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Factors Affecting Outcomes of Salvage Micro Testicular Sperm Extraction Following a Failed Testicular Sperm Extraction

Başarısız Testiküler Sperm Ekstraksiyonu Sonrası Yapılan Kurtarma Mikro Testicular Sperm Extraction Sonuçlarını Etkileyen Unsurlar

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Abstract

Aim: To investigate the likelihood of sperm retrieval in repeated micro-testicular sperm extraction (micro-TESE) in non-obstructive azoospermia (NOA) patients.

Methods: Data of 310 patients, who underwent a micro-TESE procedure at a center experienced in *in vitro* fertilization between January 2015 and July 2019, was evaluated retrospectively. Seventy-three patients who had a previous failed sperm retrieval procedure (33 micro-TESE, and 40 TESE) were included in the study. The patients were divided into two groups (group 1: successful, group 2: failure) according to sperm retrieval in salvage micro-TESE. The groups were compared in terms of demographic characteristics and hormonal and histological features.

Results: The mean age of the patients was 36.71±8.1 (25-45) years and duration of infertility was 59.45±21.4 (22-247) months. The sperm retrieval rate in patients who underwent salvage micro-TESE was 36.99% (27/73). Sperm retrieval rates were 8/43, 8/16 and 11/14 for patients diagnosed with Sertoli Cell-only syndrome (SCOS), maturation arrest and hypospermatogenesis. The rate of patients with SCOS was significantly higher in the failure group (p<0.01).

Conclusion: Salvage micro-TESE provides an important opportunity in patients with NOA with a history of unsuccessful micro-TESE. Sertoli cell-only syndrome seems to have a negative effect on the success of the procedure.

Keywords: Azoospermia, sertoli cell-only syndrome, spermatozoa, sperm retrieval

Öz

Amaç: Daha önce başarısız testiküler sperm ekstraksiyonu (TESE) öyküsüne sahip non-obstrüktif azospermi (NOA) hastalarında kurtarma mikro-TESE işleminin sperm bulma olasılığını etkileyen faktörleri analiz etmektir.

Yöntemler: Ocak 2015 ve Temmuz 2019 tarihleri arasında *in vitro* fertilizasyon konusunda deneyimli bir merkezde TESE uygalanan 310 hastanın verileri incelendi. Daha önce başarısız mikro-TESE öyküsü olan 73 hasta (33 mikro-TESE, 40 TESE) çalışmaya dahil edildi. Kurtarma mikro-TESE işleminde sperm bulunan hastalar "başarılı"; bulunamayanlar ise "başarısız" olarak sınıflandırıldı. Gruplar demografik veriler, hormonal durum ve histolojik bulgular açısından karşılaştırıldı.

Bulgular: Hastaların ortalama yaşı ve infertilite süresi sırasıyla 36,71±8,1 (25-45) yıl ve 59,45±21,4 (22-247) ay idi. Kurtarma mikro-TESE sonrasında sperm elde etme oranı %36,99 (27/73) idi. Sperm elde etme oranları Sertoli Cell-only Syndrome (SCOS), maturasyon arresti and hipospermatogenesis için sırasyla 8/43, 8/16 ve 11/14 idi. Gruplar arasında yaş, infertilite süresi, kurtarma mikro TESE öncesi hormon parametreleri açısından anlamlı farklılık izlenmedi. Başarısız grupta anlamlı olarak SCOS oranı daha yüksek idi (p<0,01).

Sonuç: Kurtarma mikro-TESE daha önce başarısız TESE öyküsü olan hastalarda önemli bir uygulama olarak düşünülmelidir. İlk işlemde SCOS saptanan hastalarda kurtarma mikro-TESE işleminde sperm bulma olasılığı belirgin olarak azalmaktadır.

Anahtar Sözcükler: Azospermi, sertoli cell-only sendromu, spermatozoa, sperm eldesi

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Introduction

A conventional testicular sperm extraction (TESE) technique or testicular sperm aspiration (TESA) can be used for testicular sperm retrieval in patients with non-obstructive azoospermia (NOA) with success rates of 10 to 50% and 10 to 20%, respectively (1). Micro-TESE procedure may allow better visualization of larger tubules with increased opacity. Micro-TESE procedure is presented as a safe procedure with minimal complications together with yields of higher sperm retrieval rates as compared to conventional TESE and TESA (2).

