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Clinical outcomes after elective double-embryo transfer in frozen cycles for women of advanced maternal age

A retrospective cohort study

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Abstract

We aimed to determine the clinical outcome of double cleavage-stage embryo transfers in frozen-thawed embryo transfer cycles for older women.

This study analyzed a total of 8189 cleavage-stage frozen-thawed embryo transfer cycles between January 2013 and December 2017 at Sir Run Run Shaw Hospital. All cycles were sorted into 3 groups based on patient age: ≤35 years (Group A), 36 to 37 years (Group B), and ≥38 years (Group C). The clinical pregnancy rate (CPR), implantation rate (IR), live birth rate (LBR), miscarriage rate, multiple pregnancy rate (MPR), preterm birth rate, and low-birth-weight rate were compared between the 3 groups.

Significant differences in CPR, IR, LBR, MPR, and premature birth rate were found among the 3 groups. The CPR, IR, LBR, and MPR in Group A were higher than those in Group C. Transfers of 2 high-quality embryos resulted in significant differences in CPR, IR, LBR, MPR, and neonatal weight among the 3 groups, but no differences in premature birth and abortion rates were observed. Transfers with 1 high-quality and 1 fair-quality embryo resulted in significant differences in CPR, IR, and LBR among the 3 groups. Comparison of transfers of 2 high-quality embryos with 1 high-quality and 1 fair-quality embryo showed that the CPR and LBR were significantly lower for the latter in Groups A and C, but Group B had no salient changes.

Higher IR and LBR and lower MPR may be achieved by selection of optimal embryo types for patients of different ages. Two high-quality embryos need to be transferred in women older than 38 years. For women aged 36 to 37 years, 1 high-quality embryo or 1 high-quality plus 1 fair-quality embryo should be singled out for transfer. For women younger than 35 years, a single high-quality embryo should be selected for transfer.

Abbreviations: BMI = body mass index, CPR = clinical pregnancy rate, FET = frozen-thawed embryo transfers, HCG = human chorionic gonadotropin, HMG = human menopausal gonadotropin, ICSI = intracytoplasmic sperm injection, IR = implantation rate, IVF = in vitro fertilization, LBR = live birth rate, MPR = multiple pregnancy rate.

Keywords: advanced age, cleavage embryo, frozen-thaw embryo transfer, live birth rate

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1. Introduction

In vitro fertilization (IVF) is an effective method for treating infertility. There are more than 200,000 women in China seeking IVF treatment each year. The cryopreservation of embryos is increasingly important in assisted reproductive technology; this is because older women with decreased ovarian reserve often need to undergo multiple ovarian stimulation cycles to accumulate embryos for transfer. Hormone replacement cycles can precisely regulate the synchronization of embryo and endometrium and significantly improve pregnancy rates. Therefore, the number of frozen-thawed embryo transfers (FET) is increasing worldwide.[1,2] According to the Society for Assisted Reproductive Technology, the percentage of FET increased 82.5% from 2006 to 2012.[3] With the implementation of the 2-child policy in China, diagnosed infertility in women over 35 years of age has increased. Because age is crucial to the final outcome of IVF, it is important to develop embryo transfer protocols specifically for advanced-age patients to obtain good pregnancy outcomes while reducing multiple birth rates. The risk of adverse outcomes for both mother and fetus increases in multiple pregnancies, and so choosing the appropriate number of embryos to transfer can effectively avoid such outcomes.[4] However, women with advanced maternal age have considerably lower clinical pregnancy and live birth rates (LBRs) compared with younger

groups. A selective embryo transfer strategy for older women remains undetermined to date. Our research aimed to determine the clinical outcomes from double cleavage-stage embryo transfer in FET cycles for women of advanced maternal age. We focused on implantation rate (IR), clinical pregnancy rate (CPR), multiple pregnancy rate (MPR), LBR, number of gestational days, and neonatal weight.

2. Methods

2.1. Ethics statement

This study was approved by the institutional ethical review board of Sir Run Run Shaw Hospital, the Affiliated Hospital of the College of Medicine, Zhejiang University.

