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Micro-TESE strategy in patients with NOA caused by AZFc deletion: synchronous or asynchronous?

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Summary

In the treatment of infertile patients with non-obstructive azoospermia (NOA) caused by the deletion of the azoospermia factor c region (AZFc) on the Y chromosome, synchronous and asynchronous surgical strategies are discussed. Clinical data from NOA patients with the AZFc deletion who underwent micro-TESE were analyzed retrospectively. The sperm retrieval rate (SRR) and sperm utilization rate of synchronous and asynchronous operation groups were followed up and compared. The fertilization rate, high-quality embryo rate, clinical pregnancy rate, abortion rate, and cumulative live birth rate of ICSI in patients with successful sperm retrieval were compared between the two groups. The two groups had sperm utilization rates of 98.9% (93/94) and 50.0% (14/28), respectively. The asynchronous group's sperm consumption rates were much lower than those of the synchronous operation group. Fertilization rate, high-quality embryo rate, clinical pregnancy rate of fresh transfer cycle, abortion rate, and cumulative live birth rate of patients in the synchronous operation group with fresh sperm, and the asynchronous operation group with thawed sperm, respectively, were 30.6% vs 33.8%, 33.8% vs 40.7%, 40.0% vs 12.5%, 30.4% vs 7.1%. Between the two groups, there was no significant difference. This suggests that individuals with NOA caused by the AZFc deletion have a high possibility of successfully acquiring sperm using micro-TESE and ICSI to conceive their own offspring. Synchronous micro-TESE is recommended to improve sperm utilization rate and the cumulative live birth rate.

Introduction

Infertility affects roughly 10–15% of couples, according to clinical epidemiological research, and approximately half of infertile couples have male infertility causes (Kumar and Singh, 2015). In total, 10–15% of male infertility patients are azoospermic. Obstructive azoospermia and non-obstructive azoospermia (NOA) are the two forms of azoospermia. Klinefelter's syndrome and deletions of the Y chromosome are the most common genetic variables in NOA patients, accounting for 30% of the cases (Cocuzza *et al.*, 2013). Deletions of the Y chromosome are found in 10–15% of NOA patients, with the AZFc deletion being the most common type (Colaco and Modi, 2018; Yuen *et al.*, 2021).

Microscopic orchidopexy offers a higher sperm acquisition rate and less surgical damage than standard testicular puncture/incisional sperm extraction for patients with definite NOA diagnosis (Ishikawa, 2012; Dabaja and Schlegel, 2013). Approximately 50–80% of patients with NOA due to AZFc deletion can successfully obtain testicular sperm using micro-TESE that, in combination with ICSI, can give infertile couples a chance to conceive their own offspring (Goncalves *et al.*, 2017).

The scheduling of microscopic sperm retrieval surgery at our Center for individuals with NOA can be done in two ways. The first is an asynchronous operation in which the husband receives micro-TESE and, if sperm are detected, they are frozen and maintained, whereas the spouse undergoes controlled ovulation at a later date and sperm are thawed for ICSI to aid in conception. The second method is a synchronous operation in which micro-TESE is performed on the same day as or the day before the woman's egg retrieval. If sperm are found during the operation, fresh sperm are used for ICSI to help with conception. The asynchronous approach risks sperm quality and clinical outcomes of subsequent ICSI-assisted conception due to sperm freezing and thawing recovery, whereas the synchronous protocol eliminates this risk, but risks sperm not being detected on the day of egg retrieval using the female partner. Both therapy strategies have benefits and drawbacks, and their clinical application is a source of debate (Cissen *et al.*, 2016).

Our research analyzed the clinical outcomes of NOA patients with the AZFc deletion who underwent micro-TESE, followed up on the clinical outcomes of patients who found sperm intraoperatively for further treatment after ICSI; compared the therapeutic effects of both synchronous and asynchronous surgical strategies on patients; and provided clinical management guidance for NOA patients with the AZFc deletion.

