

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; however, few studies exist regarding the difference between fresh and frozen-thawed single blastocyst transfer. To provide further clinical strategies for single blastocyst transfer, the purpose of this retrospective analysis was to compare the pregnancy outcomes of young patients who received fresh or frozen-thawed single blastocyst transfer.

Methods: A retrospective analysis of patients, aged ≤ 35 years, who underwent single blastocyst transfer was conducted from January 2018 to December 2018 in the reproductive center of the Second Affiliated Hospital of Wenzhou Medical University (Wenzhou, China). In total, 901 patients were included and were divided into two groups, based on the type of transfer cycle: the fresh embryo transfer cycle group (group A; n=693) and the frozen-thawed embryo transfer cycle group (group B; n=208). The laboratory and pregnancy outcomes were compared between the groups.

Results The number of oocytes retrieved in group B was significantly higher than in group A ($P < 0.05$). The early miscarriage rate in group B was significantly higher than in group A and was significantly different ($P < 0.05$). Frozen-thawed single blastocyst transplantation was an independent risk factor for early miscarriage. Other basic conditions and obstetric pregnancy outcomes were not significantly different between the two groups.

Conclusions Favourable pregnancy outcomes could be obtained with fresh and frozen-thawed single blastocyst transfer in young patients. However, because the early miscarriage rate was higher in the frozen-thawed embryo transfer group, further research is needed to determine the cause and possible solutions.

Background

In the past decades, several studies^[1,2] have demonstrated that human embryos can be cultured to the blastocyst stage in vitro and successfully result in a clinical pregnancy after blastocyst implantation. With the continuous improvement in the cultivation condition in vitro, the blastocyst formation rate has also increased. Compared to embryo transfer in the cleavage stage, blastocyst culture is a process of 'survival of the fittest', which shares higher synchronism physiological with endometrial development, and thereby increases the embryo implantation rate and clinical pregnancy rate^[3]. However, with the increasing application of blastocyst transfer in clinical practice, a higher clinical pregnancy rate is also accompanied by a higher multiple pregnancy rate. In recent years, how to reduce the multiple pregnancy rate while achieving satisfactory pregnancy outcomes has become a research hotspot. Previous research has revealed that single blastocyst transfer can achieve similar satisfactory pregnancy outcomes as double cleavage-stage embryo transfer^[4]. Therefore, single blastocyst transfer should be the ideal selection. At present, substantial research has been conducted regarding differences in pregnancy outcomes between fresh embryo transfer and frozen-thawed embryo transfer, although few studies have

investigated the differences between fresh and frozen-thawed single blastocyst transfer. In view of this, to provide further clinical strategies for single blastocyst transfer, the purpose of this study was to compare the pregnancy outcomes of young patients undergoing fresh or frozen-thawed 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 trophectoderm (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 ovarian hyperstimulation syndrome (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 ≥ 8 mm and serum progesterone levels ≥ 1.5 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 ≥ 15 mlU/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 g was considered as low birth weight infants, while ≥ 4000 g was fetal macrosomia in China^[6]. Gestational hypertension^[7] was defined as the first occurrence of hypertension after 20 weeks of pregnancy, with systolic blood pressure ≥ 140 mmHg (1 mmHg=0.133kPa) and/or diastolic pressure ≥ 90 mmHg, which will return to normal within 12 weeks after delivery; Preeclampsia^[7] referred to systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg after 20 weeks of pregnancy, accompanied by any of the following: urine protein ≥ 0.3 g/24h, or urine protein/creatinine ratio ≥ 0.3 , or random urine protein $\geq (+)$ (test method when urine protein cannot be quantified); no proteinuria but with any of the following organ or system involvement: heart, lung, liver, kidney and other important organs, or blood system, abnormal changes in the digestive system and nervous system, placenta-fetal involvement and so on. Pregnant women receive oral glucose tolerance test (OGTT) for the first visit after 24-28 weeks or 28 weeks of gestation. The fasting blood glucose before taking 75g glucose is measured, and the

blood glucose is measured after 1 hour and 2 hours after taking the glucose. These three blood glucose values should be lower than 5.1, 10.0, 8.5mmol/l (92, 180, 153mg/dl), gestational diabetes mellitus(GDM) can be diagnosed if one of the blood glucose levels meets or the above standards^[8]. Birth defect was abnormality in the structure, function, or metabolism that occur before the baby is born^[9]. Common birth defects include: anencephaly, hydrocephalus, open spina bifida, meningocele, cleft lip, cleft palate, congenital heart disease, trisomy 21, and conjunctivitis.

