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Microdissection TESE (MD-TESE) Does not Improve Sperm Retrieval Rate but Contributes to Favorable Pregnancy Rate in Non-Obstructive Azoospermic (NOA) Patients

Hatsuki Hibi[1*], Tadashi Ohori[1], Yoshiaki Yamada[2], Nobuaki Honda[2], Chikako Ito[3], Miho Sano[4], Yoshiki Hashiba[4], Yoshimasa Asada[4]

[1]Department of Urology, Kyoritsu General Hospital, Nagoya, Japan

[2]Department of Urology, Aichi Medical University School of Medicine, Aichi, Japan

[3]Narita Hospital, Nagoya, Japan

[4]Asada Lady's Clinic, Nagoya, Japan

Abstract

Background and Aims: Microdissection testicular sperm extraction TESE (MD-TESE), which can improve surgical sperm retrieval rate is widely applied in sperm retrieval surgery in non-obstructive azoospermic (NOA) patients. We assessed whether MD-TESE can improve sperm retrieval rate as well as Intracytoplasmic sperm injection (ICSI) results.

Methods: A retrospective comparative study involving 86 NOA patients who underwent 6-8 conventional multiple TESE (C-TESE) procedures was conducted. Another 116 NOA patients underwent MD-TESE. C-TESE and MD-TESE were employed for the bilateral testis via a median raphe incision under spinal or local anesthesia with spermatic block.

Results: Motile sperm recovery was achieved in 26 (30.2%) and 30 (25.8%) patients with C-TESE and MD-TESE, respectively. Collected sperm was cryopreserved. All couples in which motile sperm was recovered received ICSI utilizing frozen-thawed sperm samples. Overall pregnancy rates of the C-TESE and MD-TESE groups were 23% (6/26) and 40% (12/30), respectively. Abortion was not observed in either group and healthy deliveries resulted. Statistical differences in sperm recovery and pregnancy rate were not evident between the groups (P=0.495, P=0.182, respectively). Moreover, no successful pre-operative predictors of sperm recovery were identified.

Conclusions: These data demonstrated that MD-TESE does not improve sperm recovery rate; however, MD-TESE contributes to favorable pregnancy rate in NOA patients.

Keywords: Non-Obstructive Azoospermia, Conventional TESE, Microdissection TESE, Sperm Recovery Rate, Pregnancy Rate

Introduction

Surgical sperm retrieval methods such as microscopic epididymal sperm aspiration and testicular sperm extraction were initially applied to the obstructive azoospermia (OA). Theoretically, the OA patient is a suitable candidate for microsurgical seminal

**Correspondence:*
Hastuki Hibi, MD
Department of Urology, Kyoritsu General Hospital, 4-33 Goban-cho, Atsuta-ward, Nagoya, 456-8611, Japan
Tel: +81-52-654-2211
Fax: +81-52-651-7210
E-mail:hibih@quartz.ocn.ne.jp
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re-anastomosis, except in cases involving the irreparable congenital absence of seminal tract or failed anastomotic surgery. Intracytoplasmic sperm injection (ICSI) is a highly effective treatment; therefore, there is no doubt about it as an essential therapy for male infertility. In contrast, non-obstructive azoospermia (NOA) is characterized by hypergonadotropic hypogonadism in which bilateral atrophic testes and elevated levels of follicle stimulating hormone (FSH) are observed. In these patients, testicular sperm extraction (TESE) is the only available intervention. TESE followed by ICSI has become a standard therapeutic procedure for NOA patients. Thus, ICSI has allowed some NOA patients to father offspring with a small number of spermatozoa.

Needle biopsy or an open biopsy to obtain one sample (simple TESE) can lead to rare foci of spermatogenesis. The number of testicular samples that need be obtained in order to achieve successful sperm retrieval in NOA patients varies. Simple TESE does not improve the outcome with respect to high success rate of sperm recovery in comparison with multiple TESE. Multiple TESE is more effective than needle biopsy in terms of sperm retrieval in NOA patients (1). In NOA patients, diffuse distribution of spermatogenesis, which is observed throughout the testis, is not limited to a specific region. Consequently, multiple biopsies or a recent microsurgical approach to TESE is recommended.