2.2. Study design

This retrospective study reviewed a total of 8189 cleavage-stage FET cycles between January 2013 and December 2017 at the Affiliated Sir Run Run Shaw Hospital, Zhejiang University. The inclusion criteria were: chromosomally normal couples with IVF/intracytoplasmic sperm injection (ICSI) indication, first FET cycle, cleavage-stage embryo transfers, and 1 or 2 high-quality embryos per transfer. The exclusion criteria were: previous history of IVF/ICSI treatment, previous history of FET, blastocyst-stage embryo transfer, 1 fair-quality embryo alone or more than 2 embryos transferred. All cycles were categorized into 3 groups based on patient age: $\leq$35 years (Group A), 36 to 37 years (Group B), and $\geq$38 years (Group C). Every patient, nearly all couples, acknowledged that they fully understood the protocol and then signed the informed consent form.

2.3. Endometrial preparation

In frozen-thawed cycles, 3 major clinical protocol categories was applied to endometrial preparation: natural cycles, hormone replacement treatment cycles, or low-dose human menopausal gonadotropin (HMG) cycles. For natural cycles, embryo transfer was planned for 3 days after ovulation, or 4.5 days after the luteinizing hormone peak. For hormone replacement treatment cycles, oral estradiol valerate tablets (6–8 mg/d) were administered for 14 to 20 days. Transvaginal ultrasound scanning is an effective modality for assessing endometrial thickness. If the endometrium thickness were $\geq$8 mm and the serum estradiol concentration reached 300 ng/L, injections of 80 mg/d of progesterone were provided for 3 days before embryo transfer. For low-dose HMG cycles, mild stimulation was initiated with a low dose of HMG on days 8 to 11 of the menstrual cycle, and dosage was adjusted according to follicle development. Ovulation was induced by human chorionic gonadotropin (HCG) injection, followed by 40 mg/d progesterone injections. Embryo transfer was performed 4.5 days after HCG administration, and 60 mg/d progesterone was injected for luteal support after transfer.

2.4. Embryo freezing and thawing

Patients were treated with conventional long or short gonadotropin-releasing hormone agonist regimens for controlled ovarian hyperstimulation. Oocytes were fertilized using either conventional IVF or ICSI. Day 3 embryos were suspended in equilibration solution for 9 minutes at room temperature, and then transferred to vitrification solution for 30 to 60 seconds. Embryos were loaded on a Cryotop strip (Kitazato Supply Co., Fujinomiya, Japan), and then immediately put into liquid nitrogen for cryopreservation. For warming, the Cryotop strip was taken out of the liquid nitrogen and directly inserted into thawing solution for 1 minute at 37°C. Warmed embryos were transferred to diluent solution for 3 minutes and then washed in washing solution for 5 minutes and 1 minute. Embryos were subsequently placed in G2 medium containing 12% human serum albumin and cultured for 2 to 4 hours prior to transfer.

2.5. Assessment of embryo quality

According to the uniformity of blastomeres, the number of blastomeres and degree of cytoplasmic fragmentation, we evaluated embryonic development on Day 3 (70–72 hours). Four grades of embryos were defined: grade 1, embryo contained 6 to 12 cells of the same size without cytoplasmic fragmentation; grade 2, embryo contained 6 to 12 cells with cytoplasmic fragmentation $\leq$20% and the same or different size; grade 3, embryo contains 4 to 12 cells, with a fragmentation rate of 21% to 49%, and cells of equal or different sizes; grade 4, embryo had $>$50% cytoplasmic fragmentation. Thawed embryos with at least half of the cells remaining intact were considered viable embryos. Therefore, only grade 1 to 3 embryos could be transferred. Grade 1 to 2 embryos were considered high-quality embryos, and grade 3 embryos were considered fair-quality embryos. Embryos were selected for transfer to the mid endometrium using a Wallace transfer tube under abdominal ultrasound guidance.

2.6. Definition of outcome

Pregnancy is usually predicted by measuring serum HCG levels on the 12th day after frozen embryo transfer. Clinical pregnancy was defined by ultrasound observation of a gestational sac 35 days after embryo transfer. The CPR was defined as clinical pregnancy cycles/transfer cycles. The definition of the IR was the number of intrauterine gestational sacs/number of embryos transferred. LBR was defined as the number of fetuses born alive/transfer cycles. MPR was the number of multiple pregnancy cycles/number of live-birth cycles.

