Clinical Outcomes and Live Birth Rate (LBR) of Micro-TESE With ICSI-IVF in 968 Non-obstructive Azoospermia Patients From One Reproductive Medicine Center: a Retrospective Cohort Study

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Research

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Abstract

Background: Most of data available in the literature reported the sperm retrieval rate (SRR) and ICSI results of microdissection testicular sperm extraction (micro-TESE) in non-obstructive azoospermia (NOA) patients with different etiologies. Unfortunately, there is currently a lack of comprehensive Intent-to-treat (ITT) data to guide clinicians in conducting comprehensive consultations with NOA patients. The aim of current study was to obtain more comprehensive evidence-based data and clinical outcomes for better consultation of NOA patients who opted to undergo micro-TESE combined with ICSI-IVF.

Materials and methods: A retrospective study involved 968 NOA patients underwent micro-TESE during the period between January 2015 and December 2019. Those who had successful sperm retrieval and performed ICSI-IVF cycles were included in intent-to-treat (ITT) analysis. The primary outcome measure was the live birth rate (LBR). The cumulative pregnancy or live birth was defined as clinical pregnancy or at least one live-born baby resulting from an ICSI initiated cycle. Two kinds of stratification analyses were performed based on different etiologies of NOA and various amounts of sperm retrieved. The 'Student's t-test' was used for comparison of continuous variables. One-way ANOVA was used to assess outcomes among more than two groups. Chi-squared (χ^2) or Fisher's exact test was used for proportions.

Results: The SRR of all 968 NOA patients undergoing micro-TESE was 44.6% ($n=432$). ITT analysis was performed in 424 patients, and ICSI-IVF was applied in 362 couples, leading to 171 clinical pregnancies (40.3%) and 161 live-birth deliveries (38.0%) in the first embryo transfer cycle. No significant difference was observed for per-protocol analysis between the groups of frozen sperm and fresh sperm in cumulative clinical pregnancy rate (CPR, 51.0% vs. 45.2%) and live-birth rate (LBR, 47.5% vs. 42.9%). NOA patients with Y chromosome azoospermia factor c (AZFc) microdeletions had the lowest rate of the high-score embryo on day 3 (4.4%, $P<0.05$) and the lowest cumulative CPR (22.2%, $P<0.05$). NOA patients with lower sperm count had significantly lower cumulative LBR than those with higher sperm count (25.0% vs. 49.2%, $P<0.05$).

Conclusions: Micro-TESE is an effective sperm retrieval technique for NOA patients. Our data indicated no significant difference in the LBR between ICSI-IVF cycles using frozen or fresh testicular sperm.

Background

Non-obstructive azoospermia (NOA) is the most severe form of male infertility and is characterized by the testis's inability to produce mature sperm, and NOA accounts for 60% of all patients with azoospermia [1]. Based on the different causes of non-obstructive azoospermia, non-genetic etiologies include cryptorchidism, heat exposure, infections, and chemoradiotherapy. The most common genetic causes are Y chromosome microdeletions and chromosomal abnormalities [2, 3]. However, most NOA patients have the unknown cause of their azoospermia [4]. Because of spermatogenesis, couples with NOA used donor sperm or opted for adoption to have children before current sperm retrieval methods [5, 6].

The development of intracytoplasmic sperm injection (ICSI) in 1992 provided a novel opportunity for azoospermia patients to become fathers [7, 8]. Sperm obtained through testicular sperm extraction (TESE) was first performed on NOA patients for ICSI in 1995 [9]. TESE-ICSI then became a routine procedure to treat NOA patients. Studies reported that the sperm retrieval rate (SRR) of TESE in the NOA population was approximately 30–50%. However, these studies' selective bias makes numbers controversial [10–12]. Subsequently, various surgical techniques, such as multiple testicular biopsies and fine-needle aspiration, were used to improve SRR and reduce complications in NOA patients, but each has its limitations [13–15]. In the past 20 years, microdissection testicular sperm extraction (micro-TESE) has gradually become a popular surgical technique with a high SRR and low tissue loss [16–19]. However, most of the previous studies were focus on SRR, and few could provide comprehensive follow-up data on ICSI-IVF outcomes of NOA patients, explicitly focusing on the use of fresh or frozen sperm, cumulative pregnancy rate (PR), and live birth rate (LBR), not alone birth defect rate (20–24).

Our goal was to obtain more comprehensive evidence-based data and clinical outcomes for better preoperative consultation and counseling of NOA patients who opted to undergo micro-TESE combined with ICSI-IVF. This retrospective study aimed to analyze the effects of frozen or fresh sperm, different etiologies, and retrieved sperm quantity on ICSI-IVF outcomes after micro-TESE.

