as Day 3 embryos, but result in a greater chance of transfer than Day 3.

Keywords: Assisted reproductive technology; Embryo transplantation; Blastocyst culture; Birth follow-up

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Introduction

In recent years, the incidence of infertility among the Chinese population of childbearing age has risen to 10% to 15%, and the need for assisted reproductive technology such as IVF-ET (*in vitro* Fertilization—Embryo Transfer) has become a grim reality for an increasing number of families. Embryo culture and transfer are important components of Assisted Reproductive Technology (ART), and the right timing of transfer during embryo development can increase the clinical pregnancy rate for infertile couples. Each reproductive medicine center differs in the choice of embryo age for fresh cycle embryo transfer, with some centers choosing oogenesis embryos and others blastocyst transfer. However, Day 4 embryos are less commonly transferred in fresh cycles than in other transfer models [1,2], and Day 4 embryos are mostly used in resuscitation cycles to achieve better pregnancy outcomes [3,4]. There is a paucity of information on the outcome of Day 4 transfers in fresh cycles, and it is worthwhile to investigate whether there are differences in clinical outcomes compared to Day 3 transfers. In this study, we retrospectively analyzed the clinical outcomes of fresh transfer cycles in the last three years at our reproductive medicine center to explore the value of a day-four embryo transfer strategy in fresh transfer cycles.

Materials and Methods

Materials: Retrospective analysis of fresh transfer cycles performed at the Reproductive Medicine Center of Hangzhou Women's Hospital from January 1st, 2019 to December 31st, 2021.

Exclusion criteria: 1. A female partner aged 40 years; 2. Chromosomal abnormalities in either spouse; and 3. Congenital abnormalities in the female uterus, such as a small uterus, a longitudinal uterus, and other congenital uterine abnormalities [5-7]. A total of 768 cycles met the requirements

and were included in the study. The embryos were grouped according to the number of days of development of the transferred embryos, with the cycles in which Day 3 embryos were transferred being the D3 group (n=640) and the cycles in which Day 4 embryos were transferred being the Day 4 group (n=128). Differences in clinical data, AMH (Anti-Müllerian Hormone), number of embryos transferred, clinical pregnancy rate, implantation rate, early miscarriage rate, and births were counted between the groups. 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.

Methods: After egg retrieval and fertilization, embryos were cultured to Day 3 or 4 for transfer. Endometrium, ovarian size, and pelvic fluid were examined by ultrasound prior to the transfer procedure. Transfer can only be performed if certain conditions are met, such as uniform endometrial echogenicity, thickness greater than 8 mm, the ovaries are not prone to overstimulation, and no uterine fluid accumulation. Blood beta-HCG was checked 14 days after transplantation; if positive, luteal support was continued, and an ultrasound was performed 28 days after transplantation to observe the condition of the gestational sac.

Outcome Determination Criteria [8].

Day 3 quality embryo determination criteria: Refer to the WHO morphological scoring method; grades I and II are considered quality embryos.

Day 4 embryo transfer criteria: Partial or complete fusion of 14 or more cleft globes.

Clinical pregnancy determination: blood beta-HCG is checked 14 days after transfer, and the gestational sac is visible under ultrasound 28 days after transfer (clinical pregnancy includes ectopic pregnancy).

Clinical pregnancy rate = number of pregnancy cycles/number of transplant cycles × 100%

Fertility rate = number of gestational sacs/number of embryos transferred × 100%; monozygotic twins are counted as 1 gestational sac.

Early abortion rate = number of spontaneous abortion cycles within 12 weeks or number of clinical pregnancy cycles × 100%.

Live birth rate = number of live birth cycles/number of transferred cycles × 100%.

Statistical methods

Analysis was performed using IBM SPSS 17.0 software. Count data were expressed as a rate (%), and statistics were calculated using the X^2 test. $p < 0.05$ was considered a statistically significant difference.

Results

1. The results of the clinical data were: average age (31.91 *vs.* 32.41) years; average years of infertility (3.21 *vs.* 3.12) years; average blood AMH value (3.83 *vs.* 3.99) ng/mL; percentage of primary infertility (50.32% *vs.* 43.75%); endometrial thickness on the day of transplantation (10.35 *vs.* 10.43); and infertility factors in both groups, none of which were statistically different (p>0.05); see Table 1.

2. Transfer of embryos and clinical outcome There were differences in the average number of eggs obtained between groups D3 and D4 (9.58 *vs.* 9.33, p=0.044). However, there were differences in the average number of embryos transferred (1.78 *vs.* 1.74), clinical pregnancy rate (64.06% *vs.* 60.16%), implantation rate (47.23% *vs.* 46.19%), multiple birth rate (31.22% *vs.* 33.77%), early miscarriage rate (9.02% *vs.* 14.29%), live birth rate (55.78% *vs.* 47.66%), birth weight (1855.13 *vs.* 1814.71) etc. There were no statistical differences (p>0.05); see Table 2.