The number of patients, who request second or third micro-TESE procedure following an unsuccessful TESE procedure and do not wish to use donor sperms, increases gradually. Data on the outcome of patients in this specific group is limited (3,4). Previous studies reported that repeated TESE procedures should be performed with caution due to the increased risk of testicular damage associated with the operations (5,6). However, current studies reported that sperm retrieval could be safely repeated several times if required (7).

This study aimed to investigate the likelihood of sperm retrieval in repeated micro-TESE in NOA patients who had undergone an unsuccessful sperm recovery attempt previously. In addition, factors including testicular histopathology, follicle-stimulating hormone (FSH) level, patient age, duration of infertility, testosterone level and results of genetic evaluation, which may be related to successful sperm retrieval were evaluated.

Methods

Study Design

The study was approved by the Institutional Ethics Committee (approval no: 366/2019). Data of 310 patients who underwent micro-TESE procedure between January 2015 and July 2019 in a center specialized in *in vitro* fertilization was evaluated retrospectively. Seventy-three patients who had a failed sperm retrieval procedure previously (33 micro TESE, and 40 TESE) were included in the study. The patients were divided into two groups (group 1: successful, group 2: failure) according to sperm retrieval rates in salvage micro-TESE. The groups were compared in terms of demographic characteristics and hormonal and histological features.

Pre-operative Assessment

Pre-operative clinical work-up included physical examination, testicular ultrasound, hormonal assessment (luteinizing hormone LH, FSH, and testosterone levels), and genetic analysis (karyotype and Y chromosome micro-deletion). Salvage micro-TESE procedure was performed at least six months after the previous sperm retrieval procedure. A period of 3 to 6 months since the primary surgery has been reported to be associated with higher sperm retrieval rates (8). Patients who were detected to have an azoospermia factor (AZF) a or b micro-deletion pre-operatively were not operated on because the sperm retrieval rate in these patients has been reported to be zero (9).

Micro-TESE Technique

All patients underwent a micro-TESE procedure under general anaesthesia in the day oocyte retrieval was performed to their partner (10). A floor-standing operating microscope (Leica M500; Leica Microsystems Pty Ltd, Gladesville, NSW, Australia) was used throughout the procedures. Sperm extraction was performed at x20 or x40 magnification. A transversal incision of the tunica albuginea was made either equatorially or in the cranial part of the testis. The fragments were washed in human tubal fluid medium to remove the blood, and given to the biologist for microscopic examination. Afterwards, the testicular tissue surfaces were irrigated with Ringer solution containing 80 mg gentamicin/100 mL for antisepsis. Hemostasis was then performed pressing the testicular tissue gently for 2 min, using gauze wet with an antiseptic solution, which then followed by very limited and careful bipolar micro coagulation. After administering 1.5 mg betamethasone into the tunica vaginalis to prevent pain and tunica vaginalis adhesions, the tunica vaginalis was repaired by a continuous Vicryl 5/0 or 4/0. The contralateral testis was exposed with the same technique in cases where spermatozoa were not found.

All salvage micro-TESE procedures were performed by an experienced andrologist who is an expert in microsurgery and performs more than 80 micro-TESE procedures annually. Post-operative complications were followed up for at least six weeks after surgery.

Statistical Analysis

Statistical analysis was conducted using the IBM SPSS software (v. 22.0). Continuous variables, such as patient age, duration of infertility, results of genetic evaluation, and histopathological features, were presented as mean ± standard deviation (SD). Categorical variables were presented as numbers and percentages. A p value of less than 0.05 was considered statistically significant.

Results

Table 1 presents preoperative characteristics of the patients. The mean age of the patients and infertility duration were 36.62±7.5 (25-45) years and 60.1±30.3 (22-247) months, respectively. The sperm retrieval rate in patients, who had a history of unsuccessful micro-TESE procedure and underwent a salvage micro-TESE,

was 24.42% (8/33). However, the sperm retrieval rate was 47.5% (19/40) in patients who had undergone an unsuccessful TESE previously and then underwent salvage micro-TESE. This difference was statistically significant (p=0.03). There was no statistically significant difference between the groups in terms of mean age, infertility duration, mean FSH levels and inability to find sperm on salvage micro-TESE.

