Comparison of the Histopathological Findings of Testis Tissues of Non-Obstructive Azoospermia with the Findings after Microscopic Testicular Sperm Extraction

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Purpose: To investigate the relationship between the histopathological findings of testis tissue samples and sperm retrieval success of micro-TESE in non-obstructive azoospermia (NOA) patients.

Method: Histopathological examination results of the testis tissue samples of 795 NOA patients who underwent micro-TESE operation in our clinic between 2003 and 2014 were included. Histopathological findings were grouped as hypospermatogenesis, incomplete spermatocytic arrest, complete spermatocytic arrest, Sertoli cell only syndrome (SCOS), and fibrosis/atrophy. Chi-square analysis was used to compare the histopathological findings with the sperm retrieval rates of micro-TESE.

Result: Sperm was found in 341 (42,9%) patients following micro-TESE compared to 454(57,1%) patients where sperm were not detected ($P < 0.001$). Sperm retrieval rates of micro TESE were significantly higher in hypospermatogenesis and incomplete maturation arrest groups (93.2% ($P < 0.001$) and 72.5% ($P < 0.001$), respectively). Complete maturation arrest, SCOS and fibrosis/atrophy were determined at significantly higher rates in patients (220.2%) with no sperm found compared to patients with sperm ($P < 0.001$).

Conclusion: The findings of this study are consistent with those of previous studies in the literature. Testicular histopathological findings can provide additional data when informing NOA patients about the expected success of further micro-TESE operations.

Keywords: Non-obstructive azoospermia; micro-TESE; testicular sperm extraction; pathology

INTRODUCTION

Infertility is defined as the inability to become pregnant of a sexually active couple despite one year of unprotected intercourse[(1)]. Azoospermia is defined as the absence of sperm in ejaculate, and is seen in 10%-15% of patients with complaints of infertility. The absence of spermatozoa in the ejaculate due to failure of spermatogenesis or a very low number of mature sperm in the testes is known as non-obstructive azoospermia (NOA)[(2)].

The causes of NOA are anorchia, acquired testis trauma, testis torsion, undescended testis, Klinefelter syndrome, germ cell aplasia, focal hypospermatogenesis, maturation arrest, orchitis, radiation, testicular temperature increase, gonadotoxic agents, systemic diseases such as cirrhosis and kidney failure, testis tumors, varicocele, surgical interventions, and idiopathic reasons[(3,4)]. In some NOA cases, rare foci of spermatogenesis may be present and mature sperm cells can be obtained from these foci with a microsurgery method, known as microscopic testicular sperm extraction (micro-TESE)[(5)]. Knowing the effect of testicular histopathological findings on micro-TESE outcomes can provide additional data when informing NOA patients about the expected success of further micro-TESE operations.

The aim of this study was to investigate the relationship between histopathological findings of testis tissue samples and sperm retrieval success of micro-TESE in NOA patients.

MATERIALS AND METHODS

Approval for this retrospective cohort study was granted by the Ankara Baskent University Ethics Committee (14/05/2014, KA14/162). Obstructive azoospermic patients were excluded from the study. The study included 795 NOA patients who underwent micro-TESE operation and whose testis tissue samples were examined histopathologically at the Urology Clinic of Baskent University Hospital between 2003 and 2014. The histopathological findings were recorded as hypospermatogenesis, incomplete spermatocytic arrest, complete spermatocytic arrest, Sertoli cell only syndrome (SCOS), and fibrosis/atrophy. The presence or absence of sperm were also recorded.

In the micro-TESE operation, a single longitudinal incision was made passing the scrotal raphe. By opening the layers of the scrotum with blunt and sharp dissections, the testis was exposed on the side where the procedure was to be applied. The avascular area was identified under the microscope and the tunica albuginea was opened with a transverse or longitudinal incision with a No. 15 scalpel. Enlarged seminiferous tubules that were

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Table 1. The histopathological results of the testis tissues examined after the micro-TESE operation and the SRR results

	Micro-TESE Result		
Histopathological Findings	Sperm (+)	Sperm (-)	Total
Hypospermatogenesis*	41 (93.2%)	3(6.8%)	44(100%)
Incomplete spermatocytic arrest*	147 (72.5%)	56 (27.5%)	203 (100%)
Complete spermatocytic arrest*	80 (32.3%)	168 (67.7%)	248 (100%)
Sertoli cell only*	51 (27.5%)	135 (72.5%)	186 (100%)
Fibrosis/Atrophy*	22 (20%)	92 (80%)	114 (100%)
Total	341 (42.9%)	434 (57.1%)	795(100%)

*P-Value < 0.001

thought to have active spermatogenesis at x20 or x40 magnification were extracted. (**Figure 1**).

