Pregnancy outcomes of the first thawing cycle in “freeze-all” strategy of infertility patients with fever during oocyte recruitment: a matched-pair study

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Abstract

Background: It is currently unknown whether patients with a fever after controlled ovulation during egg retrieval could increase the risk of pelvic infection or not, and fever itself may affect endometrial receptivity or embryo quality with poor pregnancy outcomes. The aim of this study was to analyze the outcomes of patients with fever during oocyte retrieval after the first frozen-thawed embryo transfer (FET) cycle.

Methods: This was a 1:3 retrospective paired study matched for age. In this study, 58 infertility patients (Group 1) had a fever during the control ovulation, and the time of the oocyte retrieval was within 72 hours, they underwent ovum pick up and whole embryo freezing (“freeze-all” strategy). The control subjects (Group 2) are 174 patients matched for age who underwent whole embryo freezing for other reasons. The baseline characteristics, clinical data of ovarian stimulation, and outcomes, such as the clinical pregnancy rate, ongoing clinical pregnancy rate were compared between the two groups in the subsequent FET cycle.

Results: All patients had no pelvic inflammatory disease after oocyte retrieval. Anti-Mullerian hormone (AMH) levels (4.2 vs. 2.2, P <0.001) were higher in group 2, and the number of oocytes retrieved, and fertilization rate were lower in group 1 (P< 0.001), but the endometrial thickness, the number of embryo transfers, and the type of luteal support supplementation were similar between the two groups. Regarding pregnancy outcomes in the subsequent FET cycle, the implantation rate, clinical pregnancy rate, early spontaneous rate, ectopic pregnancy rate, and ongoing pregnancy rate were all not significantly different. Further regression analyses showed that the clinical pregnancy rate and ongoing pregnancy rate were also not significantly different.

Conclusions: Transvaginal ultrasound-guided follicular puncture for oocyte retrieval is a safe and minimally invasive method for patients with fever. Moreover, the fever had almost no effect on embryo quality.

Keywords: Fever; Oocyte retrieval; Infertility; Pregnancy outcomes

Introduction

Human infertility is defined as the inability of a couple to conceive following at least 12 consecutive months of unprotected sexual intercourse [1,2]. Infertility is a medical distressing chronic condition that is appraised to affect 9% to 30% of reproductive-aged couples worldwide [3,4]. It is well known that assisted reproduction technology (ART) is currently an effective method for treating infertility. Many of them seek to achieve pregnancy via assisted reproductive technologies (ART). Transvaginal ultrasound-guided follicular puncture for oocyte retrieval is a highly efficient and minimally invasive method for assisted reproductive techniques.

ART is complex and costly, and each ART cycle consists of several steps, such as downregulation with agonists or antagonists, ovarian stimulation, ovulation triggering, oocyte retrieval, laboratory phase, and embryo transfer. If one of the steps is incorrectly applied, the conception may not occur and cost a lot. With this in mind, it is important to ensure that each step involved in ART is...
Thus, we design this study to consensus guidelines available on the management of others in every center of the world. To date, there are no as having fever before oocyte retrieval during control despite the fact that some patients are inevitably recognized supported by good evidence from well-designed studies,[5] Chinese Medical Journal 2021;134(7) www.cmj.org cycles,[6] pregnancy outcome than that of the fresh transplantation and the rapid development of the current overall freezing ovariano pedicle torsion. as ovarian hyper-stimulation syndrome, thrombus, and severe ovulation complications might also increase, such as ovarian hyper-stimulation syndrome, thrombus, and ovarian pedicle torsion.

In order to reduce the occurrence of these complications, and the rapid development of the current overall freezing technology, which gradually has a similar to or even better pregnancy outcome than that of the fresh transplantation cycles[6–9] strictly disinfecting and oral antibiotics were applied for prevention. More importantly, whole embryo freezing (“freeze-all” strategy) was conducted in all the patients for the subsequent frozen-thawed embryo transfer (FET) cycle.

Methods

Ethics approval
The study was approved by the local Ethics Committee of Peking University Third Hospital (No. 2008013). Informed written consent was obtained from all patients before enrollment in this study.