Y chromosome microdeletion was detected in five patients, and Klinefelter's syndrome was found in eight patients. Sperm was found in one of the patients with Kleinefelter's syndrome (12.5%). Four patients had Y chromosome AZF c microdeletion and sperm was found in one (25%).

Table 2 presents the hystological features of the previous TESE and micro-TESE results which are Sertoli cell-only syndrome (SCOS) in 58.9% (n=43), maturation arrest (MA) in 21.9% (n=16)% and hypospermatogenesis in 19.10% (n=14) of the patients. The sperm retrieval rate was 18.6% (8/43) in SCOS, 50% (8/16) in MA and 78.57% (11/14) in hypospermatogenesis histopathology of the previous microTESE (Table 1). The sperm detection rate was significantly higher in patients without SCOS (p<0.01). No major complications occurred. Mild abdominal pain was observed most commonly in the early postoperative period and resolved with a pain killer (paracetamol).

Discussion

The sperm retrieval rate in NOA patients who underwent the first micro-TESE procedure in our unit between 2015 and 2019 was 47.42% (147/310). This rate is comparable with the literature (2,9,10). Kalsi et al. (4) reported a sperm retrieval rate of 46.5% in NOA patients who underwent unsuccessful multiple biopsy TESE or TESA. In another study, the sperm retrieval rate after unsuccessful TESE procedure was reported to be 45.7% (11). In the present study, the sperm retrieval rate was 36.99% in the salvage micro-TESE group. This rate is lower than the rate reported by Kalsi et al. (10) (46.5%) and Tsujimura et al. (11) (45.7%). These lower rates may be explained by the technique used for sperm retrieval in the first TESE procedure. In our study, most of the patients underwent a microTESE as the first procedure (45.2%) instead of TESA or multiple biopsy TESE. In subgroup analysis of patients who previously underwent a TESE procedure, the sperm retrieval rate was found to be 47.61% after salvage micro-TESE. Bernie et al. (12) suggested that the success rate of micro-TESE was 17% higher than the conventional TESE, and furthermore, the conventional TESE procedure was two times more successful for sperm retrieval as compared with TESA for NOA patients in the first attempt. These findings may explain the lower sperm retrieval rates after the salvage micro-TESE procedures in our study.

Previous studies reported that the success rate of sperm retrieval during the random testis biopsy decreased in patients with increased FSH levels (13,14). FSH exerted its function by binding to Sertoli cell receptor, and an increased level of FSH would tend to indicate a global failure of spermatogenesis. However, Bromage et al. (15) found no association between FSH levels and advanced stages of spermatogenesis. Therefore, the FSH level may not be a good predictor for the determination of isolated areas of mature spermatogenesis within the testis. Other

Table 1. Pre-operative characteristics of the salvage micro-TESE patients

	Successful (n=27) (37.84%)	Unsuccessful (n=46) (62.16%)	p
Mean Age (years) ± SD	36.71±8.1	36.50±5.57	0.95
Mean duration of infertility (months) ± SD	59.45±21.4	61.4±23.5	0.68
Mean FSH (IU/L) ± SD	18.01±5.4	20.01±9.07	0.492
Mean testosteron level (nmol/L) ± SD	37.07±23.81	21±17.46	0.393
Hormone therapy, n (%)	2 (25)	8 (75)	0.17

TESE: Testicular sperm extraction, SD: Standard deviation, FSH: Follicle-stimulating hormone, n: Number

Table 2. Hystologic analysis of the salvage micro-TESE patients

		Successful (n=27) (37.84%)	Unsuccessful (n=46) (62.16%)	p
Histology, n (%)	Sertoli Cell-only, 43 (58.9)*	8 (18.6)	35 (81.4)	p<0.01
	Hypospermatogenesis, 14 (19.1)	11 (78.5)	3 (21.5)	
	Maturation arrest, 16 (21.9)	8 (50)	8 (50)	

TESE: Testicular sperm extraction, n: Number
*Sertoli cell-only syndrome was compared to those with and without

studies demonstrated that the correlation between the ability to retrieve sperm and the FSH level was weak (2,16). Similar to the literature, we did not find any association between sperm retrieval and FSH level in sperm retrieved and not retrieved groups.

Testicular biopsies or conventional TESE procedures give some clues about the predominant histology of the testis but do not represent the histology of the whole testicular tissue. A previous study has suggested that pre-operative histopathology is the most important factor in predicting sperm retrieval rates in men on repeat biopsy (10).

