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*Research Article*

# Outcomes of Microdissection Testicular Sperm Extraction/ Intracytoplasmic Sperm Injection in Cases of Nonobstructive Azoospermia: A Retrospective Study

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The outcomes and safety of intracytoplasmic sperm injection (ICSI) using testicular sperm have been controversial. We evaluated ICSI results, pregnancy outcomes, and neonatal health conditions using testicular sperm from patients with obstructive (OA) or nonobstructive (NOA) azoospermia. We further compared the ICSI outcomes after use of fresh versus cryopreserved testicular sperm from men with NOA. We included 314 men with NOA who underwent microdissection testicular sperm extraction (mTESE) and 303 with OA who underwent testicular sperm aspiration; both groups underwent ICSI. Therefore, 101 and 329 ICSI cycles were performed for mTESE and aspirated sperms, respectively. Furthermore, fresh and thawed embryos from both groups were transplanted to evaluate fertilization and pregnancy rates (NOA, 15 fresh and 123 thawed; OA, 59 fresh and 393 thawed). Finally, of the 101 ICSI cycles performed for NOA patients, 56 fresh-sperm cycles and 45 thawed-sperm cycles were performed to evaluate ICSI outcomes. Fertilization rates and two-pronuclear (2PN) fertilization rates were significantly lower in the NOA group than in the OA group. However, the 2PN cleavage rate, the high-quality embryo rate, the blastocyst formation rate, and the available blastocyst rate showed no significant intergroup differences. In addition, the pregnancy outcomes and neonatal health conditions were statistically similar. Finally, compared with thawed sperm, the fresh-sperm group had a higher 2PN fertilization rate and a higher high-quality embryo rate. However, blastocyst formation and available blastocyst rates were similar between the two groups. Patients with NOA achieved the same favorable results in embryo development, clinical pregnancy, and live birth capability as did OA patients. Neonatal conditions were not affected by type of azoospermia (NOA versus OA). For patients with NOA, fresh testicular sperm is superior to frozen-thawed testicular sperm in embryo development as evaluated at the cleavage stage, but we find no superiority evaluating at the stage of blastocyst formation.

## 1. Introduction

Infertility is found in at least 30% of men globally, and azoospermia accounts for 10% to 15% of male infertility [1, 2]. Azoospermia, defined as the complete absence of ejaculated sperm, is the most severe form of infertility. It can be roughly divided into obstructive azoospermia (OA) and nonobstructive azoospermia (NOA). In OA, testicular spermatogenesis function is preserved, and azoospermia is caused by the mechanical obstruction of any region along the reproductive tract. In NOA, testicular defects are present, and sperm production is remarkably impaired [3].

Even men without sperm in their ejaculated semen due to obstructions or problems in sperm production can benefit from therapeutic retrieval of testicular spermatozoa ([4]). Microdissection testicular sperm extraction (mTESE) is the preferred method of sperm extraction in cases of NOA in many reproductive centers, and testicular sperm aspiration (TESA) is used for OA [5, 6]. Intracytoplasmic sperm injection (ICSI) has been proven effective in treating male infertility and in overcoming poor-quality and low-quantity sperm ([7]). mTESE or TESA combined with ICSI allows azoospermic men to father biological children [5].

Recently, concerns have been raised about the relationship between spermatozoa obtained from men with some etiologies of sterility and the outcomes of assisted reproductive technology (ART). Many studies have estimated the

clinical outcomes of ICSI using testicular sperm in patients with azoospermia. However, it is unclear whether sperm extracted from men with NOA or OA can affect embryonic development and clinical outcomes, such as fertilization rate, blastocyst development, and pregnancy rate.

Therefore, we undertook this retrospective study to evaluate outcomes of ICSI using testicular spermatozoa from men with NOA versus OA. We also compared the outcomes of ICSI using fresh versus cryopreserved spermatozoa, both retrieved from patients with NOA. Use of cryopreserved spermatozoa is advantageous in cases of NOA to avoid repeat surgery if the first treatment cycle is unsuccessful.

