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ICSI outcomes of fresh or cryopreserved spermatozoa from micro-TESE in patients with nonobstructive azoospermia

CONSORT

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Abstract

The aim of this study was to evaluate intracytoplasmic sperm injection (ICSI) outcomes of fresh and cryopreserved sperm via microdissection testicular sperm extraction (micro-TESE) in patients with nonobstructive azoospermia (NOA).

From March 2016 to February 2020, a total of 244 men with NOA underwent micro-TESE at the Center for Reproductive Medicine, First Hospital of Jilin University, P. R. China. These cases included 40 patients who underwent 40 ICSI cycles with fresh spermatozoa from micro-TESE (Group A) and 30 patients who underwent 30 ICSI cycles with cryopreserved spermatozoa from micro-TESE (Group B). The characteristics, embryonic development, and ICSI outcomes of patients were compared between groups A and B.

Our sperm retrieval rate (SRR) by micro-TESE in patients with NOA was 35.25%. No statistical differences in the patient characteristics and fertilization or quality embryo rates were observed between Groups A and B. Higher miscarriage rates and lower live births were observed in Group B than in Group A (both $P < .05$).

Fresh testicular spermatozoa seem to produce better ICSI outcomes than cryopreserved testicular spermatozoa from patients with NOA in the micro-TESE-ICSI cycle.

Abbreviations: 2PN = two-pronuclear, AMH = anti-Müllerian hormone, BMI = body mass index, FSH = follicle-stimulating hormone, hCG = human chorionic gonadotropin, HEPES = 2-hydroxyethyl, ICSI = intracytoplasmic sperm injection, IVF = in vitro fertilization, LH = luteinizing hormone, micro-TESE = microdissection testicular sperm extraction, NOA = non-obstructive azoospermia, PR = clinical pregnancy rate, PVP = polyvinylpyrrolidone, SRR = sperm retrieval rate, T = testosterone.

Keywords: cryopreservation, ICSI, micro-TESE, testicular spermatozoa

1. Introduction

The most severe form of male infertility is nonobstructive azoospermia (NOA), where spermatogenesis is severely impaired or absent.[1] NOA can be categorized as congenital, acquired, or idiopathic in terms of etiology, and these patients are predicted to comprise 60% of azoospermia cases.[2] If some testicular spermatozoa can be retrieved, men with NOA can become biological fathers using in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI).[1] Microdissection testicular sperm extraction (micro-TESE) is applied to men with NOA, which is an advanced microsurgical technique to expose seminiferous tubules that are most likely to contain sperm.[3,4]

ICSI has resulted in high fertilization and pregnancy rates by using fresh sperm from micro-TESE in patients with NOA.[5,6] However, in some affected couples, ICSI treatment cycles might be canceled if no spermatozoa are found in testicular biopsy specimens.[7] Some patients might have to freeze the oocyte if they do not wish to conceive using donor spermatozoa. This might not only bring psychological pressure and economic burden to patients but could also be problematic for therapy, particularly for men with NOA.[8]

Until now, methods of cryopreservation for small numbers of spermatozoa are still being studied by some authors.[9,10] Some studies reported similar clinical outcomes using fresh and cryopreserved testicular sperm for ICSI.[11–15] In this paper, we aimed to determine the practicality of cryopreservation of micro-

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The study was received approval from the Medical Ethics Committee of the First Hospital of Jilin University, P. R. China, and informed consent was acquired from all patients (2016-455).

The authors declare that there is no conflict of interest.

All data generated or analyzed during this study are included in this published article [and its supplementary information files];

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TESE retrieved spermatozoa and to estimate ICSI outcomes of fresh and cryopreserved spermatozoa from micro-TESE in patients with NOA.

2. Methods

2.1. Patients

Between March 2016 and February 2020, we retrospectively analyzed 244 men with NOA who underwent micro-TESE and 65 male patients who underwent 70 ICSI cycles with testicular spermatozoa at the Center for Reproductive Medicine, First Hospital of Jilin University, P. R. China. These cases included 40 patients who underwent 40 ICSI cycles with fresh spermatozoa from micro-TESE (Group A) and 30 patients who underwent 30 ICSI cycles with cryopreserved spermatozoa from micro-TESE (Group B). Among these 40 patients in Group A, 5 patients underwent both fresh and cryopreserved spermatozoa ICSI cycles.

All patients underwent complete physical and medical examinations; we assessed their endocrine profile and age, and conducted a urologic evaluation and genetic analysis. Testicular volume using standard ultrasonic testing was also noted. The volume of both testes was included in the analysis. The surgical arrangements were decided after the clinical team assessed the results of the patients. In the TESE procedure, a maximum of 2 aspirations per testicle was suggested to minimize testicular injury.

