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Retrospective Study to Compare Frozen-Thawed Embryo Transfer with Fresh Embryo Transfer on Pregnancy Outcome Following Intracytoplasmic Sperm Injection for Male Infertility

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Background: With the development of assisted reproductive technology, there is increasing evidence that frozen-thawed (FT) embryo transfer achieves a better outcome when compared with fresh embryo transfer in different types of infertile individuals. This aim of this study was to investigate the effect of FT embryo transfer for intracytoplasmic sperm injection (ICSI) on pregnancy outcome in assisted reproductive technology for male infertility.





Material/Methods: A total of 516 embryo transfer cycles (286 fresh embryo transfer cycles and 230 FT embryo transfer cycles) were studied, in which ICSI treatment was performed for the first time because of male infertility. The women in the study were normal or had Fallopian tube abnormalities. The clinical data and pregnancy outcomes of these two study groups were compared.

Results: The implantation rate, pregnancy rate, and multiple birth rate of the FT embryo transfer group were similar when compared with the fresh embryo transfer group. The live birth rate of the FT embryo transfer group was significantly greater when compared with the fresh embryo transfer group ($P < 0.05$). The rate of miscarriage of the FT embryo transfer group was 6.52%, which was significantly less than that of the fresh embryo transfer group (14.01%) ($P < 0.05$). The gestational age and neonatal birth weight were not significantly different between the two groups ($P > 0.05$).

Conclusions: FT embryo transfer was an effective and safe treatment for patients undergoing ICSI, which improved the live birth rate and reduced the rate of miscarriage.

MeSH Keywords: Embryo Transfer • Sperm Injections, Intracytoplasmic • Treatment Outcome

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Background

With the continued improvement in assisted reproductive technology, the effectiveness and safety of this technology have become established in reproductive medicine. There is growing evidence that frozen-thawed (FT) embryo transfer may achieve the same, or even better, outcome when compared with fresh embryo transfer in different infertile populations. A recent randomized controlled trial has shown, that for patients with polycystic ovary syndrome (PCOS), FT embryo transfer significantly improved the live birth rate following *in vitro* fertilization (IVF) cycles [1].

For patients with ovarian hyperstimulation, whole-embryo freezing and embryo transfer has become accepted [2]. For patients with an endometrium measuring less than 7 mm in thickness, the pregnancy rate is significantly reduced, but FT embryo transfer with hormone replacement therapy has been shown to improve the endometrial receptivity and the pregnancy rate [3]. Furthermore, for patients with repeated embryo implantation failure, the whole-embryo freezing program for pre-implantation genetic diagnosis or pre-implantation genetic screening is recommended [4]. FT embryo transfer has been shown to result in similar live birth rates and to improve infant outcomes when compared with fresh embryo transfer [5].

Although some authors believe that singleton pregnancy outcomes and perinatal outcomes between FT and fresh embryo transfer procedures are similar [6], other authors have argued that the cryopreservation cycle could increase the risk of complications during pregnancy [7]. However, high estrogen status follows controlled ovarian hyperstimulation, and prenatal exposure to abnormal hormone levels could affect the pregnancy outcome, and even lead to fetal disease. High maternal estrogen levels in early pregnancy have been shown to be associated with an increased risk of small for gestational age (SGA) newborns [8]. Also, some studies have found that a high estrogen environment increased the risk of thyroid damage in children, but FT embryo transfer could reduce the risk of thyroid dysfunction in children [9].

In China, intracytoplasmic sperm injection (ICSI) is mainly used to treat male infertility, with most of the women partners having normal fertility and ovarian activity, and so these women are prone to high estrogen status following controlled ovarian hyperstimulation. Therefore, for these couples FT embryo transfer would be more likely to improve ICSI treatment for these couples, and the outcome of this form of assisted reproductive technology should be explored in more depth.

The aim of this study was to evaluate IVF therapy for couples who chose ICSI treatment for the first time due to male infertility, by comparing the pregnancy outcome of ICSI treatment

between fresh embryo transfer and FT embryo transfer. The findings of this study may provide additional evidence in the development of an optimal strategy for male infertility in China.