We evaluated patients referred to our clinic for azoospermic infertility (nonobstructive and obstructive) who underwent mTESE and TESA procedures.

## 2. Materials and Methods

*2.1. Patients.* We retrospectively analyzed 314 men with NOA who underwent mTESE and 303 men with OA who underwent TESA between March 2016 and December 2021 at the First Hospital of Jilin University, China.

All patients underwent rigorous medical examinations and diagnoses, and their ages and endocrine profiles were recorded in detail. Testicular volumes on both sides were tested using a standard ultrasonic instrument. The surgeries were performed after consideration of the patients' clinical profiles.

*2.2. Testicular Sperm Retrieval.* After assessment and approval by gynecologists, urologists, and embryologists, mTESE or TESA surgery was performed. We performed mTESE in all nonobstructive patients and TESA in all patients with obstruction. The detailed procedures for mTESE have been described previously [8, 9]. Briefly, surgeons made a transverse midscrotal incision over the left or right testis under general anesthesia. The tunica vaginalis was then opened to visualize the tunica albuginea. Next, an equatorial incision was extended over the tunica albuginea under an operative microscope. Finally, the larger and more opaque seminiferous tubules were identified by microdissection at 12x to 18x magnification under an operating microscope. The obtained testis tissues and seminiferous tubules were collected in culture dishes in 2-hydroxyethyl- (HEPES-) buffered medium and sent to the in vitro fertilization (IVF) laboratory for immediate microscopic examination. Next, the extracted tubules were minced, and the cell suspension was examined under an inverted microscope for sperm. The embryologist informed the surgeon if any sperm were found and ensured that sufficient sperm were collected for the ICSI procedure.

*2.3. Spermatozoa Frozen and Thawed in Straw.* At our IVF center, spermatozoa retrieved from mTESE or TESA is routinely cryopreserved in separate straws before ovarian stimulation and oocyte retrieval from females or after the current ICSI cycle.

Straws were supplied by Cryo BioSystem, France, and were made of transparent polymerized resin. Sperm samples were mixed 1 : 1 with sperm cryopreservation medium (SAGE BioPharma, Bedminster, NJ, USA) and then loaded into the straws for freezing. They were exposed to liquid-nitrogen vapor for approximately 5 min and then immersed in liquid nitrogen for storage at -196°C.

For spermatozoa thawing, each straw was quickly removed from liquid nitrogen, transferred into a sterile culture dish on a 37°C constant-temperature platform, and swiftly placed under a microscope. Spermatozoa were then individually moved to an insemination medium containing polyvinylpyrrolidone (PVP) to prepare for ICSI.

*2.4. Outcome Measures.* For laboratory outcomes, fertilization rate was defined as the ratio of the number of fertilized oocytes to the number of oocytes used for ICSI. The two-pronuclear (2PN) fertilization rate was calculated as the ratio of 2PN zygotes to the number of ICSI-treated oocytes. The 2PN cleavage rate was defined as the ratio of the number of cleavage-stage embryos that had developed from 2PN zygotes by day 3 to the number of 2PN zygotes. A high-quality embryo was defined as containing 2–5 blastomeres and <20% fragments on day 2 and 6–9 blastomeres with <20% fragments on day 3. The high-quality embryo rate was the ratio of the number of high-quality embryos on day 3 to the number of cleavage-stage embryos. Blastocyst formation rate was defined as the ratio of the number of blastocysts to the number of day 3 embryos in continued culture. Finally, the available blastocyst rate was defined as the ratio of available blastocysts to the number of blastocysts formed on days 5 or 6.

