Comparison of Pregnancy Outcomes in Young Patients Following Fresh Versus Frozen Single Blastocyst Transfer: A Retrospective Study

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Research article

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Abstract

Background Previous studies have shown that in young women, single blastocyst transfer can achieve satisfactory pregnancy results, but there are few studies on the difference between fresh and frozen-thawed single blastocyst transfer. In this retrospective analysis, the purpose of this study was to compare the pregnancy outcomes of young patients who received fresh or frozen-thawed single blastocyst transfer, so as to provide further clinical strategies for single blastocyst transfer.

Methods: A retrospective analysis of patients aged ≤ 35 years old undergoing single blastocyst transfer was performed from January 2018 to December 2018 in the reproductive center of the Second Affiliated Hospital of Wenzhou Medical University. A total of 901 patients were involved and were divided into two groups according to the type of transfer cycle, 693 of which were in the fresh embryo transfer cycle (group A) and 208 of which were in the frozen-thawed embryo transfer cycle (group B). The laboratory and pregnancy outcomes were compared between the groups.

Results The number of oocytes retrieved in group B was significantly more than in group A ($P < 0.05$). The early miscarriage rate in group B was higher than in group A, with significant difference ($P < 0.05$). There were no significant differences in other basic conditions and obstetric pregnancy outcomes between the two groups.

Conclusions Favorable pregnancy outcomes could be obtained in both fresh and frozen-thawed single blastocyst transfer in young patients without increasing the birth defect rate. For patients with risk factors of OHSS such as more oocyte retrieval, ovarian hyper-response, or risk factors of ectopic pregnancy such as tubal infertility and the history of ectopic pregnancy, frozen-thawed single blastocyst transfer could be highly recommended in clinical practice. However, since the early miscarriage rate is higher in frozen-thawed embryo transfer group, further research is needed to figure out the cause and possible solutions.

Background

In the past decades, several studies^[1,2] have found that human embryos could be cultured to the blastocyst stage in vitro, and successfully obtained clinical pregnancy after blastocyst implantation. With the continuous perfection of cultivation condition in vitro, the blastocyst formation rate has also increased. Compared with embryo transfer in cleavage stage, blastocyst culture is a process of survival of the fittest which shares higher synchronism with endometrial development physiologically, thus increasing embryo implantation rate and clinical pregnancy rate^[3]. However, with the increasing application of blastocyst transfer in clinical practice, higher clinical pregnancy rate is also accompanied with higher multiple pregnancy rate. How to reduce multiple pregnancy rate while achieving satisfactory pregnancy outcomes has become a research hotspot in recent years. Previous research has uncovered that single blastocyst transfer can achieve similar satisfactory pregnancy outcomes as double cleavage-stage embryo transfer^[4]. Therefore, single blastocyst transfer should be an ideal selection. At present, although quite a lot of researches about the differences of pregnancy outcomes between fresh embryo

transfer and frozen-thawed embryo transfer have been done, there are few studies aimed at the differences between fresh and frozen-thawed single blastocyst transfer. In view of this, the purpose of this study is to compare the pregnancy outcomes of young patients undergoing fresh or frozen-thawed single blastocyst transfer, thus providing further clinical strategies for single blastocyst transfer.

Methods

Research objects

A retrospective analysis of patients undergoing single blastocyst transfer was performed from January 2018 to December 2018 in the reproductive center of the Second Affiliated Hospital of Wenzhou Medical University. The inclusion criteria of this study were: (1) age $\leq$ 35 years old; (2) first embryo transfer cycle; (3) normal basal hormone levels. The exclusion criteria include (1) Uterine abnormality under ultrasound such as endometrial polyps, endometrial fibroids, intrauterine adhesion or uterine malformation; (2) endometriosis or adenomyosis; (3) alcoholism or drug addiction. A total of 901 patients were involved and were divided into two groups according to the type of transfer cycle, 693 of which were in the fresh embryo transfer cycle (group A) and 208 of which were in the frozen-thawed embryo transfer cycle (group B).

