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Microsurgical testicular sperm extraction for testicular failure: the South African experience and first successful pregnancy

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Background: In men with non-obstructive azoospermia (NOA), biological fatherhood is only possibly by specialised microsurgical sperm retrieval techniques (micro-TESE), only recently introduced to South Africa. This study aimed to analyse the spectrum of causes of NOA and the outcomes of micro-TESE, including live births, following the use of this technique in South Africa.
Methods: This was a retrospective review of all micro-TESE cases performed in South Africa by a single surgeon from 2014 to 2018. Data collected prospectively included: patient demographics, preoperative blood results, cause of azoospermia, intraoperative findings and postoperative complications. The primary outcome measured was surgical success of micro-TESE, which was defined as testicular sperm successfully retrieved and cryopreserved. Subsequent live births from assisted reproductive technology (ART) using the cryopreserved sperm were also documented.
Results: Twenty-six men with NOA underwent micro-TESE between May 2014 and April 2018. Mean preoperative total testosterone level was 12.0 nmol/l (IQR 5.2) and follicle-stimulating hormone level 23.5 IU/l (IQR 15.6). Genetic testing was performed as part of the preoperative work-up in only 10 of the 26 patients. A specific cause of NOA was identified in 9 of the 26 patients and included Klinefelter syndrome (1 patient), Y-chromosome AZFc microdeletion (1 patient), undescended testicles (5 patients) and chemotherapy (2 patients). The average testicular volume was 9.05 ml (IQR 5.6), and the mean duration of surgery 95.8 minutes (IQR 28.0). The overall sperm retrieval rate was 34.6%. A single pregnancy and subsequent live birth were recorded from a total of eight cycles of intracytoplasmic sperm injection (ICSI): four female partners had one ICSI cycle each and two females underwent two cycles each. Frozen and thawed sperm was used in seven of the ICSI cycles and fresh sperm in one cycle.
Conclusion: In this South African series, sperm retrieval rates of micro-TESE for non-obstructive azoospermia were comparable to those reported internationally. Preoperative genetic testing should be increased to optimise the selection of surgical candidates.
Keywords: microsurgical testicular sperm extraction, testicular failure, first successful pregnancy

Introduction

Azoospermia due to severely impaired or absent sperm production (non-obstructive azoospermia/NOA) affects approximately 1% of the male population and 15% of men who seek fertility evaluation.[1] The most common causes of NOA are cryptorchidism, varicocele, epididymitis, mumps, testis torsion, chemotherapy and genetic abnormalities (Klinefelter syndrome, Y-chromosome microdeletions).

Until the late 1990s, no treatment was available for men with NOA and biological fatherhood was not a possibility. These patients were often referred to as being 'sterile' or having testicular failure. The only way for these couples to have children was through adoption or the use of donor sperm. However, over the last three decades, several innovations have changed the approach to NOA and its management.[2]

Jow et al.[3] reported in 1993 that direct evaluation of testis biopsy specimens in men with NOA often demonstrated the presence of sperm in isolated parts of the testicular parenchyma despite a background of severe defects in spermatogenesis. This observation was interpreted to mean that a low level of sperm production may be present in some areas of the testes of men with NOA, but that these very low sperm numbers did not survive epididymal transit and ejaculation.

Silber et al.[4] quantified this further: in men with severe oligozoospermia more than three mature spermatids per seminiferous tubule could be found in the testes. This was seen as merely a quantitative variant of azoospermia where the amount of spermatogenesis was not necessarily zero but simply below the threshold of three mature spermatids per tubule required for the sperm to 'spill over' into the ejaculate.[4] Silber described minute 'islands' or clusters of spermatogenesis in the testes of a reasonable proportion of so-called azoospermic men, confirming that these patients were actually not absolutely azoospermic.

Based on these observations, investigators then moved in men with NOA to perform assisted reproductive technology (ART) procedures using sperm from testicular sperm extraction (TESE) for intracytoplasmic sperm injection (ICSI) into the ova that resulted in successful live births. In 1999, Schlegel[5] described microdissection testicular sperm extraction (micro-TESE): a procedure which proved to dramatically increase the success rates of obtaining sperm from the testes of men with NOA.

In Africa, the burden of male infertility is higher than many developed nations but access to appropriate treatment is limited.[6] In June 2014, after undergoing training in male infertility microsurgery abroad, the author (AZ) started performing micro-TESE in South Africa.

The aim of this study was to analyse the South African experience with micro-TESE and to compare the primary outcome of successful sperm retrieval to international standards.