Microdissection TESE (MD-TESE), which can improve sperm retrieval rate and which requires the resection of a reduced volume of testicular tissue is widely applied in sperm retrieval surgery in NOA patients. The objective of this study was to determine whether MD-TESE can improve sperm recovery and pregnancy rates when compared with the conventional multiple TESE. Whether MD-TESE can improve sperm retrieval rate as well as ICSI results was evaluated at our institute.

Materials and Methods

A retrospective analysis of 202 NOA patients was conducted between 1997 and 2007. One hundred and sixteen NOA patients underwent MD-TESE, whereas conventional multiple TESE (C-TESE) was performed in 86 subjects. Each patient received a history and physical examination. Azoospermia was determined on the basis of at least two semen analyses. Testicular volume was measured with an orchidometer. Endocrine panel, e.g., luteinizing hormone (LH), FSH and testosterone, was performed in all the patients, whereas karyotyping was conducted in males characterized by any feature suggestive of an abnormality.

Surgical procedure

MD-TESE was employed essentially for bilateral testis via a median raphe incision under spinal or local anesthesia with spermatic block. Scrotal content was delivered to the side of the larger testis; subsequently, the tunica vaginalis was opened. Under operative microscopy, a fully vertical incision was made to the tunica albuginea. Control of bleeding was achieved with bipolar cautery, application of which was minimized to avoid local temperature elevation, which exerts a harmful effect on spermatozoa. Under an operative microscope, testicular parenchyma was examined at 20-25 x magnification to identify dilated white opaque tubules. This examination was conducted from the surface to deeper levels as well as from the upper to the lower portion through the testicular parenchyma repeatedly (at least twice).

On the other hand, C-TESE was employed for essentially bilateral testis via a manner identical to that of MD-TESE. C-TESE consisted of 3-4 randomly standard biopsies per testicle.

Excised tissues were shredded and minced with sterile glass slides. Subsequently, smaller pieces

Table 1. Patient characteristics

	Age	Spouse age	Testis (ml)	LH (mIU/ml)	FSH (mIU/ml)	T (ng/ml)
MD-TESE (n=116)	34.0±4.8	31.8±3.5	7.1±3.7/ 6.4±3.6	10.0±6.2	26.8±13.3	3.94±1.74
C- TESE (n=86)	33.1±4.9	30.1±3.3	7.7±4.4/ 6.7±3.3	8.3±5.5	29.7±12.6	4.53±1.70
	NS	NS	NS/NS	NS	NS	NS

*These values are expressed as Mean±SD

MD-TESE, microdissection testicular sperm extraction; **C-TESE,** conventional testicular sperm extraction; **Testis,** testicular volume right/left; **T;** testosterone.

were generated to facilitate the release of spermatozoa from the tubules utilizing microscissors. Samples were collected in test tubes filled with sperm washing medium (modified human tubal fluid; Irvine Scientific Co., Santa Ana, CA, USA) and transferred to the IVF laboratory. The specimens were then finely minced again and cryopreserved for ICSI when sperm was evident.

The retrieval process was terminated when adequate motile sperm was present or when further procedure was considered to be harmful to the testicular blood supply. The tunica albugenia and vaginalis were sutured with running absorbable 4-0 and skin closure was affected with a stapler. A drainage tube was not maintained and scrotal support was provided.

Statistics

Statistics analysis was performed using ANOVA. Results, which were subjected to Fisher's test, were considered significant at $P<0.05$. All data were expressed as Mean±SD.

Results

Average ages of the patients and spouses undergoing MD-TESE were 34.0 ± 4.8 and 31.8 ± 3.5 years, respectively. On the other hand, average ages of the patients and spouses undergoing C-TESE were 33.1 ± 4.9 and 30.1 ± 3.3 years, respectively. Details of patient characteristics are presented in Table 1. No significant difference was observed in age, testicular volume or endocrine panel between the MD-TESE and C-TESE groups. Chromosomal analysis was

Table 2. The difference between motile sperm retrieval and no sperm obtained by microdissection TESE

	Age	Testis (ml)	LH (mIU/ml)	FSH (mIU/ml)	T (ng/ml)
Motile sperm (n=30)	34.5±5.0	5.6±3.1/ 5.7±3.8	5.7±3.8	27.4±10.7	4.36±1.91
No sperm (n=86)	34.0±4.9	7.7±3.7/ 6.9±3.6	9.5±5.9	26.2±14.7	3.87±1.72
	NS	NS/NS	P=0.006	NS	NS

*These values are expressed as Mean±SD

conducted in 72 patients; 18 subjects demonstrated non-mosaic Klinefelter syndrome (KS). No other chromosomal abnormality was observed in either group.

