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¹Urology Clinic, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina

²Bahçeci BIH IVF Center, Sarajevo, Sarajevo, Bosnia and Herzegovina

³Department of Physiology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

⁴Clinic for Anesthesia and Resuscitation, Clinical Center Sarajevo, Bosnia and Herzegovina

Corresponding author: Jasmin Alic, MD. Urology Clinic, Clinical Center University of Sarajevo, Bolnicka 25, 71 000 Sarajevo, Bosnia and Herzegovina. Tel. 00 387 61 390 200. Tel./Fax. 00 387 33 297 130. E-mail: jasmin.alic@gmail.com. ORCID ID: <http://www.orcid.org/0000-0002-7435-1810>.

"Second-look" Micro Testicular Sperm Extraction (MicroTESE) in Patients with Non-obstructive Azoospermia Following Histopathological Analysis

Hajrudin Spahovic¹, Jasmin Alic¹, Ümit Göktolga², Zahid Lepara¹, Orhan Lepara³, Admir Rama², Ismet Suljevic⁴

ABSTRACT

Introduction: Microdissection testicular sperm extraction (microTESE) is considered the gold standard method for surgical sperm retrieval among patients with non-obstructive azoospermia (NOA). **Aim:** This study aimed to evaluate the correlation between histopathological findings after failed microTESE procedure and outcomes of the „second-look“ procedure and to provide insight into the most common histopathological patterns after testicular biopsy within our population. **Methods:** The retrospective study included 33 selected patients with NOA, who had undergone unsuccessful sperm retrieval. The diagnosis of NOA was made after the assessment of the patient's history data, a physical examination, semen analysis, the hormonal profile, and genetic studies. After negative sperm retrieval, histopathological report has been analyzed for „second-look“ microTESE attempt. **Results:** Five testicular histopathological patterns were found: hypospermatogenesis (9,1%), Sertoli cell-only syndrome (43%), germ cell maturation arrest (15%), seminiferous tubule hyalinization (15%), mixed pattern (21%). Y-microdeletions were detected in 5 patients, of which 3 patients showed AZFc region deletions. Only 3 patients (9,1%) underwent a „second-look“ procedure after the evaluation of histopathological reports. After the stimulation therapy and „second-look“ procedure, we had a positive outcome in a single patient (33,3%). Mean FSH value in patients with confirmed spermatogenesis was 17.26 ± 3.11 IU/l, while mean FSH value in patients without presence or germ cell statistically significantly exceeded and was 24.28 ± 4.71 IU/L ($p=0.038$). **Conclusion:** Histopathological reports following the microTESE procedure are obligatory for the proper selection of patients who are candidates for the „second-look“ microTESE attempt. Patients with Sertoli cell-only syndrome and hypospermatogenesis particularly can benefit from the „second-look“ procedure.

Keywords: Azoospermia, nonobstructive; sperm retrieval; microdissection testicular sperm extraction (microTESE); „second-look“ microTESE; male infertility.

1. INTRODUCTION

Infertility is a major health problem and affects more than 50 million couples worldwide (1). Azoospermia is present in 10-15% of all infertility cases and affects about 1% of the men in the general population (2). It is defined as the absence of spermatozoa in the ejaculate following two separate semen analyses (3). Unlike obstructive azoospermia (OA), in non-obstructive azoospermia (NOA), sperm production in the testis is reduced, which is typically associated with dysfunction along the hypothalamic-pituitary-testis axis (3, 4).

Clinical conditions associated with NOA include genetic abnormalities (Y-microdeletions, Klinefelter syndrome), congenital abnormalities (cryptorchidism), post-infectious (mumps orchitis), post-traumatic and surgery (vasectomy), exposure to toxins (chemotherapy, radiotherapy), and idiopathic causes. In contrast to OA, patients with NOA are usually present with high plasma gonadotrophins levels especially follicle-stimulating hormone (FSH) and low testosterone levels. Testicular volume tends to be significantly reduced and testicular consistency soft (1-4).