Study design and participants
This study was a retrospective 1:3 matched-pair study, and the data collection protocol was approved by the Ethics Committee of the Medical Center of Peking University Third Hospital (PUTH). Information on infertility patients who underwent whole embryo freezing cycles (“freeze-all” strategy) were collected from 1st Jan 2018 to 31st June 2019 in PUTH. The patients taken “freeze-all” strategy due to having a fever during the oocyte retrieval period before oocyte pick-up within 72 hours were included in the study group (group 1). The control group (group 2) was 1:3 matched with the age of whole embryo freezing cycles because of ovarian hyperstimulation syndrome (OHSS), high progesterone value ($P > 6 \text{ nmol/L}$), or personal reasons in the same period. All patients underwent oocyte retrieval under strict disinfection and received oral antibiotics for three days to prevent pelvic infection. The available embryos were frozen for subsequent FET. The outcomes of the first thaw cycle between the two groups were compared. The exclusion criteria were as follows: (1) patients with abnormal uterus cavity, such as endometrial polyps, uterus malformation, intrauterine adhesion, and submucosal myoma; (2) endometritis; (3) untreated hydrosalpinx; (4) preimplantation genetic diagnosis cycles; (5) endocrine disorders, such as hyperprolactinemia.

Controlled ovarian stimulation and embryo transfer
The selection of the controlled ovarian stimulation (COS) program should be based on the patient’s basic conditions, age, patient’s wishes and economic conditions, and the experience of the doctor. All patients received standardized, controlled ovarian stimulation in the cycle. The gonadotropin releasing hormone agonist analogue (GnRHa) long protocol, GnRHa short protocol, GnRHa prolonged protocol, GnRH antagonist protocol, and Mild-stimulation Cycles/Luteal phase stimulation.[10] Human menopausal gonadotropin (Menopur, Ferring; hMG, Lizhu, China) could be added if needed. The doses of the medications were adjusted according to the ovarian response. Recombinant human chorionic gonadotropin (rhCG) at a dose of 4000 to 10,000 IU was administered to induce oocyte maturation when at least two leading follicles achieved 18 mm in diameter (or 17 mm in GnRH antagonist protocol). Oocyte retrieval was performed 36 to 38 hours after recombinant human chorionic gonadotropin (rhCG) injection. All the embryos were in vitro fertilization and cultured for three days (D3) or 5 days (D5), all the D3 and D5 embryos were freezing in the group 1 and group 2 for canceling the fresh transfer cycle (“freeze-all” strategy). The pregnancy outcomes of the first FET cycle were analyzed retrospectively.

FET and Luteal supplementation
Performing FET in a monitored natural cycle has the advantage that no medications are used, making such cycles preferable to many women.[11] In the natural cycle FET (NC-FET), the detection of ovulation is a marker for the timing of thawing and transfer. The moment of ovulation can be estimated based on the detection of the luteinizing hormone (LH) surge in either urine or blood (constituting “true” NC-FET) or after triggering ovulation of the dominant follicle using human chorionic gonadotrophin (hCG) (“modified” NC-FET). The luteal support method was 20 mg of oral dydrogesterone, twice a day, until 8 to 10 pregnancy weeks, which is the same in the ovulation induction cycle FET. Artificial cycle FET (AC-FET) mimics the natural menstruation cycle by the administration of consecutive estrogen and progesterone. Otherwise, 90 mg of vaginal progesterone gel daily (Crinone gel 8%, Merck Serono SA, Geneva, Switzerland), they also were provided 6 mg of oral estradiol daily and 20 mg of oral dydrogesterone two times a day and the dose gradually reduce until 12 pregnancy weeks.

Definition of clinical outcomes and follow-up
Clinical pregnancy was defined as the presence of one or more intrauterine gestational sacs. Early miscarriage is defined as pregnancy loss during the first trimester of pregnancy (less than 12 weeks of gestation). Spontaneous abortion rate is expressed per clinical pregnancy cycle. The ectopic pregnancy rate is expressed per clinical pregnancy cycle. The ongoing clinical pregnancy, defined as pregnancy gestational weeks, was more than 20 weeks.
**Statistical analysis**

Descriptive data for baseline characteristics were compared using the Student’s *t* test (normal data distribution) or the Mann-Whitney *U* test (skewed data). One-way ANOVA test, independent *t* test, and two-tailed χ² analysis were performed to compare data between the groups. All statistical analysis was performed using the IBM SPSS Statistics Package (IBM SPSS V.20; IBM Corporation Inc., Chicago, USA). A *P* value of 0.05 is used as the cutoff for statistical significance.

**Results**

Data were extracted from clinical records, including age, duration of infertility, infertility type, basal estradiol (E2), basal follicle-stimulating hormone (FSH), basal LH, the primary cause of infertility, ovulation protocol, total gonadotropin (Gn) dosage, number of oocytes retrieved, number of available embryos (D3 and D5), number of embryos transferred (D3 and D5), and endometrial thickness. Reproductive outcomes included clinical pregnancy rate, early spontaneous abortion rate, and the persistent clinical pregnancy rate.

