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Original Research

Effects of Repeated Cryopreservation of Cleavage-Stage Embryos on Pregnancy and Perinatal Outcomes of Single Embryo Transfer

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Abstract

Background: Embryo cryopreservation is an important part of assisted reproductive technologies to increase cumulative pregnancy rate in clients. However, in clinical settings, embryos can be subjected to repeated cryopreservation-warming cycles due to certain clinical circumstances. There are limited data on the effect such cycles may have on the success of embryo transfer procedures. In this study, we investigated the effect of repeated cryopreservation of cleavage-stage embryos on the pregnancy and perinatal outcomes of frozen embryo transfer (FET). **Methods**: We retrospectively collected data on single cleavage-stage embryo transfer (ET) cycles performed in our center from January 2017 to August 2021 and identified 98 cycles of repeated cryopreservation-warming in cleavage-stage ET cycles. Propensity score matching was conducted according to the age of the patients, body mass index, endometrial thickness, type of oocyte retrial cycle, and quality of the transplanted embryos to match once-cryopreserved cleavage-stage ET cycles in our center at a ratio of 1:3 (caliper value 0.01). Finally, 294 once-cryopreserved cleavage-stage ET cycles were set as controls. Clinical pregnancy, early miscarriage, live birth rates, and other pregnancy and perinatal outcomes were compared between the two groups. **Results**: Similar success rates were obtained with twice-cryopreserved embryo transfers (n = 98) and with once-cryopreserved embryo transfers (n = 294). Importantly, there was no significant difference in the clinical pregnancy, early miscarriage, and live birth rates as well as perinatal outcomes such as preterm birth and pregnancy complication rates between the two groups. There were no birth defects in the two groups. **Conclusions**: Repeated cryopreservation may not negatively affect pregnancy and perinatal outcomes of single cleavage-stage ET and may present a safe option for resuscitated cleavage-stage embryos that have been canceled for transfer.

Keywords: cryopreserved cleavage-stage embryo; thawing; frozen-thawed embryo transfer; intracytoplasmic sperm injection; *in vitro* fertilization

1. Introduction

In recent years, embryo cryopreservation has played an increasingly important role in assisted reproductive technologies. This technique preserves embryos to increase the cumulative pregnancy rate of a single ovarian stimulation cycle, helps avoid unfavorable embryo transfer timings due to poor endometrial receptivity and high risk of ovarian hyperstimulation, and improves the pregnancy rate of a single transplant. Embryo cryopreservation has been used for 38 years and has been widely recognized by domestic and foreign reproductive centers [1]. Frozen-thawed embryo transfer (FET) cycles can achieve similar pregnancy and perinatal outcomes, and similar offspring health status as fresh embryo transfer cycles [2,3]. Currently, vitrification is the most widely adopted embryo cryopreservation technique.

However, in clinical practice, resuscitated embryos are occasionally frozen again on the same day to be thawed and transferred into the uterus in the subsequent cycle due to special patient circumstances such as sudden diarrhea, fever, and vaginitis. Currently, there are limited and controversial data on FET outcomes of embryos undergoing repeated cryopreservation-warming cycles, especially cleavage-stage embryos. Therefore, the impact of repeated cryopreservation-warming procedures on clinical pregnancy and perinatal outcomes needs to be further elucidated. In this study, we retrospectively analyzed the pregnancy and perinatal outcomes of repeated cryopreservation-warming single cleavage-stage embryo transfers at our center from 2017 to 2021.

2. Materials and Methods

2.1 Patients

A total of 98 cycles of repeated cryopreservation-warming single cleavage embryo transfer performed at our center from January 2017 to August 2021 were included in this study. Only *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles were included in this study, and preimplantation genetic testing (PGT) cycles were excluded. Propensity score matching was conducted according to the age of the patients, body mass index, endometrial thickness, type of oocyte retrial cycle (IVF or ICSI), and quality of the transplanted embryos to match once-cryopreserved single cleavage embryo FET cycles in our center during the same period at a ratio of 1:3 (caliper

value 0.01). Finally, 294 once-cryopreserved single cleavage embryo FET cycles were used as controls.