However, a testicular biopsy can cause fibrosis, haematoma, inflammatory changes, or atrophy (6). Therefore, an isolated testicular biopsy is not recommended before the surgery. A success rate of 40% in sperm retrieval in patients with SCOS was only achieved via salvage micro-TESE with concurrent testicular biopsy. This study concluded that in a significant number of patients, sperm would have been found irrespective of the histopathological diagnosis of SCOS. Furthermore, hypospermatogenesis was associated with a higher success rate (75%) as compared with patients with MA; there only 36% success rate could be achieved (4).

The sperm retrieval rate in our study population with hypospermatogenesis and MA was comparable to that in the study by Kalsi et al. (10) whereas the sperm retrieval rate was lower in patients with SCOS in our study. Our sperm retrieval rate was 18.6% whereas Kalsi et al. (10) achieved a sperm retrieval rate of 40%. In our series, an important part of previous sperm retrieval interventions was micro-TESE; however, in the study by Kalsi et al. (10), all previous sperm retrieval interventions were TESE or TESA instead of microTESE. Therefore, we think that in the cohort of Kalsi et al. (10), the first sperm retrieval approach was less successful. The sperm retrieval rate was higher in SCOS cases during the salvage microTESE procedure owing to the more precise surgery under the microscope with better visualization and more detailed seminiferous tubules structure.

Schlegel stated that in complete azoospermia factor AZFa, AZFb deletions and absence of AZFa-c, the seminal phenotype of the patient is azoospermia and, as a result, the sperm retrieval rate with TESE was very low in such patients (17). In the present study, there were four patients with AZFc deletions and sperm was obtained from one of them.

Karyotype analyses have been an important predictor of the success of micro-TESE. Sperm retrieval rate in Klinefelter's syndrome was reported to be between 41% and 72% (18,19). This rate was found to be 30% in the first micro-TESE procedure in our patients with Klinefelter's syndrome. In our series, eight patients with Klinefelter's syndrome underwent salvage microTESE procedures, and in one, sperm retrieval was successful (14%).

In a study by Shiraishi and Matsuyama (20), 48 men with NOA who had a negative sperm retrieval with micro-TESE were divided into two groups. Twenty-eight patients received daily injections of hCG for 4-5 months before a second micro-TESE procedure, whereas twenty patients did not receive any hormonal therapy. Sperm was obtained successfully in the second micro-TESE from six patients who had received hormonal therapy (21%), whereas no sperm was retrieved from patients who did not receive treatment. In our study, the sperm retrieval rate was 24.42% (8/33) after salvage micro-TESE in patients who had undergone microTESE procedure previously. Only two of the patients with positive sperm retrieval were under hormonal treatment.

There was no significant complication after the salvage micro-TESE procedure. However, half of the patients experienced mild abdominal pain after the procedure which was managed with paracetamol. It might be thought that the risk of hypogonadism may increase after repeated micro-TESE; however, no patient required hormone replacement therapy during follow-up.

Concordant with our study, it has been reported that testosterone level return to 80-93% of the pre-operative value in a period of 12 months, approximately (10). However, the Klinefelter's status and the histology of the patient seem to affect the return of the testosterone level, partially. Therefore, we recommend a post-operative hormone evaluation for all patients who have undergone a micro-TESE procedure. Our protocol includes measurements of LH, FSH and testosterone levels at 6-9 months following micro-dissection sperm retrieval.

Study Limitations

Some limitations regarding this study should be noted. First of all, the primary endpoint of this study was the sperm retrieval rate instead of pregnancy or delivery rates. The retrospective nature of the study is another limitation. Furthermore, most of the patients underwent the first micro-TESE or TESE procedure in different centers whereas they applied to our unit mostly for salvage microTESE. Small sample size is a further limitation of this study.

Conclusion

Salvage micro-TESE provides an important opportunity for patients with NOA with a history of unsuccessful micro-TESE. Sperm retrieval rate in salvage micro-TESE was higher in patients who had undergone conventional TESE instead of micro-TESE. Our study shows that sperm can be found despite high FSH levels. Furthermore, in

histopathological findings of NOA patients, SCOS seems to have a negative effect.