There were 4312 patients taken the “freeze-all” strategy from 1st January 2018 to 31st June 2019. Based on the exclusion criteria, 2561 patients were included. Fifty-eight patients were included in the study group (group 1) due to fever during oocyte retrieval. All patients had a fever between 37.4°C and 40°C, and upper respiratory tract bacterial infection was the main reason for fever in 53 patients (91.4%), and 5 fever patients for other reasons (such as pneumonia and acute gastroenteritis). With the 1:3 ratio paired of age, 174 patients were enrolled in the control group (Group 2) from the 2503 patients taken “freeze-all” strategy for other reasons (not fever) in the same period. None of the patients had a pelvic inflammatory disease after oocyte retrieval.

The basic characteristics of patients are shown in Table 1. Age was already matched. There were no significant differences in body mass index (BMI), basal FSH, basal LH, basal E2, and infertility type (primary or secondary). However, the anti-mullerian hormone (AMH) levels were higher in the control group compared to the study group (4.2 ng/mL vs. 2.2 ng/mL, *P* < 0.001), and the infertility time was longer in the control group (3 years vs. 2 years, *P* = 0.035).

Table 2 shows the clinical data after the ovarian stimulation; there were no significant differences in the ovarian stimulation protocol, the usage of Gn (both time and dose), and the intracytoplasmic sperm injection (ICSI) rate between the two groups. The number of oocytes retrieved (10.9 ± 4.6 vs. 17.4 ± 5.9) and the fertilization rate (81.0% vs. 90.6%) were lower in the study group, with a significant difference (*P* < 0.001), but the two pronucleate (2PN) rate was higher in the study group (66.7% vs. 57.4%, *P* < 0.001) and the number of available embryos (D3 and D5) were similar in the two groups (*P* > 0.05).

Regarding pregnancy outcomes, 58 patients in Group 1 had 58 FET cycles with no cycle canceled, and 174 patients in Group 2 had 173 FET cycles with one cycle canceled because of all frozen embryo damage after thawing (Table 3). The endometrial thickness (10.3 ± 2.0 mm vs. 9.9 ± 1.5 mm), the number of embryo transfer (D3 or D5), the type of luteal support supplementation was similar between the two groups. The implantation rate (36.4% vs. 40.2%), clinical pregnancy rate (48.3% vs. 52.0%), early spontaneous abortion rate (17.9% vs. 13.3%), and ongoing pregnancy rate (37.9% vs. 44.5%) were comparable with no significant difference. After taking into account the effects of age, BMI, AMH, transfer D3 or D5 numbers, endometrial thickness, we performed regression analyses, which are shown in Table 4. Clinical pregnancy and ongoing pregnancy rates were also not significantly different after balance the confounding variables.

The patients with T ≤ 37.2°C, low fever (T < 38°C) and higher fever (T ≥ 38°C) had no significant difference in clinical pregnancy rate and ongoing pregnancy rate (*P* > 0.05, Table 5).

Table 1: Baseline characteristic of the infertility patients taken whole embryo freezing cycles with fever during oocyte retrieval.

| Variables                  | Group 1 (n = 58) | Group 2 (n = 174) | *P*  |
|----------------------------|------------------|-------------------|------|
| Age (years)                | 31.4 ± 5.3       | 31.3 ± 5.2        | 0.98 |
| Type of infertility        |                  |                   | 0.88 |
| Primary                    | 58.6 (34/58)     | 60.9 (106/174)    |      |
| Secondary                  | 41.4 (24/58)     | 39.1 (68/174)     |      |
| Duration of infertility (years) | 2.00 (1.25, 3.75) | 3.00 (2.00, 5.00) | 0.04 |
| Nulliparity                | 86.2 (50/58)     | 93.1 (162/174)    | 0.10 |
| BMI (kg/m²)                | 22.40 ± 3.60     | 22.40 ± 3.33      | 0.06 |
| Basal FSH (IU/L)           | 6.70 ± 3.64      | 6.30 ± 2.01       | 0.40 |
| Basal LH (IU/L)            | 4.00 ± 2.70      | 4.80 ± 2.75       | 0.05 |
| Basal E2 (nmol/L)          | 181.7 ± 88.06    | 163.9 ± 62.9      | 0.16 |
| AMH (ng/mL)                | 2.20 (1.35, 4.18) | 4.20 (2.25, 6.42) | <0.001 |

Data are presented as % (n/N), median (range) or mean ± standard deviation. AMH: Anti-mullerian hormone; BMI: Body mass index; E2: Estradiol; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone.
Discussion

The body temperature exceeds the normal body temperature by 0.5°C, which is called fever. In clinical, it is generally considered that T ≥ 37.3°C. According to the armpit temperature, it is divided into low fever (<38°C), medium fever (38–39°C) and high fever (39–40°C), and ultra-high fever (>40°C). In our study, patients had a fever within 72 hours before egg retrieval and lasted from 24 hours to 72 hours. The average duration was 29.4 ± 11.9 hours. The maximum body temperature fluctuated from 37.4°C to 40°C, and the average maximum body temperature was 38.1 ± 0.6°C.

