REVIEW

Microdissection testicular sperm extraction for non-obstructive azoospermia: The assessment of serum hormone levels before and after procedure

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Introduction

Microdissection testicular sperm extraction (micro-TESE) has become a recognized procedure for men with nonobstructive azoospermia (NOA). Micro-TESE and intracytoplasmic sperm injection (ICSI) cycles expose the couple to an emotional and financial burden, so it would be beneficial to predict the success of sperm retrieval using noninvasive parameters before attempted procedure.

It has been noted that micro-TESE done while observing the seminiferous tubules under an operating microscope could minimize the damage to testicular tissue, while maximizing sperm recovery, compared to random biopsies [1]. Micro-TESE has also been shown to be more successful in sperm retrieval than a single biopsy or multiple random biopsies [2, 3]. In addition, micro-TESE improves the yield of spermatozoa per biopsy, results in less tissue removal (and loss of testicle), and allows identification of blood vessels within the testicle, minimizing the risk of vascular injury and loss of other areas of the testis [1]. Thus, micro-TESE is based on the principle of identifying the most advanced pattern, not necessarily the predominant pattern, of spermatogenesis in the testis. Although FSH reflects the predominant pattern of spermatogenesis, it may not reflect isolated areas of spermatogenesis within the testis.

Klinefelter syndrome (KS) is the most common sex-chromosome disorder, with a prevalence of 1 in 660 men [4], and is a frequent cause of hypogonadism and infertility. In all patients in whom micro-TESE was successful we could identify focal spermatogenesis in dilated and opaque seminiferous tubules surrounded by shrunken tubules or fibrous tissue. Micro-TESE is particularly helpful for successful sperm retrieval in KS cases.

Although numerous studies have compared conventional versus micro-TESE [2, 3, 5], there are a few large comprehensive study analyzing the hormonal changes after micro-TESE. In addition, especially KS patients, the study of hormonal change after micro-TESE is lacking. Hypogonadism in KS is relative rather than absolute and has been found to be an independent risk factor for development of abdominal adiposity [6]. Hypogonadism is also associated with metabolic syndrome and type 2 diabetes [7].

We reviewed the outcomes of micro-TESE, primarily the sperm retrieval in patients with elevated serum FSH. We also analyzed the predictive value of gonadotropin and biopsy histology for retrieving testicular sperm by micro-TESE in NOA patients. In addition, we reviewed complications in this procedure for the patients between 46XY males with NOA and KS during follow-up.

Sperm retrieval technique

The sperm extraction procedures have been extensively described already. In cases of NOA, the procedure was often extensive, involving general anesthesia and micro-TESE until sufficient spermatozoa were extracted for ICSI. Micro-TESE was used in which seminiferous tubule are directly examined throughout the testis using an operating microscope and selectively biopsied for all of the NOA patients as below in Schlegel's method. Briefly, a midline incision was

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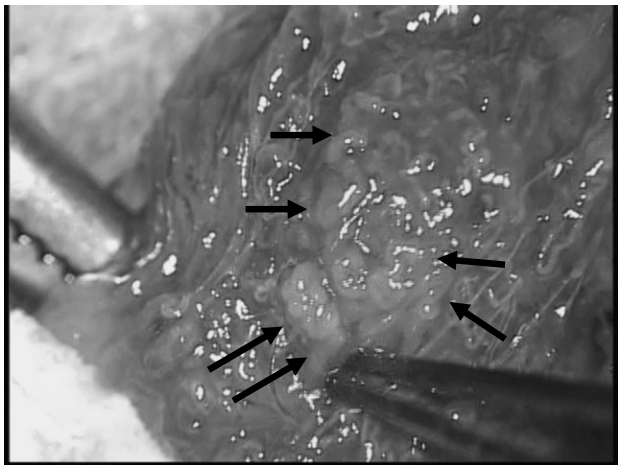

Fig. 1

made in the scrotum, and the tunica vaginalis was opened and the testis covered with the tunica albuginea was visualized. The remainder of the procedure was performed under an operative microscope. After the tunica albuginea was opened, direct examination of the testicular parenchyma was performed at x15-25 magnification (Fig. 1). Small samples (5-10mg) were excised from the tubules. If no spermatozoa were identified in the initial sample, subsequent samples were taken from the same testis and, if needed, from the contralateral testis. Dissection was performed through all regions of testicular tissue, preserving the testicular blood supply. The procedure was terminated when spermatozoa were retrieved or when further dissection was thought likely to jeopardize the testicular blood supply [8]. In general, we define success of sperm retrieval as the recovery of at least one spermatozoa.

