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Outcome of Microdissection Testicular Sperm Extraction in Non-Obstructive Azoospermic Patients

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ABSTRACT

Background: Micro-TESE (Microdissection testicular sperm extraction) is a best technique of sperm retrieval for non-obstructive azoospermic patients. This study is carried out to evaluate the possibility of successful retrieval of sperm and the relation of its sperm retrieval rate with patients' characteristics.

Materials and methods: We surveyed50 patients with non-obstructive azoospermia who underwent micro-TESE from June 2016 to July 2017 at Centre for Assisted Reproduction, Vietnam Military Medical University. Medical documents were reviewed for the outcome of the average patients' age, duration of infertility, testicular volume, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone levels, AZF microdeletions analysis and the overall sperm retrieval rate.

Results: The average patients' age was 32.34 ± 5.27 years, duration of infertility 4.72 ± 3.48 years; testicular volume 6.76 ± 3.15 ml. Six (12%) patients had AZF microdeletions. Patients' age, testicular size, serum LH, testosterone and AZF microdeletions showed no significant effect on sperm retrieval rate. FSH levels differ significantly in patients with whom sperm is retrieved versus patients where sperm is not retrieved with micro TESE. The overall sperm retrieval rate was 32%.

Conclusion: Microdissection testicular sperm extraction (micro-TESE) has a high sperm retrieval rate. FSH levels may be able to foretell the possibility of getting spermatozoa in patients with non-obstructive azoospermia.

Keywords: Microdissection testicular sperm extraction, Non-obstructive azoospermia, Sperm retrieval

INTRODUCTION

Azoospermia is explained as the lack of spermatozoa in the ejaculate after the assessment of centrifuged semen on at least two occasions. It is noticed in 1% of the general population and in 10%-15% of infertile men [1].

Surgical sperm retrieval and intracytoplasmic sperm injection (ICSI) have revolutionized the management of non-obstructive azoospermia (NOA) [2]. Fine-needle aspiration (FNA), percutaneous testis biopsy and open testicular biopsy or testicular sperm extraction (TESE) can be used to retrieve testicular spermatozoa [3]. Failure to extract spermatozoa may happen in up to 57% of TESE attempts [4]. Focal testicular spermatogenesis accounts for the failure rate of these procedures [5]. Moreover, multiple testicular biopsies can ensue in the loss of testicular tissue and can interrupt the testicular blood supply underneath the tunica albuginea with uncertainty of testicular devascularization and atrophy of the testis [6]. Microdissection TESE (micro-TESE) was introduced to try to sample focal healthy looking tubules, thus to maximize the yield of spermatozoa, lessen the amount of testicular tissue removed, make sperm retrieval rate (SRR) better and keep away from subtunical vessels [7].

We performed the study to evaluate the possibility of successful retrieval of sperm and the relation of its sperm retrieval rate with patients' characteristics.

MATERIALS AND METHODS

Study population

This study was approved by the institutional review board of

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Vietnam Military Medical University (IRB-VN01015) and written informed consent was obtained from all patients.

We surveyed 50 patients with non-obstructive azoospermia with healthy female partners who had undergone micro-TESE from June 2016 to July 2017 at Centre for Assisted Reproduction, Vietnam Military Medical University.

All patients were diagnosed on the basis of a complete history, physical examination, and endocrine profile. Medical documents were evaluated for follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone levels, AZF analysis. Testicular volume was taken the measurements with a Praderorchidometer.

Seminal study

Semen samples were made by masturbation after 3-5 days of sexual abstinence and gathered into sterile containers. The presence of azoospermia was recorded in at least two semen specimens more than 2 weeks apart, all processed with centrifugation at 3000 g and extensive examination of the re-suspended pellet. A repeat analysis was also carried out on the morning of the planned sperm retrieval.

Hormonal measurements

Serum FSH, LH and total testosterone were taken the measurements and documented preoperatively, at 3 months (early) and more than 1 year (late) follow up visits.

Screening method for AZF microdeletions

Polymerase chain reaction (PCR) amplification using specific sequence-tagged sites (STS) was carried out to screen for AZF region microdeletions of the Y chromosome.

Microdissection testicular sperm extraction

Procedures were carried out under regional anesthesia, with the patient positioned on an operating table in a supine position. The operating microscope (Karl Zeiss, Germany) was used throughout the procedures. After skin disinfecting and draping, the scrotal skin was stretched over the anterior surface of the testis and a 2.5 cm midline raphe longitudinal incision was placed. The incision was performed through the dartos muscle and tunica vaginalis. The tunica was opened and its bleeders cauterized. The testis was taken extra vaginally and the tunica albuginea was examined. A single large longitudinal intra-polar incision was made on an avascular area in the tunica albuginea under 6-8x magnification and the testicular parenchyma was widely revealed. Dissection of the testicular parenchyma was then undertaken at 16-25x magnifications searching for enlarged tubules, which are more likely to have capacity for germ cells. The superficial and deep testicular regions were inspected, as needed, and microsurgical-guided testicular biopsies were carried out by carefully removing enlarged and opaque tubules using microsurgical forceps. If enlarged tubules were not seen, then two to three random micro-biopsies were carried out at the upper, medium and lower testicular poles. The excised specimens were put into the center well of petri dishes containing phosphate-buffered saline (PBS) in room temperature and processed as described below. The tunica albuginea was closed using continuous non-absorbable 5-0 polydioxanone sutures suture. Following hemostasis, the tunica vaginalis was closed in a running fashion using similar suture, after that the dartos muscle was closed with interrupted Vycril sutures. Eventually, the skin was closed with continuous subcuticular 5-0 monocryl suture and a fluffy-type dressing and scrotal supporter were put. The procedures were performed at the contralateral testicle, as needed, when an insufficient number or no sperm have been found at initial laboratory examination. Patients were discharged same day of surgery. Success was explained as the presence of a sperm that could be either preserved or used for ICSI.