Materials And Methods

Patients selection

This retrospective study included 968 men with NOA who underwent micro-TESE in the Reproductive Medicine Center of Third Affiliated Hospital of Guangzhou Medical University between January 2015 and December 2019 (Fig. 1). All semen samples were centrifuged at 3,000 g for 30 minutes to confirm azoospermia and on at least three separate occasions. NOA patients underwent a complete clinical evaluation to determine the etiology of azoospermia, including clinical history, physical examination, testicular volume ultrasound, evaluation of sex hormone levels [follicle stimulating hormone (FSH), luteinizing hormone (LH) testosterone (T), estradiol (E2), and prolactin (PRL)], karyotyping and Y chromosome microdeletion analysis. Patients with a history of micro-TESE, anejaculation, or hypogonadotropic hypogonadism were excluded. The same surgeon (GA) performed all micro-TESE procedures. The Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University approved this protocol.

All NOA patients were offered the option to receive fresh or frozen testicular sperm for ICSI cycles before surgical sperm retrieval and be informed of their respective advantages and disadvantages. Controlled ovarian stimulation (COS) will be initiated after the sperm is frozen. However, the simultaneous ICSI cycles of fresh sperm and egg collection may result in passive egg freezing due to micro-TESE failure or unimplemented surgery.

Micro-TESE

A single surgeon (GA) performed the micro-TESE surgical procedures as described in the previous literature [25], a few minor and technical modifications, including the use of saline instead of Ringer's solution as the rinsing fluid and suturing the albuginea testis with a 5-0 polypropylene suture instead of 6-0. Instead of the surgeon grinding the testicular tissue on the operating table, an embryologist grind the testicular tissue and microscopically search sperm on a bench setting near the operating table [26]. If no sperm was identified in one testis, micro-TESE of the contralateral testis was performed immediately.

Evaluation of sperm quantity and quality

Sperm count was determined by examining the processed homogenate under high power magnification (200×), and 20 high power fields (HPF) were microscopically totaled. Motile spermatozoa rate was determined (motile spermatozoa rate = motile sperm/total sperm × 100%). If the total number of sperm was greater than 100, the number of motile sperm was quantified in 100 sperm. The sperm deformity rate was assessed in the same manner as the motile spermatozoa rate.

Testicular tissue suspension cryopreservation and thawed

After testicular tissue was retrieved from the testis and transferred to the embryology laboratory, a freezing process was immediately performed. First, testicular tissue was further processed to release spermatozoa. Procedures were performed at room temperature as follows: wash testicular tissue with 1 ml G-MOPS-Plus fluid (Vitrolife, Sweden) to remove the red blood cells; Move testicular tissue into a new petri dish and add 1 ml new G-MOPS-Plus fluid and grind the tissue into tiny patches by using microscopic forceps to release spermatozoa; Transfer all fluid and tissues into a 15 ml centrifuge tube; Add 1 ml fresh G-MOPS-Plus fluid to the petri dish to wash additional tissue from the dish and transfer into the 15-ml tube; Incubate at room temperature for five minutes; Aspirate the supernatant to a fresh new 15-ml centrifuge tube; Add 1ml G-MOPS-Plus fluid to the pellet, and transfer the supernatant to the fresh new 15-ml tube. Centrifuge the tube at 400g for 10 minutes, and remove all the supernatant. Next, the freezing procedures were performed as follows: add freezing medium (Test York Buffer with Gentamicin Sulfate) (Irvine Scientific, USA) and G-MOPS-Plus in a 1:1 ratio into a pellet and resuspend, aliquot resuspended pellet into 2-4 cryovials; and place at t 4°C for 30 minutes; Resuspend the mixture prior to placing in liquid nitrogen for 1 hour. Transfer the cryovials into a liquid nitrogen container for long-term cryopreservation. The thawing process was performed as follows. Remove the cryovial from the liquid nitrogen container, place it at room temperature for 10 minutes; Transfer the thawed liquid to a fresh 15ml centrifuge tube; Add drops of IM washing buffer (Vitrolife, Sweden) to the tube, and mix gently. Centrifuge at 400g for 10 minutes, and then remove the supernatant. Resuspend the pellet with 1-2 ml IM washing buffer. After the second wash, resuspend the sperm pellet with 50-100 µl G-MOPS-Plus fluid. The count, motility, and deformity rate of thawed sperm were reassessed.

Ovarian stimulation and oocytes retrieval

In couples who had testicular sperm was retrieved and frozen, or in couples undergoing a synchronous micro-TESE-ICSI procedure, female partners underwent ovarian stimulation using recombinant FSH or

hMG combined GnRH antagonists or GnRH-a [27]. Oocyte–cumulus complexes were recovered 36h after administering 5000 or 10 000 IU of hCG.

