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Clinical outcomes of microdissection testicular sperm extraction-intracytoplasmic sperm injection with fresh or cryopreserved sperm in patients with nonobstructive azoospermia

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We performed this study to evaluate the clinical outcomes of microdissection testicular sperm extraction-intracytoplasmic sperm injection (micro-TESE-ICSI) treatment that used fresh or cryopreserved sperm in patients with nonobstructive azoospermia (NOA). A total of 338 NOA patients with 344 consecutive cycles received treatment in the reproductive medicine center of Peking University Third Hospital in Beijing, China, from January 2014 to December 2017. Fresh oocytes and fresh sperm were used in 222 patients with 234 cycles (Group A). Fresh oocytes and cryopreserved sperm were used in 116 patients with 110 cycles (Group B). We compared patient characteristics, embryonic development, and pregnancy outcomes between Groups A and B. There was no statistical difference in the patient characteristics, and no differences were observed with fertilization or quality embryo rates between Groups A and B. The rates of clinical pregnancy and live birth were both higher for Group A than those for Group B (both $P < 0.05$). In conclusion, fresh testicular sperm appears to produce better ICSI outcomes than cryopreserved testicular sperm in patients with NOA.

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INTRODUCTION

Azoospermia may cause up to 10% of male infertility cases and can be classified into obstructive azoospermia (OA) and nonobstructive azoospermia (NOA).[1] Surgical sperm retrieval is indicated in men with OA if reconstruction is not possible or has failed. In men with OA, sperm may be retrieved by percutaneous epididymal aspiration. According to the etiology, NOA can be classified as congenital, acquired, or idiopathic. Causes of congenital NOA mainly include genetic abnormalities such as Klinefelter syndrome, azoospermia Y chromosome factor c microdeletions, and cryptorchidism; those of acquired NOA mainly include trauma, testicular torsion, varicocele, inflammation, drugs, radiation, and testicular spermatogenetic dysfunction caused by other factors; and those of idiopathic NOA are unknown, and this condition is diagnosed when known etiologies are excluded. Patients with NOA are estimated to account for 60% of azoospermia cases.[2] Microdissection testicular sperm extraction (micro-TESE) is a procedure performed in men with NOA that results in high spermatozoa retrieval rates and minimal tissue trauma.[3–5] Micro-TESE is an advanced version of TESE that uses a microsurgical technique to identify the individual seminiferous tubules that are most likely to contain sperm.

Intracytoplasmic sperm injection (ICSI) using fresh sperm obtained by micro-TESE has been commonly applied in patients with NOA, resulting in high fertilization and pregnancy rates.[6,7] Traditional ICSI is performed with fresh sperm and oocytes. In practice, however, fresh sperm sometimes cannot be retrieved on the same day as the ICSI procedure. For example, to avoid a failed sperm extraction, some couples undergo micro-TESE before ovarian stimulation and oocyte retrieval. When the sperm are successfully retrieved, they are cryopreserved, and then ovarian stimulation and oocyte retrieval are performed. These patients receive ICSI with cryopreserved sperm and fresh oocyte. Oates *et al*.[8,9] first described the use of cryopreserved sperm for ICSI. Some studies reported clinical outcomes following the use of cryopreserved sperm for ICSI, with most reporting similar clinical outcomes following ICSI with cryopreserved-thawed testicular sperm and ICSI with fresh sperm.[10–15] Therefore, we performed this study to systematically evaluate clinical outcomes after the use of fresh or cryopreserved sperm from patients with NOA after micro-TESE-ICSI.

