

# Predictive factors of successful sperm retrieval on microdissection testicular sperm extraction in Japanese men

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## Abstract

**Purpose** To evaluate retrospectively the outcomes of microdissection testicular sperm extraction (micro-TESE) in men with nonobstructive azoospermia (NOA) and to identify the parameters predicting successful sperm retrieval in this cohort of patients.

**Methods** After excluding patients with normal testicular volume and serum follicle-stimulating hormone (FSH) level who received conventional TESE, this study included 329 consecutive NOA patients undergoing micro-TESE at our institution. The significance of several factors, including age, testicular volume, etiology and serum levels of FSH, luteinizing hormone (LH) and serum testosterone (T), as predictors of successful sperm retrieval, was evaluated.

**Results** Of the 329 men included in this series, 246 (74.8 %), 40 (12.2 %), and 43 (13.1 %) were pathologically diagnosed with Sertoli cell only, maturation arrest, and hypospermatogenesis, respectively. Spermatozoa were retrieved in 97 (29.5 %) of these 329 men by micro-TESE. Older age and non-idiopathic etiology were significantly associated with the probability of successful sperm retrieval; however, there were no significant effects of testicular volume as well as serum levels of FSH, LH, and T on sperm retrieval outcome. Furthermore, Johnsen score of the micro-TESE specimen showed a significant association with whether spermatozoa were successfully retrieved. Univariate analysis of preoperative parameters identified older age and non-idiopathic etiology as significant

predictors of successful sperm retrieval, of which only etiology appeared to be independently related to successful sperm retrieval on multivariate analysis.

**Conclusions** Spermatozoa are significantly less likely to be successfully retrieved by micro-TESE in men with idiopathic azoospermia.

**Keywords** Male infertility · Microdissection testicular sperm extraction · Nonobstructive azoospermia · Predictive factor · Sperm retrieval

## Introduction

Microdissection testicular sperm extraction (micro-TESE) with intracytoplasmic sperm injection (ICSI) has been regarded as the first-line treatment for men with nonobstructive azoospermia (NOA). However, the successful sperm retrieval rates by micro-TESE in men with NOA remain unsatisfactory, being reported to range between 20 and 60 % [1–4]. Furthermore, micro-TESE and subsequent ICSI involve the couple being exposed to physical, emotional, and financial burdens; therefore, it is necessary to determine the proper indications for micro-TESE by identifying parameters that exactly predict the outcome of this procedure.

There have been several studies exploring the predictive factors associated with successful sperm retrieval by micro-TESE in men with NOA. For example, Bromage et al. identified a significant correlation between an elevated serum level of follicle-stimulating hormone (FSH) and successful sperm retrieval in men using random biopsy TESE techniques [5], while Tsujimura et al. reported that the concentrations of total testosterone (T) and inhibin B, in addition to that of FSH, were shown to be significantly

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influential predictive factors of a successful outcome in men with NOA undergoing micro-TESE [4]. However, Tunc et al. found no significant differences in the serum FSH and inhibin B levels between patients with and without successful sperm retrieval by TESE [6]. In addition, despite being a very helpful parameter for predicting the outcome of micro-TESE [7], AZF microdeletions are positive in only 6–16 % of men with NOA [8–10]. Collectively, these findings suggest a failure to identify parameters with reliable predictive values that could provide enough information on the routine clinical practice of micro-TESE.

In this study, therefore, we conducted a retrospective assessment of the clinical outcome of micro-TESE in 329 consecutive patients with NOA in order to identify predictive parameters associated with successful sperm retrieval by this procedure.

## Materials and methods

At our institution, upon being diagnosed with azoospermia, patients with normal testicular volume and serum FSH level were usually treated by conventional TESE. Hence, after excluding patients receiving conventional TESE, this study included a total of 329 consecutive men who were diagnosed with NOA and subsequently underwent micro-TESE. Semen collected by masturbation following a 3-day abstinence period was analyzed at least twice prior to micro-TESE. In order to exclude OA and hypogonadotropic hypogonadism, endocrine evaluation was performed for all patients by measuring serum FSH, luteinizing hormone (LH), and T levels. For all patients, a chromosomal survey using the G-banding technique was carried out. The patients with Y chromosome AZFa and/or AZFb microdeletions were excluded from surgical indication.

Micro-TESE was performed based on the previously described technique [11]. Briefly, one sagittal incision in the tunica albuginea was made to allow visualization of the testicular parenchyma without affecting the testicular blood supply. Direct examination of the testicular parenchyma was then performed with a surgical microscope at 20× magnification. Small samples were excised containing tubules that were particularly large and opaque. The procedure was terminated upon obtaining spermatozoa or when it was considered that further dissection would damage the testicular blood supply. We defined the success of sperm retrieval as obtaining at least one spermatozoon. This study contains the result of micro-TESE performed by six surgeons belonging to our

hospital. Surgeons who have >50 cases of micro-TESE were considered experienced surgeons based on the previous report [1].