2.2 Ovulation Stimulation, Fertilization, and Embryo Culture

All patients underwent controlled ovulation stimulation using either a long-acting gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist regimens. When the diameter of the dominant follicle monitored by B-ultrasound was $\geq$18 mm, intramuscular injection of human chorionic gonadotropin (hCG) was administered, and 36 h later, ultrasound-guided transvaginal ovarian oocyte retrieval was performed. The retrieved oocytes were fertilized using conventional IVF or ICSI. Two pronuclei (2PN) and two polar bodies were observed at 16–18 h after normal fertilization. Zygotes were cultured in a cleavage medium until cleavage embryos formed on the third day after oocyte retrieval. Cleavage embryo morphology scoring is based on the criteria issued by the Society for Assisted Reproductive Technology (SART), which evaluates the morphology of the cleavage embryo based on three aspects: the number of blastomeres, the uniformity of blastomere size, and the number of fragments [4]. Good-quality day 3 embryos were defined as embryos at the cleavage stage with 7–9 blastomeres, cell fragmentation grade $\leq$2, and cell symmetry grade $\leq$2 (symmetric or moderately asymmetric).

2.3 Vitrification of Cleavage Embryos

The vitrification kit used was purchased from Kitazato Co. (Fuji, Japan). Cleavage-stage embryos with a normal fertilization source, $\geq$4 cells, $\leq$20% cell debris, and uniform or mildly heterogeneous blastomere size can be frozen. The cleavage embryos were equilibrated in an equilibration solution (ES, Kitazato, Japan) for 8 min, then transferred to a vitrification solution (VS, Kitazato, Japan) for equilibration for 45–60 s, then quickly placed in the carrier, and stored in liquid nitrogen.

2.4 Warming of Cleavage Embryos

We used sucrose (VWR Chemicals, Solon, OH, USA) and HEPES buffer (SAGE, Trumbull, NY, USA) containing 15% human serum albumin (SAGE, Trumbull, NY, USA) to prepare warming solutions with different sucrose concentrations: WS1 (1M sucrose), WS2 (0.5M sucrose), WS3 (0.25M sucrose), WS4 (no sucrose). WS1 warming solution was balanced overnight in an incubator at 37 °C. After being placed in WS1 warming solution for 30–60 s, the embryos were immediately transferred to WS2, WS3, and WS4 warming solution in sequence, for 3 min each. The criterion for embryo resuscitation survival was the following: the number of surviving blastomeres after thawing $>$50%. The cleavage embryo that survived thawing was cultured for 2 h and transplanted.

2.5 Frozen-Thawed Embryo Transfer Cycle

Patients with regular menstrual cycles and normal ovulation use their natural cycles for endometrial preparations. The patients began to receive transvaginal ultrasound monitoring from the 10th to the 12th day of menstruation. When the average diameter of the dominant follicle was $\geq$14 mm, luteinizing hormone (LH) levels were monitored daily to determine the time of ovulation. Frozen-thawed cleavage embryos were transferred on the third day after ovulation.

Hormone replacement cycles are used for endometrial preparation in patients with irregular menstrual periods and ovulation. Oral estradiol valerate (Proganol, Bayer, Germany) was administered in increments, starting at 4 mg/day and being increased to 6–8 mg depending on the thickness of the endometrium. When the maximum thickness of the endometrium was $\geq$8 mm, 40 mg of progesterone was injected intramuscularly daily for two days, and then the dose was increased to and maintained at 60 mg. Embryo transfer was performed three days after progesterone administration.

Biochemical pregnancy was confirmed on the 14th day after embryo transfer if the blood βhCG test was positive. On the 35th day after embryo transfer, the gestational sac and the original cardiac pulsation were observed on ultrasound, confirming a clinical pregnancy. Luteal support can be gradually reduced after intrauterine pregnancy is confirmed and can be stopped at 10–12 weeks of gestation.

2.6 Outcome Parameters

Pregnancy and perinatal outcomes were compared between the two groups. Pregnancy outcomes included embryo implantation, as well as biochemical, clinical, and ectopic pregnancies, early miscarriage, and live birth rates. The implantation rate was defined as the ratio of the number of implanted embryos to the number of transplanted embryos. Biochemical pregnancy was defined as a positive blood βhCG test on the 14th day after embryo transfer. Clinical pregnancy was defined as the presence of a gestational sac and primitive cardiac duct pulses on the 35th day after embryo transfer. Early miscarriage was defined as pregnancy loss before 12 weeks' gestation. Late miscarriage was defined as pregnancy loss during 12–28 weeks' gestation. Live births were defined as surviving neonates that were delivered at 28 weeks of gestation. Ectopic pregnancy was defined as the presence of a gestational sac outside the uterine cavity, confirmed by ultrasonography.