Material and Methods

Patients

This retrospective clinical study was undertaken at the First Affiliated Hospital of Anhui Medical University from 1st January 2014 to 30th September 2015 and was approved by the local Committee on Medical Ethics. Written informed consents were obtained from all study participants.

A total of 516 embryo transfer cycles (286 fresh embryo transfer cycles and 230 FT embryo transfer cycles) were studied, in which intracytoplasmic sperm injection (ICSI) treatment was performed for the first time due to male infertility. The women in the study were normal or had Fallopian tube abnormalities. The clinical data and pregnancy outcomes of the two study groups, the fresh embryo transfer group (N=286) and the frozen-thawed (FT) embryo transfer group (N=230), were compared.

The FT embryo transfer group received frozen embryos following ICSI treatment, and the women who received the embryos all had natural menstrual cycles, without hormone treatment before the embryo transfer.

The study inclusion criteria for men enrolled in this study were oligoasthenoteratospermia or obstructive azoospermia. The women were normal with normal menstrual cycles or had Fallopian tube abnormalities. Women with endometrial abnormalities, ovarian dysfunction, hyperprolactinemia, recurrent miscarriage, chromosomal abnormalities, or other factors that could potentially affect clinical outcome, were excluded.

Ovarian stimulation and embryo transfer

All the female study participants received a standardized ovarian stimulation protocol, oocyte retrieval, and fertilization, followed by transfer of one or two 5-day-old embryos. Ovarian stimulation was performed by the standard protocols during all the cycles. All patients were treated with a gonadotropin-releasing hormone (GnRH) agonist, and exogenous gonadotropins, including human menopausal gonadotropins (hMG) and recombinant follicle-stimulating hormone (FSH) for controlled ovarian hyperstimulation with the doses were adjusted according to the measurement of serum sex steroids and ovarian response. The ovarian response was monitored by transvaginal ultrasound (US). When the leading follicles were 18–20 mm in diameter, 5000–10000 IU urinary human chorionic gonadotropin (HCG) was administered. Between 34–36

Table 1. Characteristics of the patients included in the study.

Characteristic	Fresh embryo transfer group	FT embryo transfer group	P-value
	(N=286)	(N=230)	
Age (yrs)	27.91±3.34	27.41±3.19	0.083
BMI (kg/m ²)	22.01±3.15	21.74±2.79	0.197
Infertility duration (yrs)	3.71±2.34	3.48±2.25	0.264
Male infertility factors			
OA (n%)	56/286 (19.58%)	61/230 (26.52%)	0.061
OAT (n%)	230/286 (80.42%)	169/230 (73.48%)	0.061
Baseline FSH (IU/L)	6.80±1.82	6.61±1.78	0.233
Baseline LH (IU/L)	5.09±3.00	5.00±2.10	0.684

Values represent mean ±SD or n (%). BMI – body mass index; OA – obstructive azoospermia; OAT – oligoasthenoteratospermia; FSH – follicle stimulating hormone; LH – luteinizing hormone; FT – frozen-thawed.

hours later, oocyte retrieval was performed, followed by ICSI between 2–4 hours later.

For the fresh embryo transfer group, one or two high-quality embryos were selected for fresh transfer five days later. Intramuscular progesterone was administered for luteal-phase support, beginning on the day of oocyte retrieval until 12 weeks after conception. For the FT embryo transfer group, the whole embryos were cryopreserved for later transfer. A natural cycle was identified in the second or third menstrual cycle after oocyte retrieval. On the tenth day of menstruation, the endometrial thickness and follicle size were monitored by transvaginal US. Then, one or two embryos were thawed and transferred five days after natural ovulation. The luteal-phase support with oral progesterone continued until 12 weeks after conception.

