The clinical outcomes of fresh versus frozen embryos transfer in women ≥40 years with poor ovarian response

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Objective

To compare the clinical outcomes of fresh embryo transfers (ETs) and frozen-thawed embryo transfers (FETs) after a freeze-all cycle in women ≥40 years old with poor ovarian response (POR).

Methods

We performed a single-center, retrospective, case-control study of patients who underwent *in vitro* fertilization between January 2014 and June 2019. We included a total of 192 patients aged 40 years or older from whom 3 or fewer oocytes had been retrieved and who were receiving cleavage-stage ET in this study. Of these patients, 101 and 91 patients underwent fresh ET and FET, respectively. The primary outcome was the live birth rate (LBR) after the first ET. Logistic regression analysis was used to compare the IVF outcomes and neonatal characteristics between the fresh ET and FET groups, adjusting for maternal age, body mass index, luteinizing hormone, and the number of good quality embryos transferred.

Results

The mean maternal ages and number of oocytes retrieved (43.2 years and 2.3 in both groups, *P*=0.902 and *P*=0.927, respectively) were similar in the fresh ET and FET groups. No significant difference was observed between the LBRs of the fresh ET and FET groups (adjusted odds ratio, 1.28; 95% confidence interval, 0.29–5.70). The clinical pregnancy and miscarriage rates, and neonatal characteristics (birth weights and premature infant rates) were similar between the 2 groups.

Conclusions

FET after the freeze-all strategy had no beneficial impact on the clinical outcomes of women ≥40 years with POR.

Keywords: Maternal age; Infertility; *In vitro* fertilization; Cryopreservation; Embryo transfer

Introduction

With the development of *in vitro* fertilization (IVF) techniques over the past decades, clinicians are constantly seeking ways to optimize IVF outcomes and minimize the treatment risks [1]. The "freeze-all" strategy, which involves the freezing of all of the embryos in a fresh cycle and subsequent frozen-thawed embryo transfer (FET), has been reported to increase IVF efficacy and has become an essential aspect of assisted reproductive technology (ART) [2].

In the last century, there have been extensive studies that have compared the IVF outcomes of fresh embryo transfer (ET) and FET [3]. A previous randomized controlled trial (RCT) from China indicated that patients with polycystic ovary syndrome (PCOS) who underwent FET had a higher live birth

rate (LBR), and lower risks of ovarian hyperstimulation syndrome (OHSS) and pregnancy loss compared with patients

Obstetrics & Gynecology Science

Conghui Liu, et al. Aged POR women will not benefit from FET

who underwent fresh ET. However, whether the strategy is suitable for other patients who are undergoing IVF, such as women with advanced age or poor ovarian response (POR) is yet to be elucidated [4]. In contrast, according to a recent meta-analysis of 11 RCTs that included 5,379 patients, the significant increase in the FET LBR was only noted in hyper-responders with an increased risk of pre-eclampsia; however, the researchers did not analyze the differences among the patients with POR [5].

Recently, a few observational studies compared the fresh ET and FET outcomes in poor ovarian responders and the results were equivocal. A retrospective cohort study that included 82,935 cycles showed that both the clinical pregnancy rate (CPR) and LBR were higher after fresh ET compared with FET in intermediate (6–14 oocytes retrieved; 49.6% vs. 44.2% and 41.2% vs. 35.3%, respectively) and low responders (1–5 oocytes retrieved; 33.2% vs. 15.9% and 25.9% vs. 11.5%). However, the results derived from both the cleavage-stage embryo and blastocyst transfer data exhibited high heterogeneity [6]. Berkkanoglu et al. [7] demonstrated that the freeze-all strategy should be made available to poor responders as it resulted in improved pregnancy outcomes; however, the mean age in their study was only 35 years old with 4 or fewer oocytes collected, which did not meet the POR criteria proposed by Bologna [8]. In contrast, another retrospective cohort study from Brazil demonstrated that the freeze-all strategy had no impact on IVF outcomes in POR patients; however, the primary outcome in their study was the ongoing pregnancy rate but not the LBR [9].