The perinatal outcome indicators included gestational age at birth, preterm birth rate, neonatal sex ratio, neonatal length and weight, low-birth-weight infants, macrosomia rate, small/large gestational age rate, and fetal birth defect rate. A low-birth-weight infant was defined as a fetus with a birth weight of $<$2500 g. Macrosomia was defined as a fetal birth weight $\geq$4000 g. Small for gestational age was

Table 1. Comparison of the baseline characteristics of the two groups.

	Twice-cryopreserved (n = 98)	Once-cryopreserved (n = 294)	*p*-value
Age of female patients (years)	35.91 ± 4.56	36.26 ± 5.11	0.551
BMI (kg/m^2)	21.07 ± 2.44	21.35 ± 2.50	0.340
IVF cycles (%)	64.3 (63/98)	63.6 (187/294)	0.903
ICSI cycles (%)	35.7 (35/98)	36.4 (107/294)	0.903
Duration of infertility (years)	4.00 ± 2.62	4.24 ± 3.26	0.508
Endometrial thickness (mm)	9.56 ± 1.85	9.66 ± 1.72	0.612
Previous embryo transfer cycles	2.02 ± 0.99	0.68 ± 1.01	<0.001
Good-quality embryo rate, n (%)	53.1 (52/98)	54.8 (161/294)	0.770

BMI, body mass index; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

defined as a fetal birth weight below the 10th percentile of the mean fetal weight for the same gestational age. Large-for-gestational-age was defined as a fetal birth weight above the 90th percentile of the mean fetal weight for the same gestational age.

2.7 Statistical Analysis

All statistical analyses were performed using SPSS software (version 26.0, IBM Corp., Chicago, IL, USA). Measurement data were expressed as mean ± standard deviation (x ± SD) Measurement data that conformed to normal distribution were compared using Student's *t*-test, and non-normally distributed measurement data were compared using the Mann-Whitney U test. Counting data were expressed as case number (percentage) [N (%)], and comparisons between groups were performed using the Pearson χ^2 test or Fisher's exact test. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Patient Baseline Characteristics

There were no significant differences in female age, body mass index, duration of infertility, type of oocyte retrial cycle, or endometrial thickness between the two groups (Table 1). The twice-cryopreserved group had significantly more previous number of embryo transfer cycles than the once-cryopreserved group, (2.02 ± 0.99 *vs* 0.68 ± 1.01, $p < 0.001$). There was no statistical difference in the rate of good-quality embryos between the groups ($p = 0.770$).

3.2 Resuscitation of Cryopreserved Embryos

In the twice-cryopreserved group, a total of 250 cleavage embryos were thawed in the initial cryopreservation–warming cycles, 242 survived, with a survival rate of 96.80%. Eight embryos from 7 patients failed to survive. In the second cryopreservation–warming cycles, 98 patients thawed one embryo each, and a total of 98 embryos survived, with a survival rate of 100.00%. The survival rate of the initial cryopreservation–warming cycles was slightly lower than that of the second cryopreservation–warming cycles (96.80% *vs* 100.00%, $p = 0.163$), although the difference was not statistically significant. In the once-cryopreserved group, of the 322 thawed cleavage embryos, 310 survived with a survival rate of 96.27%. Nevertheless, there was no significant difference in the survival rates between the two groups ($p = 0.111$).

3.3 Pregnancy Outcomes

The pregnancy outcomes of the two groups are shown in Table 2. There were no significant differences in the biochemical pregnancy, clinical pregnancy, early miscarriage, late miscarriage, and live birth rates between the groups ($p > 0.05$). Two cases of labor induction occurred in the twice-cryopreserved group due to severe fetal cleft lip and palate and fetal chromosomal abnormalities. Two cases of labor induction occurred in the once-cryopreserved group because of fetal tetralogy of Fallot and fetal chromosomal abnormalities. The induced labor rates in the two groups were similar ($p > 0.05$). There were no ectopic pregnancies in either group.