Clinical pregnancy was defined as the presence of a fetal heartbeat at 6–8 weeks on ultrasonography. Implantation rate was calculated as the number of gestational sacs divided by the number of transferred embryos. Clinical pregnancy rates and live birth rates were calculated as clinical or live birth events *per* embryo transfer. Finally, the cumulative clinical pregnancy rate was calculated by dividing the number of ovarian stimulation cycles that achieved clinical pregnancy by the total number of ovarian stimulation cycles recorded over a follow-up period of two years.

*2.5. Statistical Analysis.* All statistical data were analyzed with SPSS version 17.0 (IBM, Armonk, NY, USA) Quantitative data, such as testis volume, age, and hormone levels, were compared by independent-samples $t$-test. Qualitative variables, such as fertilization and live birth rates, were evaluated using the chi-square or Fisher's exact test. Statistical significance was set at $P < 0.05$.

## 3. Results

We observed no statistical differences between the NOA and OA groups in the female characteristics. Of the 314 NOA patients who underwent mTESE, 103 (32.8%) had successfully retrieved spermatozoa. All 303 patients with OA (100%) yielded spermatozoa by TESA. In men with NOA who underwent mTESE, no difference was found between the sperm retrieval rate- (SRR-) positive and negative groups in terms of age and levels of FSH, LH, or estradiol. The negative SRR group had higher left and right testicular volumes

Table 1: Clinical characteristics of men with nonobstructive azoospermia (NOA) with positive and negative sperm retrieval rate (SRR).

| Variable | Positive SRR | Negative SRR | *P* value |
|---|---|---|---|
| Patients (*n*) | 103 | 211 | — |
| Male age (years) | 30 (27.5–34) | 30 (28–32) | 0.569 |
| Left testicular volume (mL) | 5.00 (2.00–8.00) | 7.15 (4.00–10.00) | 0.015* |
| Right testicular volume (mL) | 5.00 (2.35–8.95) | 7.20 (4.00–10.00) | 0.010* |
| FSH (mIU/mL) | 24.14 (13.70–31.55) | 20.85 (13.73–28.59) | 0.056 |
| LH (mIU/mL) | 11.68 (7.19–17.02) | 10.11 (6.85–15.80) | 0.117 |
| Estradiol, E2 (pmol/L) | 27.68 (19.60–37.29) | 27.00 (19.22–35.20) | 0.736 |

Data are presented as median (interquartile range); $^*P < 0.05$.

than did the positive SRR group: 7.15 (4.00–10.00) vs. 5.00 (2.00–8.00) and 7.20 (4.00–10.00) vs. 5.00 (2.35–8.95), respectively, $P < 0.05$ (see Table 1).

The ICSI outcomes of men with NOA with successful sperm retrieval and men with OA are provided in Table 2. A total of 101 ICSI cycles were performed using sperm retrieved from patients with NOA, and 329 ICSI cycles were performed using sperm retrieved from patients with OA. In these two groups, the infertility duration, punctured follicle numbers, and total oocytes were not different. The female age was lower in the NOA group than in the OA group: 29 (26–31.5) vs. 31 (28–35), $P < 0.01$. The number of MII oocytes and the number of oocytes used for ICSI were significantly higher in the NOA group: 11 (9–15) vs. 10 (6–14.5), $P = 0.038$; and 11 (9–15) vs. 10 (6–14), $P = 0.049$, respectively. The fertilization rates and two-pronuclear (2PN) fertilization rates were significantly lower in the NOA group than in the OA group: 65.04% vs. 78.57%, $P < 0.01$; and 59.40% vs. 70.68%, $P < 0.01$, respectively. However, the 2PN cleavage rate, the high-quality embryo rate, the blastocyst formation rate, and the available blastocyst rate were not significantly different between the two groups.