To the best of our knowledge, there are no studies that have previously evaluated the clinical outcomes of fresh ET and FET after use of the freeze-all strategy in advanced maternal age (AMA) patients with POR. This study aimed to investigate whether AMA patients with POR can benefit from the freeze-all strategy.

Materials and methods

1. Study population

This was a retrospective case-control study that analyzed the data of AMA patients with POR who underwent fresh ET and FET after the "freeze-all" strategy from January 2014 to June 2019 at the Reproductive Centre of the 901th Hospital of the Joint Logistics Support Force of The People's Liberation Army.

All the patients provided written informed consent. The eligibility criteria were as follows: 1) aged 40 years or older; 2) 3 or less oocytes retrieved after controlled ovarian stimulation (COS) including the gonadotropin-releasing hormone (GnRH) antagonist, GnRH agonist short, mild stimulation, and medroxyprogesterone acetate (MPA) pituitary down-regulation protocols [10]; 3) at least one available embryo for transfer after COS; 4) had undergone cleavage-stage ET. The exclusion criteria were: 1) oocyte donation cycles; 2) embryo biopsy; 3) uterine malformations; 4) intrauterine adhesions. None of the patients were excluded due to male factors. The subjects assigned to the FET group were mostly those who received the MPA pituitary down-regulation protocol (54.9%) and who had an endometrial thickness that was less than 8 mm (13.2%; Table 1).

A total of 192 patients who met the criteria were included in this study. Of these patients, 101 and 91 received fresh ETs and FETs after undergoing the freeze-all strategy, respectively. We collected the patients' demographic information from medical records, including their age; infertility duration and type; body mass index (BMI); basic follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E$_2$), and prolactin levels; and antral follicle count (AFC).

2. Ovarian stimulation

All of the patients received COS. The starting gonadotropin dose was 150–300 IU FSH based on the patient's age, hormone profile, AFC, and BMI. The dose was adjusted according to the ovarian response through serial ultrasonography and serum FSH, LH, E$_2$, and progesterone level monitoring. The doctors would add 75 IU human menopausal gonadotropin (Lizhu Pharmaceutical Trading Co., Ltd., Zhuhai, China) at their discretion. When one or 2 leading follicles reached

Table 1. Reason for frozen embryo transfer (n=91)

Reasons	Value
MPA pituitary down-regulation protocol	50 (54.9)
Endometrial thickness less than 8 mm	12 (13.2)
AMA per se	11 (12.1)
Level of progesterone over 1.5 ng/mL	9 (9.9)
Personal choice	5 (5.5)
Others	4 (4.4)

Values are presented as number (%).
MPA, medroxyprogesterone acetate; AMA, advanced maternal age.

18 mm or more in diameter, 250 μg recombinant human chorionic gonadotropin (The Merck Group, Darmstadt, Germany) was administered to induce oocyte maturation. All the oocytes that were ≥12 mm in diameter were transvaginally retrieved 36 hours later.

3. Embryo culture and evaluation

All of the oocytes that were retrieved were fertilized with conventional IVF or intracytoplasmic sperm injection (ICSI) 2–4 hours after oocyte retrieval was performed, according to the sperm quality. The embryos were graded according to the system proposed by Cummins et al. [11] The available embryos were defined as normally fertilized embryos with 6 or more blastomeres and <50% fragmentation on day 3. Good quality embryos were defined as those with a grade equal or superior to 7/II on day 3.

4. Embryo transfer and luteal support

For the patients who received fresh ETs, ≤3 available embryos were transferred into the uterine cavity under ultrasonographic guidance on day 3 after the oocyte retrieval. A daily intramuscular injection of 40 mg progesterone (Zhejiang Xianju Pharmaceutical Co Ltd., Taizhou, China) was used for luteal support from the day of oocyte retrieval until 10 weeks of gestation after clinical pregnancy was confirmed (defined as the presence of a gestational sac on ultrasound 30–35 days after ET).