If sufficient mature spermatozoa were found, a testicular tissue sample for histopathological examination was obtained and the procedure was terminated. When sperm could not be found, the other testis was examined. After obtaining the testicular tissue samples with appropriate microdissection techniques, the incision in the tunica albuginea was sutured with an absorbable 5-0 or 4-0 suture and the scrotal layers were sutured appropriately. (**Figure** 2)

Chi-square analysis was used to compare the histopathological findings with the sperm retrieval rates (SRR) of micro-TESE. Statistical analyses of the date were performed using Statistical Package for Social Sciences (SPSS Inc. Chicago, IL) v.22. Statistical significance was determined as $p < 0.05$ for all analyses.

RESULTS

Of 795 patients with NOA, sperm was found in 341 (42.9%) cases who underwent micro-TESE operation. The overall SRR was calculated as 42.9% in this study. According to the histopathological diagnoses, SRR was 93.2% in hypospermatogenesis, 72.5% in incomplete maturation arrest, 32.3% in complete maturation arrest, 27.5% in SCOS, and 20% in the fibrosis/atrophy group.

The histopathological findings of the testis tissue samples and the SRR are presented in **Table 1**.

Of 201 patients who had previously undergone testis biopsy, sperm was found in 58 (28%). The mean age of the patients was 34.7(± 6.30) years. Mean serum FSH, LH and testosterone levels were 17.70(± 13.45) mIU/mL, 8.88(± 7.89) mIU/mL, and 4.33(± 1.90) ng/mL, respectively. According to Chi-square analysis, the SRR of micro TESE was significantly higher in the hypospermatogenesis and incomplete maturation arrest groups (93.2% ($P < 0.001$) and 72.5% ($P < 0.001$), respectively). Complete maturation arrest, SCOS and fibrosis/atrophy were found to have a significant negative effect on the sperm retrieval success of micro-TESE (32.3% ($P < 0.001$), 27.5% ($P < 0.001$) and 20%($P < 0.001$), respectively).

DISCUSSION

NOA is determined in 80%-85% of azoospermic patients[6-8]. Micro-TESE is considered to be the gold standard surgical method for sperm retrieval in NOA patients[9,10]. The aim of the present study was to examine the testicular histopathological findings after micro-TESE in NOA patients and to investigate the relationship between histopathological findings and SRR micro-TESE operation.

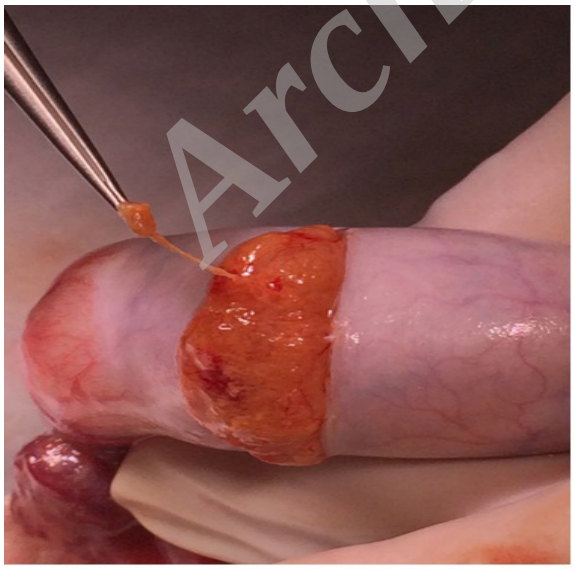

Figure 1. The identification and collection of tubules potentially with sperm, determined dilated and yellow-coloured during the micro-TESE operation (Image reproduced with the permission of Baskent University, Adana Training and Research Hospital Urology Clinic).