2.7. Statistical analysis

Statistical calculations were performed using the SPSS Statistics for Windows, version 17.0 (SPSS, Inc., Chicago, IL). Measurement data were analyzed using the ANOVA test. Count data were analyzed using the chi square test or Fisher exact test (2-tailed). $P < .05$ was regarded as statistically important. The data of 8189 cleavage-stage FET cycles were examined. Of these, 7009 cycles had 2 cleavage-stage embryos per transfer, and 1180 cycles had single high-quality embryos per transfer.

3. Results

3.1. Comparison of the clinical outcomes of double cleavage-stage embryo transfer

A total of 7009 double cleavage-stage FET cycles were analyzed; 4479 cycles resulted in pregnancy (63.9% TPR). A total of 14,018 FETs were examined, in which 6048 implanted (43.1% total IR). From the FETs, 3973 live births were obtained (56.7%

Table 1

Clinical outcomes of the 3 age groups.

	≤35 years (average age 29.92 years)	36–37 years (average age 36.44 years)	≥38 years (average age 40.36 years)	*P* value
Embryo transfer cycles	5963	475	571	
Cause of infertility				<.05
Tubal	4054 (68.0%)	324 (68.2%)	385 (67.4%)	
Endometriosis	254 (4.3%)	21 (4.4%)	11 (1.9%)	
Ovulatory disorder	285 (4.8%)	6 (1.3%)	5 (0.9%)	
Decline in ovarian reserve	202 (3.4%)	32 (6.7%)	89 (15.6%)	
Male factor	1023 (17.2%)	55 (11.6%)	63 (11.0%)	
Unexplained	855 (14.3%)	37 (7.8%)	18 (3.2%)	
E2 on the day of FET	481.84	491.41	515.97	>.05
P on the day of FET	24.29	23.84	21.62	.024
Duration of infertility (yr)	3.7±0.1	5.9±0.5	7.7±0.8	<.001
No. thawed embryo	11,054	909	1093	
No. survived embryo	10,756	888	1064	
Embryo survival rate	10,756 (97.3%)	888 (97.7%)	1064 (97.3%)	>.05
Endometrium preparation				
HRT	5181 (86.9%)	437 (92.0%)	547 (95.8)	<.001
NC	124 (2.1%)	5 (1.1%)	2 (0.4%)	
HMG	658 (11.0%)	33 (6.9%)	22 (3.8%)	
No. high-quality embryo transfer	9096 (76.3%)	718 (75.6%)	825 (72.2%)	.01
Endometrium thickness (mm)	9.4±0.0	9.3±0.1	9.1±0.1	.001
Pregnancy rate per cycle	4003 (67.1%)	254 (53.5%)	222 (38.9%)	<.001
Implantation rate per cycle	5449 (45.7%)	327 (34.4%)	272 (28.9%)	<.001
Live birth rate per cycle	3554/5963 (59.6%)	229/475 (48.2%)	190/571 (33.3%)	<.001
Multi pregnancy rate (%)	1250/3554 (35.2%)	62/229 (27.1%)	36/190 (18.9%)	<.001
Gestational days (d)	263.7±0.8	263.5±2.8	265.3±4.0	.929
Neonatal weight (g)	3007.2±14.3	3137.6±66.9	3196.9±72.4	.005
Preterm birth (<37 wks)	456/3554 (12.8%)	31/229 (13.5%)	29/190 (15.3%)	.604

E2 = estradiol, FET = frozen-thawed embryo transfers, HMG = human menopausal gonadotropin, HRT = hormone replacement therapy, NC = natural cycle, P = progesterone.

LBR). There were 1348 multiple births (33.9% multiple birth rate).

As shown in Table 1, there were 5963, 475, and 571 cycles in Groups A, B, and C, respectively. For Group A, the average age was 29.92 years; for Group B, the average age was 36.44 years; for Group C, the average age was 40.36 years. All 3 groups showed significant differences in clinical pregnancy, implantation, LBR, and MPR ($P < .05$). The CPRs were 67.1%, 53.5%, and 38.9% in Groups A, B, and C, respectively. The IRs in Groups A, B, and C were 45.7%, 34.4%, and 28.9%, respectively. The LBR s in Groups A, B, and C were 59.6%, 48.2%, and 33.3%, respectively. The MPRs were 35.2%, 27.1%, and 18.9% in Groups A, B, and C, respectively.