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 \pm SDs, number or percentage. The Student's t-test was applied if the continuous variables were normally distributed. Otherwise, the Dunnett's 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. A multivariate logistic regression analysis was conducted by using group as the independent variable and various indicators of pregnancy outcomes as the dependent variable. Multivariate analysis adopted binary logistic regression model, the method was forward LR method. In this research, $P \leq 0.05$ was considered statistically significant.

Results

Comparison of baseline characteristics and laboratory outcomes

No significant differences existed between the two groups in terms of age, infertility duration, body mass index, cause of infertility, fertilization method, basal hormone levels, proportion of good-quality blastocysts, and incidence of moderate to severe ovarian hyperstimulation syndrome (OHSS) ($P > 0.05$). The number of oocytes retrieved in group B was significantly higher than in group A ($P \leq 0.05$) (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 (mIu/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.

Group A means fresh embryo transfer cycle,group B means frozen-thawed embryo transfer cycle

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

^:IVF+ICSI means operating IVF and ICSI at the same time

a:heterogeneity of variance

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

Comparison of pregnancy outcomes

The positivity rate of the human chorionic gonadotrophin (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 low-birth-weight infant, neonatal death rate, foetal sex ratio, birth defect rate and incidence of obstetric complications were comparable between the two groups (P>0.05). However, the early miscarriage rate was significantly higher in group B than in group A(P<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 (%)	9.84(42/427)	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
			0.630
34 weeks ≤ gestational age <37 weeks	69.60(16/23)	80.00(4/5)	
28 weeks ≤ gestational age <34 weeks	30.40(7/23)	20.00(1/5)	
			0.292
Spontaneous preterm delivery (%)	82.60(19/23)	60.00(3/5)	
Iatrogenic preterm delivery (%)	17.40(4/23)	40.00(2/5)	
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
Neonatal birth age (weeks)^a	39.43(38.57,40.14)	39.71(38.86,40.43)	0.826
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
preeclampsia	0.23(1/427)	0(0/128)	1.000
Gestational Diabetes mellitus	6.32(27/427)	3.90(5/128)	0.303

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

a: heterogeneity of variance

Multiple linear regression analysis of the effect of transplantation cycle on pregnancy outcomes

The results showed that the risk of early miscarriage was significantly increased in group B compared to group A ($P=0.05$). However, the other pregnancy outcomes were not significantly different between the two groups ($P>0.05$) (Table 3).

Table 3 Multiple linear regression analysis of the effect of transplantation cycle on pregnancy outcomes

Pregnancy outcome indicators	B	S.E.	Walds	P	OR	95%CI (Lower limit)	95%CI (Upper limit)
Positive rate of hCG test (%)	0.04	0.17	0.07	0.799	1.05	0.75	1.47
Clinical pregnancy rate	-0.25	0.16	2.56	0.110	0.78	0.57	1.06
Embryo implantation rate	-0.25	0.16	2.56	0.110	0.78	0.57	1.06
Biochemical pregnancy rate	0.14	0.29	0.23	0.637	1.14	0.65	2.00
Early miscarriage rate	-0.85	0.27	9.65	0.002*	0.43	0.25	0.73
Ectopic pregnancy rate	15.84	3552.59	0.00	1.000	7602234.57	0.00	-
Late miscarriage rate	0.08	0.52	0.02	0.878	1.08	0.39	2.98
Multiple pregnancy rate	0.41	0.78	0.28	0.597	1.51	0.33	7.00
Preterm birth rate	0.38	0.50	0.58	0.447	1.47	0.55	3.92
Live birth rate	-0.24	0.16	2.33	0.127	0.79	0.58	1.07
Incidence of macrosomia	0.10	0.44	0.05	0.820	1.10	0.47	2.60
Incidence of Low birth weight infants	0.42	0.50	0.71	0.401	1.52	0.57	4.05
Neonatal death rate	-1.34	1.42	0.89	0.346	0.26	0.02	4.24
Neonatal sex ratio	0.17	0.23	0.56	0.455	1.19	0.76	1.85
Birth defect rate	0.06	0.80	0.01	0.942	1.06	0.22	5.07
Gestational hypertension	0.71	0.74	0.92	0.338	0.49	0.12	2.10
Severe preeclampsia	15.15	3552.59	0.00	0.997	3792194.64	0.00	-
Gestational Diabetes	0.51	0.50	1.04	0.308	1.66	0.63	4.40

*:the risk of early miscarriage increased in Group B, which was statistically different from the Group A(P<0.05)

-:no data

Discussion

The purpose of this retrospective analysis was to compare the pregnancy outcomes of young patients who received fresh or frozen-thawed single blastocyst transfer. We found that, with the exception of early miscarriage, favourable pregnancy outcomes could be obtained with fresh or frozen-thawed single blastocyst transfer.