Discussion

In assisted reproductive technology, the majority of fresh cycle embryos are transferred on the fourth day after egg retrieval (Day 3 embryos), chosen to mimic the timing of tubal fertilization and uterine implantation in reproductive physiology [9]. *In vitro* fertilization embryo culture is the process of embryo development from a fertile egg to a blastocyst. The embryo itself is a superior process, and sperm malformations, chromosomal abnormalities, and egg quality can affect the development of the embryo. The timing of the transfer is an important factor in obtaining a clinical pregnancy. The literature shows that there are many studies related to fresh cycle D3 embryo transfer and blastocyst transfer [10], but there are fewer studies related to fresh cycle D4 embryo transfer, and the results are not validated by a large amount of data for the time being. The D4 stage embryo is a solid individual formed by the fertilized egg dividing by 8 to 16 cells, and the embryo is shaped like a mulberry fruit, hence the name mulberry embryo. The results of this study showed no difference between the D4 transferred embryos and the D3 group in terms of clinical case composition, AMH, or average number of embryos transferred. There were differences in the number of eggs obtained between the two groups, with a difference of 0.25 eggs, which may be related to the lower age of patients in the D3 group compared to the D4 group. As the D4 transplant cycle is not a transplant strategy routinely used in our center, it takes longer to obtain more cycles for exploration. Analysis of the transfer outcomes of the last three years of fresh cycles shows that at this stage, D4 embryo transfer is safe and can have the same pregnancy outcomes as D3 embryos. The shorter culture time for Day 4 embryos compared to blastocyst transfer reduces the risk of cycle cancellation due to the

Table 1: Clinical data and embryos transferred during the transfer cycle.

	D3 group	D4 group	F/χ^2	*p*
Cases (n)	640	128		
Female age (y)	35.91 ± 3.92	32.14 ± 4.33	0.754	0.385
Infertility years (y)	3.21 ± 2.55	3.12 ± 2.12	3.605	0.058
AMH (ng/mL)	3.83 ± 2.29	3.99 ± 2.29	0.110	0.740
Primary infertility (%)	50.31	43.75	1.840	0.175
Inner membrane thickness (mm)	10.35 ± 2.16	10.43 ± 2.31	2.360	0.108
Infertility factors (%)				
Fallopian tube factors	34.38	39.84	2.014	0.156
Male factors	15.47	13.28	0.398	0.528
Factors for both sides	26.88	28.13	0.084	0.771
Unspecified	11.09	8.59	0.699	0.403
Ovulation disorders	8.44	7.81	0.055	0.815
Uterine adhesions	3.75	2.34	0.622	0.430
Harvested eggs (n)	9.58 ± 3.32	9.33 ± 3.74	4.084	0.044
Fertilization rate (%)	71.26	71.36	0.990	0.320
Transferred embryos (n)	1.78 ± 0.41	1.74 ± 0.44	3.118	0.078

Table 2: Follow-up outcomes of the transplantation cycle.

	D3 group	D4 group	F/χ^2	*p*
Cases (n)	640	128		
Clinical pregnancy rate (%)	64.06	60.16	0.702	0.402
Implantation rate (%)	47.26	46.19	0.082	0.775
Multiple pregnancy rate (%)	31.22	33.77	0.194	0.659
Early miscarriage rate (%)	9.02	14.29	2.02	0.155
Live birth cycles (n)	356	61	1.562	0.211
Live birth rate (%)	55.78	47.66	0.702	0.402
Premature birth rate (%)	22.19	29.51	1.562	0.211
Birthweight (g)	1855.12 ± 1482.76	1814.71 ± 1415.33	2.09	0.089

prolonged in vitro culture time for blastocyst culture and reduces the emotional, psychological, and financial burden on the patient [11]. Again, the presentation of D4 embryos to the compaction and morula stages already provides a clear choice. In the present study, it was not possible to confirm whether there were differences in blastocyst transfer because there were no fresh cycles of blastocyst transfer in our center for comparison [12-14]. The high rate of multiple births is the most common complication of assisted reproductive technology and cannot be completely resolved; it can only be reduced by reducing the number of embryos transferred [15,16]. The timing and advantages of D4 embryonic development can make it an effective timing option for a single embryo transfer strategy. The shorter culture time of D4 embryos compared to blastocysts reduces the incidence of cancelled cycles due to blastocyst culture [17], and the incidence of monozygotic twins at the oogenesis stage is significantly lower than that of blastocysts [15,18]. Selective single embryo transfer of D4 embryos at the compaction/morula stage reduces the number of embryos transferred at the cleavage stage and reduces the number of multiple pregnancies caused by blastocyst transfer [19]. Since the cryo-resuscitation outcome of embryonic D4 freezing has been less studied, consider the safety of the offspring, we will the remaining D4 embryos for blastocyst culture, ceases the unknown risk of D4 freezing.

In summary, D4 embryo transfer can be used as alternatives for fresh cycle transfer, achieving a similar clinical outcome to D3 embryo transfer while increasing the patient's chances of transfer. Selective single embryo transfer of D4 embryos at the compaction/morula stage reduces the number of embryos transferred at the cleavage stage and reduces the number of multiple pregnancies caused by blastocyst transfer. The D4 embryo transfer protocol also can be used to address the social and personal factors that prevent patients from transferring on time on day 3, such as busy work schedules, poor transport, and other social factors, as well as stressful situations such as mental stress and high blood pressure that make D3 patients unsuitable for transfer and can reduce the emotional burden on patients. As a next step, we will explore whether D4 embryos can achieve better pregnancy outcomes than D5/6 blastocysts and increase the carryover rate, and whether D4 embryos can be used as an alternative to single embryo transfers to reduce multiple pregnancies and achieve healthier offspring.

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