Authorship Contributions

Concept: M.B., O.Ö., A.Ç. Design: M.B., O.Ö., A.Ç. Data Collection or Processing: M.B., O.Ö., A.Ç. Analysis or Interpretation: M.B., O.Ö., A.Ç. Literature Search: M.B., O.Ö., A.Ç. Writing: M.B., O.Ö., A.Ç.

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References

1. Gil-Salom M, Romero J, Minguez Y, Molero MD, Remohi J, Pellicer A. Testicular sperm extraction and intracytoplasmic sperm injection: a chance of fertility in nonobstructive azoospermia. J Urol 1998;160:2063-7.
2. Schlegel PN. Nonobstructive azoospermia: a revolutionary surgical approach and results. Sem Reprod Med 2009;27:165-70.
3. Kızılay F, Semerci B, Şimşir A, Kalemci S, Altay B. Analysis of factors affecting repeat microdissection testicular sperm extraction outcomes in infertile men. Turk J Urol 2019;45:S1-S6.
4. Kalsi JS, Shah P, Thum Y, Muneer A, Ralph DJ, Minhas S. Salvage micro-dissection testicular sperm extraction; outcome in men with non-obstructiveazoospermia with previous failed sperm retrievals. BJU Int 2015;116:460-5.
5. Ron-El R, Strauss S, Friedler S, Strassburger D, Komarovsky D, Raziel A. Serial sonography and colour flow Doppler imaging following testicular and epididymal sperm extraction. Hum Reprod 1998;13:3390-3.
6. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. Hum Reprod 1997;12:1688-92.
7. Vernaeve V, Verheyen G, Goossens A, Van SA, Devroey P, Tournaye H. How successful is repeat testicular sperm extraction in patients with azoospermia? Hum Reprod 2006;21:1551-4.
8. Amer M, Haggar SE, Moustafa T, Abd El-Naser T, Zohdy W. Testicular sperm extraction: impact of testicular histology on outcome, number of biopsies to be performed and optimal time for repetition. Hum Reprod 1999;14:3030-4.
9. Choi DK, Gong IH, Hwang JH, Oh JJ, Hong JY. Detection of Y Chromosome Microdeletion is Valuable in the Treatment of Patients With Nonobstructive Azoospermia and Oligoasthenoteratozoospermia: Sperm Retrieval Rate and Birth Rate. Korean J Urol 2013;54:111-6.
10. Kalsi J, Thum MY, Muneer A, Abdullah H, Minhas S. In the era of micro-dissection sperm retrieval (m-TESE) is an isolated testicular biopsy necessary in the management of men with non-obstructive azoospermia? BJU Int 2012;109:418-24.
11. Tsujimura A, Miyagawa Y, Takao T, Takada S, Koga M, Takeyama M, et al. Salvage microdissection testicular sperm extraction after failed conventional testicular sperm extraction in patients with nonobstructive azoospermia. J Urol 2006;175:1446-9.
12. Bernie AM, Mata DA, Ramasamy R, Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: a systematic review and meta-analysis. Fertil Steril 2015;104:1099-103.
13. Shiraishi K, Ishikawa T, Watanabe N, Iwamoto T, Matsuyama H. Salvage hormonal therapy after failed microdissection testicular sperm extraction: A multi-institutional prospective study. Int J Urol 2016;23:496-500.
14. Tournaye H, Verheyen G, Nagy P, et al. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients? Hum Reprod 1997;12:80-6.
15. Bromage SJ, Falconer DA, Lieberman BA, et al. Sperm retrieval rates in subgroups of primary azoospermic males. Eur Urol 2007;51:534-9.
16. Ramasamy R, Lin K, Gosden LV, et al. High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. Fertil Steril 2009;92:590-3.
17. Schlegel PN. Nonobstructive azoospermia: a revolutionary surgical approach and results. Semin Reprod Med 2009;27:165-70.
18. Friedler S, Raziel A, Strassburger D, et al. Outcome of ICSI using fresh and cryopreserved-thawed testicular spermatozoa in patients with non-mosaic Klinefelter's syndrome. Hum Reprod 2001;16:2616-20.
19. Schiff JD, Palermo GD, Veeck LL, et al. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. J Clin Endocrinol Metab 2005;90:6263-7.
20. Shiraishi K, Matsuyama H. Gonadotoropin actions on spermatogenesis and hormonal therapies for spermatogenic disorders [Review]. Endocr J 2017;64:123-31.