2.2. Micro-TESE surgical technique

After approval from gynecologists, urologists, and embryologists, the Micro-TESE process was performed at a set date to avoid the second invalid ovarian stimulation cycle. General anesthesia was applied during the surgical procedure. The process of Micro-TESE was carried out as described by other authors in detail.[8] The incision into the tunica albuginea of the left or right testicle was made to avoid vascular injury, and areas of spermatogenesis were searched for and samples were retrieved under an operating microscope.

The obtained tissue was sent to an embryologist who confirmed spermatozoa cells which were isolated under a microscope (magnification $\times 400$). The testis tissue was placed in a dish with 2-hydroxyethyl (HEPES) buffered medium and sent to the embryo laboratory for immediate examination. Spermatozoa were retrieved by centrifuging to separate spermatozoa cells.

2.3. Spermatozoa frozen and thawed in straw

In our IVF center, the cryopreservation of spermatozoa from micro-TESE was carried out after fresh micro-TESE ICSI cycles or before ovarian stimulation and oocyte retrieval in the females.

Straws were supplied by the Cryo BioSystem, France. The inner tube (diameter 1.5 mm, length 30 mm) with a fine end (diameter 0.2–0.28 mm) is made of transparent polymerized resin. To carry sperm, the tubular carrier is cut in half by sterile scissors and formed into a U-shaped structure about 2–3 cm long. Before loading the sperm samples into straws, they were mixed 1:1 with sperm cryopreservation medium (SAGE BioPharma, Bedminster, NJ). Using a micropipette, the spermatozoa were loaded into straws. These were exposed to liquid nitrogen vapor for up to 5 minutes and capped before being plunged into liquid nitrogen for storage at -196°C.

Spermatozoa frozen in straws were thawed at a 37°C constant-temperature platform while being monitored by a binocular dissecting microscope. Each straw was unscrewed from its cap under liquid nitrogen and swiftly shifted under the microscope over a sterile culture dish such that it touched the bottom of the dish. Spermatozoa were individually moved to a fresh drop of medium, washed, and then transferred to insemination medium containing polyvinylpyrrolidone (PVP) before ICSI.

2.4. Outcome measures

Clinical pregnancy was defined as the presence of a fetal heartbeat at 6 to 8 weeks of pregnancy determined by ultrasonography. Neonatal outcome data were obtained via telephone interviews of the parents after delivery, and the live birth rate was counted as birth events per embryo transfer.

2.5. Statistical analysis

Statistical data were analyzed with IBM SPSS 23.0 software (SPSS Inc., Chicago, IL). Data are presented as percentages and mean ± standard deviation (SD). Student t test was used to confirm numerical data, and Pearson Chi-squared test was used to confirm categorical data. Statistical significance was set at $P < .05$.

3. Results

A total of 244 men with NOA underwent micro-TESE. Of these, 86 men were micro-TESE positive, whereas 158 men were micro-TESE negative. The sperm retrieval rate (SRR) by micro-TESE in patients with NOA was 35.25%. Five patients underwent 5 unsuccessful fresh testicular spermatozoa ICSI cycles. These patients then used surplus frozen testicular spermatozoa in 5 new ICSI cycles, and 2 patients achieved a successful pregnancy.

There was no significant difference in the age of male and female participants in the two groups ($P > .05$). No statistical differences were found in other patient characteristics, including body mass index (BMI) and levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) ($P > .05$). The characteristics of all patients are summarized in Table 1.

Before and after the freeze-thaw process, motile sperms were found in 25 cases, while none were found in 5 cases. The two-pronuclear (2PN) zygote rate of the immotile sperm group was significantly lower than that of the motile sperm group (30.84% vs 69.91%, $P < .05$) (Table 2). There was no two-pronuclear zygote after 10 immotile sperms injected in 1 case.

No differences were observed in the average number of oocytes retrieved and MII oocytes, fertilization, or good quality embryo rates between Groups A and B ($P > .05$), as summarized in Table 3. There were 2 cases of cancelled embryo transfer in Group B, including a case for all embryos with more than 50% fragment and no transferable blastocyst and another case that had no 2PN zygote.

The ICSI outcomes of all patients are summarized in Table 4. The mean number of embryos transferred was higher in Group A than in Group B ($P < .05$). There were significant higher miscarriage rates and lower birth rates in Group B (23.81% and 50.00%, respectively) than in Group A (0% and 75.00%, respectively, $P < .05$). No significant differences were found

Table 1

Baseline characteristics of Group A and Group B.