3.4 Prenatal Outcomes

A total of 11 and 35 newborns were born in the twice-cryopreserved group and in the once-cryopreserved group, respectively (Table 3). There were no significant differences in perinatal outcomes, such as gestational age, preterm birth rate, neonatal length and weight, low-birth-weight infant rate, macrosomia rate, small/large-for-gestational-age infant rate, and fetal birth defect rate between the two groups ($p > 0.05$). The rates of pregnancy complications were similar in both groups ($p > 0.05$).

4. Discussion

Embryo cryopreservation is an important part of assisted reproductive therapy. The earliest method for cryopreservation of human embryos was "slow freezing" to ensure the safety of the embryo through gradual cooling. However, this process can form crystals that may damage the cell structure, resulting in a poor embryo survival rate. Vitrification, also known as rapid freezing, is based on rapid cooling (>20,000 °C/min) combined with a high

Table 2. Comparison of pregnancy outcomes of the two groups.

	Twice-cryopreserved (n = 98)	Once-cryopreserved (n = 294)	*p*-value
Biochemical pregnancy rate, n (%)	22.4 (22/98)	25.9 (76/294)	0.501
Clinical pregnancy rate, n (%)	17.3 (17/98)	19.7 (58/294)	0.604
Early miscarriage rate, n (%)	11.8 (2/17)	22.4 (13/58)	0.535
Late miscarriage rate, n (%)	0 (0/17)	6.9 (4/58)	0.618
Induced labor rate, n (%)	11.8 (2/17)	3.4 (2/58)	0.466
Live birth rate, n (%)	11.2 (11/98)	11.9 (35/294)	0.856
Ectopic pregnancy rate, n (%)	0 (0/98)	0 (0/294)	-

BMI, body mass index; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection.

Table 3. Comparison of prenatal outcomes of the two groups.

	Twice-cryopreserved (n = 11)	Once-cryopreserved (n = 35)	*p*-value
Gestational age (weeks)	38.03 ± 1.43	38.08 ± 2.35	0.963
Early preterm birth rate/live birth, n (%)	27.3 (3/11)	22.9 (8/35)	1.000
Neonatal sex			0.371
Male, n (%)	72.7 (8/11)	51.4 (18/35)	
Female, n (%)	27.3 (3/11)	48.6 (17/35)	
Mode of delivery			1.000
Vaginal delivery rate/live birth, n (%)	27.3 (3/11)	22.9 (8/35)	
Cesarean section rate/live birth, n (%)	72.7 (8/11)	77.1 (27/35)	
Fetal length (cm)	49.73 ± 1.35	48.86 ± 2.48	0.273
Fetal Weight (g)	3198.18 ± 379.63	3048.57 ± 560.41	0.414
LBW rate, n (%)	0 (0/11)	17.1 (6/35)	0.337
Macrosomia rate, n (%)	0 (0/11)	0 (0/35)	-
SGA rate, n (%)	0 (0/11)	5.7 (2/35)	1.000
LGA rate, n (%)	9.1 (1/11)	11.4 (4/35)	1.000
Fetal birth defect rate/live birth, n (%)	0 (0/11)	0 (0/35)	-
Pregnancy complication rate/clinical pregnancy, n (%)	35.3 (6/17)	15.5 (9/58)	0.148
Pregnancy-induced hypertension/clinical pregnancy, n (%)	5.9 (1/17)	3.4 (2/58)	1.000
Gestational diabetes mellitus/clinical pregnancy, n (%)	17.6 (3/17)	3.4 (2/58)	0.131
Premature rupture of membranes/clinical pregnancy, n (%)	11.8 (2/17)	6.9 (4/58)	0.887

LBW, low-birth-weight; SGA, small-for-gestational-age; LGA, large-for-gestational-age.

concentration of cryoprotectant. In this process, the water in cells is quickly removed, and the cell components instantly turn into highly viscous solid amorphous glassy substances, which prevents the formation of ice crystals in cells and greatly reduces the possibility of cell damage. Vitrification has greatly increased the safety of embryo cryopreservation and is a simple, time-saving operation with a good resuscitation effect unlike the slow freezing method. Current evidence indicates that vitrification-based FET cycles have success rates similar to those of fresh embryo transfer cycles [5]. Therefore, vitrification is gradually becoming the mainstream method of embryo cryopreservation worldwide.

