

Original Research

# Fresh Gametes Might Get Better Clinical Results than Cryopreserved Sperm or Oocytes for Nonobstructive Azoospermia Patients Underwent micro-TESE

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## Abstract

**Background:** micro-testicular sperm extraction (TESE) has been the first choice for Nonobstructive azoospermia (NOA) patients for its higher sperm retrieval rate under microscope, meanwhile, sperm or oocyte cryopreservation are widely applied in *in-vitro* fertilization (IVF) treatment. But few researches have systematically explored the effect of oocyte and sperm cryopreservation in one study. **Methods:** we retrospectively collected and analyzed the data of fertilization and pregnancy of patients who underwent micro-TESE using fresh or vitrified-warmed gametes in our center to assess the effect of gametes cryopreservation. **Results:** we compared the clinical results using fresh or vitrified-warmed gametes in NOA patients after micro-TESE, respectively. We found that the rates of fertilization and good-quality embryo using fresh gametes were  $52.37 \pm 24.25\%$  and  $64.00 \pm 36.18\%$ , while these using vitrified sperm or oocyte were  $46.00 \pm 22.70\%$  and  $68.00 \pm 34.6\%$ ,  $45.51 \pm 25.19\%$  and  $38.57 \pm 31.08\%$ , respectively; the rates of clinical pregnancy and implantation using fresh gametes were  $50.0\%$  and  $32.5\%$ , while these using vitrified sperm or oocytes were  $39.5\%$  and  $32.9\%$ ,  $37.5\%$  and  $24.1\%$ , respectively. The rates of good-quality embryo and clinical pregnancy of the fresh gamete were above these of the vitrified, but there was no statistic difference. The live birth rate using fresh gamete was higher than that using the vitrified ( $47.1\%$  verse  $32.6\%$ ,  $31.3\%$ ). **Conclusions:** The live birth rate using fresh gamete was higher than that using vitrified gametes. Fresh gametes showed better clinical outcomes than vitrified gametes in micro-TESE-ICSI treatment for NOA patients.

**Keywords:** nonobstructive azoospermia; micro-TESE; sperm cryopreservation; oocyte cryopreservation

## 1. Introduction

Nonobstructive azoospermia (NOA) refers to the complete absence of spermatozoa in ejaculate [1]. NOA accounts for 60% of azoospermia, which constitutes 10–15% of male infertility [2]. According to the etiology, NOA can be divided into 3 types: congenital, acquired and idiopathic [3]. Besides surgical sperm retrieval, few options are left for NOA patients.

Testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI) has been largely applied in NOA patients. Compared with conventional TESE (cTESE), micro-TESE has a higher sperm retrieval rate (SRR) and less tissue loss by the guide of microscope [4,5]. It's reported that the SRR of micro-TESE after a failed cTESE was 45.7% [6]. Traditionally, most patients with NOA were performed with ICSI using fresh sperm and oocytes to avoid gamete cryopreservation [7,8]. But the practice is not always as good as we wish. Vitrified oocytes

or sperm were often utilized in clinical practice. For example, the oocyte has to be cryopreserved at the oocyte retrieval day after a failed cTESE. Some cost-sensitive patients would like to process micro-TESE first in case of sperm retrieval failure. In these specific situations, gametes would be vitrified first and then used at last if micro-TESE performed successfully. Although many studies have suggested that the results of fertilization and pregnancy using vitrified-warmed gametes were similar with these using fresh gametes [9–11], the potential damage of the cryoprotectant and the freezing-thawing process were consistently concerned [12–14].

Many reports have investigated the effect of gametes cryopreservation on fertilization and pregnancy in micro-TESE. But all these reports analyzed the effect of sperm and oocyte cryopreservation separately [9,15,16]. To best of our knowledge, there is no research which systematically analyzes the effect of oocyte and sperm cryopreservation in



one study. In this study, we analyzed the fertilization and pregnancy outcomes of micro-TESE patients using fresh or cryopreserved gametes to explore the effect of gamete cryopreservation on fertilization and pregnancy.