ICSI procedure, embryo culture, and transfer

For couples who had sperm cryopreserved, sperm were thawed only when females had oocytes retrieved. For couples who underwent synchronous micro-TESE-ICSI treatment, oocytes were vitrified if no sperm retrieved. Next, ICSI-IVF and fertilization assessment were performed as previously described by Liu [27]. Fertilization rates were expressed as the percentage of oocytes with two distinct pronuclei per injected metaphase II oocytes. Embryos were scored by their morphological appearance according to the Society for Assisted Reproductive Technology scoring system [28]. Normally cleaving embryos with ≥ 5 cells and $\leq 20\%$ fragmentation were considered eligible for transfer. Up to two embryos were transferred into the uterine cavity on day 3 (preferred) or day 5 (blastocyst culture was carried out only when no embryos were available on day 3) after injection. The remaining embryos were frozen directly for the next thawed transfer cycles.

Pregnancy follow-up

Pregnancy was diagnosed by elevated serum hCG levels ($\geq 25\text{IU/L}$) 14 days after embryo transfer. Clinical pregnancy was defined as a visible gestational sac at transvaginal ultrasound 4-5 weeks after embryo transfer. Pregnancy loss was defined as the loss of a clinical pregnancy before 28 weeks of gestation. Live birth was defined as the birth of at least one living child, irrespective of gestation duration. The cumulative pregnancy or live birth was defined as clinical pregnancy or at least one live-born baby resulting from an ICSI-IVF initiated cycle.

Statistical analysis

Statistical analyses were performed with SPSS statistical software for Windows, version 22.0 (SPSS, Chicago, IL, USA). Continuous variables were expressed as mean $\pm$ SD. The 'Student's t-test was used for comparison of continuous variables. One-way ANOVA was used to assess outcomes among more than two groups. Chi-squared (χ^2) or Fisher's exact test was used for proportions. Differences were considered statistically significant when the p -value was <0.05 .

Results

Micro-TESE and sperm recovery

A total of 968 patients with NOA underwent micro-TESE, and 432 had sperm retrieved (sperm retrieval rate, SRR = 44.6%). A total of 424 patients with sperm retrieved were included in intent to treat (ITT) analysis, and 299 patients were defined as the per-protocol (PP) analysis set (Fig. 1 and Table 1). The SRR showed significantly different among the different etiologies, including orchitis (81.2%), Klinefelter Syndrome (KS) (43.6%), Y chromosome azoospermia factor c (AZFc) microdeletions (68.6%), cryptorchidism (62.4%), and idiopathic (31.1%) (Additional file 1: Table S1) ($p < 0.01$).

Table 1
Basic characteristics and ICSI outcomes of the NOA patients with sperm retrieved by micro-TESE

ITT analysis set			
	Frozen sperm (n = 323)	Fresh sperm (n = 101)	P- value
Male	n = 323	n = 101	/
Age (yr.)	31.37 ± 4.95	30.88 ± 4.35	0.378
BMI (kg/m ²)	24.43 ± 3.38	24.46 ± 3.74	0.947
Left Testicular volume (ml)	7.06 ± 3.77	7.26 ± 3.83	0.640
Right Testicular volume (ml)	7.01 ± 3.81	7.22 ± 3.92	0.628
Hormone profile	/	/	/
FSH (IU/L)	19.57 ± 10.92	17.62 ± 10.09	0.112
LH (IU/L)	9.49 ± 5.73	8.52 ± 5.17	0.130
T (ng/ml)	10.72 ± 6.95	11.52 ± 6.90	0.317
Female	n = 301 ^{#1}	n = 101	/
Age (yr.)	29.12 ± 3.96	28.87 ± 3.86	0.589
AMH (ng/ml)	5.15 ± 3.84	5.30 ± 3.91	0.727
BMI (kg/m ²)	21.87 ± 3.38	21.73 ± 3.02	0.705
Infertility type	/	/	0.925
Primary %	84.8(267/315)	85.1(86/101)	/
Secondary %	15.2(48/315) [⊠]	14.9(15/101)	/
COS-ICSI outcomes	n = 261 ^{#2}	n = 101	/

Abbreviation: ITT, intent-to-treat; BMI, body mass index; FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone; T, testosterone; AMH, anti-mullerian hormone.

¹ Computational formula: number of available embryos on day3 / number of MII oocytes for ICSI.

² Computational formula: number of high-score embryos on day 3 / number of 2PN.

#1: There were 22 female without information about their spouses.

#2: There were 62 female did not receive ICSI treatment.

* Significantly different.