The pregnancy outcomes of men with NOA with successful sperm retrieval and men with OA are provided in Table 3. In the NOA group, we analyzed 15 transplantation cycles with fresh embryos and 123 with thawed embryos. In the OA group, the figures were 59 fresh and 393 thawed. The number of embryos transferred and the embryo stage at transfer were not significantly different between the groups. The implantation rate *per* cycle (35.74% vs. 38.28%, $P = 0.483$), clinical pregnancy rate (50.00% vs. 51.11%, $P = 0.820$), miscarriage rate (17.339% vs. 9.96%, $P = 0.091$), and live birth rate (72.62% vs. 70.75%, $P = 0.739$) did not show intergroup differences.

We also analyzed the cumulative clinical pregnancy rate and cumulative live birth rate. In our NOA patients, seven ovarian stimulation cycles still had embryos left, but patients had not achieved pregnancy or live birth before our data deadline, and another seven ovarian stimulation cycles achieved clinical pregnancy but had not delivered before deadline. In patients with OA, these numbers were 20 and 46 data points, respectively. After removing the invalid cases, the cumulative clinical pregnancy rate (69.15% vs. 70.23%, $P = 0.842$) and cumulative live birth rate (57.47% vs. 60.46%, $P = 0.623$) were not significantly different between the two groups (Table 3).

In the live births, the conditions of the neonates in the NOA and OA groups are provided in Table 4; groupwise comparison did not reveal significant differences in either neonate length or weight.

Of the 101 ICSI cycles in the data of patients with NOA, 56 used fresh sperm, and 45 used thawed sperm (see Table 5). The infertility duration, punctured follicle numbers, total oocytes, MII oocytes, and oocytes used for ICSI were similar between the two groups. However, the fertilization rate (74.92% vs. 52.86%, $P < 0.001$), the 2PN fertilization rate (68.27% vs. 48.47%, $P < 0.001$), and the high-quality embryo rate (52.06% vs. 43.82%, $P = 0.038$) were higher in the fresh-sperm group than in the thawed group. The 2PN cleavage rate was not significantly different between the two groups. The number of 2PN cleavages was significantly higher in the fresh-sperm group than in the thawed group: 7 (6–9) vs. 5 (2.5–8), $P = 0.010$. However, the two groups had similar results for blastocyst formation rate and available blastocyst rate: 55.79% vs. 54.30%, $P = 0.774$, and 73.85% vs. 70.73%, $P = 0.620$, respectively (Table 5).

## 4. Discussion

NOA accounts for 10–15% of clinical cases of male infertility [10], and mTESE improves the frequency of sperm retrieval in such cases [8]. mTESE was initially described in 1999 as an effective alternative method for retrieving sperm from men with NOA and allows these patients to possibly father their children. The SRR of mTESE in our study was 32.80% (103/314), which is slightly lower than in previous reports [3, 11]. Achermann and coworkers [3] summarized data from 4075 patients from twenty-four retrospective and five prospective studies and showed that mTESE successfully retrieved sperm in 45.1% of cases. However, this value could be affected by patient characteristics and varied considerably across studies.

Recent studies have focused on identifying factors predictive of successful retrieval of sperm from patients with NOA. Testis volumes (left and right) were the only significant predictive factors for successful SRR found among several clinical and biochemical parameters [11]. Our results

Table 2: Outcomes of intracytoplasmic sperm injection (ICSI) in men with NOA with successful sperm retrieval and in men with obstructive azoospermia (OA).