Statistical comparisons of clinical parameters between patients with successful and unsuccessful sperm retrieval were performed by Student's *t* test or Chi-squared test. Univariate and multivariate logistic regression models were constructed to analyze the association of several parameters with the outcome of micro-TESE. *p* values <0.05 were considered to be significant.

## Results

Of the 329 men included in this series, 246 (74.8 %), 40 (12.2 %), and 43 (13.1 %) were diagnosed with Sertoli cell only, maturation arrest, and hypospermatogenesis, respectively, by histopathological examination. Spermatozoa were successfully retrieved in 97 (29.5 %) of these 329 men by micro-TESE. Table 1 summarizes the characteristics of the 329 patients included in this study according to the outcome of micro-TESE. Karyotype abnormality other than Klinefelter syndrome included six patients with chromosomal translocation, three patients with additional chromosome, two patients with derivative chromosome, and one patient with Y chromosome AZFc microdeletion.

Among the preoperative parameters, age at micro-TESE in the group with successful sperm retrieval was significantly older than that in the group with unsuccessful sperm retrieval; however, there were no significant differences in the remaining parameters, including the testicular volume and serum levels of FSH, LH, and T, between these two groups. Upon dividing the included patients by the etiology of NOA, sperm was significantly more likely to be successfully retrieved in men with non-idiopathic etiology than in those with idiopathic etiology. In addition, Johnsen score of the micro-TESE specimen in the group with successful sperm retrieval was significantly higher than that in the group with unsuccessful sperm retrieval. The sperm retrieval rate by experienced surgeons (31.7 %) was slightly higher than that of less experienced surgeons (27.7 %). However, there was no significant difference between the two groups.

Univariate and multivariate logistic regression analyses were subsequently conducted to identify preoperatively available factors predicting the outcome of micro-TESE for men with NOA. As shown in Table 2, univariate analysis identified age and idiopathic etiology as significant predictors of successful sperm retrieval, of which only idiopathic etiology appeared to be independently associated with successful sperm retrieval.

**Table 1** Baseline clinical characteristics and comparison of successful and unsuccessful sperm retrieval groups

	Overall ( <i>n</i> = 329)	Sperm retrieval (%)		<i>p</i> value
		Successful ( <i>n</i> = 97)	Failure ( <i>n</i> = 232)	
<b>Preoperative factors</b>				
Age	33.9 ± 5.3	35.0 ± 5.6	33.2 ± 4.9	0.013
Testicular volume (ml)	10.9 ± 5.1	11.2 ± 5.4	10.8 ± 5.1	0.27
FSH (IU/l)	22.5 ± 12.2	22.3 ± 14.8	22.9 ± 10.6	0.42
LH (IU/l)	8.9 ± 6.1	9.1 ± 6.9	8.7 ± 5.7	0.30
T (ng/l)	4.3 ± 1.9	4.2 ± 1.8	4.3 ± 1.9	0.42
<b>Etiology</b>				
Idiopathic	194	41 (21.1)	153 (78.9)	0.003
Klinefelter syndrome	65	26 (40.0)	39 (60.0)	
Varicocele <sup>a</sup>	36	14 (38.9)	22 (61.1)	
Other karyotype abnormalities	12	7 (58.3)	5 (41.7)	
Other <sup>b</sup>	22	9 (40.9)	13 (59.1)	
<b>Histologic finding</b>				
Sertoli cell only	246	48 (19.5)	198 (80.4)	<0.001
Maturation arrest	40	11 (27.5)	29 (72.5)	
Hypospermatogenesis	43	38 (88.3)	5 (11.7)	
<b>Experience of surgeon</b>				
≤50 cases	184	51 (27.7)	133 (72.3)	0.12
>50 cases	145	46 (31.7)	99 (68.3)	

Values are expressed as mean ± standard deviation

FSH follicle-stimulating hormone, LH luteinizing hormone T testosterone

<sup>a</sup> Five patients of post-varicocelectomy and 31 patients with untreated varicocele

<sup>b</sup> Eight patients of post-inguinal surgery, seven patients of post scrotal surgery, five patients of post-chemotherapy, and two patients of Addison’s disease

**Table 2** Univariate analysis of several parameters as predictors of sperm retrieval on micro-TESE

	Univariate analysis		Multivariate analysis	
	Odds ratio	<i>p</i> value	Odds ratio	<i>p</i> value
Age (<35 vs. ≥35)	1.45	0.01	1.43	0.15
Testicular volume (ml) (<12 vs. ≥12)	1.07	0.78	–	–
Idiopathic vs. non-idiopathic	1.78	0.006	1.65	0.01
FSH (IU/l) (<20 vs. ≥20)	1.28	0.33	–	–
LH (IU/l) (<10 vs. ≥10)	1.21	0.48	–	–
T (ng/ml) (<3 vs. ≥3)	1.23	0.46	–	–