ITT analysis set			
Days of ovarian stimulation	10.61 ± 2.29	10.63 ± 2.04	0.913
Total gonadotropin dose (IU)	1915.86 ± 957.17	1915.37 ± 1096.30	0.997
Oestradiol level on HCG trigger day (pmol/L)	10747.84 ± 4511.79	12049.94 ± 4732.90	0.016*
Progesterone level on HCG trigger day (nmol/L)	2.35 ± 1.29	2.55 ± 1.26	0.181
The endometrial thickness on HCG trigger day (mm)	10.56 ± 2.07	10.61 ± 1.86	0.852
Number of oocytes retrieved	13.80 ± 7.12	15.59 ± 7.96	0.038*
Number of MII oocytes for ICSI	10.96 ± 8.25	10.85 ± 6.04	0.906
Fertilization rate (%)	70(2003/2860)	71.5(784/1096)	0.355
2PN rate (%)	61.7(1764/2860)	62.0(679/1096)	0.874
2PN cleavage rate (%)	80.8(1619/2003)	80.2(629/784)	0.719
Day 3 utilization rate of MII eggs for ICSI (%) ¹	28.6(818/2860)	27.8(305/1096)	0.629
Rate of high-score embryo on day 3 (%) ²	12.4(219/1764)	13.3(90/679)	0.576
No. of cycles without available embryos / ICSI cycles (%)	11.5(30/261)	13.9(14/101)	0.536
Number of available embryos on day3	3.13 ± 2.88	3.02 ± 2.95	0.788
Number of high-score embryos on day 3	0.84 ± 1.15	0.89 ± 1.15	0.910
Abbreviation: ITT, intent-to-treat; BMI, body mass index; FSH, Follicle-Stimulating Hormone; LH, Luteinizing Hormone; T, testosterone; AMH, anti-mullerian hormone.			
¹ Computational formula: number of available embryos on day3 / number of MII oocytes for ICSI.			
² Computational formula: number of high-score embryos on day 3 / number of 2PN.			
#1: There were 22 female without information about their spouses.			
#2: There were 62 female did not receive ICSI treatment.			
* Significantly different.			

Sperm quality and quantity were evaluated under a microscope before freezing and after thawing, and assessment included a total of sperm count, percentage of motile sperm, and the percentage of abnormal

sperm (teratozoospermia). No significant differences were found in the total sperm count, motility, or morphology of testicular sperm between the different groups (Additional file 1: Table S2) ($p > 0.05$).

Outcomes of ICSI-IVF

ICSI-IVF was performed in 362 couples in the ITT set, leading to 171 clinical pregnancies and 161 live-birth deliveries in the first embryo transfer cycle (Fig. 1). All patients had completed at least one embryo transfer by the end of this follow-up, and 25 couples have not yet achieved live birth but still have cryopreserved embryos. All couples undergoing the ICSI-IVF cycle were divided into frozen sperm group and fresh sperm group (323 cases with frozen sperm vs. 101 cases with fresh sperm (Fig. 1). In ITT analysis, baseline demographic data were comparable between the two groups and fertilization rate, 2 primary nucleus (PN) rate, numbers of available and high-score embryos on day 3 (Table 1). Though the clinical pregnancy rate (CPR) in the first embryo transfer cycle was lower in frozen sperm than that in the fresh sperm group (37.5% vs. 49.5%, $p < 0.05$, Table 1), there was no significant difference in live birth rate (LBR) between these two groups (35.9% vs. 44.6%, $p > 0.05$, Table 1).

In the PP set, 299 couples were undergoing ICSI-IVF treatments (257 cases with frozen sperm vs. 42 cases with fresh sperm, Fig. 1), leading to 136 clinical pregnancies and 130 live-birth deliveries in the first embryo transfer cycle. There were no significant differences in outcomes of embryo culture, including fertilization rate, day 3 utilization rate of MII eggs for ICSI, and high-score embryo rate on day 3, between these two groups (Table 3). Besides, the percentage of patients with no available embryo was low in both frozen and fresh sperm groups and was not significantly different between the two groups (11.3% vs. 14.3%, Table 3). Then, accumulative embryo transfer cycles were analyzed in PP set, and there was no significant difference in cumulative CPR between the frozen and fresh groups (51.0% vs. 45.2%, $p = 0.491$). Also, no significant difference was observed in the cumulative LBR (PP: 47.5% vs. 42.9%, $p = 0.579$) between the two groups (Table 3).

There were three cases of birth defects recorded. One case is a cardiovascular malformation, one is a cleft lip and palate, and the other is congenital hypospadias (Table 2). There were no stillbirths, pregnancy complications, or neonatal complications. Follow-up data showed that compared to the fresh sperm group, singleton newborns of frozen sperm group have heavier weight ($3197.57 \pm 402.64\text{g}$ vs. $3080.36 \pm 482.22\text{g}$, $p < 0.05$) in the ITT set (Table 2) and higher height ($49.84 \pm 2.04\text{cm}$ vs. $48.50 \pm 3.03\text{cm}$, $p < 0.05$) in PP set (Table 3). Overall, LBR was 17.6% (170/968) in the first embryo transfer cycle for NOA patients with different etiologies who underwent the micro-TESE procedure for the first time, regardless of fresh or frozen testicular spermatozoa (Fig. 1).