Microdissection testicular sperm extraction operative treatment (microTESE) is a standard procedure for the treatment of patients with NOA (3). Sperm retrieval is successful in up to 50% of men with NOA (5). Common evaluation of the patient includes a complete history, physical examination

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with ultrasound testicular volume determination, hormonal profile (FSH, LH, testosterone, prolactin, estrogen), and genetic analysis (karyotype and Y-microdeletion analysis).

After the diagnosis of NOA is made, preparation for microTESE with hormonal treatment follows in selected patients. Hormonal therapy often includes human chorionic gonadotropin (hCG), follicle-stimulating hormone (FSH), clomiphene, and anastrozole as treatment options (2).

Among other factors (testis size, FSH level, inhibin beta, genetic alterations), testicular histopathology has been found to be the most reliable predictor of successful sperm retrieval in NOA patients (7-12). Analysis of these findings can provide precious pieces of information when considering the „second-look“ microTESE procedure.

Testicular biopsy specimen from NOA shows different histopathological patterns. Normal spermatogenesis, hypospermatogenesis, germ cell maturation arrest (GSMA), Sertoli cells-only syndrome (SCOS), seminiferous tubule hyalinization and fibrosis/atrophy can be seen in the histopathological reports. In hypospermatogenesis, a total number of germ cells is reduced but all stages of spermatogenesis are present. These patients may be azoospermic or oligozoospermic. In maturation arrest, primary spermatocytes or late spermatids are seen. In SCOS (germinal aplasia) only Sertoli cells line is present in seminiferous tubules.

This condition is characterized by small testicular volumes and high FSH levels. Seminiferous tubule hyalinization is characterized by extensive intratubular and peritubular hyalinization with an absence of germ cells (13-22).

2. AIM

This study aimed to evaluate the correlation between histopathological findings after failed microTESE procedure and outcomes of the „second-look“ procedure. Also, this study should provide insight into the most common histopathological patterns after testicular biopsy within our population.

3. MATERIAL AND METHODS

Patient's population

From all the amount of microTESE procedures (59 patients) in a single IVF center, evaluation comprised 33 selected patients (56%) with non-obstructive azoospermia (NOA), who had undergone unsuccessful sperm retrieval.

Diagnosis of azoospermia was confirmed by analysis of 2 different centrifuged semen samples according to WHO criteria. Patients with obstructive azoospermia were excluded, as well as patients with complete AZFa or AZFb microdeletions.

Samples were collected after obtaining informed consent from all individuals and all procedures were performed in accordance with the applicable ethical standards.

Patient's evaluation

The diagnosis of NOA was made after the assessment of the patient's history data, a physical examination, semen analysis, hormonal profile, and genetic studies.

The analyzed history data included age, weight and height, smoking and drinking habits, and history of trauma, cryptorchidism, varicocele, orchitis, environmental, radiation and chemotherapy exposure, prescribed drug use, and previous surgical procedures. All patients underwent a physical examination and scrotal color Doppler ultrasound scan to measure testicular volume and to exclude the presence of epididymis or/and vas deferens morphological abnormalities or/and varicocele.

Laboratory and genetic assessment

The hormonal profile included serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), prolactin (P), and estrogen (E). Genetic studies included karyotype and analysis of Y-microdeletions (AZF a, b or c).

Surgical procedure

MicroTESE procedure was performed under general anesthesia as it was previously described by Schlegel (23, 24). The procedure began on the testis with larger volume or on the right one if there was no difference between the two testicles. After a single incision is made through the median raphe of the scrotum, the testis is delivered and the vaginal layer of the tunica albuginea is opened. Testicular parenchyma is observed at $\times 20$ and $\times 40$ magnification to locate and collect the wider seminiferous tubules which are more likely to contain areas of active spermatogenesis. If sperm could not be found, the contralateral testis was examined. The procedure is terminated when sperm is retrieved or when further dissection is no longer technically possible. Successful retrievals were defined as the detection of sperm (25). A testicular tissue sample was fixed in Bouin's solution for histopathological examination. The incision in the tunica albuginea was sutured with an absorbable 5-0 suture together with the scrotal layers.