Tissue processing and sperm retrieval

Testicular tissues obtained at the procedure were put into 2 ml of PBS medium supplemented with 0.8 mg/ml collagenase Type IA (Sigma). The tissue samples were digested in an incubator at 37°C for 2 h. To facilitate complete enzymatic digestion, the samples were vibrated every 10-15 min during this incubation period. The cell suspension (supernatant) having capacity for the loose cells was then cleaned with PBS medium and centrifuged for 10 min at 2000 g. The supernatant was removed and the pellet re-suspended in 50-100 µl. A drop of 5 µl from each of the suspensions representing one biopsy was taken for examination on a glass slide with coverslip under a microscope.

STATISTICS

Descriptive statistics were illustrated as the mean (standard deviation) and percent. The value of $p<0.05$ was considered statistically significant for all tests carried out using the Predictive Analysis Software version 19.0 (SPSS Inc., IBM, Chicago, Illinois, USA).

RESULTS

A total of 50 patients underwent micro-TESE. The average age of the patients was 32.34 ± 5.27 years. The average duration of infertility was 4.72 ± 3.48 years. The average testicular volume was 6.76 ± 3.15 ml. Clinically, 39 (78%) testes were small sized (Testicular volume ≤ 8 ml) (**Table 1**).

Table 1. Age, duration of infertility and testicular volume of patients.

	Mean ± SD	Max	Min
Age (years)	32.34 ± 5.27	47	25
Duration of infertility (years)	4.72 ± 3.48	19	1
Testicular volume (ml)	6.76 ± 3.15	16	2

The sperm retrieval was successful in 16 (32%) patients and unsuccessful (no sperm found) in 34 (68%) patients **(Table 2)**.

Table 2. Sperm retrieval rate.

	n	%
Successful group	16	32
Unsuccessful group	34	68
Total	**50**	**100**

Their average serum FSH, LH and testosterone were 22.69 ± 15.32 mIU/ml, 10.18 ± 5.87 mIU/ml and 3.47 ± 2.33 ng/ml **(Table 3)**.

Table 3. Endocrine of patients.

Variables	Mean ± SD	Max	Min
FSH (mIU/ml)	22.69 ± 15.32	62.85	1.94
LH (mIU/ml)	10.18 ± 5.87	30.36	1.02
Testosterone (ng/ml)	3.47 ± 2.33	15	0.32

Patients' age, duration of infertility, testicular size, serum LH, testosterone and AZF microdeletions showed no significant effect on sperm retrieval rate. But FSH levels may be able to foretell the **possibility** of getting spermatozoa in patients with non-obstructive azoospermia (p=0.016) **(Table 4)**.

Table 4. Relation of age, duration of infertility, testicular volume, endocrine and AZF microdeletionsto sperm retrieval rate.

Variables	Microdissection testicular sperm extraction		p
	Successful group	Unsuccessful group	
Age (years)	33.56 ± 4.47	31.76 ± 5.57	0.26
Duration of infertility (years)	5.38 ± 4.56	4.41 ± 2.86	0.37
Testicular volume (ml)	6.41 ± 3.44	7.50 ± 2.34	0.197
FSH (mIU/mL)	15.17 ± 10.47	26.22 ± 16.08	0.016
LH (mIU/mL)	7.90 ± 3.67	11.26 ± 6.43	0.059
Testosteron (ng/ml)	2.74 ± 0.77	3.82 ± 2.72	0.129
AZF microdeletions (%)	0/16	6/34	0.073

DISCUSSION

The introduction of ICSI and the application of different testicular sperm retrieval techniques have revolutionized treatment in patients with NOA [2]. Different methods can be used to retrieve testicular spermatozoa, including FNA, open testicular biopsy and percutaneous biopsy [3]. The introduction of micro-TESE has made sperm retrieval rate

better, maximized the yield of spermatozoa per biopsy, resulted in removal of less testicular tissue and had fewer acute and chronic complications than conventional procedures [8].

Sperm retrieval rate between 33.3% and 63% have been notified after micro-TESE [9,10]. In our series, sperm retrieval rate was 32%. Serum FSH can be used as predictive factors of success. Our outcome concur with previously published studies that showed patients' age, duration of infertility, testicular size, serum LH, testosterone and AZF microdeletions had no effect on sperm retrieval rate **(Table 4)**.

Our study had some limitations. First, there were only a limited number of patients, limited number of relevant studies, so we analysed some parameters as predictors for sperm retrieval rate, such as average patients' age, duration of infertility, testicular volume, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone levels, AZF microdeletions. Second, the measurement methods and reference levels of both FSH and testicular volume varied among different studies, which might have an effect on the results of our analysis.

This is the first study evaluating the possibility of successful retrieval of sperm on Microdissection testicular sperm extraction in non-obstructive azoospermic Vietnamese. In this study, the testicular tissue samples were digested by collagenase Type IA (0.8 mg/ml). The enzymatic digestion process take a long time but this process is easy to perform, and does not damage cells.

CONCLUSION

Micro-TESE (Microdissection testicular sperm extraction) has a high sperm retrieval rate (32%), minimal postoperative complications. Patients' age, duration of infertility, testicular size, serum LH and testosterone showed no significant effect on sperm retrieval rate. FSH levels may be able to foretell the possibility of getting spermatozoa in patients with non-obstructive azoospermia.

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