For the patients who underwent FET, hormone replacement treatment was carried out for endometrial preparation. Briefly, 4–8 mg oral E_2 valerate tablets (Progynova; Bayer Schering Pharma, Beijing, China) were administered from day 3 of the second menstrual cycle after oocyte retrieval or later. An intramuscular progesterone injection at a dose of 40 mg per day was added for 3 days when the endometrial thickness reached 8 mm or more. For patients that had an endometrial thickness that was less than 8 mm after prolonged oral E_2 valerate tablet usage (18 days) and that did not have any disorders identified by hysteroscopy, endometrial transformation with 40 mg progesterone were also performed. The frozen embryos were thawed with a commercial warming kit (Kitazato Biopharma Co., Yanagishima, Japan), and ≤3 surviving embryos (defined by at least 50% retention of intact cells) were transferred into the uterine cavity under ultrasonographic guidance. Luteal support with the original E_2 valerate dose and intramuscular progesterone injections were continued until 10 weeks of gestation once clinical pregnancy was confirmed.

5. Outcomes

The primary outcome in this study was the LBR. Live birth was defined as the birth of at least one newborn that exhibited any signs of life. The secondary outcomes were the biochemical pregnancy, clinical pregnancy, and miscarriage rates. Biochemical pregnancy was defined as an human chorionic gonadotropin level more than 20 IU/L 14 days after ET. Miscarriage was defined as the loss of a clinical pregnancy at <24 weeks' gestation.

The mean birth weights, premature infant rates, number of multiple pregnancies, and numbers of neonates with low birth weights and fetal macrosomia were recorded. A premature infant was defined as preterm birth at <37 weeks' gestation. Multiple pregnancy was defined as at least 2 detectable gestational sacs or heartbeats on ultrasound. Low birth weight and macrosomia were defined as birth weights <2,500 g and ≥4,000 g, respectively.

6. Statistical analysis

Continuous variables are expressed as the mean with the standard deviation or the median with the interquartile range, categorical variables are expressed as numbers (percentages). Comparisons between the patients who underwent fresh ET and FET were performed using the *t*-test for continuous variables when the distribution and variance met the conditions. Otherwise, the Kruskal-Wallis test was used.

To identify the differences in the reproductive and neonatal characteristics between the fresh ET and FET groups, multivariate logistic regression analysis was used to derive the odds ratios (ORs) and 95% confidence intervals (CIs). The potential confounders were variables that resulted in more than 10% variation in the coefficient of the principal study factor when added into the model. Results were presented after adjusting for maternal age, BMI (model 1), LH, and the number of good quality embryos transferred (model 2).

A 2-tailed *P*-value that was less than 0.05 was considered statistically significant. All analyses were performed using STATA 16.0 (StataCorp, College Station, TX, USA).

Results

1. Patient characteristics

The patient characteristics are shown in Table 2. There were no significant differences in the maternal ages; infertility durations and types; basal FSH, LH, E$_2$, and prolactin levels; and AFCs between the fresh ET and FET groups. However, the BMI was higher for patients in the fresh ET group compared with the FET group (25.0 kg/m^2 vs. 23.9 kg/m^2, P=0.044).

2. Controlled ovarian stimulation outcomes

The outcomes are shown in Table 3. The number of days of ovarian stimulation and gonadotropin dose were similar between the fresh ET and FET groups. No significant differences were identified between the numbers of oocytes retrieved, available embryos, and good quality embryos between the 2 groups. Although the numbers of embryos transferred were similar between the 2 groups, the FET group had a greater number of good quality embryos transferred and had thinner endometria compared with the fresh ET group (1.6 vs. 0.9 and 7.9 mm vs. 9.6 mm, respectively; P<0.001 for both).