3.2. Comparison of the clinical outcomes of patients with 2 high-quality embryos transferred

As indicated in Table 2, the 3 groups showed significant differences in clinical pregnancy, implantation, live birth and MPRs, and neonatal weight ($P < .001$) after transfer of 2 high-quality embryos. However, the preterm birth rate and gestational days showed no difference between the groups. Although the

Table 2

Comparison of the clinical outcomes from 2 high-quality embryos per transfer.

	≤35 years (average age 29.92 years)	36–37 years (average age 36.44 years)	≥38 years (average age 40.36 years)	*P* value
Embryo transfer cycles	3758	296	318	
Duration of infertility (yrs)	3.4±0.1	5.8±0.8	7.8±1.3	<.001
Embryos from ICSI	1103 (29.4%)	77 (26.0%)	116 (36.5%)	.01
Endometrium thickness (mm)	9.4±0.0	9.3±0.1	9.1±0.1	.026
Pregnancy rate per cycle	2734 (72.8%)	174 (58.8%)	143 (45.0%)	<.001
Implantation rate per cycle	3831 (51.0%)	232 (39.2%)	173 (27.2%)	<.001
Live birth rate per cycle	2416/3758 (64.3%)	161/296 (54.4%)	123/318 (38.7%)	<.001
Multi pregnancy rate (%)	942/2416 (39.0%)	48/161 (29.8%)	24/123 (19.5%)	<.001
Gestational days (d)	263.1±1.1	260.5±5.1	268.5±1.4	.61
Neonatal weight (g)	2969.8±17.8	3141.8±83.3	3268.6±86.5	.001
Preterm birth (<37 wks)	312/2416 (12.9%)	22/161 (13.7%)	13/123 (10.6%)	.713

ICSI = intracytoplasmic sperm injection.

Table 3

Comparison of the clinical outcomes from 1 high-quality plus 1 fair-quality embryo per transfer.

	≤35 years (average age 29.92 years)	36–37 years (average age 36.44 years)	≥38 years (average age 40.36 years)	*P* value
Embryo transfer cycles	1576	126	189	
Duration of infertility (yrs)	3.9 ± 0.2	5.7 ± 0.9	8.1 ± 1.4	<.001
Embryos from ICSI	556 (35.3%)	45 (35.7%)	69 (36.5%)	.944
Endometrium thickness (mm)	9.4 ± 0.0	9.2 ± 0.2	9.1 ± 0.1	.018
Pregnancy rate per cycle	963 (61.1%)	61 (48.4%)	67 (35.4%)	<.001
Implantation rate per cycle	1230 (39.0%)	76 (30.8%)	86 (22.8%)	<.001
Live birth rate per cycle	867/1576 (55.0%)	55/126 (43.7%)	57/189 (30.2%)	<.001
Multi pregnancy rate (%)	236/867 (27.2%)	14/55 (25.5%)	10/57 (17.5%)	.272
Gestational days (d)	263.7 ± 1.3	265.3 ± 2.5	268.7 ± 4.2	.68
Neonatal weight (g)	3077.2 ± 26.9	3101.7 ± 140.7	3094.0 ± 131.8	.005
Preterm birth (<37 wks)	108/867 (12.5%)	8/55 (14.5%)	14/57 (24.6%)	.032

ICSI = intracytoplasmic sperm injection.

CPRs in Groups B and C (58.8% and 45.0%, respectively) were significantly lower compared with Group A, the numbers were relatively high.

3.3. Comparison of the clinical outcomes of patients with 1 high-quality embryo plus 1 fair-quality per embryo transfer

Following the transfer of 1 high-quality plus 1 fair-quality embryo, significant differences in clinical pregnancy, embryo implantation, and LBRs were found among the 3 groups ($P < .001$), as shown in Table 3. The CPR was 61.1%, 48.4%, and 35.4% in Groups A, B, and C, respectively. The IRs were 39.0%, 30.8%, and 22.8% in Groups A, B, and C, respectively. LBRs/cycle in Groups A, B, and C were 55.0%, 43.7%, and 30.2%, respectively. A pairwise comparison between the groups revealed that clinical pregnancy, IR, and LBR were significantly higher in Group A than Groups B and C, but were equivalent between Groups B and C.