Histopathological assessment

Histopathological analysis of biopsy samples was performed by a single pathologist. After the microTESE procedure was done, both side testicular biopsy was performed. Tissue samples were stained with hematoxylin and eosin (HE) and histological examination by light microscopy was performed. The evaluation included presence or absence of seminiferous tubule (size and number), basement membrane thickening and tubular hyalinization, number of germ, Sertoli and Leydig cells. Based on the main morphological pattern, the testicular pathohistological findings were categorized as follows: hypospermatogenesis, germ cell maturation arrest (GCMA), Sertoli-cell only syndrome (SCOS), seminiferous tubule hyalinization and mixed pattern. The mixed pattern is presented as more than one different pathological pattern seen in the testicular specimen.

Statistical analysis

Data were provided as a median and interquartile range, and the Shapiro-Wilk test was used for the data distribution analysis. Data were analyzed with Student

t-test, including age and hormone levels. P values <0.05 were considered statistically significant. Statistical analysis was performed using IBM SPSS 23.0 statistical software system (IBM Corporation, Chicago, Illinois).

4. RESULTS

We have evaluated 33 selected cases of sperm retrieval with negative microTESE operative outcomes (100%). Histopathological testicular tissue analysis was performed in a total of 33 patients (n=33). In 3 cases (9.1%) spermatogenesis cells were found, while in 30 (90.9%) patients the sample was free of germ cells. After applying the squared test, a statistically significant difference was established ($\chi^2 = 22.091$; $p = 0.001$).

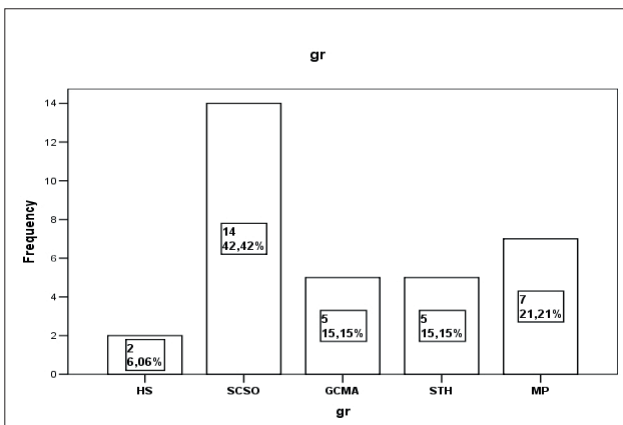


Figure 1. HS- hypospermatogenesis; SCSO–Sertoli cell; syndrome; GCMA–germ cell maturation arrest; STH–seminiferous tubule hyalinization; MP–mixed pattern

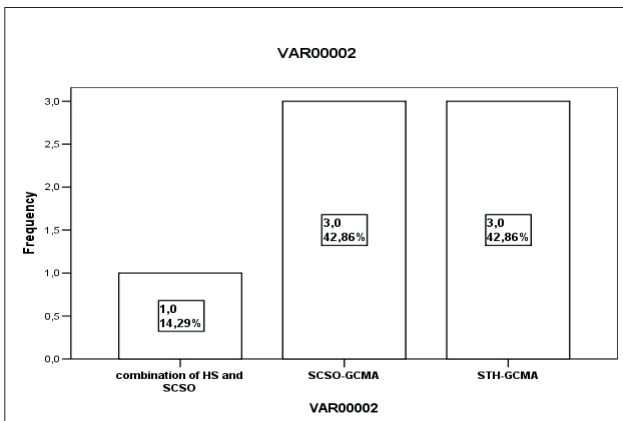


Figure 2. HS- hypospermatogenesis; SCSO–Sertoli cell; syndrome; GCMA–germ cell maturation arrest; STH–seminiferous tubule hyalinization; MP–mixed pattern