Methods

This was a retrospective review of all micro-TESE cases performed in South Africa by a single surgeon from 2014 to 2018. Data collected prospectively included patient demographics, preoperative blood results, cause of azoospermia, intraoperative findings and postoperative complications. The primary outcome measured was surgical success of micro-TESE, which was defined as testicular sperm successfully retrieved and cryopreserved. Subsequent live births from ART using fresh or cryopreserved sperm were also documented.

Surgical technique

Procedures were performed on an outpatient basis with patients under general anaesthesia. Both a surgical microscope (6X to 40X magnification) and light microscope (400X magnification) were used for all cases. Surgical access was by means of a single vertical midline scrotal incision of ± 20 mm. Once delivered from the scrotum, the testicle was bivalved through an equatorial incision (Figure 1). The tunica albuginea of the testicle was opened using the surgical microscope and the seminiferous tubules of the entire testicular parenchyma were meticulously and systematically inspected for the presence of visibly dilated tubules (Figure 2) which are the ones likely to harbor islands of spermatogenesis. Single tubules considered to be dilated were removed (Figure 3).

The small amount of testicular tissue removed was placed in HEPES buffer, which acted as a sperm preservation medium. Processing of the biopsy material was performed in the operating room; the aim of processing is to liberate sperm from the seminiferous tubule and this was accomplished by manual disruption of the tubules in a petri dish using fine-tipped scissors. This was followed by fixing two drops of the mixture on a glass microscope slide and subsequent light microscopy to check for the presence of sperm. Retrieved sperm was transported to a fertility laboratory where it was either cryopreserved for ART at a later stage or alternatively used immediately for a cycle of ART/ICSI.

If no sperm was found initially, dissection of the contralateral testicle was also performed. At the end of the procedure the tunica albuginea of the testicle was closed using a running, non-absorbable suture and thereafter the scrotum was closed in two layers. No wound drains were used and all patients were discharged home on the same day.

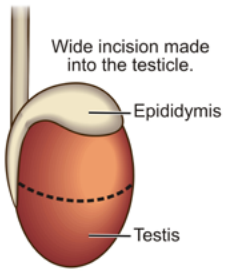

Figure 1: The testicle is bivalved using a transverse (equatorial) incision

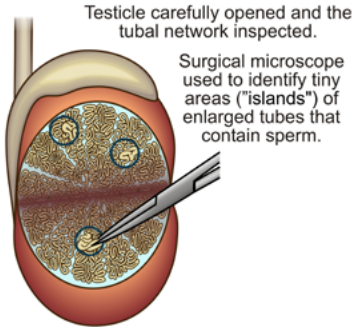

Figure 2: Inspecting seminiferous tubules to identify dilated tubules likely to harbour spermatogenesis

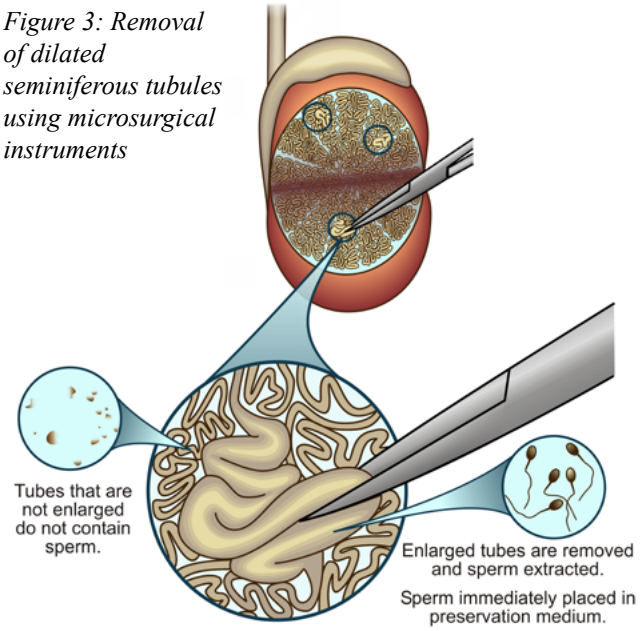

Figure 3: Removal of dilated seminiferous tubules using microsurgical instruments

Patients were contacted on day seven postoperatively to check for early complications. Invasive testicular surgery may cause long-term decline in testicular androgen production and for this reason patients were requested to have serum testosterone (TT) levels checked at six, 12 and 18 months post-procedure. Demographic and intraoperative data was prospectively collected and updated postoperatively.

Results

Twenty-six patients with NOA underwent micro-TESE, performed by a single surgeon, from May 2014 to April 2018. The average patient age at the time of surgery was 37.1 years (IQR 5.6). The mean body mass index (BMI) was 28.6 kg/m^2.