| Variable | NOA | OA | *P* value |
|---|---|---|---|
| Number of cycles | 101 | 329 | — |
| Female age | 29 (26–31.5) | 31 (28–35) | <0.01* |
| Infertility duration (years) | 3 (2–6) | 3.5 (2–6) | 0.265 |
| Follicles punctured (*n*)† | 17.92 ± 7.75 | 16.09 ± 9.40 | 0.075 |
| Total oocytes (*n*)† | 14.18 ± 5.90 | 12.96 ± 7.78 | 0.096 |
| MII oocytes (*n*) | 11 (9–15) | 10 (6–14.5) | 0.038* |
| Oocytes used for ICSI (*n*) | 11 (9–15) | 10 (6–14) | 0.049* |
| Zygotes (*n*) | 8 (4–10) | 7 (4–11) | 0.385 |
| Fertilization rate (%) | 65.04% (761/1170) | 78.57% (2771/3527) | <0.01* |
| Two-pronuclear (2PN) zygotes (*n*) | 7 (4–9) | 7 (4–10.5) | 0.368 |
| 2PN fertilization rate (%) | 59.40% (695/1170) | 70.68% (2493/3527) | <0.01* |
| 2PN cleavages (*n*) | 3.5 (7–9) | 4 (7–10) | 0.395 |
| 2PN cleavage rate (%) | 98.85% (687/695) | 97.99% (2443/2493) | 0.136 |
| High-quality embryos (*n*) | 2 (1–5) | 3 (1–6) | 0.211 |
| High-quality embryo rate (%) | 49.05% (337/687) | 51.58% (1260/2443) | 0.243 |
| Blastocysts formed (*n*) | 1 (0–3.5) | 2 (0–4) | 0.357 |
| Blastocyst formation rate (%) | 55.21% (212/384) | 56.72% (814/1435) | 0.595 |
| Available blastocysts (*n*) | 0 (0–2) | 1 (0–2) | 0.323 |
| Available blastocyst rate (%) | 72.64% (154/212) | 70.39% (573/814) | 0.521 |

†Mean ± standard deviation; all other data are presented as median (interquartile range) or percentage (number ratio); *$P < 0.05$.

Table 3: Pregnancy outcomes for men with NOA with successful sperm retrieval and for men with OA.

| Variable | NOA | OA | *P* value |
|---|---|---|---|
| Embryos transferred, *n* (%) | | | 0.932 |
| Single | 41 (29.71%) | 136 (29.96%) | |
| Double | 97 (70.29%) | 316 (70.04%) | |
| Embryo stage at transfer, *n* (%) | | | 0.241 |
| Cleavage | 86 (62.32%) | 306 (67.70%) | |
| Blastocyst | 52 (37.68%) | 146 (32.30%) | |
| Embryo transfer strategy, *n* (%) | | | 0.477 |
| Single cleavage | 7 (5.07%) | 37 (8.19%) | |
| Double cleavage | 79 (57.25%) | 269 (59.51%) | |
| Single blastocyst | 34 (24.64%) | 99 (21.90%) | |
| Double blastocyst | 18 (13.04%) | 47 (10.40%) | |
| Implantation rate (%) | 35.74% (84/235) | 38.28% (294/768) | 0.483 |
| Clinical pregnancy rate (%) | 50.00% (69/138) | 51.11% (231/452) | 0.820 |
| Cumulative clinical pregnancy rate (%) | 69.15% (65/94) | 70.23% (217/309) | 0.842 |
| Miscarriage rate (%) | 17.339% (12/69) | 9.96% (23/231) | 0.091 |
| Live birth rate (%) | 72.62% (61/84) | 70.75% (208/294) | 0.739 |
| Cumulative live birth rate (%) | 57.47% (50/87) | 60.46% (159/263) | 0.623 |

Notes: data are presented as number (percentage) or percentage (number ratio).

seem to favor the selection of smaller-volume testes for successful retrieval. However, the results of prior studies on levels of hormones such as FSH, LH, and estradiol (E2) are controversial. Many researchers have proposed FSH as predictive of positive SRR [12], while other authors disagree [13, 14], and several studies have speculated that ethnicity might influence SRR in cases of NOA.

Many sterile couples facing azoospermia rely on assisted reproductive techniques (ART) as the first-line treatment for achieving biological parenthood [15]. Through ICSI, the

Table 4: Conditions of neonates ensuing from ICSI in cases of NOA versus OA.

| Variable | NOA | OA | *P* value |
|---|---|---|---|
| Number of neonates (*n*) | 61 | 208 | — |
| Sex (male/female) | 35/26 | 114/94 | — |
| Length (cm) | 50 (48–50) | 50 (48–50) | 0.260 |
| Weight (g) | 3100 (2725–3620) | 3100 (2650–3500) | 0.260 |

Notes: data are presented as median (interquartile range).