3.4. Comparison of the clinical outcomes between patients with single high-quality embryo and double embryo transfers

As indicated in Table 4, for Group A, the pregnancy and LBRs were significantly lower ($P < .001$) with single high-quality compared with double embryo transfer cycles (50.9% vs 67.1% and 45.9% vs 59.6%, respectively). Single high-quality and double embryo transfers showed no difference in Group B ($P > .05$). For Group C, the pregnancy and LBRs were significantly lower ($P < .001$) with single high-quality embryo transfer than those of double embryo transfer.

4. Discussion

This study showed that clinical pregnancy and LBRs were affected by age and embryo quality. The clinical pregnancy and LBRs decreased as the patients got older. Conversely, the pregnancy rate and LBR increased with the increase in the number of high-quality embryo transfers. This study found that patients 36 to 37 years old or >38 years old had significantly diminished clinical birth and LBRs compared with those younger than 35 years. In the same age grouping, more high-quality embryo transfers guaranteed higher clinical pregnancy and LBRs. In comparison, single high-quality embryo and double embryo transfers had similar clinical pregnancy and LBRs in the 36- to 37-year-old group, and the clinical results of single high-quality embryo transfer in the ≤35-year-old group remained high compared with older groups. However, single high-quality embryo transfers significantly reduced clinical pregnancy and

Table 4

Comparison of the clinical outcomes from single high-quality embryo transfers and double embryo transfers.

	Single high-quality embryo	Two embryos	*P* value
≤35 years (average age 29.92 years)			
Embryo transfer cycles	956	5963	
Pregnancy rate per cycle	487/956 (50.9%)	4003 (67.1%)	<.001
Implantation rate per cycle	439/956 (45.9%)	3554/5963 (59.6%)	<.001
Preterm birth (<37 wks)	51/439 (11.6%)	456/3554 (12.8%)	.543
36–37 years (average age 36.44 years)			
Embryo transfer cycles	94	475	
Pregnancy rate per cycle	41/94 (43.6%)	254 (53.5%)	.09
Implantation rate per cycle	36/94 (38.3%)	229/475 (48.2%)	.09
Preterm birth (<37 wks)	5/36 (13.9%)	31/229 (13.5%)	1
≥38 years (average age 40.36 years)			
Embryo transfer cycles	130	571	
Pregnancy rate per cycle	20/130 (15.4%)	222 (38.9%)	<.001
Implantation rate per cycle	17/130 (13.1%)	190/571 (33.3%)	<.001
Preterm birth (<37 wks)	2/17 (11.8%)	29/190 (15.3%)	1

LBRs in the ≥38-year-old group, indicating that double embryo transfer may be a more desirable choice.

A study by Sallem et al[5] showed that patient's age and quality of embryos were the 2 most crucial predictive variables of birth and multiple birth, similar to our findings. Our results showed that clinical pregnancy, embryo IR, and LBR gradually decreased with patient's age. Fertility declined significantly in women of childbearing age but >35 years.[6] Reduced estrogen levels and increased gonadotropin levels in older women have been reported to decrease the quantity and quality of oocytes, therefore affecting the quality of embryos.[7] The main reasons for the age-related decrease in oocyte and embryo quantity and quality include increased chromosomal abnormalities and apoptosis in oocytes, decreased mitochondrial number impacting function, and insufficient energy supply eventually leading to reduced oocyte number and viability.[8,9] Decreased ovarian function not only affects the quantity and quality of oocytes and embryos,[10] but it also may impair endometrial receptivity.[11] Decreased ovarian reserve and endometrial injury caused by abortion and endometritis after delivery may also explain reduced endometrial receptivity. Some studies have reported that the pregnancy rate and LBR of patients over 40 years old with frozen embryo transfer have increased significantly.[12] Therefore, patients of advanced age can choose FET to improve pregnancy outcomes.