In many studies, recurrent implantation failure was reported to be related to age, BMI, smoking, embryo, and endometriosis, which can be an extremely stressful experience for couples undergoing in vitro fertilization (IVF) and embryo transfer (ET) cycles as well as to clinicians. Endometrial receptivity is widely regarded as a key factor in the success of IVF. The current study determined that certain genital tract infection (GTI) may contribute to failure in patients undergoing IVF. Many women who have experienced recurrent implantation failure (RIF) have also been found to have chronic endometritis (CE) from bacterial colonization. In fact, Romero et al. reported that the prevalence rate of CE was as high as 42% in patients with recurrent implantation failure (RIF) underwent in vitro fertilization (IVF) cycles. However, we did not know whether having a fever during oocyte retrieval would affect the embryo quality and endometrial receptivity (such as increased chronic endometritis), which may increase implantation failure.

Table 2: Clinical data of the infertility patients after ovarian stimulation.

| Clinical data                                      | Group 1 (n = 58) | Group 2 (n = 174) | P      |
|---------------------------------------------------|-----------------|------------------|--------|
| Ovarian stimulation protocol                      |                 |                  |        |
| GnRHa long protocol                              | 25.9 (15/58)    | 26.4 (46/174)    | 0.92   |
| GnRHa prolonged protocol                         | 12.1 (7/58)     | 14.4 (25/174)    |        |
| GnRHa short protocol                             | 1.7 (1/58)      | 4.0 (7/174)      |        |
| GnRH antagonist protocol                         | 58.6 (34/58)    | 54.0 (94/174)    |        |
| Mild-stimulation stimulation                      | 1.7 (1/58)      | 1.1 (2/174)      |        |
| Total gonadotrophin dose (IU)                     | 2595 ± 994.1    | 2399 ± 947.7     | 0.18   |
| No. days of stimulation (d)                       | 11.1 ± 2.6      | 11.1 ± 2.1       | 0.97   |
| Number of oocytes retrieved                       | 10.9 ± 4.6      | 17.4 ± 5.9       | <0.001 |
| ICSI rate                                         | 29.3 (17/58)    | 37.9 (66/174)    | 0.24   |
| Fertilization rate                                | 81.0 (511/631)  | 90.6 (2689/2968) | <0.001 |
| 2PN rate                                          | 66.7 (421/631)  | 57.4 (1705/2968) | <0.001 |
| Available embryos number                          |                 |                  |        |
| D3                                                | 4 (1, 12)       | 6 (2, 20)        | 0.07   |
| D5                                                | 2 (1, 8)        | 2 (1, 12)        | 0.89   |

Table 3: Outcomes of first frozen-thawed embryo transfer cycle of the infertility patients.

| Outcomes                                      | Group 1 (n = 58) | Group 2 (n = 174) | P    |
|-----------------------------------------------|-----------------|------------------|------|
| Cancelled FET cycles                           | 0               | 1                |      |
| FET cycles                                    | 58              | 173              |      |
| Embryos transferred number                     |                 |                  |      |
| D3                                            | 2 (1, 2)        | 2 (1, 2)         | 0.14 |
| D5                                            | 1 (1, 1)        | 1 (1, 2)         | 0.29 |
| Endometrial thickness                         | 10.3 ± 2.0      | 9.9 ± 1.5        | 0.16 |
| Luteal support supplementation                |                 |                  |      |
| NC-FET cycle                                  | 60.3 (35/58)    | 50.3 (87/173)    |      |
| AC-FET cycle                                  | 25.9 (15/58)    | 38.7 (67/173)    |      |
| Ovulation induction cycle                      | 13.8 (8/58)     | 11.0 (19/173)    |      |
| Implantation rate                              | 36.4 (32/88)    | 40.2 (104/259)   |      |
| Clinical pregnancy rate                        | 48.3 (28/58)    | 52.0 (90/173)    |      |
| Early spontaneous abortion rate                | 17.9 (5/28)     | 13.3 (12/90)     |      |
| Ectopic pregnancy rate                        | 3.6 (1/28)      | 1.1 (1/90)       |      |
| Ongoing pregnancy rate                         | 37.9 (22/58)    | 44.5 (77/173)    |      |