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PATIENTS AND METHODS

Patients

We conducted a retrospective study in the reproductive medicine center of Peking University Third Hospital in Beijing, China, from January 2014 to December 2017. Azoospermia was defined according to the World Health Organization guidelines[16] and confirmed in at least three semen samples. A physical examination and an assessment of sex hormone levels, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T), were conducted in all patients before the micro-TESE treatment. Testicular volume in men with NOA was determined by physical examination and ultrasound examination. We excluded couples with symptoms of female infertility including anovulation, hormonal infertility, tubal endometriosis, and preexisting chronic diseases such as diabetes mellitus, hypertension, and heart disease. The inclusion criteria for males and females included ages from 23 to 35 years and body mass index (BMI) from 18 to 30 kg m^{-2}. Between January 2014 and December 2017, 780 patients with NOA received micro-TESE treatment in our center. Of these, sperm were successfully retrieved from 338 patients, and the sperm retrieval rate was 43.3%. In total, 338 NOA patients with 344 consecutive cycles were evaluated. Two groups were formed based on the state (fresh or cryopreserved) of the sperm. Fresh oocytes and fresh sperm were used in 222 patients with 234 cycles (Group A or fresh group). Fresh oocytes and cryopreserved sperm were used in 116 patients with 110 cycles (Group B or cryopreserved sperm group). In Group B, frozen-thawed samples from 11 (9.5%, 11/116) patients had no motile sperm that could be selected for ICSI. Our study was approved by the ethics committee for reproductive medicine of Peking University Third Hospital (No. 2020SZ-003). Written informed consent was obtained from all participants in our study. All participants were followed until their delivery.

Micro-TESE, cryopreservation, and warming of sperm

Micro-TESE was performed under general anesthesia, and a mid-line scrotal incision was made on the median raphe of the scrotum. The testicular parenchyma was exposed and directly examined under an operating microscope (OPMI Vario, Carl Zeiss, Jena, Germany) for dilated tubule areas. The seminiferous tubules were teased with 21-gauge needles and placed into G-MOPS-plus buffer (Vitrolife, Goteborg, Sweden). The tubules obtained were finely minced into a homogeneous suspension and observed using an inverted microscope (TS100, Nikon, Tokyo, Japan) for the presence of spermatozoa. Results were positive when at least one sperm was identified. If fresh oocytes could be obtained on the same day of sperm retrieval, fresh sperm was used for the ICSI procedure. If not, the sperm were cryopreserved with sperm freezing solution (Vitrolife). No sperm-egg pairs were handled in a "delayed fresh" manner in which sperm were retrieved 24–48 h before the eggs and then incubated until use but not frozen. The testicular suspension was diluted 1:1 with sperm freezing solution (Vitrolife) in a 2-ml straw. The straw was left at room temperature for 10 min and was then placed horizontally on a styrofoam board and left in a liquid nitrogen bath for 30 min. After 30 min, the straw was stored in liquid nitrogen vapor. When thawing, the straws were put in a 37°C incubator for 15 min and sperm were separated from the cryoprotectant by washing in culture medium followed by centrifugation for 10 min at 300*g* twice (BY-160A, BY-CENTRIFUGE, Beijing, China). The resulting samples were cultured in culture medium (Vitrolife) for later use. Only motile sperm were used in all cases (both fresh and frozen-thawed).

Ovarian stimulation and oocyte retrieval

Ovarian stimulation was performed by treating the patients with a combination of gonadotropin-releasing hormone (GnRH) analogs (Cetrotide, Merck Serono, Amsterdam, the Netherlands), human menopausal gonadotropin, and human chorionic gonadotropin (HCG; Choriogonadotropin alfa, Merck Serono). Oocyte retrieval was performed by vaginal ultrasound-guided puncture of the follicles at 36–38 h after HCG administration. The oocytes were maintained in G-MOPS-plus (Vitrolife) and placed in a 37°C incubator with 5% CO_2, 5% O_2, and 90% N_2. Cumulus cells were removed by pipetting and exposure to hyaluronidase (diluted, 8 IU ml^{-1}) 2 h after retrieval (Type VIII; Sigma Chemical Company, St. Louis, MO, USA).

ICSI and embryo transfer

ICSI was performed as described in detail elsewhere.[17] Fertilization was assessed 17–19 h after insemination by the presence of two pronuclei (2pn) and two polar bodies. Embryo transfer was performed on day 3 after oocyte retrieval. Embryos were scored according to the Society for Assisted Reproductive Technology (SART) scoring system,[18] and only the best embryos were selected for transfer. The number of transferred embryos was usually limited to two to reduce the risk of multiple pregnancy.