Repeated freezing-thawing is often necessary to avoid wasting human embryos. However, the developmental potential of embryos subjected to repeated freezing and thawing could be impaired despite the use of vitrification, a safe cryopreservation protocol. At present, most studies have observed that the pregnancy rate of twice-vitrified embryo transfer is comparable to the implantation and pregnancy rates of once-vitrified embryo transfer, which supports the safety and reliability of repeated vitrification [6–10]. In contrast, Aluko *et al*. [11] have observed that in the PGT-A (preimplantation genetic testing for aneuploid cycle), biopsied blastocysts subjected to double vitrification had lower clinical pregnancy and live birth rates than once-vitrification blastocysts. Wang *et al*. [12] have shown that the implantation, clinical pregnancy, and live birth rates of twice-vitrified embryos were significantly lower than those of once-vitrified embryos, and the miscarriage rate was slightly higher. Additionally, their logistic regression analysis showed that repeated cryopreservation could increase the risk of embryo implantation failure (OR ratio = 1.79) [12].

These contradictory findings suggest that further studies are needed to verify the effects of repeated vitrification on the reproductive potential of embryos. Furthermore, a majority of the current research focuses on the blastocyst FET cycle. In this study, we assessed the effect of repeated vitrification on the outcome of FET in cleavage-stage embryos and found that the clinical pregnancy, early miscarriage, and live birth rates after the transfer of twice-vitrified cleavage embryos were similar to those of once-vitrified cleavage embryos. Our study suggests that repeated cryopreservation of cleavage-stage embryos may not reduce the success rate of single embryo transfer. Nevertheless, there is evidence suggesting that cryopreservation may impair the physiological function of embryos; studies have suggested that high concentrations of cryoprotectants used in vitrification may cause toxic effects and osmotic shock in embryos [13]. Wang *et al*. [12] observed that embryos were morphologically intact after repeated cryopreservation; however, they suggested that the ultrastructure of the embryo cells might be damaged. Shaw *et al*. [14] observed differences in gene expression between frozen and fresh embryos. Animal studies have also shown that repeated freezing-thawing procedures can lead to an abnormal increase in chromosomal ploidy in oocytes or embryos [15,16]. To date, the safety of repeated vitrification of embryos has not been fully established, and its impact on embryonic developmental potential remains to be verified. Therefore, the decision for repeated cryopreservation of cleavage-stage embryos must be made cautiously after informing the patient of the benefits and disadvantages of the procedure.

We noted that in the twice-cryopreserved group, the survival rate of the initial cryopreservation–warming cycles was slightly lower than that of the second cryopreservation–warming cycles (96.8% *vs* 100.0%), although the difference was not statistically significant. In a study based on blastocyst transfer cycles, it was also observed that the survival rate of the initial cryopreservation–warming cycles was lower than that of the second ones (94.6% *vs* 98.9%) [11]. This may be because the most vulnerable embryos may not survive the first thawing. Twice freezing-thawing cycles may have led to twice viability screening on the embryos, which made us wonder whether this mitigated the negative effects of cryopreservation on pregnancy outcomes in this group. This is an interesting question that deserves further exploration.

Additionally, offspring safety is crucial in the field of assisted reproductive technology. Therefore, perinatal outcomes are an important measure of the safety of repeated cryopreservation procedures. In this study, a total of 46 singletons were successfully delivered, of which, 11 were from the twice-cryopreserved group. We analyzed sex, gestational age, birth weight, cesarean section, and neonatal complications, including preterm birth, low-birth-weight, and birth defect rates, and found no significant differences between the two groups. Our results are consistent with previous studies suggesting that repeated cryopreservation does not negatively affect neonatal outcomes [12,17,18]. However, these studies are limited and have small sample sizes; therefore, studies with larger sample sizes are needed to determine whether repeated cryopreservation is a perinatal risk factor for neonatal health.