Fresh Single blastocyst Transfer Cycle

Long GnRH agonist protocol during early follicular phase or mini-stimulation protocol was adopted as the ovarian stimulation protocol in this study. When 2-3 follicles reached 18mm in diameter or one dominant follicle reached 20mm, intramuscular injection of 4000-10000IU human chorionic gonadotropin (hCG, 10000 IU, LIZHU Pharmaceutical Factory, China) at 21:00 to 22:00 that night was executed (trigger). Ultrasound-guided transvaginal oocyte retrieval was performed 36-38 hours after trigger. The retrieved oocytes were subsequently fertilized by IVF/ICSI and incubated at 37°C under a humidified gas phase of a mixture of 4% O₂, 6% CO₂, and 90% N₂. Blastocyst scoring was graded according to the Gardner classification system^[5]. Score the expansion stage and incubation status of blastocysts with number 1-6. Score the inner cell mass (ICM) and trophoctoderm (TE) with letter A, B or C. A grade of "3BB" or greater was defined as "good quality" and a grade below this was defined as "poor quality". Select a good-quality blastocyst for embryo transfer on the 5th day after oocyte retrieval. If there is no good-quality blastocyst in this cycle, select a blastocyst that is available for embryo transfer. Luteal phase support was implemented with 20 mg dydrogesterone tablets (Duphaston; Solvay Pharmaceuticals B.V. Dose 10mg/tablet) administered orally twice per day and 200 mg micronized progesterone administered orally or vaginally per day (Utrogestan; Capsugel, Besins Manufacturing Belgium, Bruxelles, Belgium. Dose 0.1g/tablet).

Vitrification and Warming Procedures

Fresh embryo transfer cycle was canceled in patients with ovarian hyperresponse or high risk factors of OHSS. All the embryos were frozen for the next frozen-thawed embryo transfer cycle. The process of

ovulation trigger, oocyte retrieval and blastocyst grading was consistent with the fresh blastocyst transfer cycle. Blastocysts were vitrified and warmed by the Cryotop method (Kitazato Biopharma) and the operation was carried out in accordance with the kit instructions.

Frozen-thawed Single Blastocyst Transfer Cycle

Hormone replacement therapy (HRT) or exogenous hormone stimulation with GnRH agonist (GnRH-a) was used for endometrial preparation. HRT patients commenced oral administration of one tablet of estradiol tablet(Femoston;Abbott Biologicals B.V.Dose 2mg estradiol /tablet)twice a day on the 2nd-5th day of menstrual cycle. B-ultrasound was performed every 3 to 5 days to measure endometrial thickness and adjust the dosage of estradiol tablets accordingly. When the endometrial thickness $\geq 8\text{mm}$ and serum progesterone levels $\geq 1.5\text{ng/ml}$, take 10mg dydrogesterone tablets (Duphaston;Solvay Pharmaceuticals B,V. Dose 10mg/tablet) orally twice a day, one tablet of estradiol and dydrogesterone tablet(Femoston;Abbott Biologicals B.V.Dose 2mg estradiol and 10mg dydrogesterone/tablet)orally twice a day and 200 mg micronized progesterone administered orally or vaginally twice day(Utrogestan; Capsugel, Besins Manufacturing Belgium, Bruxelles, Belgium.Dose 0.1g/tablet).A single i.m. injection of depot GnRH agonist (Decapeptyl ; Ipsen, Milan, Italy.Dose 3.75mg/branch) was administered in the early follicular phase or mid-luteal phase of the cycle. Prepare the endometrium with HRT 4 weeks after GnRH-a injection. Select a good-quality blastocyst for embryo transfer on the 5th day after the day of endometrial transfer. If there is no good-quality blastocyst in this cycle, select a blastocyst that is available for embryo transfer. The regimen for luteal phase support was the same as the regimen after endometrial transformation.