Definitions

The fertilization rate was the number of 2pn zygotes among all mature metaphase II (MII) stage oocytes. Clinical pregnancy was defined as a rising serum HCG level at least 12 days after embryo transfer. Clinical pregnancy was confirmed by the presence of a gestational sac upon ultrasound examination in week 5 after transfer. The miscarriage rate was calculated as the number of miscarriage cycles divided by the number of clinical pregnancy cycles. Indicators evaluating embryonic development included the number of oocytes and MII oocytes that were retrieved, fertilization rate, transferred embryos, and good-quality embryo rate. Clinical outcomes included pregnancy rate, implantation rate, live birth rate, miscarriage rate, and number of birth defects.

Statistical analyses

Data were expressed as the mean ± standard deviation (s.d.), and categorical data were expressed as percentages. An analysis of variance was used for continuous variables. The Chi-square or Fisher's exact tests were used for categorical variables. All analyses were conducted using IBM SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

RESULTS

There was no significant difference in the age of male and female participants in the two groups ($P > 0.05$). No statistical differences were found in other patient characteristics, including years of infertility, BMI, or levels of FSH, LH, and T ($P > 0.05$). Characteristics of all patients are shown in **Table 1**.

The average number of oocytes retrieved and MII oocytes that were injected with sperm was higher in Group A than that in Group B ($P < 0.05$). No differences were observed in fertilization or good-quality embryo rates between Group A and Group B (both $P > 0.05$), as shown in **Table 2**.

Pregnancy outcomes of all patients are summarized in **Table 3**. The rates of clinical pregnancy and live birth were 52.3% and 48.7%, respectively, in Group A, both of which were higher than the corresponding rates in Group B (40.5% and 34.5%, respectively; both $P < 0.05$). No significant differences were found between Group A and Group B in implantation rate or miscarriage rate.

Table 1: Characteristics of Group A and Group B

Characteristics	*Group A (n=222)*	*Group B (n=116)*	*P*
Age (year)			
Male	30.61±5.37	30.06±4.59	0.361
Female	28.94±4.22	29.02±4.04	0.878
Infertile time (year)	3.43±3.83	3.19±2.64	0.44
BMI (kg m^{-2})			
Male	24.82±4.78	25.02±3.64	0.721
Female	21.73±3.40	22.30±3.16	0.148
FSH (mIU ml^{-1})	25.29±15.02	27.69±14.59	0.257
LH (mIU ml^{-1})	12.44±7.30	12.47±8.00	0.971
T (ng ml^{-1})	9.14±6.55	8.48±4.81	0.348
Testicular volume (ml)	6.08±4.52	5.71±3.33	0.385

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

Table 2: Embryonic development in Group A and Group B

Parameters	*Group A (n=222)*	*Group B (n=116)*	*P*
Cycles (*n*)	234	110	
Oocytes (*n*)	15.0±8.51	13.11±7.21	0.041*
MII oocytes (*n*)	12.35±7.12	10.44±5.97	0.015*
Fertilization rate (%)	47.68±24.73	44.25±24.83	0.233
Transferred embryos (*n*)	1.64±0.63	1.65±0.65	0.664
Good quality embryo rate (%)	52.13±32.35	53.75±38.90	0.806

*$P<0.05$, Group A versus Group B. Date are presented as mean±s.d. Group A: fresh oocytes and fresh sperm; Group B: fresh oocytes and cryopreserved sperm. MII oocytes: metaphase II oocytes; s.d.: standard deviation

Table 3: Pregnancy outcomes of Group A and Group B

Outcome	*Group A (n=222)*	*Group B (n=116)*	*P*
Cycles (*n*)	234	110	
Clinical pregnancy rate, % (*n*/total)	52.3 (116/222)	40.5 (47/116)	0.040*
Implantation rate, % (*n*/total)	36.2 (142/392)	33.5 (59/176)	0.533
Live birth rate, % (*n*/total)	48.7 (108/222)	34.5 (40/116)	0.013*
Miscarriage rate, % (*n*/total)	6.0 (7/116)	14.9 (7/47)	0.129
Birth defects (*n*)	3	0	

*$P<0.05$, Group A versus Group B. Group A: fresh oocytes and fresh sperm; Group B: fresh oocytes and cryopreserved sperm