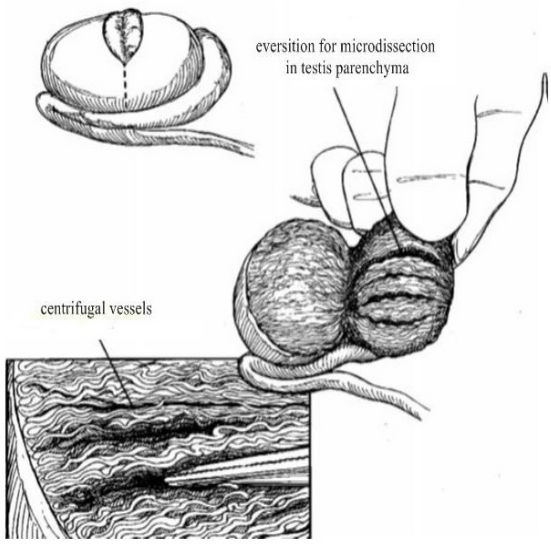

Figure 2. Schematic drawing of the incision in the micro-TESE operation, showing access to the deep parenchymal tissue

Testis biopsy is no longer used for diagnostic purposes. However testicular tissue sampling can be a part of the micro-TESE procedure. Testicular biopsy can be used to differentiate between obstructive and non-obstructive azoospermia. In conditions that lead to obstruction (such as vasectomy, vas agenesis), testis biopsy is not required. Normal spermatogenesis, maturation arrest, SCOS and fibrosis/atrophy can be determined after the histopathological examination of testis biopsy samples. In hypospermatogenesis, all germ cell counts are reduced but all stages of spermatogenesis are present. Patients with hypospermatogenesis may be azoospermic or oligozoospermic. In maturation arrest, primary spermatocytes or late spermatids are seen. In germinal aplasia, small testicular volumes and high FSH levels are present. Several studies have reported that testis biopsy applied before micro-TESE is the most important predictive parameter of SRR[6]. Diagnostic testicular biopsy performed prior to micro-TESE increases the risk of complications such as infection, bleeding, hematoma, tubular sclerosis and permanent devascularisation in the testis. It also requires patients to undergo 2 surgical interventions[11].

In a study by Salehi et al., SRR of TESE procedure was determined to be 48.8% in 170 NOA patients. According to the histopathological examination, patients were grouped as hypospermatogenesis, maturation arrest and SCOS, and SRR was determined as 94%, 43.5% and 21.6% respectively[12]. In the current study, a similar SRR of 93.2% was determined in the hypospermatogenesis group.

In a study by Kuai et al., the histopathological findings of testis tissue samples obtained from NOA patients were compared with the results of intracytoplasmic sperm injection (ICSI). There was no statistically significant difference between histopathological groups in respect of fertilisation, transferable embryos, high quality embryos and the number of transferred embryos. Although several studies have reported SCOS histopathology to be associated with low SRR in micro-TESE, ICSI success and normal birth rates after sperm retrieval are the same as for other groups[13,14].

Eken et al. investigated the effects of age, testicular volume, serum FSH, LH, testosterone levels and histopathological findings on SRR in 145 NOA patients. Among these factors, only histopathological findings were shown to significantly affect SRR. Similar to that study, SRR was also significantly higher in the hypospermatogenesis group in the present study[15]. In another study, in addition to the effect of histopathology on SRR, fertilization, pregnancy and live birth rates were also investigated in 271 patients. It was demonstrated that while SRR and fertilization were significantly affected, pregnancy and live birth rates were not affected by the histopathological groups[16].

The association between infertility and psychological state of both men and women is well known. When viewed from this aspect, the predictability of micro-TESE outcomes carries additional importance[17].

There are some limitations to this study. The retrospective design of the study is one of the limitations. Although surgical procedures and histopathological examination techniques did not change during the 11 year period of this study, the fact that the micro-TESE operation was performed by different surgeons and the histopathological findings were examined by different pathologists might have affected the results.

CONCLUSIONS

To the best of our knowledge, the present study of 795 patients is the largest patient series in the literature. Testis biopsy is still the most valuable predictive parameter for sperm retrieval success in micro-TESE, although it is not currently a part of common practice. In this context, it can be considered that the histopathological findings are the most applicable and reliable parameter when informing NOA patients about the expected success of further micro-TESE operations.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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