Materials and methods

Clinical materials

The age of the patients and their partners, the outcome of micro-TESE, and the clinical outcomes of patients with intraoperative sperm finding who underwent ICSI for pregnancy assistance were collected retrospectively from NOA patients with the AZFc deletion who underwent micro-TESE at our Center of Reproductive Medicine from January 2015 to December 2019.

Patients were examined for the *YAZF* gene and diagnosed with the AZFc deletion, as well as chromosomal karyotyping and exclusion of chromosomal abnormalities. According to the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) guidelines for molecular diagnosis of Y-chromosomal microdeletions, in total, eight loci were examined for sY14 (SRY), ZFX/ZFY, sY84, sY86, sY127, sY134, sY254, and sY255, of which both sY254 and sY255 were lacking as regards the AZFc deletion (Krausz *et al.*, 2014).

Methods

The patient's medical history, physical examination, and relevant laboratory tests were used to make the clinical diagnosis of NOA and a AZFc deletion. Patients in the study had multiple semen analyses before surgery, and no sperm were found on microscopic examination after centrifugation. Patients masturbated again before surgery to obtain semen for examination, and no sperm were found in the semen before performing microscopic sperm extraction; if sperm were found in the semen, the operation was cancelled. Patients and their families were given information on the surgical options and risks, signed an informed consent form, and were given the option of choosing between synchronous and asynchronous surgery.

The surgical procedure for micro-TESE has been published previously by Schlegel's group (Stahl *et al.*, 2010). Two senior laboratory staff members tore the spermatogenic tubules mechanically and then search for sperm under an inverted microscope (×400 magnification) during the operation. The operation was concluded if sufficient quantity and good morphology sperm were found. If no sperm were detected in one testicle, the opposite testicle was incised and thoroughly examined at the same time. If patients underwent synchronous surgery, ICSI with fresh sperm for conception was used. If patients underwent asynchronous surgery, the sperm will be frozen and stored, and the woman would undergo controlled ovulation and use thawed sperm for ICSI later.

Definitions

The number of 2PN zygotes among all mature metaphase II (MII) stage oocytes was used to calculate the 2PN fertilization rate. Between the fresh embryo transfer cycle and the frozen–thawed embryo transfer cycle, clinical pregnancy was defined as a rising serum human chorionic gonadotrophin (hCG) level at least 12 days after embryo transfer. The discovery of a gestational sac on ultrasound scan during week 5 following transfer indicated clinical pregnancy. The number of abortion cycles was divided by the number of clinical pregnancy cycles to arrive at the abortion rate. The numbers of recovered oocytes and MII oocytes, fertilization rate, and good-quality embryo rate were all used to assess embryonic progress. Pregnancy rate, live birth rate, abortion rate, and numbers of birth abnormalities were among the clinical outcomes.

Table 1. Characteristics of synchronous group and asynchronous group

Characteristics	Synchronous group	Asynchronous group	*P*
	(*n* = 93)	(*n* = 28)	
Age (year)			
Female	29.54 ± 3.5	29.8 ± 3.4	0.772
Male	30.7 ± 4.7	30.9 ± 4.6	0.684

Data are presented as mean ± SD.
P, synchronous group versus asynchronous group.

Statistics

We used IBM SPSS Statistics version 16.0 (IBM Corp., Armonk, NY, USA) for the relevant statistical work. The data were subjected to descriptive statistical analysis [mean, standard error of the mean (SEM)]. Chi-squared test was performed to determine if there was a difference in SRR and sperm utilization rate, as well as the clinical outcomes including fertilization rate, high-quality embryo rate, clinical pregnancy rate, abortion rate, and cumulative live birth rate, between the two surgical groups. To ascertain if there was a difference in age between the two surgery groups, a one-way analysis of variance (ANOVA) was used. A P-value < 0.05 indicated a statistical difference.