DISCUSSION

The development of cryopreservation techniques has enabled the cryopreservation of sperm to meet the medical and social needs of patients. Many studies have shown that cryopreservation does not increase the prevalence of sperm abnormalities and can maintain a high survival rate of sperm.[15,19–21] In practice, freezing techniques are applied to micro-TESE-ICSI treatment, because sperm and oocytes may not be obtained on the same day.[8] Some reports on the application of sperm cryopreservation in micro-TESE-ICSI treatment in patients with NOA have primarily focused on sperm retrieval rate and outcomes after ICSI-embryo transfer.[8–11,15] We analyzed the cryopreservation of sperm in the micro-TESE-ICSI treatment of NOA patients and compared outcomes between cycles using fresh sperm and cryopreserved sperm.

We observed no differences in patient characteristics, such as age or BMI, in the two groups, suggesting that two groups were comparable.

With regard to pregnancy outcomes between Group A and Group B, rates of clinical pregnancy and live birth were both higher in Group A than those in Group B, and the miscarriage rate was lower in Group A than in Group B. These differences may indicate that pregnancy outcomes in Group A were better. The most recent study on the application of sperm cryopreservation in micro-TESE-ICSI treatment in NOA patients reported that the mean numbers of embryos transferred, the rate of good-quality embryos, and the clinical pregnancy rate with fresh motile and frozen-thawed motile spermatozoa were 1.60 (s.d.: 0.49) versus 1.59 (s.d.: 1.5), 58.1% versus 51.3%, and 44.2% versus 43.6%, respectively, which were similar to our results; however, there was no significant difference in the number of MII oocytes retrieved or the clinical outcomes between the methods of sperm retrieval in that study.[17] Our results were in accordance with that report overall, although the live birth rate in our report was higher and the pregnancy outcomes evaluated in our study were more comprehensive. In further agreement with our results, other studies have demonstrated no differences in fertilization (from 44% to 63%) or clinical pregnancy rates between the use of fresh and frozen-thawed sperm in the micro-TESE-ICSI procedure in NOA patients; the reported clinical pregnancy rate per embryo transfer was approximately 30%, which was lower than our results.[11,13,14] Our study had 3 cases of birth defects in total, including one case of congenital heart disease and two cases of chromosome abnormality in Group A; all three cases resulted in induced labor. No birth defects occurred in Group B. However, opinions regarding the use of fresh or cryopreserved sperm in NOA patients have been controversial.[22,23] The variable cryopreservation and thawing methods that are used in different centers may explain these discrepancies. The differences that we report in clinical outcomes after the use of fresh or cryopreserved sperm are statistically significant, and the use of fresh sperm seems to result in better clinical outcomes.

Our results confirmed that cryopreservation techniques for testicular sperm before a micro-TESE-ICSI procedure in NOA patients are a promising and feasible option with many advantages, such as avoiding repeat biopsies for successive ICSI cycles and minimizing trauma to the testicular tissue. However, our data also suggest that if possible, fresh sperm should be given priority in the micro-TESE-ICSI procedure.

Our study had several limitations. The main limitation was the retrospective nature of our data collection and the study was completed at a single center, both of which limit the generalizability of our results. In addition, our sample size was relatively small, so some findings did not reach statistical significance. Moreover, we did not include a long-term follow-up after delivery. Therefore, we cannot confirm whether the cryopreservation technique affects neonatal outcomes or the long-term growth and development of the offspring. An additional prospective study on the effects of cryopreservation with multiple centers and larger samples may be needed in the future.

CONCLUSION

We found significant differences in clinical outcomes between fresh and cryopreserved sperm in NOA patients receiving micro-TESE-ICSI. Thus, fresh testicular sperm appears to produce better ICSI outcomes than cryopreserved testicular sperm in patients with NOA, although prospective studies conducted in multiple centers with larger sample sizes are needed to systematically explore clinical outcomes using fresh or cryopreserved sperm from NOA patients after the micro-TESE-ICSI procedure.

AUTHOR CONTRIBUTIONS

HJ conceived and designed the study. HCL supported substantial contributions to the study conception and design. DFL, LMZ, WHT, KH, LZ, and YL collected the data. JMM analyzed and interpreted the data. HLZ drafted the article. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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