Table 5: Fertilization and pregnancy outcomes of ICSI for NOA using fresh versus thawed sperm collected by microdissection testicular sperm extraction.

| Variable | Fresh sperm | Thawed sperm | *P* value |
|---|---|---|---|
| Number of cycles | 56 | 45 | — |
| Female age | 29 (26–31) | 28 (26–32.5) | 0.784 |
| Infertility duration (years) | 3 (2–6) | 3 (1.5–5.5) | 0.796 |
| Follicles punctured (*n*) | 17.5 (13–22.75) | 16 (12–22.5) | 0.475 |
| Total oocytes (*n*) | 14 (10–19) | 13 (11–17.5) | 0.959 |
| MII oocytes (*n*) | 11 (8.25–15) | 11 (9–15) | 0.921 |
| Oocytes used for ICSI (*n*) | 11 (8–15) | 11 (9–15) | 0.875 |
| Zygotes (*n*) | 8 (6.25–10) | 6 (3–9) | 0.007* |
| Fertilization rate (%) | 74.92% (484/646) | 52.86% (277/524) | <0.001* |
| Two-pronuclear (2PN) zygotes (n) | 8 (6–9) | 5 (2.5–8) | 0.008* |
| 2PN fertilization rate (%) | 68.27% (441/646) | 48.47% (254/524) | <0.001* |
| 2PN cleavages (*n*) | 7 (6–9) | 5 (2.5–8) | 0.010* |
| 2PN cleavage rate (%) | 98.87% (436/441) | 98.82% (251/254) | 0.955 |
| High-quality embryos (*n*) | 4 (2–6) | 2 (1–4) | 0.007* |
| High-quality embryo rate (%) | 52.06% (227/436) | 43.82% (110/251) | 0.038* |
| Blastocysts formed (*n*) | 1 (0–4) | 1 (0–3) | 0.370 |
| Blastocyst formation rate (%) | 55.79% (130/233) | 54.30% (82/151) | 0.774 |
| Available blastocysts (*n*) | 1 (0–2) | 0 (0–2) | 0.421 |
| Available blastocyst rate (%) | 73.85% (96/130) | 70.73% (58/82) | 0.620 |

Notes: data are presented as median (interquartile range) or percentage (number ratio); *$P < 0.05$.

testicular sperm of azoospermic males can cause normal fertilization and embryo development [3, 16]. However, reports making a clear distinction between the different types of azoospermia when comparing ICSI results, pregnancy outcomes, and the health conditions of neonates, are minimal. In this study, the OA group showed a higher fertilization rate and two-pronuclear (2PN) fertilization rate than did the NOA group, which is in agreement with previous reports by Esteves and coworkers [15], and neither group showed superiority in blastocyst formation. Nevertheless, we found that the pregnancy outcomes of men with NOA with successful sperm retrieval, including implantation rate per cycle, clinical pregnancy rate, miscarriage rate, and live birth rate, were similar to those of men with OA. In addition, we innovatively assessed the condition of the neonates and concluded that neonatal birth length and weight are comparable between the two groups. Based on the limited group of 269 neonates that we analyzed, we suggest that the neonatal profile of children does not seem to be affected by the type of azoospermia being treated.

Despite considerable research on the fertilization and pregnancy rates of ICSI with testicular sperm, few studies have compared the efficacy of using fresh versus frozen-thawed sperm in ICSI for couples affected by NOA [17]. In recent years, the development of an efficient technique for testicular sperm cryopreservation has played a crucial role in the preparations for ICSI that occur prior to oocyte collection, with the aim of providing for further treatment or a repeated cycle after an initial mTESE ICSI cycle with fresh sperm, thus avoiding repeated surgery. Therefore, many researchers have focused on estimating the clinical outcomes of fresh versus cryopreserved testicular sperm in ICSI. Some studies have reported similar results between the two groups [18, 19], but some have suggested that fresh sperm yields better clinical outcomes [20]. Our results show that fresh testicular sperm seems to produce better ICSI outcomes than does cryopreserved testicular sperm in mTESE-ICSI.