Biochemical pregnancy was defined as serum β -human chorionic gonadotropin (β -HCG) levels greater than 10 mIU/ml on the 14th day after embryo transfer. Transvaginal US was performed on the 30th day after embryo transfer. Clinical pregnancy was defined by transvaginal US imaging confirmation of one gestational sac or more in the uterine cavity. The clinical outcomes and neonatal status of all patients were evaluated by review of the medical records and follow-up.

Statistical analysis

All statistical analysis was performed using SPSS version 13.0 software. Categorical data were presented as absolute numbers and percentages. Quantitative data were presented as the mean ± standard deviation (SD). The Student's t-test was used to analyze differences between groups for quantitative variables. The analysis of the categorical variables was performed using the Chi-squared test. A P-value <0.05 was considered to be statistically significant.

Results

Patient characteristics

A total of 516 transfer cycles met the inclusion criteria, with 286 fresh embryo transfer cycles and 230 frozen-thawed (FT) embryo transfer cycles. The characteristics of study participants are detailed in Table 1.

In the fresh embryo transfer group and the FT embryo transfer group, the average age was 27.91±3.34 years and 27.41±3.19 years, the body mass index (BMI) was 22.01±3.15 kg/m² and 21.74±2.79 kg/m², the baseline follicle-stimulating hormone (FSH) level was 6.80±1.82 IU/L and 6.61±1.78 IU/L, respectively. No differences were observed in age, infertility duration, BMI, basal FSH and luteinizing hormone (LH) values (P>0.05).

The population of men who were infertile was divided into two groups, with obstructive azoospermia or oligoasthenoteratospermia. There were 56 patients (19.58%) with obstructive azoospermia and 230 patients with oligoasthenoteratospermia (80.42%) in fresh embryo transfer group. There were 61 patients (26.52%) with obstructive azoospermia and 169 patients (73.48%) with oligoasthenoteratospermia in the FT embryo transfer group (Table 1).

Controlled ovarian hyperstimulation and embryo transfer

No differences were found between the number of days of ovarian stimulation, the number of embryos transferred, and the gonadotropin dose in these two groups. The number of oocytes retrieved in the FT embryo transfer group was greater than those in fresh embryo transfer group. Patients were always advised to freeze the entire embryo to prevent ovarian hyperstimulation syndrome if there were too many oocytes

Table 2. Controlled ovarian hyperstimulation and embryo transfer.

Characteristic	Fresh embryo transfer group	FT embryo transfer group	P-value
	(N=286)	(N=230)	
No. of days of ovarian stimulation	11.97±2.237	11.86±2.00	0.562
Total gonadotropin dose (IU)	2238.42±800.02	2139.89±765.25	0.157
No. of oocytes retrieved	11.77±4.04	17.97±6.82	<0.001*
No. of embryos transferred			
Mean No. (n)	1.89±0.31	1.93±0.26	0.173
1	30/541 (5.55%)	17/443 (3.84%)	0.211
2	511/541 (94.45%)	426/443 (96.16%)	0.211
No. of good-quality embryos transferred	507/541 (93.72%)	421/443 (95.03%)	0.374

Values represent mean ±SD or (%). * P<0.05 was considered to be statistically significant. FT – frozen-thawed.

Table 3. Pregnancy and neonatal outcomes.

Outcome	Fresh embryo transfer group	FT embryo transfer group	P- value
	(N=286)	(N=230)	
Biochemical pregnancy (n%)	170/286 (59.44%)	151/230 (65.65%)	0.148
Clinical pregnancy (n%)	157/286 (54.90%)	138/230 (60.00%)	0.244
Implantation rate (n%)	230/541 (42.51%)	208/443 (46.95%)	0.163
Miscarriage rate (n%)	22/157 (14.01%)	9/138 (6.52%)	0.036*
Live births (n%)	135/286 (47.20%)	129/230 (56.09%)	0.045*
Multiple pregnancies (n%)	53/157 (33.76%)	50/138 (36.23%)	0.656
Premature births (n%)	37/157 (23.57%)	39/138 (28.26%)	0.358
Gestational age (weeks)	38.46±2.10	37.23±6.22	0.092
Singleton birth weight (g)	3305.49±604.28	3214.65±750.96	0.397
Congenital anomalies (n%)	2/188 (1.01%)	3/181 (1.66%)	0.966

Values represent mean ±SD or n (%). * P<0.05 was considered to be statistically significant. FT – frozen-thawed.

retrieved. Therefore, the number of oocytes in the FT embryo transfer group was greater than that in fresh embryo transfer group. There was no significant difference in the number of good-quality embryos transferred between these two groups (Table 2).