Sperm retrieval rate (SRR) by micro-TESE

The outcome of micro-TESE has been reported (Table 1) [1-3, 5, 8-12]. In 1999, the first report on this technique compared 22 patients whom underwent standard multiple biopsy with a group of 27 men undergoing micro-TESE. The author describes a significant improvement on the SRR when micro-TESE had been performed (63 versus 45%) [1]. A comparative study including 116 men found a significantly higher SRR with the addition of optical magnification compared with conventional TESE (47 versus 30%) [5]. To date, the largest series on Micro-TESE reported

Table1 Comparison of SRR between conventional and micro-TESE

Year	Author	Case (n)	SRR by conventional TESE(%)	SRR by micro-TESE (%)
1999	Schlegel et al.	27	45	63
2002	Amer et al.	100	30	47
2002	Okada et al.	74	16.7	44.6
2002	Tsujimura et al.	56	35.1	42.9
2004	Tsujimura et al.	180	−	44.4
2005	Ramasamy et al.	460	32	58
2005	Mulhall et al.	48	50	45
2009	Ramasamy et al.	792	−	60
2009	Ishikawa et al.	150	−	42(32, 44, 48)

TESE, testicular sperm extraction.

the results of 792 procedures achieving a SRR of 60% [11]. We reported the learning curves that SRR for NOA patients in the mid 50 cases (44%) and the latest 50 cases (48%) was significantly higher than first 50 cases (32%). SRR of micro-TESE are strongly influenced by the surgeon's case volume. A considerable amount of cases is necessary to reach an optimal plateau level of SRR in this microscopic surgery [12]. The outcome of SRR may depend on factors other than surgical technique, such as embryologists' learning curves.

Biopsy histology for SRR by micro-TESE in NOA patients

Pathological findings are important; many reports have shown a relation between successful TESE and testicular histopathology [13-15]. Tsujimura et al. [3] reported that SRR by micro-TESE for patients with Sertoli cell only syndrome (SCO) was 22.5%, whereas that by conventional TESE was 13%. Furthermore, SRR by micro-TESE for patients with hypospermatogenesis was 100%, whereas that by conventional TESE was 76.9%. Okada et al. also reported the reasonable result showing that SRR by micro-TESE for hypospermatogenesis was 100%. They also reported that SRRs by micro-TESE for maturation arrest (MA) and SCO were 75% and 33.9%, respectively, whereas those by conventional TESE were 37.5% and 6.3% [2]. Ramasamy et al. [8] reported SRRs of 81%, 44%, and 41% for hypospermatogenesis, MA, and SCO, respectively. We reported SRRs of 100%, 50%, and 27.8%, respectively [12]. Thus, it is well accepted that micro-TESE offers a great advantage for patients with all types of testicular histology.

Micro-TESE for KS

It has been reported that focal spermatogenesis can result in ejaculated spermatozoa or the retrieval of spermatozoa by micro-TESE. Several studies have been conducted with micro-TESE in patients with KS [16–18]. Schiffe et al. reported an impressive SRR (69.0%); however, these cases of KS were including mosaic type [17]. We reproted a SRR of 52.4% of 21 patients with non-mosaic KS by micro-TESE [12]. It has been reported that patient age in successful TESE for cases of KS is significantly younger than that in failed cases [16, 19, 20]. In NOA, the absence of uniformity in testicular tissue is a critical key to succeed and rationale in micro-TESE. If better portion or best portion can be identified under microscope, there is an increased chance to retrieve testicular sperm. In men with KS, the histological diagnosis was uniformly the SCO syndrome. In all patients in whom micro-TESE was successful we could identify focal spermatogenesis in dilated and opaque seminiferous tubules surrounded by shrunken tubules or fibrous tissue. Micro-TESE is particularly helpful for successful sperm retrieval in KS cases.

Predictors for sperm retrieval

Recently, the most popular treatment in patients with NOA has been micro-TESE with subsequent assisted fertilization by intracytoplasmic sperm injection (ICSI). With the spread of ICSI, the presence of a minimum number of spermatozoa is required for fertilization. Micro-TESE and intracytoplasmic sperm injection (ICSI) cycles expose the couple to an emotional and financial burden, so it would be beneficial to predict the success of sperm retrieval using noninvasive parameters before attempted procedure. An important preoperative serum parameter studied in the first years of TESE was FSH. In general, the serum concentration of FSH is inversely correlated with impairment of spermatogenesis. Recent studies have shown that elevated FSH levels have been associated with a low probability for the retrieval of spermatozoa in men [21] using random biopsy TESE techniques. Serum FSH is an indirect reflection of the spermatogenic function and histology of the testis as a whole. Therefore, FSH may predict the presence of sperm at random biopsy using conventional TESE techniques [22–25]. However, it is even more difficult to predict the presence of testicular spermatozoa in infertile men with severely impaired spermatogenesis. Serum FSH concentration is not related to the more advanced stages of spermatogenesis [26]. The relationship of FSH with spermatogenesis is not straightforward in men with NOA. Serum FSH level has a poor predictive value for successful micro-TESE.