Follow-Up

Serum hCG levels were measured 2 weeks after embryo transfer to determine pregnancy. Another 2 weeks later, clinical pregnancy was identified as the presence of gestational sac and fetal heart beat under transvaginal ultrasound. Besides, Serum hCG $\geq 15\text{mIU/mL}$ without gestational sac 45 days after embryo transfer was defined as biochemical pregnancy. Early miscarriage was defined as the fetal loss before 12 gestational weeks while late miscarriage was defined as the fetal loss after 12 gestational weeks. Premature delivery occurred between 28 to 37 gestational weeks. Neonatal birth weight $< 2500\text{g}$ was considered as low birth weight infants, while $\geq 4000\text{g}$ was fetal macrosomia.

Observation Indicators

The patients' age, infertility duration, BMI index, cause of infertility, fertilization method, basal hormone levels, the number of oocyte retrieved, proportion of good-quality blastocyst, incidence of moderate to severe OHSS, positive rate of hCG test, clinical pregnancy rate, embryo implantation rate, biochemical pregnancy rate, ectopic pregnancy rate, early miscarriage rate, late miscarriage rate, multiple pregnancy rate, preterm birth rate, live birth rate, neonatal birth weight, fetal sex ratio, birth defect rate and obstetric complications were compared between the groups.

Statistical methods

SPSS 22.0 statistical software was used for data analysis. The data were presented as the means ± SDs, number or percentage. The Student’s t-test was applied if the continuous variables were normally distributed. Otherwise, the Dunnetts T3 test was used for the variables of non-normal distribution. The chi-square test and Fisher’s exact test were used in data analysis for categorical variables. In this research, $P\leq0.05$ was considered statistically significant.

Results

Comparison of Baseline characteristics and laboratory outcomes

There were no significant differences in the age, infertility duration, BMI index, cause of infertility, fertilization method, basal hormone levels, proportion of good-quality blastocyst, incidence of moderate to severe OHSS between the two groups ($P>0.05$). The number of oocytes retrieved in group B was significantly more than in group A ($P\leq0.05$)(Table 1).

Table 1 Comparison of Baseline characteristics and laboratory outcomes

	Group A (n=693)	Group B (n=208)	P value
Age (years)	29.48±3.02	29.24±3.09	0.313
Infertility duration(years)	3.17±2.52	3.28±1.61	0.535
BMI(kg/m2)	21.75±3.09	21.36±3.38	0.114
Infertility type(%)			0.976
Primary infertility	49.64(344/693)	49.52(103/208)	
Secondary infertility	50.36(349/288)	50.48(105/208)	
Cause of infertility (%)			0.480
Tubal factor	48.20(334/693)	42.31(88/208)	
PCOS	7.36(51/693)	10.10(21/208)	
Unexplained infertility	12.55(87/693)	11.54(24/208)	
Endometriosis	3.17(22/693)	2.40(5/208)	
Male factor	16.45(114/693)	18.75(39/208)	
#Multi factors	12.27(85/693)	14.90(31/208)	
Fertilization method (%)			0.181
IVF	81.24(563/693)	75.96(158/208)	
ICSI	15.87(110/693)	19.23(40/208)	
IVF+ICSI	2.89(20/693)	4.81(10/208)	
Basal hormone levels			
LH(IU/L)	5.25±2.47	5.62±2.71	0.059
FSH(IU/L)	7.24±2.04	7.04±2.15	0.228
E2(pg/mL)	44.97±12.31	46.12±11.65	0.234
P(ng/mL)	0.57±0.45	0.58±0.23	0.698
PRL ^a (mlu/L)	10.65(8.71,14.15)	10.58(8.57,15.27)	0.109
No. of oocytes retrieved^a	13.00(10.00,16.00)	17.00(10.00,22.00)	0.001*
Proportion of good-quality blastocyst□%□	88.31(612/693)	88.94(185/208)	0.803
Incidence of moderate to severe OHSS□%□	0.43(3/693)	0(0/208)	0.209

Note: Numbers are mean±standard deviation, median(interquartile range) or percentage.

BMI= body mass index; PCOS= polycystic ovarian syndrome; LH=luteinizing hormone; FSH=follicle stimulating hormone; E2=estradiol; P=progesterone; PRL=prolactin;OHSS=ovarian hyperstimulation syndrome.