Table 2
Embryo transfer and live birth outcomes of the NOA patients with sperm retrieved by micro-TESE

ITT analysis set			
	Frozen sperm (n = 323)	Fresh sperm (n = 101)	P- value
1st embryo transfer cycle	/	/	/
No. of embryos transferred	1.56 ± 0.51	1.60 ± 0.54	0.650
Clinical pregnancy rate (%)	37.5(121/323)	49.5(50/101)	0.031*
Live birth rate (%)	35.9(116/323)	44.6(45/101)	0.118
Live birth	/	/	/
No. live birth/clinical pregnancy (%)	95.9(116/121)	90.0 (45/50)	0.137
Singleton (%)	86.2 (100/116)	91.1 (41/45)	0.397
Twins (%)	13.8 (16/116)	8.9 (4/45)	
Birth defect (%) #	1.7 (2/116)	2.2 (1/45)	0.834
Gender	/	/	0.629
Male (%)	40.9(54/132)	44.9(22/49)	/
Female (%)	59.1(78/132)	55.1(27/49)	/
Birth weight (g)	/	/	/
Singleton	3197.57 ± 402.64	3080.36 ± 482.22	0.027*
Twins	2302.50 ± 331.11	2355.63 ± 258.84	0.868
Height (cm)	/	/	/
Singleton	49.85 ± 2.02	48.90 ± 3.03	0.132
Twins	44.91 ± 3.40	45.13 ± 2.85	0.676
#: 3 cases of birth defects were recorded: one of them is a boy with congenital hypospadias whose father was cryptorchidism and come from fresh sperm. Two were all from frozen sperm: one case is a girl with cardiovascular malformation whose father was Klinefelter syndrome; and the other is also a girl with cleft lip and palate whose father was idiopathic NOA patient.			
*: Significantly different.			

Table 3
ICSI and live birth outcomes of PP set

	Frozen sperm (n = 257)	Fresh sperm (n = 42)	P value
Age (yr)	/	/	/
Male	31.58 ± 4.91	31.10 ± 3.86	0.540
Female	29.01 ± 4.07	29.64 ± 3.94	0.350
COS-ICSI outcomes	/	/	/
Days of ovarian stimulation	10.62 ± 2.29	10.50 ± 1.55	0.738
Total gonadotropin dose (IU)	1914.55 ± 959.68	1732.14 ± 617.26	0.235
Estradiol level on HCG trigger day (pmol/L)	10789.34 ± 4528.86	13282.10 ± 4550.46	0.001*
Progesterone level on HCG trigger day (nmol/L)	2.35 ± 1.30	2.77 ± 1.50	0.059
The endometrial thickness on HCG trigger day (mm)	10.56 ± 2.08	10.29 ± 1.66	0.417
Number of oocytes retrieved	13.87 ± 7.13	17.50 ± 7.23	0.002*
Number of MII oocytes for ICSI	11.02 ± 8.29	10.62 ± 5.73	0.766
Fertilization rate (%)	70.1(1985/2831)	68.2(304/446)	0.403
2PN rate (%)	61.8(1750/2831)	59.4(265/446)	0.333
2PN cleavage rate (%)	91.9(1609/1750)	95.1(252/265)	0.072
Number of available embryos on day3	3.09 ± 2.94	2.86 ± 2.25	0.625
Number of high-score embryos on day 3	0.84 ± 1.15	0.98 ± 1.05	0.474
Day 3 utilization rate of MII eggs for ICSI (%) ¹	28.7(812/2831)	26.2(117/446)	0.286
Rate of high-score embryo on day 3 (%) ²	12.3(216/1750)	15.5(41/265)	0.16
No. of cycles without available embryos / ICSI cycles (%)	11.3 (29/257)	14.3 (6/42)	0.575
1st embryo transfer cycle	/	/	/

Abbreviation: PP, per-protocol; ICSI, intracytoplasmic sperm injection; COS, controlled ovarian stimulation.

¹ and ², the definitions see Table 1.

* Significantly different.