The application of controlled ovarian hyperstimulation in assisted reproductive medicine makes obtaining a sufficient number of oocytes during one menstrual cycle possible. The number of embryos formed after fertilisation often exceeds the number required for one embryo transfer. Therefore, the remaining embryos need to be stored frozen, which has contributed to the occurrence and development of vitrification and warming technology. The frozen embryo transfer cycle enables patients to have more opportunities for embryo transfer without having to undergo repeated oocyte retrievals, thereby avoiding

the economic and mental burdens to patients and maximising the use of embryos. More importantly, frozen embryo transfer could effectively avoid the occurrence of OHSS. In a fresh embryo transfer cycle, the process of controlled ovarian hyperstimulation affects the hormone secretion. High levels of oestrogen may lead to early endometrial maturity and affect endometrial receptivity, and thus result in adverse perinatal and neonatal outcomes^[10,11]. The frozen-thawed embryo transfer cycle can avoid hyperstimulation of gonadotropins and provide a better microenvironment for embryo implantation in the uterine cavity^[12]. However, the process of embryo freezing may cause blastomere damage, and the impact on maternal and neonatal outcomes remains controversial.

Ishihara et al.^[13] conducted a retrospective study of 3,047 fresh single blastocyst transfer cycles and 11,329 frozen-thawed single blastocyst transfer cycles. They found that, compared to fresh single blastocyst transfer, frozen-thawed single blastocyst transfer can result in similar pregnancy outcomes without increasing the miscarriage rate. However, in a large sample, multicentre, randomised controlled clinical study, Wei et al.^[14] found that the embryo implantation rate, biochemical pregnancy rate, and live birth rate of frozen-thawed single blastocyst transfer were significantly higher than those of fresh single blastocyst transfer ($P < 0.05$).

In the current study, all enrolled patients were no more than 35 years old and were treated with in vitro fertilisation (IVF) for the first time, which could effectively avoid the disadvantages of advanced age, decreased ovarian reserve, and repeated implantation failure. The results were that fresh single blastocyst transfer and frozen-thawed single blastocyst transfer could achieve satisfactory pregnancy outcomes. In addition, no significant differences existed between the two groups in adverse pregnancy outcomes such as biochemical pregnancy and late miscarriage rate. However, a noteworthy finding was that the early miscarriage rate of frozen-thawed single blastocyst transfer was significantly higher than that of fresh single blastocyst transfer. Multivariate logistic regression analysis results on the pregnancy outcomes of the two groups indicated that frozen-thawed single blastocyst transplantation was an independent risk factor for early miscarriage; thus, fresh single blastocyst transfer is a protective factor for early abortion.. This conclusion conflicts with the aforementioned research. The reason for this discrepancy 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. A study^[15] demonstrated that the incidence of chromosome abnormalities is significantly higher in frozen-thawed embryos than in fresh embryos. Researchers such as Jenkins^[16] found that the risk of shortening telomeres increases after T lymphocytes are exposed to the freezing fluid; this phenomenon may also exist in other cells. In addition, genes related to foetal growth and development, at the genome-wide methylation level and the methylation level of specific sites, were disordered in the fresh embryo transfer group, and various methylation levels in the freeze-thaw embryo transfer group similar to the natural pregnancy group. Some researchers^[17] have provided an explanation for this phenomenon: freeze-thaw embryo transfer cannot reverse the effect of fresh embryo transfer on embryo gene methylation but will cause a new embryo methylation disorder. Hiura and et al.^[18] conducted microarray gene chip analysis on human placenta obtained after freeze-thaw embryo transfer (n=64), fresh embryo transfer (n=16), and natural pregnancy

(n=28). They also concluded that low temperature freezing affects the epigenetics of embryos. Although embryo freezing technology is constantly progressing and improving, embryo freezing will undoubtedly increase human intervention on embryos, which will easily cause adverse effects on embryos and may increase the risk of early abortion. Therefore, in the future, more research is needed to evaluate the impact of embryo freezing technology on the safety of the offspring so that better use can be made of embryo cryopreservation technology to serve human health.