#:Multiple factors defined as more than one reason causing infertility.

a:heterogeneity of variance

*:The No of oocytes retrieved of Group A were significantly difference from those in Group B ($P \leq 0.05$).

Coparison of pregnancy outcomes

The positive rate of hCG test, clinical pregnancy rate, embryo implantation rate, biochemical pregnancy rate, ectopic pregnancy rate, late miscarriage rate, multiple pregnancy rate, preterm birth rate, live birth rate, neonatal birth weight, incidence of macrosomia and low birth weight infants, neonatal death rate, fetal sex ratio, birth defect rate and incidence of obstetric complications were comparable between the two groups ($P \geq 0.05$). However, the early miscarriage rate in group B was higher than in group A, with significant difference($P \leq 0.05$)(Table 2).

Table 2 Coparison of pregnancy outcomes

	Group A (n=693)	Group B (n=208)	P value
Positive rate of hCG test (%)	69.26(480/693)	70.19(146/208)	0.799
Clinical pregnancy rate (%)	61.62(427/693)	61.54(128/208)	0.984
Embryo implantation rate (%)	61.62(427/693)	61.54(128/208)	0.984
Biochemical pregnancy rate(%)	7.65(53/693)	8.65(18/208)	0.637
Early miscarriage rate (%)	9.60(41/427)	20.31(26/128)	0.001*
Ectopic pregnancy rate(%)	0.70(3/427)	0(0/128)	1.000
Late miscarriage rate(%)	4.22(18/427)	3.90(5/128)	0.877
Multiple pregnancy rate (%)	2.81(12/427)	1.56(2/128)	0.405
Preterm birth rate (%)	5.39(23/427)	3.90(5/128)	0.502
Live birth rate (%)	52.67(365/693)	46.63(97/208)	0.127
Neonatal birth weight (g)	3263.92±571.58	3332.33±518.38	0.281
Incidence of macrosomia (%)	7.75(29/374)	7.07(7/99)	0.820
Incidence of Low birth weight infants (%)	7.49(28/374)	5.05(5/99)	0.398
Neonatal death rate (%)	0.27(1/374)	1.01(1/99)	0.375
Neonatal sex ratio (%)			0.455
Male	51.34(192/374)	55.56(55/99)	
Female	48.66(182/374)	44.44(44/99)	
Birth defect rate (%)	2.14(8/374)	2.02(2/99)	1.000
Obstetric complications (%)			
gestational hypertension	1.40(6/427)	3.13(4/128)	0.250
Severe preeclampsia	0.23(1/427)	0(0/128)	1.000
Gestational Diabetes	6.32(27/427)	3.90(5/128)	0.303

*:The Early miscarriage rate of Group A were significantly lower in Group B ($P \leq 0.05$).

Discussion

The application of controlled ovarian hyperstimulation in assisted reproductive medicine makes it possible to obtain sufficient number of oocytes during one menstrual cycle. The number of embryos formed after fertilization often exceeds the number required for one embryo transfer, so the remaining embryos need to be stored frozen, which contributed to the occurrence and development of vitrification

and warming technology. Frozen embryo transfer cycle enables patients to obtain more opportunities of embryo transfer without repeated oocyte retrieval, thereby avoiding the economic and mental burdens of the patients and maximizing the use of embryos. More importantly, frozen embryos transfer could effectively avoid the occurrence of ovarian hyperstimulation syndrome (OHSS). In a fresh embryo transfer cycle, the process of controlled ovarian hyperstimulation (COH) affects the hormone secretion. High levels of estrogen may lead to early endometrial maturity and affect endometrial receptivity, thus resulting in adverse perinatal and neonatal outcomes^[6-7]. Although frozen-thawed embryo transfer cycle can avoid hyper stimulation of gonadotropins and provide a better microenvironment for embryo implantation in the uterine cavity^[8], the process of embryo freezing may cause blastomere damage, and the impact on maternal and neonatal outcomes still remains controversial.