	Group A	Group B	P
ICSI cycles (n)	40	30	/
Female age	29.18±4.12	30.17±4.36	.339
AMH, ng/mL	5.85±3.07	4.79±2.63	.123
Estradiol on HCG administration day, pg/mL	3141.10±1632.85	2949.23±1198.30	.572
Progesterone on HCG administration day, pg/mL	1.00±0.47	0.90±0.71	.493
Male age	30.90±4.94	31.77±6.44	.542
Left side testicular volume, mL	4.88±3.76	5.92±4.15	.282
Right side testicular volume, mL	4.88±3.72	5.75±4.09	.365
FSH, mIU/mL	26.58±16.34	20.80±13.74	.113
LH, mIU/mL	14.33±7.46	13.11±7.87	.512
Testosterone, nmol/L	9.12±4.83	11.96±7.86	.088

Group A versus Group B. Group A: fresh testicular spermatozoa; Group B: cryopreserved testicular spermatozoa.
FSH = follicle-stimulating hormone, LH = luteinizing hormone.

between Group A and Group B with respect to implantation, biochemical pregnancy, and clinical pregnancy rates.

4. Discussion

Since 1995, men with NOA could finally get the chance to have their own biological children with the introduction micro-TESE ICSI. It is important to counsel men with NOA about the various options available for infertility treatment, with the likely outcomes of each. The SRR in patients with NOA range from 23% to 75%.[16–18] Eken et al[19] revealed no statistical difference in age, testicular volume, or hormonal levels in the micro-TESE-positive and micro-TESE-negative groups. The sperm retrieval rate by micro-TESE in patients with NOA was 35.25% in our study. This may be due to the lack of further authentication in the histopathological classification.

Micro-TESE appears to improve the frequency of successful spermatozoa retrieval in NOA patients.[2] However, these retrieval techniques are traumatic. The few spermatozoa that can be obtained by these means are very precious to the couples. Testicular sperm cryopreservation plays a crucial role before oocyte collection for future treatment and after fresh micro-TESE ICSI cycles to avoid the need for repeated surgery. Therefore, an efficient technique for the cryopreservation of very few spermatozoa that can be obtained from micro-TESE has become important.

Liu et al[9] reported the use of new "LSL" microstraws for freezing spermatozoa. The inner tube of these microstraws, with a fine end, is made of transparent polymerized resin. A mixed sample was aspirated into the inner tube using a sterile syringe. However, the semen samples were close to normal parameters and the authors did not provide data on pregnancy outcomes of ICSI for this method.[9] In contrast, our sheet microstraw has more contact surface with the medium to settle more sperm into thawed dish compared to the "LSL" micro straws from Liu et al.[9] The freezing-thawing procedure with straws enabled us to obtain spermatozoa for ICSI in all 30 samples after micro-TESE. We obtained a similar number of motile sperm before and after the freezing-thawing process among the 25 samples studied ($P > .05$). Occasionally, to match the number of oocytes of the partner of male patients, the rare spermatozoa would be damaged to confirm the motility rate by dyeing. Although immotile spermatozoa were noted in 5 ICSI cycles, no case was canceled for not finding any spermatozoa after cryopreservation.

The method strived for more time for the patients who hesitated to choose donor spermatozoa to avoid the failure of obtaining spermatozoa. Under normal conditions, only motile spermatozoa are frozen in view of cryodamage to the spermatozoa[10]; however, it is difficult to make this decision for the precious immotile spermatozoa from micro-TESE. Alrabeeah et al[10] discovered very poor ICSI outcomes of frozen-thawed testicular spermatozoa from micro-TESE and abandoned the use of spermatozoa for ICSI. Immotile testicular spermatozoa are often discarded to cryostorage and a subsequent biopsy is arranged or the donor spermatozoa are selected,[20] which can cause a loss of opportunity to have one's own biological offspring and increase the economic burden to the patients. Therefore, effective cryopreservation of immotile spermatozoa from micro-TESE is a challenge for doctors and embryologists. In the study, 1 case with immotile spermatozoa was cancelled for having no embryo from normal fertilization. Four cases resulted in embryos transferred from normal fertilization by using immotile spermatozoa for ICSI, and 2 cases resulted in live births. Cryopreservation of immotile testicular spermatozoa from micro-TESE

Table 2

Two-pronuclear zygote rate of ICSI by using cryopreserved motile or immotile sperm.