The incidence of ectopic pregnancy in IVF patients has increased nearly three times, and currently ranges between 2.1–8.6%, compared to the incidence in natural pregnancies^[19,20]. The main reason is that tubal infertility accounts for a large proportion of infertility cases. In addition, studies^[21] have shown that the incidence of ectopic pregnancy in women with tubal infertility resorting to IVF and embryo transfer could be up to 11%. Moreover, the number of embryos transferred in the early days is often ≥ 2 . While this procedure improves pregnancy outcomes, it also increases the incidence of heterotopic pregnancy. Compared to embryos in the 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 an ectopic pregnancy. Some researchers have concluded that single blastocyst transfer in the frozen-thawed cycle could significantly reduce the risk of ectopic pregnancy.^[13] Thus, the frozen-thawed cycle may be closer to the natural state^[22,23]. The increased ectopic pregnancy rate in the fresh embryo transfer cycle may be because of the hyperphysiological hormonal environment caused by ovarian stimulation^[24]. In the current study, three cases of ectopic pregnancy occurred in the fresh single blastocyst transfer group, but no cases occurred in the frozen-thawed group. The incidence of ectopic pregnancy in both groups was lower than that of natural pregnancy and were not statistically different, suggesting that single blastocyst transfer can reduce the incidence of ectopic pregnancy. For people with high-risk factors such as tubal infertility and a 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. Its incidence in IVF and embryo transfer is approximately 1–10%^[25]. 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 oocytes obtained in the fresh single blastocyst transfer group was 13 and 3 cases of moderate to severe OHSS occurred. By contrast, 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 oocytes retrieved in the two groups was statistically different, which further indicates that, for patients with ovarian hyperresponsiveness or with high-risk factors of OHSS, frozen-thawed single blastocyst transfer may be a better choice because it can avoid the occurrence of OHSS and obtain the same satisfactory clinical outcome as that of the fresh cycle.

With regard to the obstetric complications, the incidence of gestational hypertension was significantly increased in the frozen-thawed embryo transfer cycle, compared to the fresh embryo transfer cycle, based

on the findings from a meta-analysis^[26]. Some researchers^[27,28] have found that the risk of preeclampsia and early-onset severe preeclampsia is significantly increased during the frozen embryo transfer (FET) cycle without luteinisation, whereas their risk is not significantly increased with FET during the natural ovulation cycle. The reason is that the corpus luteum can secrete blood vessel active hormones such as relaxin. These hormones produce the key maternal hemodynamic changes in pregnant rat models, at least in early to mid-pregnancy. Researchers such as Allen^[29] found that the incidence of gestational diabetes in assisted reproductive technology is more than twice that of a normal pregnancy. This occurs because the process of receiving a series of treatments with assisted reproductive technology, whether fresh embryo transfer or freeze-thaw embryo transfer, using exogenous sex hormones or gonadotropins, gonadotropin-releasing hormone, etc. is necessary. This process may affect the hypothalamus–pituitary–gonad axis or cause other mechanism changes. Hormone-related complications such as gestational diabetes mellitus (GDM) may eventually occur. In addition, the route of administration of progesterone, past OHSS risk and a history of polycystic ovary syndrome are risk factors for GDM in women undergoing assisted reproductive technology^[30]. In the current study, we did not find that freeze-thaw single blastocyst transplantation increased the risk of obstetric complications such as hypertension and gestational diabetes during pregnancy. The reason may be that our reproductive centre uses the endometrial preparation plan for the freeze-thaw embryo transfer. Most people tend to choose the down-regulating combined hormone replacement programme in which a GnRH-a injection in the middle of the luteal phase accounts for the majority. Therefore, corpus luteum may exist in these patients who receive a GnRH-a injection in the middle of the luteal phase and reduce the risk of hypertension during pregnancy. However, the current conclusion is only an inference and more research is required to confirm it.