Motile sperm were recovered in 30 and 26 patients who underwent MD-TESE and C-TESE, respectively. LH values were significantly lower in the MD-TESE group. Additionally, patient age was paradoxically higher in the C-TESE group in individuals in which motile sperm was retrieved. Pre-operative predictors of success remain unclear (Table-2, 3). Only a single spermatozoon or a few immotile sperm were recovered by MD-TESE in eight subjects. However, cryopreservation for future ICSI was not indicated for this condition As a result, cryopreservation was not pursued per patient's request. These patients were not viewed as having undergone successful sperm recovery. Overall, motile sperm retrieval rates were 25.8% and 30.2% in patients in the MD-TESE and C-TESE groups, respectively. This outcome was not statistically meaningful (P=0.495).

All the couples in which motile sperm was recovered received ICSI utilizing frozen-thawed sperm. Overall, pregnancy rates of the MD-TESE and C-TESE groups were 40% (12/30) and 23% (6/26), respectively. This outcome was not statistically significant (P=0.182). Clinical abortion was not observed and all couples who achieved pregnancy delivered healthy babies.

A single individual exhibited post-operative lump hematoma; however, it resolved spontaneously. Testosterone was re-tested in 30 subjects (MD, 11 cases; C, 19 cases) one month later. Decreased testosterone levels consequent to the operative procedure were not observed.

Discussion

Since the introduction of ICSI and surgical sperm retrieval as an accepted treatment for azoospermic patients, numerous studies have reported excellent fertilization and pregnancy outcomes. Initially, sperm retrieval procedures, e.g., microscopic epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE), were conducted in obstructive azoospermic (OA) patients presenting with irreparable congenital absence of seminal tract or failed microsurgical seminal reconstruction. Theoreticallysperm can be retrieved from the seminal tract As a result, no need exists for harvest from the testis except in cases involving efferent ductal obstruction. Furthermore, the sperm retrieval rate in OA has been quoted around 100% (2, 3). Thus, TESE is not always indicated for OA. However, TESE has been widely applied in OA patients due to technical simplicity. The rationale is reliant on large meta-analysis, which revealed no significant difference in any outcome between utility of epididymal or testicular sperm in men with OA (4).

Table 3. The difference between motile sperm retrieval and no sperm obtained by conventional TESE

	Age	Testis (ml)	LH (mIU/ml)	FSH (mIU/ml)	T (ng/ml)
Motile sperm (n=26)	35.2±5.1	6.6±2.9/ 6.9±2.6	7.5±4.1	28.5±13.0	4.95±1.53
No sperm (n=60)	32.1±4.5	7.7±4.4/ 8.2±4.9	8.6±6.0	30.1±12.5	4.37±1.75
	P=0.005	NS/NS	NS	NS	NS

*These values are expressed as Mean±SD

In contrast, TESE is an essential treatment in cases of NOA. Several procedures can be employed in order to retrieve testicular spermatozoa, including open biopsy, fine needle biopsy and percutaneous biopsy. These approaches, with the exception of open biopsy, are much less likely to identify the rare foci of spermatogenesis. However, simple open TESE did not enhance the high success rate of sperm recovery upon comparison with the conventional multiple TESE. Furthermore, a prospective study comparing C-TESE involving one testis to MD-TESE involving the contralateral testis found a significantly higher sperm retrieval rate in MD-TESE (5). As a result, MD-TESE has become a powerful therapeutic procedure for NOA patients in the modern ART era. Numerous examples of successful sperm retrieval consequent to MD-TESE have been documented (6-9). Moreover, microsurgical techniques can compensate for negative aspects such as testosterone deficiency, which requires lifelong androgen replacement in conjunction with the multiple sampling.