	Number of sample	Two-pronuclear zygote rate (%)
ICSI from motile sperm	25	69.91 (158/226)
ICSI from immotile sperm	5	30.84* (33/107)

* Statistically significant ($P < .05$).

Table 3

Embryonic development in Group A and Group B.

	Group A (n = 40)	Group B (n = 30)	P
ICSI cycles (n)	40	30	/
Retrieved oocytes (n)	13.88±6.08	13.40±6.32	.753
MII oocytes (n)	11.95±5.21	11.20±5.05	.325
Two-pronuclear zygote rate (%)	65.69 (314/478)	60.12 (202/336)	.121
High-quality embryo rate (%)	51.59 (162/314)	45.54 (92/202)	.207

Group A versus Group B. Group A: fresh testicular spermatozoa; Group B: cryopreserved testicular spermatozoa.
* Statistically significant ($P < .05$).

Table 4

ICSI outcomes of Group A and Group B.

	Group A	Group B	*P*
Embryo transfer cycles (n)	40	28	/
Mean number of embryos transferred	1.93 ± 0.27	1.60 ± 0.62	.011*
Implantation rate (%)	53.25 (41/77)	51.06 (24/47)	.854
Biochemical pregnancy rate (%)	10.00 (4/40)	17.86 (5/28)	.471
Clinical pregnancy rate (%)	75.00 (30/40)	75.00 (21/28)	1.000
Miscarriage rate (%)	0.00 (0/30)	23.81 (5/21)	.009*
Live birth rate (%)	75.00 (30/40)	50.00 (14/28)	.042*
Birth defect	0	0	/

Group A versus Group B. Group A: fresh testicular spermatozoa; Group B: cryopreserved testicular spermatozoa.

* Statistically significant ($P < .05$).

should be carefully chosen if the tails of spermatozoa are stiff in all samples.

Similar outcomes of embryonic development, including the number of oocytes, MII oocytes, 2PN rate, and high-quality embryo rate were achieved in the 2 groups, under the condition of no statistical differences of baseline characteristics. Although fewer embryos were transferred, no statistical differences were discovered at implantation, biochemical pregnancy, and clinical pregnancy rates in the cryopreserved sperm group than in the fresh sperm group from our study. Five patients underwent fresh spermatozoa ICSI cycle as well as cryopreserved spermatozoa ICSI cycle, and two of them resulted in live births. The 2PN fertilization rate of cryopreserved sperm from micro-TESE was similar to that of fresh micro-TESE in our study (60.12% vs 65.69%) and better than the fertilization rate (44.25%) reported by Zhang et al.[21] The reported clinical pregnancy rates have previously ranged from 30% to 40%, which was lower than that observed in our study (75%).[16,22] This is probably due to the difference in cryopreservation methods of testicular spermatozoa and micro-TESE. There were 17 neonates were born in 28 cases, which underwent ICSI cycles with cryopreserved spermatozoa from micro-TESE. No birth defects were observed in either group. Therefore, cryopreservation was useful for maintaining the vitality of the few spermatozoa obtained from micro-TESE with good recovery of motility for patients undergoing ICSI treatment. Our research increases the options for couples who desire conception but hesitate to choose donor spermatozoa, and also avoids unnecessary secondary testicular injury and oocyte cryopreservation in ICSI treatment cycles for no spermatozoa in micro-TESE.

However, there were limitations in the cryopreservation of testicular spermatozoa from micro-TESE. The most recent study reported that the rates of clinical pregnancy and live birth were both higher for the fresh sperm group (both $P < .05$).[17] Similar outcomes were observed in our study, with higher miscarriage rates and lower birth rates in the cryopreserved sperm group than in the fresh sperm group ($P < .05$). These differences may indicate that ICSI outcomes in the fresh sperm group were better. Moreover, a major limitation of this study is its retrospective design. In addition, micro-TESE is feasible, it appears effective in NOA patients, but it is also technically demanding, expensive, and time-consuming. This technique is possible in reproductive centers with more male infertility patients in synthetic hospitals. Current data is limited, and hence, it is recommended that the safety of offspring analyzed by such techniques is followed closely.

In conclusion, this study verified the utility of cryopreserving testicular spermatozoa from micro-TESE. At the same time, we found that fresh testicular spermatozoa seemed to produce better ICSI outcomes compared to those obtained with cryopreserved testicular spermatozoa from patients with NOA via the micro-TESE-ICSI cycle. Prospective studies systematically conducted with fresh or cryopreserved sperm from micro-TESE-ICSI in NOA patients and involving larger sample sizes and multiple centers are warranted to ascertain the safely of the offspring.

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