Furthermore, the baseline characteristics did not differ significantly between the two groups, yet fertilization rate, 2PN fertilization rate, 2PN cleavage rate, and high-quality

embryo rate were all higher in the fresh testicular sperm group. However, note that the blastocyst formation rate and available blastocyst rate were similar between the two groups. This means that fresh sperm had no advantage in terms of clinical outcomes once the embryos had formed blastocysts.

Although the current data on postnatal condition and pregnancy outcomes following ICSI with testicular sperm are reassuring, the limited sample population of this investigation calls for continued monitoring of outcomes. Moreover, studies on the growth and developmental condition of the children ensuing from these techniques are lacking. Therefore, future research should focus on long-term outcomes in such children.

## 5. Conclusions

This study verified the feasibility of ICSI using testicular sperm retrieved by mTESE from patients with NOA. Patients with NOA achieved the same favorable results in embryo development, clinical pregnancy, and live birth capability as did the OA group. Moreover, the neonatal profile of the ensuing children was not affected by the type of azoospermia: NOA versus OA. In patients with NOA, fresh testicular sperm appears superior to frozen-thawed testicular sperm for embryo development, if evaluated at the cleavage stage, but we find no advantage when evaluating at the blastocyst stage.

## Data Availability

Readers can access the data supporting the study's conclusions by contacting authors through email.

## Conflicts of Interest

The authors declare that there are no conflicts of interest in connection with this article.

## Authors' Contributions

Xiaoming Sun and Haibo Zhu conceived the study, analyzed the data, and drafted the article. Xiao Yang and Ruixue Wang acquired the data. Ruizhi Liu and Lili Luo acquired the funding. All the authors provided the final approval of the final manuscript.

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## References

[1] A. Agarwal, A. Mulgund, A. Hamada, and M. R. Chyatte, "A unique view on male infertility around the globe," *Reproductive Biology and Endocrinology*, vol. 13, no. 1, p. 37, 2015.

[2] J. P. Jarow, M. A. Espeland, and L. I. Lipshultz, "Evaluation of the azoospermic patient," *Journal of Urology*, vol. 142, no. 1, pp. 62–65, 1989.

[3] A. P. P. Achermann, T. A. Pereira, and S. C. Esteves, "Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to non-obstructive azoospermia: summary of current literature," *International Urology and Nephrology*, vol. 53, no. 11, pp. 2193–2210, 2021.

[4] Y. R. Tsai, F. J. Huang, P. Y. Lin, F. T. Kung, Y. J. Lin, and K. C. Lan, "Clinical outcomes and development of children born to couples with obstructive and nonobstructive azoospermia undergoing testicular sperm extraction- intracytoplasmic sperm injection: a comparative study," *Taiwanese Journal of Obstetrics & Gynecology*, vol. 54, no. 2, pp. 155–159, 2015.

[5] A. M. Bernie, D. A. Mata, R. Ramasamy, and P. N. Schlegel, "Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: a systematic review and meta-analysis," *Fertility and Sterility*, vol. 104, no. 5, pp. 1099–1103.e3, 2015.

[6] Y. Hu, S. Cao, S. Wu, J. Zhao, S. K. Arhin, and D. Shan, "Comparison of the effects of different testicular sperm extraction methods on the embryonic development of azoospermic men in intracytoplasmic sperm injection (ICSI) cycles: a retrospective cohort study," *BioMed Research International*, vol. 2021, Article ID 5515247, 7 pages, 2021.