Under operative microscopy, a fully vertical inci sion was made to the tunica albuginea. Originally, Schlegel (6) reported the opening of the tunica albuginea in an equatorial plane as a vertical incision may be harmful to the testicular artery. However, Silber (7) noted that a wide antimesenteric incision allows extensive visualization of the seminiferous tubules and subtunical vessels during surgery. This approach can reduce the risk of vascular injury with careful attention under the operative microscope. If the blood supply to the testes is similar on both the right and left sides, the same manner of incision may be detrimental to the bilateral testis. Based on this consideration, we recently employed a vertical incision to the larger testis, followed by a transverse incision to the contralateral testis. Diagnosis of NOA, which is clinically characterized by elevated FSH values and atrophic testes, should be confirmed histologically. However, no precise definition in terms of NOA exists. Consequently, FSH value and testicular size, which varied widely, were often not described (1, 5). Furthermore, examples of "normal histology" were included with documented NOA cases (10), which might affect differences with respect to success retrieval rate. NOA patients exhibit differing degrees of impaired spermatogenesis, ranging from varying extent of maturation arrest to Sertoli cell only syndrome. Inhibin is thought to be a parameter associated with maturation arrest and is correlated with impaired spermatogenesis more closely than is FSH value. However, it is not diagnostic (11). Although an accurate prediction of testicular sperm retrieval is essential, no non-invasive criteria have been established which can predict accurately the presence of sperm. Thus, whether to implement therapeutic sperm extraction without preliminary diagnostic biopsy is subject to debate. In the current series, LH values were significantly lower in the MD-TESE group and patient age was paradoxically higher in the C-TESE group in individuals in which motile sperm was retrieved. Successful predictors still remain unclear.

The sperm recovery rate achieved with MD-TESE was the focal point in the literature; in contrast, subsequent clinical pregnancy rate was rarely discussed (6, 8, 9). A failed sperm extraction carries with it significant emotional and financial implications for the couple. To be sure, data with which to inform individual NOA patients were insufficient. In terms of counseling these couples prior to sperm retrieval surgery, it is of paramount importance that a realistic estimation of the actual chance of success is provided and that the patients are well informed regarding the risks of ICSI, including ovarian hyperstimulation, multiple pregnancies, low birth weight and slightly increased risk of sex chromosomal aneuploidy.

Testicular sperm retrieved from NOA subjects demonstrated poor motility and morphology. The decision is further confounded by the apparent paradox that ongoing pregnancy rates are unaffected by cryopreservation but implantation is significantly

impaired by the use of frozen-thawed testicular sperm (4). Some patients are eager to undergo ICSI treatment in the presence of only a single testicular chronous TESE in conjunction with the egg harvest. However, great risk is considered with unnecessary ovarian stimulation. A single spermatozoon or a spermatozoon. However, this scenario is unrealistic and prognosis is poor. The sole alternative is syn few immotile spermatozoa were recovered by MD-TESEin eight subjects, whereas no patients who showed such condition observed in C-TESE group in this series. Under such circumstances, a strong possibility exists that viable sperm cannot be recovered from frozen-thawed specimens. ICSI was not tenable in this situation. Additionally, cryopreservation was not allowed in such cases. We believe that this severe condition should not be viewed as "successful" sperm retrieval.

Successful outcome of ICSI as measured by live birth rates has been shown to be independent of the type of procedure. Meta-analysis of published data (4) confirmed that the etiology of azoospermia and cryopreservation of surgically retrieved sperm impacts ICSI outcome, which affords several recommendations for clinical practice. Origin of sperm, particularly in men displaying similar etiology, does not affect outcome. In this series, no significant difference was observed in pregnancy and subsequent live birth rates between the MD-TESE and C-TESE groups; however, 40% of live births were achieved with MD-TESE.

Conclusions

In conclusion, this study demonstrated that MD-TESE does not improve sperm recovery rate; however, MD-TESE contributes to the favorable pregnancy rate in NOA patients.

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Conflicts of Interest

The authors have nothing to disclose.

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