Results

In total, 181 patients who had a AZFc deletion underwent micro-TESE, with 122 of them finding sperm with an overall SRR of 67.4% (122/181). In 133 cases of synchronous operation, the SRR was 69.9% (93/133), and in 47 cases of asynchronous operation, the SRR was 59.6% (28/47), with sperm identified successfully in one patient with a thawed egg cycle. There was no statistically significant difference in age between the two groups for males and females (Table 1).

One of the patients who received testicular sperm through synchronous surgery had poor sperm quality and was unable to undertake ICSI. The remaining 92 patients had fresh testicular sperm–ICSI-assisted conception with 98.9% sperm utilization rate with, in total, 40 fresh and 44 thawed cycles implanted, leading in 28 live births and 14 healthy male and 17 healthy female infants. There were no discernible changes between the two groups when we compared the oocytes, MII oocytes, 2PN fertilization rate, and good-quality embryo rate (Table 2).

In the 28 patients who successfully acquired testicular sperm using asynchronous surgery, 14 failed to use testicular sperm for later conception, and 14 had ICSI for conception after thawing sperm, with 50% sperm utilization rate. In total, eight fresh and three thawed transplantation cycles were performed, resulting in one live birth of one infant and a cumulative live birth rate of 7.1% (1/14). We followed up on the clinical treatment outcomes of the 28 patients who found sperm intraoperatively because of the lower sperm utilization rate in the asynchronous group.

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Table 2. Embryonic development in synchronous group and asynchronous group

Parameters	Synchronous group	Asynchronous group	*P*
	($n = 93$)	($n = 28$)	
Cycles (*n*)	92	14	
Oocytes (*n*)	16.5 ± 7.9	12.0 ± 4.3	0.056
MII oocytes (*n*)	13.2 ± 6.7	9.5 ± 4.4	0.131
2PN fertilization rate (%)	30.6% (372//1216)	33.8% (45/133)	0.442
Good-quality embryo rate (%)	47.6% (257/540)	40.7% (22/54)	0.336

Data are presented as mean ± SD.
P, synchronous group versus asynchronous group.
2PN fertilization rate, two pronuclei fertilization rate; MII oocytes, metaphase II oocytes; SD, standard deviation.

In total, 11 patients who chose synchronization following asynchronized surgery were discovered intraoperatively and treated with fresh sperm–ICSI. With a cumulative live birth rate of 27.3%, three cases were successfully conceived and one boy and two girls were delivered (Figure 1).

The SRR was 69.9% (93/133) vs. 59.6% (28/47), with no significant difference between the two groups, whereas the sperm utilization rate was 98.9% (92/93) vs. 50.0% (14/28), with the asynchronous group being significantly lower than the synchronous surgery group.

We compared the clinical outcomes of ICSI-assisted conception in patients who obtained sperm during their first micro-TESE, using 92 ICSI cycles and fresh sperm in 92 patients who underwent the simultaneous procedure and 14 ICSI cycles using thawed sperm in 14 patients who underwent the asynchronous operation in patients who successfully obtained sperm during their first micro-TESE. In patients who underwent synchronous versus asynchronous procedures, the fertilization rate, high-quality embryo rate, clinical pregnancy rate in fresh transfer cycles, abortion rate, and cumulative live birth rate were 30.6% vs 33.8%, 33.8% vs 40.7%, 40.0% vs 12.5%, and 30.4% vs 7.1%, respectively, with no significant differences between the two groups (Table 3).