Six of the 26 patients gave a history of having mumps in childhood, but none were able to definitively deny or confirm secondary mumps orchitis. One patient had a history

of significant testicular trauma requiring hospitalisation. Two patients had a history of cancer and chemotherapy: one testicular cancer and one lymphoma; the time from chemotherapy to micro-TESE was 15 and 33 years, respectively. Five patients gave a history of undescended testicles (four bilateral, one unilateral). All but one had corrective surgery in childhood. Only one patient in this series had previously undergone micro-TESE.

Karyotyping was performed in eight of the 26 patients preoperatively: a normal chromosome complement was found in seven and one patient was diagnosed with 47 XXY Klinefelter syndrome. Six patients underwent testing for AZF microdeletions prior to surgery and one positive AZFc microdeletion was found. A specific cause of NOA was identified in nine of the 26 patients: five undescended testicle, one AZFc microdeletion, two chemotherapy, and one Klinefelter syndrome. The remaining 17 patients were considered to have idiopathic NOA.

Preoperative

Mean preoperative total testosterone (t-TT) level was 12.0 nmol/l (IQR 5.2; normal range 5.6–22.9 nmol/L) and follicle-stimulating hormone (FSH) level 23.5 IU/l (IQR 15.6; normal range 1.5–12.4 IU/L).

Fifteen of the 26 patients gave a history of previously undergoing a diagnostic conventional testis biopsy (TESE) but in only 10 of these were the histopathological reports available: Sertoli cell-only pattern (SCO) was reported in seven, diffuse maturation arrest in two and focal maturation arrest in one.

Intraoperative

The average testicular volume measured at the time of surgery was 9.05 ml (IQR 5.6; normal range 18.6 ± 4.8). The overall sperm retrieval rate (SRR) for the entire cohort of patients was 34.6% (9 of 26 patients). For the first 13 cases, the SRR was 23.1% (3/13), and for the last 13 cases, the SRR was 46.2% (6/13). Sperm was frozen in all the successful micro-TESE cases except for two, where the patients specifically requested fresh ICSI cycles. The average duration of surgery was 95.8 minutes (IQR 28.0).

Pregnancy outcome

A single pregnancy was recorded from a total of eight ICSI cycles: four female partners had one ICSI cycle each and two females underwent two cycles each. Frozen and thawed sperm was used in seven of the ICSI cycles and fresh sperm in one cycle. The patient in whom the pregnancy was achieved had a previous successful micro-TESE 12 months earlier but unsuccessful ICSI cycle using frozen and thawed sperm. He had SCO pattern on testicular histology, normal chromosomes, no AZF microdeletions and an FSH level of 30.3 IU/l. His preoperative testis sizes were 8.5 ml and 6.5 ml on the right and left, respectively.

The pregnancy resulted in a live birth of a healthy boy at term. This represents the first successful pregnancy and live birth in South Africa as a result of micro-TESE.

Discussion

This study represents the first data available in South Africa on the microsurgical treatment of patients with NOA. The causes of NOA were identified in nine of the 26 patients in the study. Other causes of NOA include varicocele, idiopathic hypogonadotropic hypogonadism, pituitary surgery, systemic disease, Kallmann syndrome, XX male and primary hypogonadism of unknown cause.[7] In 65% of patients in our study, a cause for NOA could not be identified, though in most reports, idiopathic causes make up only 30% of cases. Genetic testing (karyotyping and Y-chromosome microdeletion analysis) is recommended in all men with severe male factor infertility prior to micro-TESE.[8] In our study, only eight of the 26 patients had karyotyping, and six had Y-chromosome microdeletion analysis performed, mainly due to patients declining as a result of high costs associated with these tests.

Micro-TESE is an invasive procedure on the testicle that carries the risk of testicular compromise that may require lifelong testosterone supplementation postoperatively. Unfortunately, accurately predicting which patients are likely to have sperm successfully retrieved during micro-TESE is problematic. The following preoperative parameters have been investigated but have not proven to be reliable in predicting successful sperm retrieval during micro-TESE surgery:[9-12] age, testicular size, serum FSH levels, cryptorchidism. In the current study, the same trends were observed in these parameters and no significant differences demonstrated when comparing the patients who had successful sperm retrieval to those where no sperm was found.