3. *In vitro* fertilization and neonatal outcomes

Table 4 shows that the LBRs were similar between the fresh ET and FET groups (5.9% vs. 6.6%). When the FET and fresh ET groups were compared, the unadjusted OR was 1.12 (95%

Table 2. Characteristics of patients

Characteristics	Fresh ET group (n=101)	Frozen ET group (n=91)	*P*-value
Age (yr)	43.2±2.6	43.2±2.2	0.902
Infertility duration (yr)	4.9±4.7	5.7±4.9	0.276
Infertility type			
Primary infertility	13 (12.9)	12 (14.5)	0.755
Secondary infertility	88 (87.1)	71 (85.5)	
BMI (kg/m^2)	25.0±4.1	23.9±3.1	0.044
Basal FSH (IU/L)	13.1±7.3	13.7±9.2	0.668
Basal LH (IU/L)	5.2±3.2	6.5±6.3	0.087
Basal oestrogen (pg/mL, median [IQR])	47.5 (53)	55.5 (63.5)	0.513
Basal prolactin (ng/mL, median [IQR])	11.2 (7.1)	12.0 (9.0)	0.598
AFC	4.9±3.1	4.7±3.7	0.673

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
ET, embryo transfer; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; IQR, interquartile range; AFC, antral follicle count.

Table 3. The outcomes of controlled ovarian stimulation

Characteristics	Fresh ET group	Frozen ET group	*P*-value
Days of ovarian stimulation (day)	7.7±3.2	7.6±3.3	0.804
Total gonadotropin dose (IU)	1,933.8±877.2	1,911.9±1,054.2	0.877
Endometrial thickness (mm)	9.6±2.6	7.9±2.7	<0.001
Oocytes retrieved	2.3±0.7	2.3±0.7	0.927
Available embryos	1.8±0.7	1.9±0.6	0.616
Good quality embryos	1.5±0.8	1.6±0.7	0.396
Embryos transferred	1.8±0.7	1.9±0.5	0.687
Good quality embryo transferred	0.9±0.8	1.6±0.6	<0.001

Values are presented as mean±standard deviation.
ET, embryo transfer.

Table 4. The outcomes of *in vitro* fertilization and neonate

Characteristics	Fresh ET group	Frozen ET group	Unadjusted OR (95% CI) in frozen ET group	P-value	Model 1 OR (95% CI) in frozen ET group	P-value	Model 2 OR (95% CI) in frozen ET group	P-value
Biochemical pregnancy	16/101 (15.8)	14/91 (15.4)	0.97 (0.44–2.11)	0.931	1.07[a)] (0.47–2.41)	0.878	1.28[b)] (0.45–3.62)	0.641
Clinical pregnancy	11/101 (10.9)	11/91 (12.1)	1.13 (0.46–2.73)	0.795	1.17[a)] (0.46–2.95)	0.745	1.16[b)] (0.36–3.69)	0.802
Miscarriage rate	5/11 (45.5)	5/11 (45.5)	1.00 (0.19–5.36)	1.000	1.58[a)] (0.20–12.49)	0.665	1.01[b)] (0.03–35.25)	0.995
Live birth rate	6/101 (5.9)	6/91 (6.6)	1.12 (0.35–3.60)	0.852	1.24[a)] (0.38–4.11)	0.719	1.28[b)] (0.29–5.70)	0.750
No. of neonate	6	6	-	-	-	-	-	-
Birth weight (g)	3,583.3±467.6	3,116.7±468.7	-	-	-	-	-	-
Premature infant rate	1/6 (16.7)	2/6 (33.3)	2.50 (0.16–38.60)	0.512	1.81[c)] (0.52–6.23)	0.349	-	-

Values are presented as number (%) or mean±standard deviation.

ET, embryo transfer; OR, odds ratio; CI, confidence interval.

[a)] Adjusted for maternal age, body mass index; [b)] Adjusted for maternal age, body mass index, luteinizing hormone and the number of good quality embryo transferred; [c)] Adjusted for maternal age.

CI, 0.35–3.60). After adjusting for the age and BMI, the OR rose 1.24 (95% CI, 0.38–4.11), but this value was still not statistically significant. After further adjusting for LH and the number of good quality embryos transferred, there was little difference in the OR (OR, 1.28; 95% CI, 0.29–5.70). There were no significant differences between the groups regarding the biochemical pregnancy, clinical pregnancy, and miscarriage rates before and after the adjustment.