Premature birth is the main cause of perinatal morbidity and mortality. The upper limit of the definition of preterm birth is globally unified: delivery is less than 37 weeks of gestation, and the lower limit is set according to the level of neonatal treatment. In developed countries such as Europe and the United States, the lower limit of gestational week for a preterm delivery is 20 or 24 weeks. China sets the lower limit of gestational week for preterm delivery at 28 weeks. Live births within 28 weeks of gestation are not included in the category of preterm delivery^[31]. Preterm labour is divided, based on cause, into spontaneous preterm labour and iatrogenic preterm labour. Studies^[32,33] have shown that the iatrogenic preterm birth of a singleton pregnancy after IVF is significantly higher than preterm birth in a natural pregnancy. Iatrogenic preterm birth is more common after frozen transplantation than after fresh transplantation. In our study, no significant differences in the incidence of preterm birth and iatrogenic preterm birth existed between the frozen-thawed single blastocyst transplantation group and the fresh single blastocyst transplantation group. The reason may be that iatrogenic preterm birth is often caused by one or more factors. These factors include hypertension during pregnancy, extrauterine infection, chorioamnionitis, placental factors, and medical and surgical diseases during pregnancy^[34]. This study shows no statistical difference existed between the two groups in obstetric complications such as hypertension during pregnancy. This finding may eventually result in inconsistencies between the two groups in preterm delivery, especially iatrogenic preterm delivery. In the current study, most cases of spontaneous preterm birth had no obvious cause. Some patients reported regular contractions after

emotional or moderate exercise. Late abortions (i.e. gestational age, ≥ 34 weeks) accounted for 72.73% of cases (n=16). Cases of iatrogenic preterm delivery comprised three cases of hypertension during pregnancy, two cases of gestational diabetes, and one case of placental factors; the rate of late abortion (66.67% [four cases]) was similar to the rate of spontaneous preterm birth. These findings suggested that, in addition to reducing pregnancy complications and improving the treatment of pregnancy complications, the gestational week should be delayed as much as possible and the proportion of iatrogenic preterm births should be reduced. This effort is important to reduce the incidence of preterm births.

Researchers have reported that the birth defect rate is higher when using the frozen-thawed cycle^[35]. However, some researchers have found that the frozen-thawed cycle actually did not significantly increase the birth defect rate in singletons^[36]. In this study, the birth defects in the fresh single blastocyst transfer group included cardiac malformations (two cases), hand malformation (one case), pulmonary sequestration (one case), ear malformation (one case), biliary atresia (one case) and favism (one case), whereas in the frozen-thawed group, birth defects included hypospadias (one case) and congenital torticollis (one case). Both groups had one case of new-born death. No statistical differences existed between the two groups in terms of birth defect rate and neonatal death rate; however, large sample randomized controlled studies with a large sample size are needed to confirm this finding.

A meta-analysis^[26] also showed a higher incidence of macrosomia and large-for-gestational age infants in the frozen-thawed embryo transfer cycle group than in the fresh embryo transfer cycle group, whereas the incidence of premature, low-birth-weight infants, and small-for-gestational age infants were lower in the frozen-thawed cycle group. The neonatal birth weight was different between the two groups. Some researchers^[37] believe that this finding may be related to epigenetic changes that occur during frozen-thawed embryo transfer and maternal hyperoestrogenemia during fresh embryo transfer. However, in the current study, the neonatal birth weight, neonatal sex ratio, incidence of macrosomia, small-for-gestational age, and preterm birth were comparable between the two groups. The reason may be that, in the process of ovarian stimulation, patients with ovarian hyperresponsiveness or high-risk factors for OHSS were usually recommended to forego the fresh embryo transfer approach and consider the freeze-all approach to reduce adverse pregnancy outcomes caused by maternal hyperoestrogenemia. In addition, single blastocyst transfer also greatly reduces the incidence of a 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 needs further research.

This study has some limitations that should be considered when interpreting the results. This was a single-centre retrospective study with a small sample size, especially for birth defects. The sample size was insufficient to fully investigate fresh embryo transfer and FET. A multicentre, prospective, randomised controlled trial currently underway. In addition, this study only followed up to the outcome of the birth of the baby in the first transplant cycle, did not calculate the cumulative pregnancy rate, and did not track

the development of the new-born after birth. Future studies should include these factors with a greater sample size.

Conclusions

Favourable pregnancy outcomes could be obtained with fresh or frozen-thawed single blastocyst transfer in young patients . Frozen-thawed single blastocyst transfer could be highly recommended in clinical practice for patients with OHSS risk factors such as more oocyte retrieval and ovarian hyperresponsiveness, or patients with risk factors for ectopic pregnancy such as tubal infertility and a history of ectopic pregnancy. In 2018, the Chinese Expert Consensus on the number of embryos transferred proposed the practice of selective single embryo transfer. Given the continuous improvement in blastocyst culture, now is an appropriate time for China to implement single blastocyst transfer is mature.. However, because the early miscarriage rate was higher with frozen-thawed embryo transfer, therefore further research is needed to determine 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

Declarations

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.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

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

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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Author's contributions

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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