	Frozen sperm (n = 257)	Fresh sperm (n = 42)	P value
No. of embryos transferred	1.56 ± 0.51	1.61 ± 0.55	0.533
Clinical pregnancy rate (%)	45.9 (118/257)	42.9 (18/42)	0.712
Live birth rate (%)	44.0 (113/257)	40.5 (17/42)	0.672
One ICSI cycle	/	/	/
Cumulative pregnancy rate per women (%)	51.0 (131/257)	45.2 (19/42)	0.491
Cumulative live birth rate per women (%)	47.5 (122/257)	42.9 (18/42)	0.579
No. live birth/clinical pregnancy (%)	93.1 (122/131)	94.7 (18/19)	0.793
Singleton (%)	86.9 (106/122)	88.9 (16/18)	0.813
Twins (%)	13.1 (16/122)	11.1 (2/18)	
Birth defect (%) #	1.6 (2/122)	0 (0/18)	0.584
Gender	/	/	0.941
Male (%)	39.1 (54/138)	40.0 (8/20)	/
Female (%)	60.9 (84/138)	60.0 (12/20)	/
Birth weight (g)	/	/	/
Singleton	3194.67 ± 405.16	3038.44 ± 495.32	0.166
Twins	2302.50 ± 331.11	2527.50 ± 197.55	0.196
Height (cm)	/	/	/
Singleton	49.84 ± 2.04	48.50 ± 3.03	0.024*
Twins	44.91 ± 3.40	47.50 ± 1.00	0.143
Abbreviation: PP, per-protocol; ICSI, intracytoplasmic sperm injection; COS, controlled ovarian stimulation.			
¹ and ² , the definitions see Table 1.			
* Significantly different.			

Next, a stratification analysis based on different etiologies of NOA was performed. The results showed a statistical difference in day 3 utilization rate of MII eggs for ICSI among each group, with the lowest percentage observed in patients with Y chromosome AZFc microdeletions (22.3%), followed by KS (24.4%), cryptorchidism (27.2%), orchitis (30.8%), and was observed to be the highest in patients with

idiopathic NOA (31.7%). The percentage of patients with no available embryo was significantly different among the five etiologies of NOA, with the highest percentage in patients with Y chromosome AZFc microdeletions (25.8%) (Additional file 2: Fig. S1). Besides, the lowest rate of the high-score embryo on day 3 (4.4%), lowest cumulative CPR (22.6%), lowest cumulative LBR with successful sperm retrieval (19.4%), and the highest rate of premature birth (50%) were observed in patients with Y chromosome AZFc microdeletions ($p < 0.05$) (Additional file 1: Table S3).

Furthermore, a stratification analysis based on the total sperm retrieved was performed and were separated into lower sperm count (LSC, ≤ 20 sperms) and higher sperm count (HSC, > 20 sperms) groups. No significant difference was observed in male or female age between the two groups ($p > 0.05$). NOA patients with LSC showed higher FSH levels, and their female partners underwent more partial oocyte freezing (23.4%, $p < 0.05$). In all initial ICSI cycles (including cycles without available embryos on day 3) in the PP set, couples with LSC had significantly lower 2PN cleavage rate, lower day 3 utilization rate of MII eggs for ICSI, a lower rate of the high-score embryo on day 3, lower cumulative CPR (29.7% vs. 55.7%, $p < 0.05$), lower cumulative LBR (28.1% vs. 51.9%, $p < 0.05$) and a significantly higher risk of no available embryos on day 3 compared with couples with HSC ($p < 0.05$). Additionally, significantly lower cumulative CPR (37.5% vs. 56.5%, $p < 0.05$) was observed in couples with LSC than couples with HSC when patients who had no available embryos were excluded (Additional file 1: Table S4).

Discussion

Our study aimed to comprehensively analyze the outcomes of the ICSI-IVF cycle of NOA patients who underwent micro-TESE. This study provides a tremendous clinical basis for reproductive medicine specialists to better understand the clinical Outcome of micro-TESE combined with ICSI-IVF cycle, which allows for more comprehensive preoperative clinical consultation and preparations.

For NOA patients, SRR has been the most studied clinical outcome. Previously, SRR has often been different in studies examining outcomes after micro-TESE, likely and mainly due to the limited amount of data and patient selection bias [19, 29]. In this study, we comprehensively analyzed micro-TESE data from 968 men with NOA diagnosis, and found that the SRR was 44.6%. The SRR did differ in NOA patients with different etiology. The SRR was highest in orchitis with 81.2%, followed by Y chromosome AZFc microdeletions (68.6%), cryptorchidism (62.4%), KS (43.6%), and lowest in idiopathic NOA (31.1%).

Not all NOA patients have access to fresh sperm, and couples who want to synchronize the ICSI-IVF cycles with fresh sperm and oocyte have to face the risk of oocyte freezing and sperm donation-IVF. Therefore, sperm cryopreservation before ICSI may be more reasonable and reduce unnecessary risks for females. Cryopreservation of testicular sperm has long been used in assisted reproductive technology [30, 31]. Previous studies confirmed significant differences between fresh and frozen sperm by ejaculation, regardless of total sperm count, motility, or morphology of sperm [32]; however, the differences contributed little to the outcome of ICSI cycles [33, 34]. Due to controversial evaluations with

NOA patients, ICSI-IVF outcomes are controversial because of different cryopreservation methods [20, 35–37].