Six neonates were born after fresh ET and FET each. The mean birth weights were 3,583.3 g and 3,116.7 g in the fresh ET and FET groups, respectively (*P*=0.115). The premature infant rate was higher in the FET group compared with the fresh ET group, but this difference was not statistically significant (33.3% vs. 16.7%). The adjusted OR was 1.81 in the FET group when compared with the fresh ET group (95% CI, 0.52–6.23; *P*=0.349). Neither of the groups had low birth weight or macrosomic neonates (Table 4).

Discussion

With the rapid increase in the success rate with vitrified-thawed embryos, the number of autologous FETs increased over 2.5-fold from 2004 to 2013; however, the number of fresh ETs was approximately the same [12]. The increased use of FET corresponded with a significant increase in the LBR (from 16.9% in 2006 to 31.5% in 2012) and a decrease in the OHSS risk, especially for women with PCOS [4,13]. The potential mechanism for the decreased LBR after fresh ET, when compared with FET, could be the supraphysiologic hormonal levels during COS resulting in endometrial implantation window advancement and alterations in the endometrial gene expression compared with the natural cycle [12,14,15]. In contrast with the previous study, the LBR in this study was comparable between the fresh ET and FET groups (adjusted OR, 1.28; 95% CI, 0.29–5.70; *P*=0.750). The possible explanation for this is that, here, the patients were 40 years or older and had POR; thus the E_2 levels in the fresh ET cycle were not high. This resulted in fewer changes to the internal environment and less endometrial receptivity in the ovarian stimulation process.

Although many studies have illustrated that IVF outcomes (LBR, implantation rate, and ongoing pregnancy) can be improved through the use of the freeze-all strategy, most of these studies were limited by the number of patients who

Obstetrics & Gynecology Science

Conghui Liu, et al. Aged POR women will not benefit from FET

were hyper- or normal ovarian responders [5,16,17]. An RCT on normal responders (8–15 AFC), that included patients with an average age of 33 years old, showed that the clinical and ongoing pregnancy rates in the frozen ET group were significantly greater than those in the fresh ET group [18]. Recently, a multicenter RCT that included 1,508 PCOS patients was conducted in China. The authors demonstrated that patients who underwent frozen ET had higher LBRs than those who underwent fresh ET (49.3% vs. 42.0%; rate ratio, 1.17; 95% CI, 1.05–1.31) with no differences in the neonatal complications; however, 5 neonatal deaths did happen in the frozen ET group [4]. They performed another study of patients aged approximately 29 years old (n=1,650) who had regular menstrual cycles and who underwent elective single blastocyst transfers. Their study also showed a higher LBR in the frozen ET group compared with the fresh ET group; however, the frozen ET group had a higher pre-eclampsia risk (3.1% vs. 1.0%) [17]. Nevertheless, in patients with POR, previous studies have had inconsistent results and have been unclear about the benefits of the freeze-all strategy. A previous cohort study that included 938 IVF cycles suggested that only patients that had 10–15 oocytes retrieved had higher implantation and ongoing pregnancy rates after FET (P=0.028 and P=0.021, respectively) compared with fresh ET, while in patients that had 4–9 oocytes retrieved, the implantation and ongoing pregnancy rates (P=0.259 and P=0.577, respectively) did not improve from FET after use of the freeze-all strategy, which suggests that the potential advantages of the freeze-all strategy decrease with worsening ovarian response [19]. Furthermore, a meta-analysis of 5 RCTs concluded that the freeze-all strategy was not advantageous when the mean number of oocytes retrieved was less than 15 [20]. To date, there have been no studies that have reported on the efficiency of frozen ET for AMA patients with POR, and whether the freeze-all strategy is suitable for all patients, irrespective of their age, ovarian response, and hormone levels during COS remains unknown [21].