Pregnancy and neonatal outcome

The rate of miscarriage in the FT embryo transfer group was lower than that of fresh embryo transfer group (6.52% vs. 14.01%). The live birth rate was greater in the FT embryo transfer group compared with the fresh embryo transfer group (56.09% vs. 47.20%). No differences were noted in the

implantation rate, biochemical pregnancy rate, clinical pregnancy rate, multiple pregnancy rates, and premature birth rate between these two groups. There were no differences in the gestational age, single neonatal weight, or congenital anomalies between these two groups (Table 3).

Discussion

With the development of assisted reproductive technology, embryo-freezing technology has become an important part of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) therapy [10]. Because cryopreservation and vitrification

technology have greatly improved the traditional freezing technology [11], and studies have shown that frozen-thawed (FT) embryo transfer can achieve the same or even better outcome when compared with fresh embryo transfer in different infertile populations [1–6].

Following controlled ovarian hyperstimulation, patients normally have a high level of serum estrogen, and prenatal exposure to an abnormal hormonal environment will increase the risks of complications of pregnancy and may even affect the neonatal outcome [12–14]. During early pregnancy, the mean serum estrogen levels of patients undergoing fresh embryo transfer have been shown to be significantly greater than those undergoing FT embryo transfer or following natural conception [8]. Compared with women undergoing natural conception, thyroid hormone levels have been shown to be significantly increased in newborns resulting from fresh embryo transfer; a similar outcome was reported in children between the ages of 3–10 years, but thyroid levels resulting from FT embryo transfer and natural conception were similar [9].

ICSI is now a validated and recommended IVF technique that is mainly used for male infertility in China and is used when female partners have normal ovarian function. Because women who undergo other forms of IVF treatment are prone to suffering from high estrogen levels during the assisted reproductive technology procedures, the exploration of optimal and safe treatment for these couples is being undertaken. For example, Shapiro et al. [15] designed a randomized controlled clinical trial to compare the success rates of fresh embryo transfer following controlled ovarian hyperstimulation and FT embryo transfer after artificial endometrial preparation. The findings of this previously published study were that the clinical pregnancy rate was significantly greater in the FT embryo transfer group compared with the fresh embryo transfer group [15]. As a result, these authors strongly recommended that patients with impaired endometrial receptivity should choose FT embryo transfer cycles with artificial endometrial preparation [15].

A further previously published study reported that the outcomes following IVF were significantly improved in the freeze-all policy group when compared with the fresh embryo transfer group [16]. The authors of this previous study speculated that endometrial receptivity might have been impaired by controlled ovarian hyperstimulation and that the freeze-all method could improve the outcomes [16]. Another observational study, involving 1,209 IVF patients, showed that FT embryo transfer had equivalent live-birth rates compared with fresh embryo transfer [5]. One recent meta-analysis that included four randomized clinical trials containing 1,892 women, compared the clinical outcome of a freeze-all embryo policy with a conventional IVF and ICSI policy [17]. In this previous study, the women in the freeze-all group experienced fewer miscarriages

but a higher rate of pregnancy complications [17]. However, no difference was found in the cumulative live birth rate between the two groups [17].

The findings of the present study are supported by these previously published studies and by the previously published meta-analysis data [15–17]. The findings of this study showed that FT embryo transfer could reduce the rate of miscarriage and increase the live birth rate when compared with that of the fresh embryo transfer. However, the implantation rate, biochemical pregnancy rate, clinical pregnancy rate, and multiple pregnancy rates were not significantly different between these two groups. Although the number of oocytes was greater in the FT embryo transfer group, natural cycles were performed in the second or third menstrual cycle after oocyte retrieval. The number of oocytes had little effect on the outcome of FT embryo transfer cycles.