Individual histopathological findings were represented as follows: hypospermatogenesis in 2 patients (6%), Sertoli cell-only syndrome (SCSO) in 14 patients (43%), germ cell maturation arrest (GCMA) in 5 patients (15%), seminiferous tubule hyalinization in 5 patients (15%) and mixed pattern in 7 patients (21%) (Figure 1). In the mixed pattern group (7 patients, 100%), a single patient (14,2%) had a combination of hypospermatogenesis and SCSO, 3 patients (42,8%) had SCSO-GCMA, and 3 patients (42,8%) had seminiferous tubule hyalinization-GCMA (Figure 2). After obtaining the histopathological report and implementing the hormonal stimulating protocol,

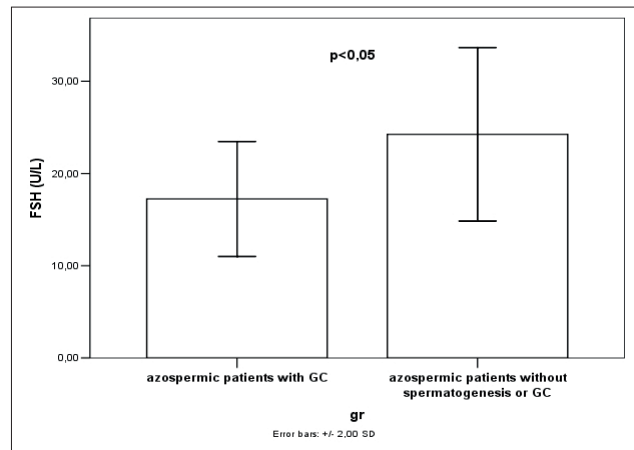


Figure 3. GC- germ cells, FSH - Follicle-stimulating hormone

we have performed a “second-look” microTESE procedure in 3 patients, 2 patients with hypospermatogenesis, and one patient with mixed pattern (hypospermatogenesis + SCSO). Only one patient (33,3%) had a positive outcome after the procedure.

Histopathological analysis following a “second-look” procedure confirmed Sertoli cell syndrome in 2 patients as a definitive report. Mean FSH value in azospermic patients with confirmed presence of germ cells in the pathological specimen, was 17.26 ± 3.11 IU/L, while mean FSH value in patients without spermatogenesis statistically significantly exceeded and was 24.28 ± 4.71 IU/L ($p=0.038$) (Figure 3).

There is no statistically significant difference in the average values of testosterone levels in the serum of patients with the presence of spermatogenesis cells in the cartilage test (14.81 ± 1.18 nmol/L), compared to the patients without spermatogenesis cells (13.90 ± 2.98 nmol/L, $p=0.511$). Mean LH and prolactin levels in patients with azoospermia showed no statistically significant difference in both groups of patients.

Ultrasound measurement of testicular volume showed lower testicular volume (less than 15 ml). The average volume of right and left testicle was 10,45 ml and 9,11 ml, respectively. All kariograms were 46 XY, and Y-deletions were detected in 5 patients (5%).

5. DISCUSSION

Infertility is defined as an inability to conceive after 12 months of regular unprotected intercourse. Non-obstructive azoospermia (NOA) remains a significant cause of male infertility and the most challenging problem in andrologist clinical practice. Most cases of NOA have a pretesticular or testicular cause (1).

Patients with small testicular size, normal or elevated FSH level, abnormal karyotype, and the presence of Y-chromosome microdeletions are highly suggestive for NOA. Schoor et al. determined that an FSH of less than 7.6 mIU/mL and testicular long axis greater than 4.6 cm predicts OA in 96% of cases, and conversely, an FSH of greater than 7.6 mIU/mL with a testicular long axis of less than 4.6 cm predicts NOA in 89% of cases (26). These patients should be offered sperm extraction reproductive treatment with therapeutic, rather than

diagnostic testicular biopsy, with cryopreservation for usage in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI) (3, 4, 26).

Since microTESE was first described by Schlegel in 1999, it has been a significant improvement over the conventional TESE procedure (23). It was accomplished by observing the heterogeneity amongst testicular seminiferous tubules and the possibility of identification of dilated seminiferous tubules that were more likely to contain areas of active spermatogenesis. By identifying and selectively removing only dilated seminiferous tubules, the sperm retrieval rate (SRR) increased from 16.7–45% to 42.9–63% (27).