## 2. Material and Methods

### 2.1 Patients

We retrospectively collected and analyzed the data of 124 patients that had undergone micro-TESE in our center. The diagnosis of azoospermia was based on the World Health Organization guidelines [17] and was further determined after three semen tests. Male or female patients with chronic diseases including diabetes, hypertension, ankylosing spondylitis, nephropathy, etc. were all excluded. Female infertility such as endometriosis, polycystic ovary syndrome (PCOS), anovulation were excluded in our study. All these 124 patients had conducted 127 oocyte retrieval cycles, in which 3 patients had both fresh and cryopreserved sperm cycles. The patients were evaluated and classified into three groups: Group A ( $n = 68$ ), patients with fresh oocytes and sperm; Group B ( $n = 43$ ), patients with fresh oocytes and vitrified sperm; Group C ( $n = 16$ ), patients with vitrified oocytes and fresh sperm. Most patients in Group C had cryopreserved oocytes for sperm acquisition failure by cTESE. All cycles in each group conducted a single stimulation. All the male patients with NOA were conducted with a physical examination. Female partners finished hormone assessment, including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. Our study was approved by the ethics committee of Peking University Third Hospital, and informed consent was provided.

### 2.2 micro-TESE, Sperm Cryopreservation and Warming

micro-TESE and sperm procession were performed just as Hong liang, Zhang *et al.* [9,15] described before. Briefly, micro-TESE was performed under anesthesia, and the larger and white seminiferous tubules were identified and removed under the operating microscope (OPMI Vario, Carl Zeiss, Jena, Germany). The tubules were immediately placed into G-MOPS-plus buffer (Vitrolife, Goteborg, Sweden) and then excised. The spermatozoa were examined by a microscope (TS100, Nikon, Tokyo, Japan). When at least one normal spermatozoa was identified, the sperm extraction operation would be considered successful. The retrieved sperm were collected for fertilization or vitrification. The cryopreservation of sperm was strictly followed the protocol of the sperm cryopreservation kit (Vitrolife). When warming, the straws which sperm vitrified in were directly placed into a 37 °C incubator for 10 min. And then the sperm was centrifuged for 10 min at 300 g twice. After centrifugation, the testicular pellet was reserved for ICSI.

### 2.3 Oocyte Retrieval and Vitrification

Oocytes were retrieved under the guidance of vaginal ultrasound 36 hours later after human chorionic go-

nadotropin (HCG) administration. After 2 hours' incubation, the cumulus cells were removed and MII oocytes were collected for ICSI or vitrification. The oocyte vitrification and warming were performed with the kit (Kitazato Biopharma, Fuji, Japan). Warming was implemented 2 hours before ICSI, and then the injected oocytes were maintained at 37 °C (6% CO<sub>2</sub> and 5% O<sub>2</sub>) for fertilization and development.

### 2.4 ICSI, Embryo Culture and Transplantation

Procedures of ICSI and embryo culture were just like described before [9,15]. Only licensed and practiced embryologist could perform ICSI using sperms retrieved by micro-TESE. The number of transferred embryos was decided according to the embryo morphology and the patient's pathography. For example, women after cesarean section would be only transferred with one embryo. Only good-quality embryo would be selected for transfer, and the definition of good-quality embryo was that the embryos consisted at least 5 blastomeres at Day 3. All the patients would be transferred with no more than two embryos to reduce the risk of multiple pregnancies.

### 2.5 Statistical Analysis

All data were collected and analyzed by Statistical Package for Social Sciences version 18 (IBM Corp., Armonk, NY, USA). The data were adjusted by SPSS 18. The Chi-square or Fisher's exact tests were used to assess the results of different groups, and  $p < 0.01$  was considered statistic difference.

## 3. Results

Totally, 127 cycles using sperm retrieved by micro-TESE were analyzed. Due to the gametes whether or not cryopreserved, we divided these patients into three groups. The characteristics of these patients were shown in Table 1, including age, body mass index (BMI), duration of infertility and female serum hormonal levels of FSH, LH, and T. No statistical difference was found among these three groups ( $p > 0.01$ ).