Y-chromosome microdeletion analysis has been found to be a good predictor of successful micro-TESE. For men with even partial deletions of the AZFb region or complete deletions of AZFa, the chances of finding sperm are exceedingly rare and patients need to be counselled appropriately prior to proceeding with surgery.[13] On the other hand, patients with deletions involving the AZFc region actually have high sperm retrieval rates which can be seen as a good prognostic sign.[14] In our study, the single patient with an AZFc deletion did have successful sperm retrieval during micro-TESE. Ideally, all patients considering micro-TESE should undergo preoperative testing for Y-chromosome microdeletions.[7] The fact that testing for Y-chromosome microdeletions was performed in only 23% of the patients in this study is of concern. Unfortunately, this is a costly laboratory test to perform in the South African setting. As neither micro-TESE nor the subsequent ICSI are covered by medical insurance, most patients do not go ahead with Y-chromosome microdeletion testing for the sake of cost-saving – even though the findings of the test may significantly affect the management plan.

The histopathology of the diagnostic testis biopsy does have some value in predicting successful sperm retrieval. Cetinkaya et al.[15] demonstrated SRRs of 36%, 48.6%, and 95.5% for SCO syndrome, maturation arrest, and hypospermatogenesis groups, respectively. In our series, the SRR for the patients with SCO histology was 42.8%. Maturation arrest was present in three of our patients: one focal and two diffuse. Sperm was found in the patient with focal maturation arrest.

Men with post-chemotherapy NOA represent a challenging group for micro-TESE as their SRRs are low.[14,16] Two patients in our study had chemotherapy-induced azoospermia and one of them (previous lymphoma) had successful sperm retrieval. The patient with a history of chemotherapy for testicular cancer had a single testis only and failed micro-TESE.

There is no consensus regarding the use of fresh versus frozen sperm for ICSI after micro-TESE. The main benefit of using fresh sperm is that the sperm are not subject to the stresses of the freeze-thaw cycle, which has been shown to substantially decrease the yield of viable sperm for ICSI.[17] On the other hand, when planning to use fresh sperm obtained from micro-TESE for an ICSI cycle, ovarian hyperstimulation will be required in all cases. This is costly considering that there is at best a 60% chance of sperm retrieval. Furthermore, in the absence of sperm freezing, subsequent ICSI cycles may only be possible after a 6-month waiting period for testicular recovery before a repeat micro-TESE procedure to obtain fresh sperm.[18] Lastly, the logistics of coordinating the female cycle, surgical procedure on the male and surgeon's schedule can also be a challenge – even more so for the large proportion of patients travelling to main centres (in our series, 31% of the patients travelled from other countries) for treatment. In our study, a fresh cycle was performed in only one of the 26 patients, at the patient's specific request and resulted in a successful pregnancy and live birth. In general, fresh and frozen ICSI cycles following micro-TESE have been shown to have equivalent pregnancy rates.[19,20]

Overall, SRRs reported in the literature vary widely and range from 30–60%.[5,11,21-24] In our study, the overall SRR was 34.6%.

Despite successful sperm retrieved during micro-TESE, not all men will become biological fathers. Dabaja and Schelegel reported the following live birth rates after micro-TESE and subsequent ICSI in their high-volume centre:[14] Klinefelter syndrome 25.8%, post-chemotherapy 19.3%, and cryptorchidism 12.2%, from 155, 114, 181 procedures respectively. Results reported by authors outside of the United States are similar.[2] In this study, sperm was available in eight patients with two patients not having undergone ICSI at the time of publication. In the remaining six patients, a total of eight ICSI cycles were performed, resulting in a single pregnancy and live birth. The fact that only a single live birth was achieved from 26 micro-TESE procedures in our series is of concern. One factor that likely contributes is the learning curve of this specialised microsurgical dissection of the testicle. In the present study, the SRR for the first 13 micro-TESE cases was 23.1% (3/13) and for the last 13 cases 46.2% (6/13). Due to the novel nature of this procedure in South Africa, a learning curve is also to be expected with regards to laboratory preparation and processing of the biopsy material, though these factors were not explored as part of the present study.

Follow-up in this study was available in 19 of the 26 patients. After undergoing micro-TESE, most patients will have a significant (though usually transient) decrease in serum testosterone levels, which may take 12 months or longer to recover.[25] In our series, more than 30% of the patients had a serum TT level lower than the laboratory reference range prior to micro-TESE. Since many men have low or borderline TT levels preoperatively, the effect of surgery on TT level is of concern postoperatively and should be discussed with the patient.

Conclusion

In this South African series, the SRRs of micro-TESE for NOA were comparable to those reported internationally. Preoperative genetic testing should be increased to optimise the selection of surgical candidates. The low live birth rate following micro-TESE in South Africa is likely related to a learning curve associated with this relatively new treatment modality.

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Conflict of interest

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Ethical approval

Ethical approval was obtained from Stellenbosch University Health Research Ethics Committee (HREC), HREC Reference #: S18/01/006

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