Notably, we observed a significant incidence of pregnancy complications/clinical pregnancy in the twice-cryopreserved group, such as pregnancy-induced hypertension, gestational diabetes mellitus, and premature rupture of membranes in the twice-cryopreserved group, although not statistically significant. Evidence from published studies currently does not support the association of embryo cryopreservation with the risk of gestational diabetes mellitus and premature rupture of membranes, but the relation between cryopreservation and Pregnancy-induced hypertension has been well established [19–21]. Opdahl *et al*. [22] compared the prenatal outcomes of 6444 singleton pregnancies after FET and 268,599 singleton pregnancies after natural pregnancy, and found that the risk of preeclampsia was significantly increased in the cryopreservation group (Odds ratio 1.41, 95% confidence interval (CI) 1.27–1.56). A multicenter randomized controlled trial based on a Chinese population also showed a significantly increased risk of preeclampsia in FET patients (Risk ratio 3.13, 95% CI 1.06–9.30, $p = 0.029$) [23]. The reasons for the increased risk of gestational hypertension in FET transplant cycles are unclear. Some scholars put forward some hypotheses for this: (1) the absence of corpus luteum in hormone replacement cycles may lead to a deficiency of vasoactive products such as relaxin, leading to an increased risk of abnormal maternal cardiovascular adaptation to pregnancy and subsequent increased risk of preeclampsia [24]; (2) the trophectoderm may be damaged during vitrification and warming, leading to abnormal placenta formation and thus an increased risk of preeclampsia [25]; (3) prematurely elevated estradiol may inhibit trophectoderm invasion of the uterine spiral arteries, thereby increasing the risk of preeclampsia during FET [26]; (4) embryo freezing-thawing procedure may cause the embryonic epigenetic/transcriptomic alterations and reduce DNA integrity, contributing to the happening of preeclampsia [27–29]. Whether the risk of pregnancy-induced hypertension increases with the times of embryo freezing-thawing cycles is an interesting question, which deserves further confirmation.

Currently, there is some evidence that some assisted reproduction procedures, such as PGT and blastocyst transfer, may increase the possibility of producing male offspring [30,31]. Therefore, it is necessary to explore the effect of repeated cryopreservation on the sex ratio. The study by Nagata *et al*. [32] showed that fresh or frozen embryo transfer did not affect the sex ratio of IVF live births. In our study, we observed that there is a higher number of males in the twice-cryopreserved group, although not statistically significant. Unfortunately, there were too few live births in

our study to draw reliable conclusions (only 11 live births in the twice-cryopreserved group), and no other studies have explored the effect of repeated cryopreservation on sex ratios. Whether the Y chromosome can help the survival of those embryos after repeated cryopreservation cycles are worth further exploration in future studies.

Previous studies on repeated cryopreservation of embryos mostly focused on blastocyst transfer cycles. However, our study revealed that for cleavage-stage embryos, which are more fragile than blastocysts, repeated cryopreservation may not reduce the implantation potential of embryos, and there was no significant negative impact on perinatal outcomes. However, because repeated cryopreservation of embryos is a relatively rare event in clinical practice, the conclusions of this study are limited by the small sample size of the twice-cryopreserved group and the fact that the study is retrospective. Moreover, we were unable to distinguish some confounding factors related to pregnancy outcomes, including infertility type, infertility cause, embryo morphology score, insemination method, endometrial preparation protocol, and the duration of cryopreservation. Large-scale prospective studies with a long-term follow-up are needed in the future to verify the feasibility and safety of repeated cryopreservation and explore its long-term impact on offspring health outcomes.

5. Conclusions

This study suggests that repeated cryopreservation may not negatively affect the pregnancy and perinatal outcomes of single cleavage embryo transfer and may be a safe option for resuscitated cleavage embryos that have been canceled for transfer. However, the reliability of the conclusions of this study is limited by the small sample size and its retrospective nature. Hence, the results need to be confirmed in future studies.

Author Contributions

YW—Conceptualization, Formal Analysis, Project Administration, Resources, Writing – Review & Editing; DC—Data Curation, Investigation, Methodology, Validation, Writing – Original Draft Preparation; BC—Writing – Review & Editing; DH—Data Curation, Formal Analysis; YX—Supervision; CD—Supervision, Writing – Review & Editing. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University and conducted in strict accordance with the Helsinki Declaration (No 2022481). The patient data required for this study were obtained from the center database and analyzed anonymously. The requirement for informed consent from the involved couples was waived because of the retrospective nature of the study.

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Conflict of Interest

The authors declare no conflict of interest.

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