Osamu Ishihara et al. conducted a retrospective study of 3047 fresh single blastocyst transfer cycles and 11329 frozen-thawed single blastocyst transfer cycles, and found that compared with fresh single blastocyst transfer, frozen-thawed single blastocyst transfer could result in similar pregnancy outcomes without increasing the miscarriage rate^[9]. However, Daimin Wei et al. have found that the embryo implantation rate, biochemical pregnancy rate and live birth rate of frozen-thawed single blastocyst transfer were significantly higher ($P \leq 0.05$) than that of fresh single blastocyst transfer through a large sample, multi-center, randomized controlled clinical study^[10]. In this study, the enrolled patients were all no more than 35 years old and treated with IVF for the first time, which could effectively avoid the disadvantages of the advanced age, decreased ovarian reserve and repeated implantation failure. The results showed that both fresh single blastocyst transfer and frozen-thawed single blastocyst transfer could achieve satisfactory pregnancy outcomes. Also, there were no significant differences in adverse pregnancy outcomes such as biochemical pregnancy and late miscarriage rate between the two groups. However, it should be noted that the early miscarriage rate of frozen-thawed single blastocyst transfer was significantly higher than that of fresh single blastocyst transfer ($P < 0.05$). This conclusion conflicts with the researches mentioned above, the reason may be that early miscarriage is generally related to the quality of the embryo. Vitrification and warming procedures of the embryo may affect the embryonic activity to varying degrees. Studies have found that the incidence of chromosome abnormalities in frozen-thawed embryos is significantly higher than that in the fresh embryos^[11]. Although embryo frozen-thawed procedures are developing towards higher efficiency and safety, the potential risks are still not negligible.

The incidence of ectopic pregnancy in IVF patients has increased nearly three times compared with normally conceived females, which ranges between 2.1%~8.6%^[12-13]. The main reason is that tubal infertility accounts for a large part of the infertility. Also, studies have shown that the incidence of ectopic pregnancy in women with tubal infertility resorting to IVF-ET could be up to 11%^[14]. Moreover, the number of embryo transferred in the early days is often ≥ 2 . While improving the pregnancy outcomes, it also increases the incidence of heterotopic pregnancy. Compared with embryos in cleavage stage, blastocysts stay in the uterine cavity for a shorter period of time, thereby reducing the probability of migration to the fallopian tube and the occurrence of ectopic pregnancy. Some researchers have concluded that single

blastocyst transfer in the frozen-thawed cycle could significantly reduce the risk of ectopic pregnancy^[9], which indicated that the frozen-thawed cycle might be closer to a natural state^[15–16]. The increased ectopic pregnancy rate in fresh embryo transfer cycle might be due to the hyperphysiological hormonal environment caused by ovarian stimulation^[17]. In this study, three cases of ectopic pregnancy occurred in the fresh single blastocyst transfer group, but no case occurred in the frozen-thawed group. The incidence of ectopic pregnancy in both groups was lower than that of natural pregnancy, with no statistical difference ($P=0.05$), which suggested that single blastocyst transfer could reduce the incidence of ectopic pregnancy. For people with high risk factors such as tubal infertility and previous history of ectopic pregnancy, single blastocyst transfer is recommended, among which the frozen-thawed cycle is more preferred.

OHSS is an iatrogenic complication caused by ovarian hyperstimulation with exogenous gonadotropins, which can be life-threatening in severe cases. The incidence in IVF-ET is about 1–10%^[18]. In order to prevent OHSS, some patients choose the freeze-all approach and wait for the next frozen-thawed cycle to reduce the production of endogenous hCG, thereby reducing the generation of vasoactive substances.. In this study, the median number of oocyte obtained in the fresh single blastocyst transfer group was 13 and there were 3 cases of moderate to severe OHSS, while the median number of oocytes obtained in the frozen-thawed group was 17 and no case of moderate to severe OHSS occurred. The number of oocyte retrieved in the two groups was statistically different, which further reminds us that for patients with ovarian hyperresponse or with high risk factors of OHSS, frozen-thawed single blastocyst transfer may be a good choice as it can not only avoid the occurrence of OHSS but also obtain the same satisfactory clinical outcome as in the fresh cycle.