[7] C. C. Tsai, F. J. Huang, L. J. Wang et al., "Clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) using extracted testicular sperm or ejaculated extreme severe oligo-astheno-teratozoospermia sperm: a comparative study," *Fertility and Sterility*, vol. 96, no. 3, pp. 567–571, 2011.

[8] P. N. Schlegel, "Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision," *Human Reproduction*, vol. 14, no. 1, pp. 131–135, 1999.

[9] Y. Yu, Q. Xi, Y. Pan et al., "Pregnancy and neonatal outcomes in azoospermic men after intracytoplasmic sperm injection using testicular sperm and donor sperm," *Medical Science Monitor*, vol. 24, pp. 6968–6974, 2018.

[10] M. H. Alkandari and A. Zini, "Medical management of non-obstructive azoospermia: a systematic review," *Arab Journal of Urology*, vol. 19, no. 3, pp. 215–220, 2021.

[11] G. Corona, S. Minhas, A. Giwercman et al., "Sperm recovery and ICSI outcomes in men with non-obstructive azoospermia: a systematic review and meta-analysis," *Human Reproduction Update*, vol. 25, no. 6, pp. 733–757, 2019.

[12] T. Ishikawa, "Surgical recovery of sperm in non-obstructive azoospermia," *Asian Journal of Andrology*, vol. 14, no. 1, pp. 109–115, 2012.

[13] D. Jezek, U. A. Knuth, and W. Schulze, "Successful testicular sperm extraction (tese) in spite of high serum follicle stimulating hormone and azoospermia: correlation between testicular morphology, tese results, semen analysis and serum hormone values in 103 infertile men," *Human Reproduction*, vol. 13, no. 5, pp. 1230–1234, 1998.

[14] H. Li, L. P. Chen, J. Yang et al., "Predictive value of FSH, testicular volume, and histopathological findings for the sperm

retrieval rate of microdissection TESE in non-obstructive azoospermia: a meta-analysis," *Asian Journal of Andrology*, vol. 20, no. 1, pp. 30–36, 2018.

[15] S. C. Esteves, C. Prudencio, B. Seol, S. Verza, C. Knoedler, and A. Agarwal, "Comparison of sperm retrieval and reproductive outcome in azoospermic men with testicular failure and obstructive azoospermia treated for infertility," *Asian Journal of Andrology*, vol. 16, no. 4, pp. 602–606, 2014.

[16] S. C. Esteves and A. C. Varghese, "Laboratory handling of epididymal and testicular spermatozoa: what can be done to improve sperm injections outcome," *Journal of Human Reproductive Sciences*, vol. 5, no. 3, pp. 233–243, 2012.

[17] S. Kanto, J. Sugawara, H. Masuda, H. Sasano, Y. Arai, and K. Kyono, "Fresh motile testicular sperm retrieved from non-obstructive azoospermic patients has the same potential to achieve fertilization and pregnancy via ICSI as sperm retrieved from obstructive azoospermic patients," *Fertil Steril*, vol. 90, no. 5, pp. 2010.e5–2010.e7, 2008.

[18] L. Desch, C. Bruno, C. Herbemont et al., "Impact on ICSI outcomes of adding 24 h of in vitro culture before testicular sperm freezing: a retrospective study," *Basic and Clinical Andrology*, vol. 25, no. 1, p. 6, 2015.

[19] A. Eken and F. Gulec, "Microdissection testicular sperm extraction (micro-TESE): predictive value of preoperative hormonal levels and pathology in non-obstructive azoospermia," *The Kaohsiung Journal of Medical Sciences*, vol. 34, no. 2, pp. 103–108, 2018.

[20] Z. Zhang, J. Jing, L. Luo et al., "ICSI outcomes of fresh or cryopreserved spermatozoa from micro-TESE in patients with non-obstructive azoospermia: CONSORT," *Medicine (Baltimore)*, vol. 100, no. 12, article e25021, 2021.