Our study reported an improved laboratory technique of testicular tissue suspension cryopreservation in NOA patients after sperm retrieval from micro-TESE. We established a technique and method to assess the quantity and quality of sperm by counting the average number of sperm in high power and calculating the percentage of motile sperm after standardizing the total volume of sperm suspension. This evaluation method is simple, reliable, and can be easily used as a conventional assessment method, even for too extremely few spermatozoa patients. We found no difference in the average number and the percentage of motile spermatozoa between frozen-thawed sperm and fresh sperm. At the same time, frozen-thawed sperm with ICSI resulted in a similar fertilization rate (70.1% vs. 68.2%, $p = 0.403$) and day 3 utilization rate of oocytes (28.7% vs. 26.2%, $p = 0.286$) compared with fresh sperm. The cumulative live birth rate (LBR) of one ICSI cycle in the frozen-thawed sperm group was 47.5%, high up to the same as conventional ICSI-IVF as reported [38].

According to previous researches, the clinical pregnancy rate between fresh sperm and frozen sperm remains controversial. Park reported that patients with frozen spermatozoa had significantly higher pregnancy and implantation rates than fresh sperm [39]. Others showed that the fertilization rate and clinical pregnancy rate were higher in fresh sperm from non-mosaic KS patients by TESE [40]. A systematic review and meta-analysis revealed that in men with NOA showed that the ICSI-IVF outcome was not affected by whether the retrieved testicular sperm is fresh or frozen [41]. Nevertheless, cumulative pregnancy or cumulative live birth was not mentioned in any of the above studies, especially the cumulative live birth. After comparing the outcomes of ICSI-IVF cycles between fresh and frozen-thawed spermatozoa, we found that there were no significant differences in all laboratory parameters, not only fertilization rate and day 3 oocytes utilization rate we mentioned above, but also cleavage rate, rate of the high-score embryo, and the ratio of patients who had no available embryos. The main results we found are consistent with previous studies [20, 37, 42]. It is worth to mention that about 1/7 to 1/9 patients may not be able to obtain available embryos according to one ICSI cycle. This detailed data will help physicians provide with sufficient counseling and avoid patients' over-high expectations of treatment.

Then, we conducted a further analysis of clinical outcomes after embryo transfer, including the first embryo transfer cycle in ITT and PP analysis and cumulative embryo transfer cycles in PP analysis. Our results showed that CPR in the first embryo transfer cycle was lower in frozen sperm with ITT analysis; however, not significantly different in PP analysis. This difference may result from social factors; for example, a high proportion of patients with frozen sperm in the ITT set who did not receive ICSI treatment after sperm acquired for divorce, singlehood, finally change for sperm donation or loss of communication. In PP analysis, the CPR or cumulative CPR and LBR showed no significant differences between the fresh and frozen groups after excluding patients with preimplantation genetic testing treatment (spouse's chromosome showed a Robertson translocation), warmed oocytes cycles, or those men whose spouse had history of recurrent pregnancy loss and uterine abnormality, and those without

ICSI treatment. Therefore, more than 40% NOA patients can achieve live birth in one ICSI cycle, no matter with frozen or fresh sperm (47.5% and 42.9%).

Few studies provided a detailed analysis of ICSI-IVF outcomes according to the different pathological types and etiologies of NOA patients [20, 21, 43]. De Croo et al. reported maturation arrest had a lower fertilization rate and clinical pregnancy rate compared with hypospermatogenesis and Sertoli Cell Only syndrome [23]. Some studies demonstrated no significant differences in ICSI-IVF outcomes between different histopathological subsets in NOA [40, 44]. However, in our study, there were significant differences in day 3 utilization rate of MII eggs for ICSI-IVF cycle among NOA patients with different etiologies (idiopathic = 31.7%, orchitis = 30.8%, cryptorchidism = 27.2%, KS = 24.4%, and lowest in Y chromosome AZFc microdeletions = 22.3%, $p < 0.05$). Some studies reported that fertilization competent, viable embryo rate, and pregnancy rate of spermatozoa retrieved from men with Y chromosome AZFc chromosome deletions were similar to men without it [45, 46]. Our results showed that lower day 3 oocytes utilization rate and high-score embryo rate and lower cumulative CPR and cumulative LBR were observed in patients with Y chromosome AZFc microdeletion. These results are also consistent with those reported by Van et al. [47], and the primary function of the AZFc region in the Y chromosome is involvement in spermatozoa quality or function than in spermatogenesis.