So far, no reliable positive prognostic factors have been identified that guarantee sperm recovery in these patients. On the other side, the only reliable negative prognostic factor is the presence of Y-chromosome AZFa and AZFb microdeletions (28). Some predictors of SRRs that have been reported include preoperative FSH levels and testicular volume. Recent research has shown that increased FSH level and smaller testicular volume do not provide an adverse prognosis for sperm retrieval (1). However, testicular histopathology has been suggested to play a role as a predictor of SSR (7). Since testicular biopsy is no longer a recommended procedure in patients with NOA, these reports are usually obtained after the initial microTESE procedure. Therefore, histopathological reports may mainly be useful for predicting SSR in patients in whom microTESE has already failed (29).

Four testicular histopathological findings are common in patients with NOA. Hypospermatogenesis, which is the least severe form of NOA, produces the highest SRR of 73–100% while late maturation arrest has an SRR of 27–86%, early maturation arrest has an SRR of 27–40%, and Sertoli cell-only syndrome (SCOS), which is the most severe form of infertility, has an SRR of 22.5–41% (5, 27, 30, 31).

Hypospermatogenesis is defined as appears of strong or moderate germinal epithelium with all stages of germ cells (spermatogonia, spermatocytes, and spermatids) present but reduced in number (15). Clinically, hypospermatogenesis can be associated with hormonal dysregulation, congenital germ cell deficiency, androgen insensitivity, and exposure to chemicals, heat, and radiation (32). Our research showed a low rate of hypospermatogenesis which was identified in 3 (9,1%) patients only. This rate may indicate a proper and minute technique of microTESE procedure. Reports about hypospermatogenesis after the initial procedure are in the range from 16 to 25%, but these studies included all azoospermic patients with pre-testicular and post-testicular etiology. Al-Rayess and Al-Rikabi in their study of 230 testicular biopsy specimens showed a 13% incidence of hypospermatogenesis (18). Other studies showed variable results with higher incidence. Jamal et al., Abdullah et al., Jamali et al., Haddad et al., reported an incidence of 24%, 29%, 36.6%, 55.8%, respectively (15, 19, 21, 22).

Sertoli cell-only syndrome (SCOS) is presented by normal or slightly narrower tubules, which contain only

Sertoli and Leydig cells but no other cells involved in spermatogenesis (15). SCOS is an irreversible condition that can be associated with cryptorchidism, orchitis, post-radiation or chemotherapy, estrogen or androgen therapy, chronic hepatopathology, and structural abnormalities of the Y chromosome, especially AZFa (16,33). In our study, SCOS was found in 14 patients (43%). A similar high incidence of SCOS (39%) was also reported by Al-Rayess (18), while other studies report a lower incidence of 16–27% (15, 19, 20, 22).

Germ cell maturation arrest (GCMA) is defined as incomplete spermatogenesis in which germ cells fail to mature. It can distinguish between early or pre-meiotic GCMA, with the presence of spermatogonia or spermatocytes only, and late or post-meiotic GCMA, in which spermatids can be detected (16, 33). Condition is associated with genetic abnormalities and/or secondary causes such as post-chemotherapy, chronic alcohol or marijuana consumption, and hypogonadotropic hypogonadism (16). In our study, GCMA was found in 5 patients (15%), and similar rates are reported in other studies (12–28%) (15, 21). Jamal et al. and Haddad et al. reported low and very low incidences of 7% and 1,7%, respectively (19, 21).

Seminiferous tubule hyalinization is also known as the "end-stage testis" or "tubular sclerosis" and it is characterized by extensive intratubular and peritubular hyalinization with the absence of germ cells. Normal tubules are not prominent. This morphological pattern may be seen in adults with Klinefelter syndrome. Seminiferous tubule hyalinization was found in 5 patients (15%), and similar rates are confirmed by other authors (15, 19, 22), while the much lower incidence was reported by Al-Rayess and Al-Rikabi (18).