It has been reported that the quantity of high-quality embryos transferred had the most impressive impact on FET pregnancy rate. Pregnancy rate increased when over 2 high-quality embryos were transferred; the multiple birth rate also increased markedly.[13] In 2012, Pandey et al[14] reported that 2-embryo transfers can effectively reduce the rate of multiple births and did not affect the rate of pregnancy. Two-embryo transfer has been previously used in most reproductive centers worldwide, and elective 2-embryo transfer in advanced-age patients results in better pregnancy outcomes. Compared with younger patients, 2-embryo transfer in older patients adds neither the risk of abortion, preterm prematurity, nor low birth weight infants.[15] The relevant studies carried out by Chinese researchers show that for women over 40 years, the MPR for 2-embryo transfers was obviously lower than that for 3-embryo transfers, indicating that decreasing the number of transferred embryos to 2 in older women does not decrease the clinical pregnancy or LBRs, but it can significantly reduce the MPR. Therefore, some adverse effects of multiple pregnancy can be avoided. In this study, pregnancy rates from the transfer of 2 high-quality embryos were 58.8% and 45.0% in the 36- to 37-year-old and >38-year-old groups, respectively. Both are significantly higher than the pregnancy rates (48.4% and 35.4%, respectively) from transferring 1 high-quality plus 1 fair-quality embryo in similar age groups. However, the effect on the MPR was not significant, corresponding to the results of previous reports.

Studies have shown that the quality of transferred embryos is one of the key factors affecting the pregnancy outcome of patients.[16] The morphological rating of an embryo reflects a very short timepoint in its development and cannot represent its entire development, but it reflects its developmental potential to some extent.[17] Clinical pregnancy and IRs improve with the embryo grade, therefore, the higher the embryo quality, the better the clinical pregnancy results. Embryo grade does not affect pregnancy outcome when implanted successfully. In this study, comparison of age groups considering the quality of the embryos showed that the pregnancy rate was significantly higher after the transfer of 2 high-quality embryos compared with transferring 1 high-quality plus 1 fair-quality embryo. The MPR was dramatically higher in patients ≤35 years old when transferring 2 high-quality embryos, while the MPR was not affected in the 36 to 37-year-old and ≥38-year-old groups.

Obtaining a single full-term live birth is the trend and goal of global assisted reproductive technology. Currently, many reproductive centers advocate single high-quality embryo transfer. The present study found that the clinical pregnancy and LBRs of patients ≤35 years of age undergoing elective single high-quality embryo transfer were dramatically lower than those of patients undergoing 2-embryo transfers, but their pregnancy and LBRs remain as high as 50.9% and 45.9%, separately. The 36 to 37-years-of-age group are literally identical, which may be connected with the small sample size. The pregnancy and LBRs of patients ≥38 years of age undergoing single high-quality embryo transfer were markedly reduced, suggesting that elective 2 high-quality embryo transfer in older patients may be more favorable to optimize the outcome of assisted reproduction treatment.

However, the research also has some limitations. Increased body mass index (BMI) is associated with adverse pregnancy outcome in women undergoing IVF treatment, including lower pregnancy and LBRs.[18] This is especially true when increased BMI is associated with polycystic ovary syndrome.[19] Conversely, in patients with endometriosis, BMI does not influence IVF outcome.[20] We have not analyzed the BMI of our patients, and we acknowledge this as a limitation of the study. Furthermore, we have not analyzed the other possible potential confounders and effect modifiers such as vitamin D,[21] free fatty acids, and phospholipids.[22] In summary, the outcome of assisted reproductive treatment has negative correlations with patients' age and positive correlations with the number and quality of the embryos transferred. For patients ≥38 years of age, transferring 2 high-quality embryos significantly improved pregnancy outcomes. For patients 36 to 37 years of age, embryo quality should be taken into consideration to decide the number of embryos to transfer. The transfer of either 1 single high-quality embryo or a combination of 1 high-quality plus 1 fair-quality embryo may help achieve high pregnancy and LBRs. Elective single high-quality embryo transfer is recommended for patients ≤35 years of age to reduce the multiple birth rate and increase obstetric safety. This single-center retrospective study only explored the clinical outcomes of cleavage-stage embryo transfer in frozen cycles involving patients of advanced reproductive age. Multicenter, prospective, randomized controlled clinical trials that incorporate fresh embryo transfer cycles should be conducted to further guide clinical work.

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