Besides, we found that compared to patients who got more sperm (> 20 approximately), NOA patients with fewer sperm (≤ 20 approximately) were detected with significantly higher serum FSH level, lower oocytes utilization rate, lower high-score embryo rate, and a higher ratio of cycles without available embryos. Some studies reported that sperm from NOA patients have aneuploidy, mosaicism, and DNA damage that contribute to decreased clinical outcomes [22, 48]. Similarly, in this study, a significantly lower clinical pregnancy rate and live birth rate were observed as expected in patients with fewer sperm. These results provide us with a better understanding of treatment outcomes for patients with different laboratory findings after testicular tissue processing.

Before micro-TESE surgery was conducted, complete clinical consulting was performed regarding the treatment protocol selection and male factors that may influence the outcome of ICSI-IVF treatment. In addition, the female's ovarian reserve would be assessed in detail before the eventual decision on the conduction of micro-TESE surgery to reduce the risk of cycle cancellation due to the female factors, especially for couples who had difficulties accepting the consequences of failure. In this study, advanced female age (≥ 40 years old, 6 cases), diminished ovarian reserve (DOR, AMH ≤ 1 ng/ml, 16 cases), or poor ovarian response (POR, ≤ 4 oocytes retrieved, 29 cases) were all defined as risk factors for adverse outcomes, and patients would be informed of high risk of ICSI-IVF failure. All of these patients treated with ICSI in this study lead to one live-birth delivery in six patients aged ≥ 40 years, three live-birth deliveries in 16 DOR patients, and 15 live-birth deliveries in 29 POR patients (data not included in the results section). The female-factor combined with male-factor assessment refined our clinical consultation on ICSI treatment for couples with NOA.

Conclusions

This study's main strength is comprehensively analyzing clinical outcomes for NOA patients undergoing micro-TESE and ICSI-IVF cycle. Information and data from both male and female sides were included in our analysis; besides, we included CPR and LBR with intent-to-treat (ITT) analysis and per-protocol (PP) analysis minimally or barely reported in the past literature. In addition, female factors that may affect the ICSI-IVF outcomes were excluded in the PP analysis, including recurrent pregnancy loss, abnormal karyotype, known uterine anomalies, and adenomyosis, improving extrapolation of our results. However, our study still has limitations, including the sample size of the fresh sperm group was not large enough, the need to supplement follow-up data on live births, and patient selection bias. In the future, multicenter data and randomized controlled trials are needed to determine clinical predictors of successful outcomes for NOA couples.

Abbreviations

NOA: non-obstructive azoospermia; Micro-TESE: microdissection testicular sperm extraction; SRR: sperm retrieval rate; LBR: live birth rate; ICSI-IVF: intracytoplasmic sperm injection in-vitro fertilization; ITT: intent-to-treat; CPR: cumulative clinical pregnancy rate; PP: per-protocol; CI: Confidential interval; OR: Odds ratio. FSH: Follicle stimulating hormone; LH: Luteinizing hormone; T: Testosterone; E2: Estradiol; PRL: Prolactin; TESE: Testicular sperm extraction; COS: Controlled ovarian stimulation; HPF: High power fields; hCG: Human chorionic gonadotrophin; GnRH: Gonadotropin-releasing hormone; IU: International unit; KS: Klinefelter Syndrome; AZFc: Azoospermia factor c; LSC: Lower sperm count; HSC: Higher sperm count; PN: Primary nucleus; DOR: Diminished ovarian reserve; POR: Poor ovarian response

Declarations

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Authors'contributions

All authors contributed substantially to this work. Geng An developed the original concept of this study. He also interpreted data, drafted the article, and critically approved the final article. Data collection was performed by Yu Lan, Xin Fu, Tianwen Peng, Chen Liao, Jianan Liu, and Min Liu; Haiyan Zheng implemented additional statistical analysis. All authors have contributed to critical discussion, reviewed the final version of the manuscript and approved, reviewed the final version of the manuscript, approved the manuscript's final version, and approved it for publication.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University (reference number 2017-055) and was carried out in accordance with the Helsinki Declaration. Due to the retrospective nature, informed consent was not required, and patients' data were used anonymously.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Figures

Abbreviation: NOA, non-obstructive azoospermia; Micro-TESE, microdissection testicular sperm extraction; ITT, intent-to-treat; PP, per-protocol; PGT, preimplantation genetic testing; ICSI, intracytoplasmic sperm injection
#: There were 8 couples without ICSI: 2 of them temporarily asked for sperm donation; 6 of them retrieved with unavailable sperm.
*:There were 66 men excluded:
4 with ICSI: 2 of them whose spouse had history of recurrent pregnancy loss; 2 of the other whose spouse had acquired uterine abnormality;
62 men without ICSI: 5 of them asked for sperm donation; 35 of them retrieved with unavailable sperm; 22 of the other were divorced or unmarried or loss of communication.

Figure 1

Flow Chart

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