Heterogeneous and mixed pathological patterns are common in histopathological reports after negative sperm retrieval procedures (9, 12, 15). McLachlan et al. studied reports of 534 patients after the bilateral testicular biopsies and indicated the relative rarity of pure phenotypes and the high frequency of hypospermatogenesis and mixed patterns (20). In our study, we found 7 patients (21%) with a mixed pattern. Therefrom, a combination of hypospermatogenesis and SCOS was found in a single patient (14,2%), SCOS and GCMA was found in 3 patients (42,8%), and seminiferous tubule hyalinization with GCMA was identified in 3 patients (42,8%).

In the current series, we have made an evaluation of negative sperm retrieval cases, especially regarding the histopathological specimen results. In the group of 33 NOA patients with negative microTESE outcomes, only 3 patients (9,1%) underwent a „second-look“ procedure after the evaluation of histopathological reports. Histopathological analysis showed the same morphological pattern equivalent to hypospermatogenesis. In all 3 cases, FSH serum level was ≥ 25 IU/L, and hormonal stimulation therapy with FSH in a dose of 5000 IU once per week and menotrophin (Merional) in a dose of 75 IJ twice per week for 3 months, was applied. After the stimulation therapy and „second-look“ procedure, we had a positive result of microTESE procedure in a single

patient (33,3%). For other patients with confirmed germ-cell free azoospermia, we did not offer a „second-look“ procedure. Therapeutic testicular biopsy is still a standard investigation for testicular causes of infertility (3, 15).

In clinical practice, karyotyping and Y chromosome microdeletion screening are recommended by the latest guidelines and these analyses lead to a diagnosis in more than 15% of cases (33). The prevalence of Y chromosome microdeletion ranges from 7 to 8% (34). Three various microdeletions have been identified: AZFa, AZFb, and AZFc (azoospermia factor a, b and c). AZFc deletions are most common (65-70%), followed by AZFb and AZFb+c or AZFa+b+c (25-30%), while AZFa deletions are rare (5%). The complete AZFa deletions lead to SCOS, while AZFb deletions lead to SCOS or early GCMA histopathological patterns. Although the AZFc phenotype is highly variable, the SRR for these patients ranges between 50-60%. The complete deletion of AZFa and/or AZFb are currently contraindications for microTESE since no reports of successful sperm retrieval have been described in those patients (33-35). In the current series, all karyograms were 46 XY and Y-microdeletions were detected in 5 patients, of which 3 patients showed AZFc region deletions.

It is certain that a single failed attempt of sperm retrieval cannot exclude the possibility of an existing area of spermatogenesis in the testes (23, 24). The results show that histopathological diagnosis of hypospermatogenesis alone or in the mixed pattern can and must be considered in the evaluation for the „second-look“ microTESE procedure. Considering the time that is required for recovery of the limited sperm production, at least 6–12 months should be allowed after microTESE, before considering the „second-look“ microTESE procedure (1). All procedures should be done in the IVF center with the possibility of specimen cryopreservation (36).

One of the strengths of the current study is that the histopathological analysis of all samples was performed by the same highly-experienced uropathologist. The limitation of our study is a relatively small sample of patients, with only 3 patients who underwent secondary sperm retrieval procedure. However, this is the first study of this kind in our region, and it can provide insight into histopathological patterns in the general population when it comes to failed sperm retrieval.

6. CONCLUSION

Histopathological reports following the initial microTESE procedure are appropriate and obligatory for the proper evaluation of the procedure outcomes. The evaluation of these reports helps in the selection of patients who are candidates for the „second-look“ microTESE attempt after the initial failure. Our research suggests that the most common histopathological findings in these patients are Sertoli cell-only syndrome (SCSO) and hypospermatogenesis. These patients in particular can benefit from the “second-look” procedure following the initial histopathological analysis. However, further

research involving studies with a higher sample is required.

- **Declaration of patient consent:** The authors certify that they have obtained all appropriate patient consent forms.
- **Author’s contribution:** H.S., J.A., O.L., and Z.L. gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work. H.S., J.A., Z.L., U.G., A.R. and I.S. had role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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