Data are presented as % (n/N), median (range) or mean ± standard deviation. ICSI: Two pro-nucleate; D3: Three days; D5: Five days; GnRHa: Gonadotropin releasing hormone agonist analogue.
Upper respiratory and digestive tract infections accompanied by fever, such as influenza and acute gastroenteritis, can occur in almost any population and are unpredictable. The infertility patients entered in the IVF cycle cannot be completely avoided from them, so doctors should think about how to deal with such patients. Transvaginal ultrasound-guided oocyte retrieval is an essential procedure for ART. It is now considered the gold standard procedure for oocyte retrieval for its simplicity, but the operation must go through the vagina that is not completely sterile. Patients with fever may increase the risk of infection during oocyte retrieval and cause endometritis, decrease in embryo quality, and pelvic inflammatory disease resulting in implantation failure. In our study, none of the patients had a pelvic inflammatory disease after oocyte retrieval using oral antibiotics. It is a relatively straightforward and safe procedure for patients with fever. As shown in Tables 1 and 2, the patients’ age, BMI, nulliparity, basal FSH, basal LH, basal E2, infertility type (primary or secondary), ovarian stimulation protocol, usage of Gn, ICSI rate, 2PN rate, and the number of available embryos (D3 and D5) were not significantly different. However, the AMH levels were higher in the control group (4.2 ng/mL vs. 2.2 ng/mL, \( P < 0.001 \)). The number of oocytes retrieved (10.9 ± 4.6 vs. 17.4 ± 5.9) and the fertilization rate (81.0% vs. 90.6%) were lower in the study group. The higher AMH and number of oocytes retrieved in the control group were because of most whole embryos freezing in the control group due to the OHSS. We can see that AMH can more sensitively respond to ovarian function than the hormones FSH, LH, and E2 in predicting the number of oocytes.

### Table 4: Binary logistic regression analysis to account for confounding variables of the infertility patients.

| Variables                        | Clinical pregnancy | Ongoing pregnancy |
|----------------------------------|--------------------|-------------------|
| Group                            | P                  | OR (95% CI)       | P                  | OR (95% CI)       |
| Age                              | 0.56               | 0.98 (0.93, 1.04) | 0.74               | 0.95 (0.89, 1.01) |
| BMI                              | 0.76               | 0.93 (0.85, 1.01) | 0.02               | 0.90 (0.83, 0.98) |
| AMH                              | 0.91               | 0.99 (0.92, 1.07) | 0.98               | 1.00 (0.93, 1.08) |
| Transfer D3 number               | 0.79               | 1.15 (0.41, 3.25) | 0.47               | 1.48 (0.52, 4.22) |
| Transfer D5 number               | 0.72               | 1.44 (0.21, 10.10)| 0.52               | 1.92 (0.27, 13.70)|
| Endometrial-thickness            | 0.51               | 0.51 (0.89, 1.25) | 1.18               | 1.13 (0.95, 1.35) |

AMH: Anti-mullerian hormone; BMI: Body mass index; CI: Confidence interval; OR: Odds ratio.

### Table 5: The outcomes of patients with \( T \leq 37.2 \, ^\circ C \), \( 37.3 \, ^\circ C \leq T < 38 \, ^\circ C \) and \( T \geq 38 \, ^\circ C \) group.

| Items                        | \( T \leq 37.2 \, ^\circ C \) | \( 37.3 \, ^\circ C \leq T < 38 \, ^\circ C \) \((n = 26)\) | \( T \geq 38 \, ^\circ C \) \((n = 32)\) | P     |
|-----------------------------|-----------------------------|-------------------------------------------------|-----------------------------|-------|
| Clinical pregnancy rate (%) | 52.0 (90/173)               | 42.3 (11/26)                                   | 53.1 (17/32)                | 0.63  |
| Ongoing pregnancy rate (%)  | 44.5 (77/173)               | 30.8 (8/26)                                    | 43.8 (14/32)                | 0.42  |

In conclusion, for patients with fever during oocyte retrieval, the transvaginal ultrasound-guided follicular puncture for oocyte retrieval is a safe and minimally invasive method. It is recommended in the “freeze-all” strategy not to cancel the oocyte retrieval cycle, but to cancel the fresh embryo transplantation cycle. FET cycles for embryo transfer would be beneficial for these patients. Although this study was not a randomized controlled trial and the sample size of our study was relatively small, this matched-pair study can be considered to be well designed. More studies should be conducted in this area.
**Conflicts of interest**

None.

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