Results of Oocyte maturation and embryo development were shown in Table 2. The fertilization rate of Group A was  $52.37 \pm 24.25\%$ , and Group B  $46.00 \pm 22.70\%$ , Group C  $45.51 \pm 25.19\%$ . The good-quality embryo rate of Group C ( $38.57 \pm 31.08\%$ ) got behind with the others (Group A =  $64.00 \pm 36.18\%$ , Group B =  $68.00 \pm 34.60\%$ ), but there was no statistic difference.

The outcomes of embryo transplantation were presented in Table 3. The live birth rate of Group A, Group B and Group C was 47.1%, 32.6% and 31.3% respectively, and Group A was higher than Group B and C. There was no statistic difference in clinical pregnancy rate and implantation rate among these groups.

**Table 1. Characteristics of Group A, Group B and Group C.**

Characteristics	Group A	Group B	Group C
Age (year)			
Male	29.91 ± 5.23	30.37 ± 4.47	30.63 ± 2.60
Female	28.16 ± 4.27	29.19 ± 4.15	30.56 ± 2.58
Duration of infertility (year)	3.73 ± 3.12	3.58 ± 2.86	3.42 ± 2.31
BMI (kg/m <sup>2</sup> )			
Male	24.50 ± 3.43	25.38 ± 3.12	25.97 ± 3.51
female	22.37 ± 3.40	23.08 ± 4.38	21.25 ± 2.74
FSH (mIU/mL)	5.47 ± 2.71	5.27 ± 2.73	5.45 ± 2.32
LH (mIU/mL)	3.25 ± 1.95	2.56 ± 1.67	3.26 ± 1.49
T (mIU/mL)	0.79 ± 0.04	0.80 ± 0.22	0.70 ± 0.01

Date are presented as mean ± s.d.. Group A: fresh oocytes and fresh sperm; Group B: fresh oocytes and cryopreserved sperm; Group C: cryopreserved oocytes and fresh sperm. BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone; s.d., standard deviation.

**Table 2. Embryonic development in Group A, Group B and Group C.**

Parameters	Group A	Group B	Group C
cycles	68	43	16
Oocytes (n)	11.57 ± 4.81	11.79 ± 5.11	11.63 ± 6.45
MII oocytes (n)	9.62 ± 4.11	9.58 ± 4.47	8.69 ± 5.25
Fertilization Rate (%)	52.37 ± 24.25	46.00 ± 22.70	45.51 ± 25.19
Transferred embryos (n)	1.81 ± 0.40	1.77 ± 0.43	1.81 ± 0.40
Good quality embryo rate (%)	64.00 ± 36.18	68.00 ± 34.60	38.57 ± 31.08

Date are presented as mean ± s.d.. Group A: fresh oocytes and fresh sperm; Group B: fresh oocytes and cryopreserved sperm; Group C: cryopreserved oocytes and fresh sperm. MII oocytes, metaphase II oocytes; s.d., standard deviation.

**Table 3. Pregnancy outcomes of Group A, Group B and Group C.**

	Group A	Group B	Group C
cycles	68	43	16
Clinical pregnancy rate (%; n/total)	50.0 (34/68)	39.5 (17/43)	37.5 (6/16)
Implantation rate (%; n/total)	32.5 (40/123)	32.9 (25/76)	24.1 (7/29)
Live birth rate (%; n/total)	47.1 (32/68) <sup>a</sup>	32.6 (14/43) <sup>b</sup>	31.3 (5/16) <sup>b</sup>
Miscarriage rate (%; n/total)	6.0 (2/34)	17.6 (3/17)	16.7 (1/6)

Date are presented as mean ± s.d. Group A: fresh oocytes and fresh sperm; Group B: fresh oocytes and cryopreserved sperm; Group C: cryopreserved oocytes and fresh sperm. <sup>a</sup><sup>b</sup>Different character means statistic difference ( $p < 0.01$ ). s.d., standard deviation.