Previous authors have proposed a whole-embryo freezing strategy in IVF. In 2015, Roque proposed that there was increasing evidence in the literature to indicate better clinical outcomes, and decreased obstetric and perinatal complications in IVF when using the freeze-all policy, instead of fresh embryo transfer [18]. More recently, in 2016, Blockeel et al. undertook a SWOT (strengths, weaknesses, opportunities, and threats) analysis that shed light on the different aspects of the freeze-all strategy in improving outcome in IVF [19].

However, the specific mechanisms by which a freeze-all policy can reduce the rate of miscarriage and improve the live birth rate remain unclear. One explanation could be the presence of a steroid hormone-related placenta-formation defect in the early stages of pregnancy [12,20]. A study compared the ultrastructure of placentas from natural conceptions with those from pregnancies resulting from assisted reproductive technology, and the findings showed that some ultrastructural differences were present with respect to the placental blood barrier [21]. In a mouse model, assisted reproductive technology procedures cumulatively increased placental epigenetic abnormalities and morphological abnormalities that resulted in adverse long-term neonatal health outcomes [22]. In the present study, for the FT embryo transfer group, embryos were transferred on the fifth day after natural ovulation, and the estrogen levels were much lower when compared with the fresh embryo transfer group. As a result, the FT embryo transfer group had a higher live birth rate and a lower rate of miscarriage.

Recently, the effect of FT embryo transfer and fresh embryo transfer on obstetric and neonatal outcome have become topical research areas. However, but the findings and opinions still vary between studies and meta-analysis. It has been previously reported that there was no significant difference in preterm delivery rates between fresh and frozen embryo transfer in

women with polycystic ovary syndrome (PCOS) [1]. Recently, Roy et al. showed that FT embryo transfer improved newborn outcomes and resulted in similar live birth rates when compared with fresh embryo transfer [5]. Some authors have argued that the FT embryo transfer cycles increased the risk of complications during pregnancy such as preeclampsia, and of congenital malformation in the fetus [1,7,23,24]. In a recent study, microRNA expression profiles were compared between placentas derived from fresh embryo transfer, FT embryo transfer, and spontaneous pregnancies [25]. The results supported the hypothesis that FT embryo transfer might increase exposure of the epigenome to external influences and could be associated with perinatal disease [25]. However, Wennerholm compared a very large population of fresh embryo transfer, FT embryo transfer, and natural conception births, and found that preterm delivery, low weight birth in frozen transfer group were significantly lower when compared with the fresh group [26]. A cohort study based on 14,262 newborns showed that the birth weight of the fresh embryo transfer group was lower when compared with the FT embryo transfer group and that the perinatal outcome was negatively affected by controlled ovarian hyperstimulation [27]. Another recent retrospective cohort study that included 4,071 patients with live singleton births showed that for normal responder patients undergoing

fresh embryo transfer cycles, estrogen levels >2,500 pg/ml on the day of transfer was an independent predictor for low birth weight [28]. The findings of this study showed that the rate of preterm birth, gestational age, and birth weight were no different in the FT embryo transfer group when compared with the fresh embryo transfer group. A recently published systematic review by Wong et al. could not conclude whether or not FT embryo transfer would bring any advantages or disadvantages to obstetric outcomes [17]. Therefore, the obstetric and neonatal changes with FT embryo transfer required further controlled clinical studies.

Conclusions

The findings of this study showed that frozen-thawed (FT) embryo transfer could improve the live birth rate and reduce the miscarriage rate when compared with fresh embryo transfer with intracytoplasmic sperm injection (ICSI) for male infertility. Furthermore, there was no significant disadvantage for the obstetric and neonatal outcomes following embryo transfer. A policy for freezing embryos prior to transfer could be an effective and safe procedure for ICSI in assisted reproduction technology, or *in vitro* fertilization.

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