POR, first described by Garcia et al. [22], represents a challenging group of patients who undergo IVF and the prognosis differs between older poor responders and young women [5,23,24]. Our previous study of patients older than 40 years demonstrated women without POR had a relatively higher cumulative LBR compared with women with POR, indicating that ovarian reserves play a significant role in female fertility [25]. Thus far, the clinical outcomes of POR and AMA

patients when choosing between fresh ET and FET remain inconsistent. A retrospective cohort study of 2,263 patients who received ICSIs demonstrated that patients who had 4 or less oocytes retrieved could also benefit from the freeze-all strategy, with better pregnancy outcomes including the pregnancy, clinical pregnancy, implantation, and LBRs compared with those who underwent fresh ET. However, the mean age in this study was 35 years old or younger, which might not have been representative of the older population as a whole [7]. In contrast, another case-control study of 259 POR patients with an average age of 36.9 years (range, 21–43 years) showed a similar LBR between the fresh ET and FET groups (21% vs. 17%, P=0.42); however, they did not consider some of the related confounders such as the BMI [26]. In this study, all the subjects were 40 years old or older, with a mean age of 43.2 years old and mean number of oocytes retrieved of 2.3 in both the fresh ET and FET groups, which was a more rational representation of older women with POR. The results showed that there were no significant differences in terms of the CPR, LBR, and neonatal characteristics (body weight and premature infant rate) between the fresh ET and FET groups, indicating that the freeze-all strategy holds no benefit over fresh ET in AMA patients with POR who are undergoing cleavage-stage ET. The results were in accordance with those from previous studies that found that the benefits of the freeze-all strategy decreased as the number of oocytes retrieved decreased.

As an independent factor for ART outcomes, age predicts over 80% of IVF success [27]. Even in poor ovarian responders, being young can act as a protective factor against the deleterious effects of POR [24]. The most probable reason for the steep decline in fertility with aging is a diminished ovarian reserve [27]. Moreover, among women with advanced age, a significant increase in the number of aneuploidy oocytes and a decrease in the potential for embryonic development are also noticeable. The number of antral follicles and the follicular microenvironment (the granulosa cells and vasculature) are also negatively affected by age [28,29]. The best assisted reproductive strategy for AMA patients is to shorten the time to get pregnant. However, the first FET with the freeze-all strategy is usually performed 2 or 3 months after oocyte retrieval, and the median time to conception is 1.4 months later compared with the fresh ET cycle [30]. In addition, the cryopreservation may have some disadvantages such as the cost, time, inconvenience, and possible alterations in the

embryo's competence for implantation and/or development derived from membrane damage and general protein denaturation in the process of embryo freezing and thawing [20]. Furthermore, FET is associated with the emotional costs of deferring ET and physical costs of additional treatments such as hormone administration, multiple injections, and medical consultations [31]. In this study, AMA patients with POR did not benefit from the freeze-all strategy, indicating there is no need to replace fresh ET with FET after use of the freeze-all strategy.

There are several limitations to this study. First, the retrospective design had inherent problems relating to selection bias; thus, the characteristics and prognoses of the patients assigned to the study may have differed between the 2 groups. However, as shown in Table 1, the patient characteristics were similar between the fresh ET and FET groups. Second, the study had a relatively small sample size; therefore, the results should be interpreted carefully. Reassuringly, the similarities in the findings regarding the biochemical pregnancy, clinical pregnancy, and miscarriage rates between the fresh ET and FET groups support our findings. Unquestionably, an RCT with a large sample size should be carried out to further examine whether the freeze-all strategy can be beneficial to AMA patients with POR. Finally, there was a significant difference between the 2 groups when analyzing the endometrial thickness and number of good quality embryos transferred. However, we only found 2.3% variation in the coefficient of the principal study factor when endometrial thickness was added into the logistic regression model; therefore we did not adjust for it. We had already excluded patients with intrauterine adhesions; thus, the influence of endometrial thickness on the clinical outcomes in this study may not be as significant as it appears. Regarding the number of good quality embryos transferred, the variation in the coefficient was 55.5% when added it into the model with no interaction (effect modification) between the different numbers. Thus, we did not perform a stratification analysis of the number of good quality embryos transferred. Moreover, stratification will further decrease the sample size in each layer, resulting in lower statistical power.

In conclusion, compared with fresh ET, FET followed by a freeze-all cycle is not beneficial for women ≥40 years old with POR. RCTs with a large sample size should be carried out to confirm this conclusion.

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Obstetrics & Gynecology Science

Conghui Liu, et al. Aged POR women will not benefit from FET

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