Hung et al. have recently reported a subset of men with normal FSH, normalsized testes, and diffuse MA, who had lower sperm retrieval rates [27]. Therefore, in this subgroup of men with diffuse MA and normal FSH, the FSH level may reflect adequate control feedback from germ cells and Sertoli cells despite the absence of sperm production. Ramasamy et al. [11] showed that sperm retrieval was higher in NOA men with FSH>15 IU/mL than those men with FSH<15 IU/mL. Also, sperm retrieval rates were maintained even when the FSH value was markedly elevated. Lower FSH may be a reflection of the larger number of Sertoli cells in a larger testis, providing more control feedback to suppress FSH production. The excellent findings with higher FSH may reflect the sensitivity of microdissection in finding small areas of sperm production. It further illustrates that FSH is not able to resolve spermatogenesis on an individual tubule level, and, therefore, they should not be used as predictors of sperm recovery.

Recent reports have suggested that the serum concentration of inhibin B may be a good non-invasive predictor of spermatozoa retrieval by TESE [28–31] The production of sufficient inhibin B to maintain detectable serum concentrations in adults depends on spermatogenic activity. Inhibin B has been found to be slightly more sensitive than FSH as an index of spermatogenic status [32]. However, the predictive value of inhibin B is not considered sufficient to exclude or include patients for TESE [33, 34]. Therefore, a prognostic parameter for successful sperm retrieval in TESE seems to be decisive for male fertility. It is important to provide predictive information to patients and their spouses preoperatively and to allow them to determine whether to undergo micro-TESE.

We conclude that at the present time there are no absolute predictors of sperm yield for micro-TESE.

Postoperative complication

Although numerous studies have compared conventional versus micro-TESE [2, 3, 5], there are a few large comprehensive study analyzing the hormonal changes after micro-TESE. In addition, especially KS patients, the study of hormonal change after micro-TESE is lacking.

Given the testicular anatomic consideration, multiple site testicular biopsy is suspected to increase the risk of testicular damage caused by interruption of branches of the testicular artery [35] or pressure atrophy from intratesticular swelling and hematoma [36].

The most important advantage of micro-TESE is that meticulous hemostasis can be achieved under clear magnified vision. Ramasamy et al. [8] reported that for NOA patients, the initial decrease was followed by a return to 95% of the pre-micro-TESE T levels at the end of 18 months. In KS but not 46XY males with NOA, a decrease in the serum T levels after micro-TESE was noted. A recent study on micro-TESE for NOA also reported no change in T after the procedure [37]. However, interestingly, our KS study series demonstrated a significant decline in T by 30-35% ($P<0.01$) at 1 to 12 months, and returned to 75% of the pre-TESE levels after 18 months. The change in T levels in KS may have been a result of the much smaller testis and Leydig cell loss near the scar after the procedure [38].

In addition, Ramasamy et al. [8] showed that for NOA patients the mean FSH levels increased after micro-TESE significantly, but no significant difference in the LH levels was noted between the preoperative and postoperative groups. In our data, in 46XY males with NOA, serum levels of FSH increased significantly during 18 months follow up after micro-TESE and serum levels of LH at 1 to 3 months after micro-TESE were significantly increased from baseline concentrations, but no significant differences were observed in the levels of LH after 6 months compared to baseline [38]. Interestingly, FSH and LH concentrations in patients with KS were not significantly changed. The change in FSH levels for 46XY males with NOA may have been a result of the scar tissue left behind in the testis with local germ cell loss near the scar after the procedure [39]. Tash et al. [39] also suggested an increase in peritubular scar tissue that may have affected Leydig cells as well as germ cell number. The changes in FSH and LH after micro-TESE may suggest that the testes of NOA patients overall may be better able to respond than KS patients. This could reflect 1) a defect in pituitary responsiveness of KS patients, or 2) better testicular response in idiopathic NOA patients.

Even Micro-TESE could be suspected to increase the risk of testicular damage caused by interruption of branches of the testicular artery, pressure atrophy from intratesticular swelling, or an increase in peritubular scar tissue that may have affected Leydig cells as well as germ cell number. This technique was developed to minimize unnecessary damage to the testis and also to improve the sperm recovery. T itself may have a central or permissive role in the pathogenesis of the metabolic syndrome and type 2 diabetes by increasing skeletal muscle tissue and decreasing abdominal obesity and nonesterified fatty acids, consequently improving insulin sensitivity [40]. Hypogonadism in KS may cause an unfavorable change in body composition and metabolic syndrome [41]. We may recommend that patients in KS with low T level after micro-TESE may be treated properly to prevent the long-term deleterious consequences of hypogonadism. We would like to add that since serum T levels are abnormal at baseline for KS patients, in general, many KS patients (before micro-TESE) were already candidates for androgen replacement.

Although the microdissection procedure is relatively safer and improves the sperm retrieval rate significantly in patients with NOA, we should take care of the hypogonadism in KS patients after even microdissection procedure.

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