It has been reported that the birth defect rate is higher in the frozen-thawed cycle^[19], but some scholars^[20] found that the frozen-thawed cycle actually did not significantly increase the birth defect rate in singletons. In this study, the birth defects in the fresh single blastocyst transfer group included cardiac malformations (2 cases), hand malformation (1 case), pulmonary sequestration (1 case), ear malformation (1 case), biliary atresia (1 case) and favism (1 case), while in the frozen-thawed group, it was consisted of hypospadias (1 case) and congenital torticollis (1 case). There was one case of newborn death in both groups. No statistical differences were found between the two groups in terms of birth defect rate and neonatal death rate($P > 0.05$), but still lacks of large sample randomized controlled studies to confirm.

With regard to the obstetric complications, the incidence of gestational hypertension in frozen-thawed embryo transfer cycle was significantly increased compared with fresh embryo transfer cycle according to a meta-analysis^[21]. This study has not yet found that frozen-thawed single blastocyst transfer would increase the risk of obstetric complications. Since whether frozen-thawed cycle would increase the risk of obstetric complications is still controversial, it could not be concluded at present. The meta-analysis also indicated that the incidence of macrosomia and large for gestational age in frozen-thawed embryo transfer cycle was higher than that of fresh embryo transfer cycle, while the incidence of premature, low

birth weight infants, and small for gestational age were lower in the frozen-thawed cycle. There was a difference in neonatal birth weight between the two groups. Some researchers^[22] believed that this might be related to the epigenetic changes during frozen-thawed embryo transfer and maternal hyperestrogenemia during fresh embryo transfer. However, in this study, the neonatal birth weight, neonatal sex ratio, incidence of macrosomia, small for gestational age and preterm birth were comparable between the two groups ($P \geq 0.05$). The reason might be that in the process of ovarian stimulation, for patients with ovarian hyperresponse or high risk factors of OHSS, it was usually recommended that patients give up fresh embryo transfer and consider freeze-all approach to reduce the adverse pregnancy outcomes caused by maternal hyperestrogenemia. In addition, single blastocyst transfer also greatly reduce the incidence of multiple pregnancy, thereby further reducing the occurrence of adverse pregnancy outcomes such as preterm birth and small for gestational age. These results provide new ideas for explaining the differences between the frozen-thawed and fresh single blastocyst transfer, which still need further research.

Conclusions

In 2018, the Chinese Expert Consensus on the number of embryo transferred has proposed the practice of selective single embryo transfer. Given continuous improvement of blastocyst culture, the time for China to implement single blastocyst transfer is mature. Favorable pregnancy outcomes could be obtained in both fresh and frozen-thawed single blastocyst transfer in young patients without increasing the birth defect rate. For patients with risk factors of OHSS such as more oocyte retrieval, ovarian hyper-response, or risk factors of ectopic pregnancy such tubal infertility and the history of ectopic pregnancy, frozen-thawed single blastocyst transfer could be highly recommended in clinical practice. However, since the early miscarriage rate is higher in frozen-thawed embryo transfer group, further research is needed to figure out the cause and possible solutions.

Abbreviations

Ovarian hyperstimulation syndrome OHSS

Follicle-stimulating hormone FSH

Luteinizing hormone LH

Estrogen-2 E2

Progesterone P

Prolactin PRL

Human chorionic gonadotropin hCG

Inner cell mass ICM

Trophectoderm TE

Body mass index BMI

Hormone replacement therapy HRT

GnRH agonist GnRH-a

Controlled ovarian hyperstimulation COH

In vitro fertilization and embryo transfer IVF-ET

Intra-cytoplasmic sperm injection ICSI

Declarations

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Author's contribution

YHW and CL designed and performed the study, analyzed the data, and wrote and edited the manuscript. JZZ and HTX conceived and participated in the study design, evaluated the results and edited the manuscript. CCS and YHF contributed to data collection and statistical analysis. All authors have read and approved the final manuscript.

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Availability of data and materials

The transcripts from which this manuscript was developed are available on request from the corresponding author.

Ethics approval and consent to participate

This study was approved by the Ethics Committee (Institutional Review Board) of the Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University and informed written consent was obtained from all participants.

Competing interests

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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