Discussion

The *AZF* gene is divided into three regions (AZFa, AZFb and AZFc) on the long arm of the Y chromosome. The majority of patients with AZFa or AZFb deletion were diagnosed with NOA (Rabinowitz *et al.*, 2021). SRR using testicular sperm retrieval is exceedingly low in patients with NOA who have AZFa or AZFb deletions, making natural conception or ICSI impossible. In this situation, invasive testicular sperm retrieval is not indicated, and donor-assisted reproduction or adoption is recommended. Because the AZFc region of the Y chromosome contains many amplicons and palindromic sequences and is especially susceptible to structural rearrangements by non-homologous recombination, deletions are more likely to occur there, accounting for 57% of all AZF deletions, and being the most common type of AZF deletion (Nailwal and Chauhan, 2017). The clinical symptoms of AZFc deletion are highly heterogeneous, with some case reports of natural conception and fertility (Deng *et al.*, 2022). However, most patients with AZFc deletion have some degree of testicular spermatogenic dysfunction, with semen analysis revealing azoospermia or oligospermia, leading to male infertility. They may, however, have the option of conceiving biological children using sperm from the testes for ICSI-assisted conception (Zhou *et al.*, 2021).

The results of the current study showed that the SRR for testicular sperm extraction in NOA patients with AZFc deletion ranged from 13% to 100%, with a mean of 47% in 32 studies (Yuen *et al.*, 2021). Micro-TESE, of course, has a larger SRR than standard testicular sperm extraction, however SRR varies from centre to centre and surgeon to surgeon. There are three important reasons to think about. The first reason is the difference in patient selection. The spermatogenic function of the testes varies greatly among patient groups due to the vast range of clinical symptoms following AZFc deletion, from hypospermatogenic to Sertoli cell only syndrome. To avoid the damage during the operation, our Center conducted several careful semen examinations and analyses before operation and, for clear NOA, we also routinely performed another masturbation for sperm extraction before the operation, and cancelled micro-TESE if usable sperm were found in the semen. The second reason could be the surgeon's lack of clinical experience. If experience is insufficient or the surgery time is too short, the area with spermatogenic function may be missed. Microscopic sperm extraction surgery requires probing the entire testicular tissue under microscope magnification, and the end of the surgery is marked by finding enough usable sperm or by probing the entire testicular tissue. The third reason is that the laboratory staff have little experience of searching for sperm intraoperatively. To improve efficiency and ensure quality, the better spermatogenic tubules obtained intraoperatively must be physically crushed and carefully searched under an inverted microscope for mature sperm. Our Center routinely assigns two senior laboratory specialists to be responsible for intraoperative spermatogenic tubule crushing and sperm searching. There were 181 cases of full NOA in this study, with a sperm acquisition rate of 67.4% (122/181), which is a high rate.

The effect of testicular pathology on the efficacy of subsequent micro-TESE in individuals with AZFc deletion and the requirement for preoperative diagnostic testicular aspiration biopsy in patients with AZFc deletion remain inconclusive. Sertoli cell only syndrome accounted for 46% of testicular pathology in 178 AZFc deletion patients from 19 studies, inhibited maturation for 38%, and hypo-spermatogenesis for 16%, according to a systematic review of testicular pathology in 178 AZFc deletion patients from 19 studies (Yuen *et al.*, 2021). However, spermatogenic function in the testis of AZFc deletion patients is highly heterogeneous, and the testis may contain three pathological tissue types at the same time, so a simple testicular pathological examination is hardly representative of the actual spermatogenic condition of the entire testis. In our study, SRR was 67.4% in 181 individuals with AZFc deletion NOA, which is nearly twice as high as that of traditional sperm retrieval via testicular puncture (Bernie *et al.*, 2015). Given the limited predictive value of testicular biopsy pathology for subsequent micro-TESE and the fact that patients who are unable to undergo conventional orchiectomy may still be able to undergo micro-TESE and find mature sperm for ICSI, we recommend direct micro-TESE for patients with NOA due to AZFc deletion to increase the likelihood of finding sperm intraoperatively and obtaining a sufficient quantity and quality of sperm for ICSI, as well as to avoid additional damage to the testicular tissue caused by testicular biopsy.