Values are expressed as mean ± standard deviation

FSH follicle-stimulating hormone, LH luteinizing hormone, T testosterone

**Discussion**

Micro-TESE combined with ICSI is currently recognized as the most common and useful treatment for men with NOA. In fact, micro-TESE, which is performed by precisely observing the seminiferous tubules under a surgical microscope, has been shown to be able to minimize the damage to testicular tissue and increase the probability of successful sperm retrieval compared with other approaches, such as biopsy and conventional TESE. However, the sperm retrieval rate by micro-TESE, ranging between 20

and 60 % [1–4], remains unsatisfactory considering the negative physical, emotional, and financial effects of this procedure. Although a new attempt that could improve the chance of successful retrieval by micro-TESE such as hormonal therapy for NOA [12] is desirable, there is no generally accepted method that could drastically improve the success rate of micro-TESE at this moment. Therefore, there is also a pressing need for a reliable marker that will help guide physicians to determine cases for which micro-TESE would be suitable. In this study, therefore, we retrospectively reviewed the clinical outcomes of micro-

TESE performed for a total of 329 consecutive men with NOA at our institution in order to identify parameters predicting the outcome of this procedure.

The sperm retrieval rate in this study was 29.5 %, which seems to be lower than those in previous studies. This could be partially explained by the indication of micro-TESE at our institution; that is, conventional TESE is usually applied to patients with normal testicular volume and serum FSH level, resulting in the high prevalence of SCO patients in this series. In fact, this study included 12 patients after the failure of sperm retrieval by conventional TESE. As a result, mean testicular volume (11.4 ml) and serum FSH level (22.3 IU/l) in the group with successful sperm retrieval in this study were markedly small and elevated, respectively, compared with those in previous studies [13, 14].

Similar to the results of the previous report, histopathological diagnosis was significantly correlated with the sperm retrieval rate [15, 16]. On the other hand, unlike the former report [1], there was no statistical difference on sperm retrieval rate by surgeon experience. This might be due to our training policy for micro-TESE; that is, in our hospital, the less experienced surgeon usually performs micro-TESE under the direct guidance of an experienced surgeon in order to maintain the quality of the operation.

In this series, older age and non-idiopathic etiology were shown to be significantly associated with the probability of successful sperm retrieval, whereas no significant effects of testicular volume and serum levels of FSH, LH, and T on the outcomes of micro-TESE were noted. Although assisted reproductive technologies are generally regarded as being less effective in the elderly than in younger patients [17], the present study provided a conflicting finding on this point. However, Ramassamy et al. recently reported that age did not negatively affect sperm retrieval in men undergoing micro-TESE, and speculated that elderly men might be more likely to have acquired rather than congenital NOA [18]. In this study, the proportion of patients  $\geq 35$  years with hypospermatogenesis and SCO were 18 and 67 %, while those patients  $< 35$  years with hypospermatogenesis and SCO were 9 and 77 %, respectively, which seems to be supportive of the above finding by Ramassamy et al.

A number of studies have evaluated the significance of several parameters as predictors for the outcome of micro-TESE; however, the outcomes of these studies remain controversial. As a result, there has been no predictive system introduced into clinical practice in men undergoing micro-TESE. Although several parameters, such as testicular volume and serum levels of inhibin B and FSH, were shown to be useful for predicting the outcome of micro-TESE [1, 4, 9–11], the association of these parameters with

the sperm retrieval outcome appeared to be less intense in men with advanced testicular dysfunction [3, 19]. In this study, only non-idiopathic etiology was identified as an independent predictive factor of successful sperm retrieval by micro-TESE. Taking these findings together, it appears necessary to perform a prospective study, including a larger number of men with NOA receiving micro-TESE, particularly those with severely impaired spermatogenesis, in order to identify reliable factors predicting the outcome of this procedure.

Here, we would like to emphasize several limitations of this study. This study included only Japanese men, who have been shown to have different characteristics regarding factors associated with testicular dysfunction from Western populations; therefore, it would be difficult to apply the present findings to the entire cohort of men with NOA. In addition, the indication of micro-TESE at our institution seems to be comparatively strict, which makes it difficult to compare the current findings directly with those of other studies. Finally, the primary objective of this kind of study should be to identify predictive factors of successful pregnancy, rather than those of sperm retrieval after micro-TESE.

In conclusion, although a prospective study including a larger number of men is required to draw a definitive conclusion, the findings of this study suggest that idiopathic etiology of azoospermia could be used as a poor predictive factor for successful sperm retrieval by micro-TESE in men with NOA.

#### Complaints of ethical statement

**Conflict of interest** Noritoshi Enatsu, Hideaki Miyake, Koji Chiba and Masato Fujisawa declare that they have no conflict of interest.

**Human right statements and informed consent** All procedures followed were in accordance with the ethical standards of the institutional ethical committee and with the Helsinki Declaration of 1964 and its later amendments. Written informed consent was obtained from all patients for being included in this study.

**Animal studies** This article does not contain any studies with animal subjects performed by any of the authors.

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