## 4. Discussion

micro-TESE has been strongly recommended to NOA patients for its higher sperm retrieval rates compared with conventional TESE, since its better identification of seminiferous tubules under microscope [18,19]. It's reported that the SRR of micro-TESE was about 40%–60% [20,21], which indicated that almost half of the patients were failed after surgical treatment. In practice, oocyte and sperm cryopreservation have been applied in micro-TESE-ICSI treatment. Many reports have explored the effect of gamete cryopreservation on fertilization and pregnancy respectively, but no study has assessed that systematically in one study.

To best of our knowledge, we for the first time analyzed the fertilization and pregnancy outcomes using cryopreserved sperm or oocytes after micro-TESE together and found that fresh gamete might get better clinical results than the cryopreserved.

Patient characteristics, such as age, BMI and hormone levels, showed no difference among these three groups. We also collected and analyzed the results of embryo development and pregnancy, and then observed similar oocyte maturation and fertilization rate. But the rate of good-quality embryo using fresh or vitrified sperm were above that using vitrified oocytes (64.00 ± 36.18%, 68.00 ± 34.60% verse

38.57 ± 31.08%) and the rates of clinical pregnancy and implantation using fresh gametes were also above these using vitrified sperm or oocytes (50% and 32.5% verse 39.5% and 32.9%, 37.5% and 24.1%, respectively) (Table 2), although there was no significant difference, and the Group A which used fresh gametes got higher live birth rate (47.1% verse 32.6%, 31.3%) (Table 3). All these results were consistent with the previous researches by our colleagues [9,15]. Our results also confirmed that gametes cryopreservation in micro-TESE-ET treatment was feasible and reliable, for it can reduce the frequency of testicular biopsies, and can get similar fertilization and pregnancy rate [9,16,22,23]. But these data also suggested that fresh gametes might be the first choice in micro-TESE-ICIS-ET treatment for NOA patients if possible. This can be partly explained by these three reasons: First, in practice we found that it's hard to select the motile sperm after cryopreservation-warming procedure, especially for the NOA patients treated after micro-TESE; Second, cryopreservation might increase the sperm DNA single-strand breaks and the degree of DNA condensation or fragmentation, but its effects on oocyte was not clear for limited research [24]. A cohort study in Italian with multi-center and large observations showed that the implantation and pregnancy rates of the vitrified oocytes were lower than these of the fresh [25,26]; Third, testicular sperm especially from NOA patients might have higher ratios of cytogenetic abnormal and chromosomal abnormalities [1,27,28]. However, the pregnancy results of our research showed no statistic difference between these three groups, which indicated that the vitrified gamete was at least not worse than the fresh for NOA patients. Plenty of researches showed similar pregnancy results using vitrified gametes compared with the fresh [9,16,22,29]. In fact, gamete vitrification provides patients with more choices and less cost in medical practice. For example, female could start ovulation stimulation until sperm retrieved successfully after micro-TESE. Oocyte could be cryopreserved to avoid waste when conventional TESE failed before micro-TESE.

Since the sample size of our research was relatively small, a larger sample size and multi-center prospective cohort study would be needed in future to illustrate the effect of gamete cryopreservation. We also ask for a long-term (even past-generation) follow-up plan to investigate the effect of gamete cryopreservation.

## 5. Conclusions

We collected and compared the fertilization and pregnancy results between fresh and vitrified gametes in NOA patients after micro-TESE-ICIS-ET treatments. The rates of good-quality embryo using fresh or vitrified sperm were above that using vitrified oocytes, but there was no statistic difference, and the fresh gamete had higher live birth rate than the vitrified. Fresh gamete showed better clinical outcomes. These results suggested that we should consider us-

ing fresh gamete for fertilization in micro-TESE-ICSI treatment for NOA patients if possible. A larger sample size and long-time follow-up study is needed in the future.

## Author Contributions

HJ and PL conceived and designed this study. XLR, SLL, and ML collected the date. YPW and DFL analyzed the date and drafted the article. All authors read and approved the final manuscript.

## Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. Our study was approved by the ethics committee of Peking University Third Hospital (No. 2020SZ-003), and informed consent was provided.

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## Conflict of Interest

The authors declare no conflict of interest.

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