This study focused on NOA patients with AZFc deletion. Only 14 cases used thawed sperm–ICSI to help conception, and one case

Table 3. Pregnancy outcomes of synchronous group and asynchronous group

Outcome	Synchronous group	Asynchronous group	*P*
	(*n* = 93)	(*n* = 28)	
Cycles (*n*)	92	14	
Clinical pregnancy rate, % (*n*/total)			
Fresh embryos transfer cycle	40.0% (16/40)	12.5% (1/8)	0.138
Frozen–thawed embryo transfer cycle	31.8% (14/44)	66.7% (2/3)	0.218
Live birth rate, % (*n*/total)	30.4% (28/92)	7.1% (1/14)	0.069
Abortion rate, % (*n*/total)	6.7% (2/30)	66.7% (2/3)	0.002*
Birth defects (*n*)	0	0	

Data are presented as mean ± SD.
P, synchronous group versus asynchronous group.
*$P < 0.05$, synchronous group versus asynchronous group.

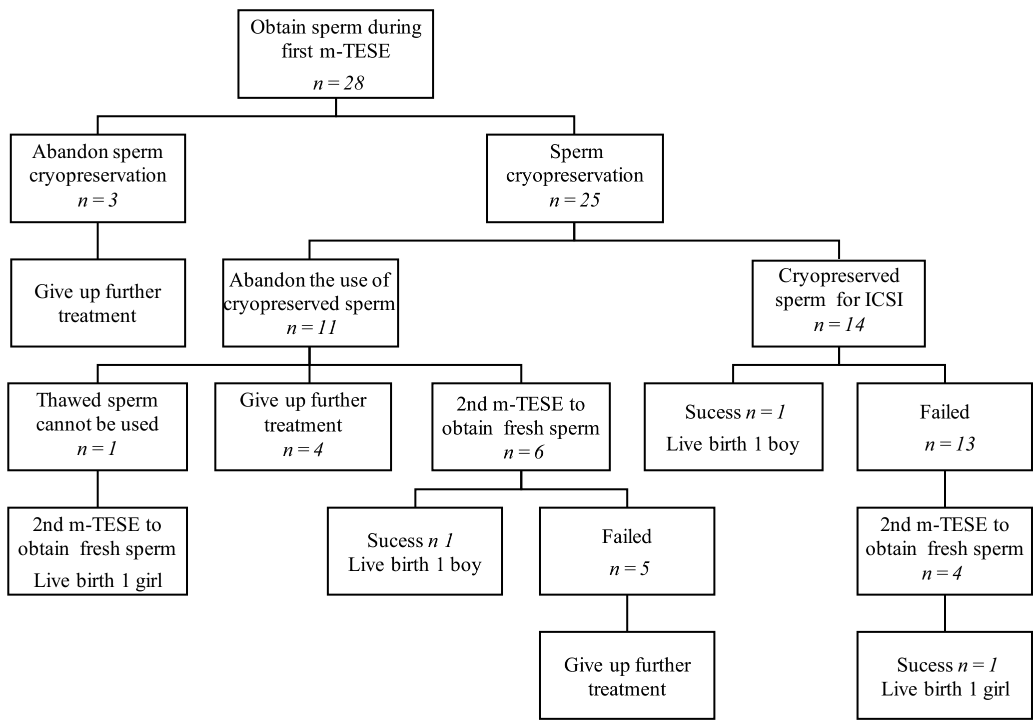

Figure 1. Flow chart of the synchronous versus asynchronous surgery research.

successfully produced a child. Of the 28 patients whose sperm were successfully found during the first micro-TESE procedure, 10.7% (3/28) abandoned sperm freezing due to poor sperm, whereas 36% (9/25) of patients with frozen sperm abandoned the use of frozen sperm. Sperm were successfully discovered intraoperatively in 11 of the 11 patients who chose a second synchronous operation using fresh sperm–ICSI, and conception was successful in three of them. As a result, we advocate the same walk-in method for NOA patients with AZFc deletion to minimize the negative consequences of sperm freezing on later treatment (Liu and Li, 2020).

Several studies have been carried out to see if ICSI for male infertility in AZFc-deficient patients affects embryo development, abortion rate and cumulative live birth rate (Zhang *et al.*, 2018). Our Center conducted a study comparing the clinical outcomes of patients with oligospermia due to AZFc deletion and patients without genetic deletion who underwent ART for pregnancy, and the results revealed no significant differences between the two groups in terms of good embryo rates, clinical pregnancy rate, ectopic pregnancy rate, abortion rate and preterm birth rate, implying that genetic deletion in patients with oligospermia due to AZFc deletion was not associated (Liu *et al.*, 2013). Another meta-analysis study compared the effects of different sources of sperm on treatment outcome in male infertility patients who had AZFc deletion and found no significant difference in clinical pregnancy rate, abortion rate and cumulative live birth rate in male infertility patients with AZFc deletion, indicating that for patients with oligospermia due to AZFc deletion, ICSI using semen sperm is advised as a first-line treatment.

AZFc deletion is inherited paternally in a Y-linked manner. As the AZFc region has the highest frequency of microdeletions and duplications and contains the most palindromic structures, AZFc deletion accounts for at least 80% of all Y-chromosomal deletions (Navarro-Costa *et al.*, 2010). Using non-allelic homologous recombination (NARH), these palindromic sequences can be paired and converted. They can also lead to structural variations in the Y chromosome, such as inversions and deletions. The four

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subtypes of the AZFc deletion – b2/b4, b1/b3, b2/b3, and gr/gr – most commonly manifest as male infertility; as a result, the deletion is frequently a *de novo* deletion. Although azoospermic or oligozoospermic patients with AZFc deletion predominate, there is still the potential to obtain offspring using ICSI and pass on the AZFc deletion vertically to the male offspring. Silber and Repping (2002) reported that vertical inheritance of AZFc deletion may have some unintended consequences for male offspring (e.g. Deleted-in-azoospermia mutation) and increase the risk of cystic fibrosis if couples are not tested beforehand. Several case studies have reported the direct natural reproduction-based vertical transmission of Y chromosome microdeletions from fathers to sons (Luddi *et al.*, 2009; Plotton *et al.*, 2010; Pan *et al.*, 2018). Chang *et al.* (1999) reported that fathers with AZFc deletion naturally gave birth to four infertile sons who inherited the AZFc deletion with an increased deletion range, suggesting that increased AZFc deletion range in male offspring may lead to infertility. However, it has also been shown that the range and type of deletions in male offspring did not expand or change (Oates *et al.*, 2002).

To avoid vertical transmission, we can also consider preimplantation genetic testing (PGT) to select female embryos (Minhas *et al.*, 2021). However, the adoption of PGT can impose an additional financial burden on the patient couple, and the abandonment of genetically defective male embryos can also affect, to some extent, the eventual conception rate of infertile couples. We can also think about PGT to choose female embryos for transfer that do not possess the damaged Y chromosome, to prevent vertical transmission. In this study, there were 99 infertile couples with an AZFc deletion who underwent ICSI using testicular sperm for conception. We provided genetic counselling and informed them of the risk of the vertical transmission of the AZFc deletion. They all underwent PGT to select female embryos.

There is a higher chance of successfully obtaining sperm in the testis through micro-TESE and producing their own offspring in combination with ICSI for patients with male infertility due to AZFc deletion. Synchronous surgical strategies using fresh sperm for ICSI is recommended, with better sperm utilization and eventual cumulative live birth rate.

Author contributions. MJM gathered clinical data, analyzed it, and authored the initial draft of the paper. DCY wrote the article's manuscript and managed its translation and retouching. The surgical surgery and data collection were carried out by ZLM, LDF, LHC, ZZ, YYZ, ZHT, HK, and LR. The study was co-designed by MJM and JH. All authors read and approved the final manuscript.

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Conflict of interest. No conflicts of interest are declared.

Ethical standards. Not applicable.

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