Optimal Number of Biopsies and Impact of Testicular Histology on the Outcome of Testicular Sperm Extraction

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Purpose: To determine the optimal number of biopsies in patients with non-obstructive azoospermia (NOA) who undergo testicular sperm extraction (TESE), and assess the impact of testicular histology on outcome.

Materials and Methods: Seven hundred and forty-one patients with NOA who underwent TESE in our institution were enrolled in the study. Testicular sperm extraction was performed applying an open surgical technique on the larger testis. The number of biopsies varied according to the presence or absence of spermatozoa. No further biopsies were obtained once spermatozoa were detected. If no spermatozoa were seen, the procedure was continued to a maximum number of 5 biopsies, including a single biopsy of the contralateral testis.

Results: Spermatozoa were obtained in 330 (44.5%) patients after a single biopsy. The success rate increased to 381 (51.4%), 416 (56.1%), 433 (58.4%), and 441 (59.5%) after the second, third, fourth, and contralateral sampling, respectively. Multiple sampling increased the success rate; however, success rate did not increase considerably after the third sampling. Performing contralateral testicular biopsy was advantageous in patients with uniform or mixed pattern hypospermatogenesis.

Conclusion: We recommend performing at least 3 biopsies in patients with NOA who undergo TESE. Further biopsies may also be advantageous when the NOA is a consequence of either uniform or mixed pattern hypospermatogenesis.

Keywords: azoospermia, infertility, histology, sperm retrieval

INTRODUCTION

Men with non-obstructive azoospermia (NOA) have long been considered irrevocably infertile. However, with the advent of testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI), these patients have the opportunity to recover from infertility. Due to existence of isolated foci of active spermatogenesis, sperm can be retrieved in some infertile men with NOA. Sperm acquisition success rate varies in different studies and ranges from 17% to 60%.[1-5]

Different prognostic factors have been recommended to predict the success rate of TESE, of which testicular histopathological pattern is more accurate.[6-8] Although various patterns of testicular histology can be treated applying TESE + ICSI, sperm acquisition success rate varies based on testicular histopathology and ranges from 15% in Sertoli cell only (SCO) syndrome to more than 90% in hypospermatogenesis.[8,9] In addition to the histologic pattern, presence of sperm in prior biopsies also predicts the success probability of the subsequent sperm retrieval procedures.[10,11]

Two different approaches have been considered to perform conventional TESE, including sampling a larger testicular tissue through a single incision and multiple biopsies through different small incisions in tunica albuginea. There is inconsistency in the literature concerning the optimal number of biopsies while applying the latter approach.[12] Multiple biopsies may be associated with a significant loss of testicular tissue, an impaired testosterone synthesis,[13,14] and increased risk of interruption of the blood supply while single biopsy may be associated with decreased success rate. Histological pattern and success or failure of previous biopsies may also affect the optimal number of biopsies.

We conducted this study to determine the optimal number of biopsies in patients with NOA to increase the likelihood of success and avoid multiple unnecessary biopsies. We also evaluated the impact of testicular histology and the success or failure of prior biopsies on the outcome of subsequent sperm retrieval procedure.

MATERIALS AND METHODS

Between March 2007 and December 2010, we reviewed the medical record of 905 patients with NOA who underwent TESE in our institution. To assess the optimal number of biopsies, data of 741 patients who underwent TESE with a uniform protocol were considered for analysis.

Four hundred and forty-one patients were referred to our institution with history of prior testicular biopsy and 300 underwent TESE + ICSI in our institution without prior diagnostic biopsy. Azoospermia was confirmed by the analysis of two centrifuged semen specimens based on World Health Organization guidelines. The study was conducted in accordance with the declaration of Helsinki, and institutional review board approved the study.

Testicular sperm extraction was performed using an open surgical technique on the larger testis. A 5-mm incision was made on tunica albuginea, and the extruded tissue was excised. The number of biopsies varied according to the presence or absence of spermatozoa. No further biopsies were obtained once adequate spermatozoa with acceptable quality were detected. Each specimen was deposited into Bouin solution. A touch-prep was prepared by blotting the cut edge of the testis with a glass slide. If no sperm was found, the specimen was crushed under coverslip and examined using light microscopy. If no spermatozoa were seen, the procedure was continued to a maximum number of 4 biopsies, and an additional single biopsy was also taken from the contralateral testis.

Thereafter, a specimen was sent to the histopathology laboratory in Bouin solution. We also reevaluated the histopathological sections if they were inconsistent with standard classification. Since the aforesaid protocol requires multiple samplings, it is not applicable in patients with very small or atrophied testes, and such patients have not been included in the analysis.

Statistical analysis was performed using SPSS software, (the Statistical Package for the Social Sciences, Version 16.0, SPSS Inc, Chicago, Illinois, USA). Comparison of qualitative variables was performed using the Chi-Square test. Two-tailed $P < .05$ was considered statistically significant.

RESULTS

Based on the histopathological findings, patients were di-

Table 1. Cumulative incidence of positive testicular sperm extraction according to the number of biopsies in different histopathological patterns.

Histopathological pattern	Number of biopsies				Contralateral biopsy
	1	2	3	4	
Uniform hypospermatogenesis	114 (73.5%)	132 (85.2%)	141 (91.0%)	147 (94.8%)	151 (97.4%)
Early maturation arrest	64 (46.4%)	75 (54.3%)	81 (58.7%)	83 (60.1%)	83 (60.1%)
Late maturation arrest	22 (20.6%)	26 (24.3%)	29 (27.1%)	29 (27.1%)	29 (27.1%)
Sertoli cell only	29 (17.8%)	35 (21.5%)	41 (25.1%)	41 (25.1%)	41 (25.1%)
Hypospermatogenesis with mixed pattern	101 (56.7%)	113 (63.5%)	124 (69.7%)	133 (74.7%)	137 (77.0%)

vided into 4 groups, including uniform hypospermatogenesis, germ cell maturation arrest, SCO appearance, and hypospermatogenesis with mixed pattern. Of 741 men who were assessed in the study period, uniform hypospermatogenesis, early maturation arrest, late maturation arrest, SCO appearance, and hypospermatogenesis with mixed pattern were evident in 155 (20.9%), 138 (18.6%), 107 (14.4%), 163 (22.0%), and 178 (24.0%) patients, respectively.

Spermatozoa were obtained in 330 (44.5%) patients after a single biopsy. The success rate increased to 381 (51.4%), 416 (56.1%), 433 (58.4%), and 441 (59.5%) after the second, third, fourth, and contralateral sampling, respectively. Although the success rate increased considerably with further biopsies, this increase was minimal after the third sampling.

We also assessed the success rate among patients with different histopathological patterns and noted a considerable higher success rate among patients with hypospermatogenesis. Although contralateral testicular biopsy yielded no spermatozoa in patients with SCO and germ cell maturation arrest histological patterns, 8 patients with hypospermatogenesis, either uniform or mixed pattern, underwent successful TESE in the contralateral testis. Table 1 shows the success rate of TESE in different histopathological patterns according to the number of biopsies.

In the second analysis, patients with prior history of testicular biopsy were divided into 2 subgroups based on the presence or absence of spermatozoa in their testicular biopsy specimen. One hundred and fifty-four patients had spermatozoa (Sp^+) in their prior biopsy and 287 patients had prior negative biopsies (Sp^-). Sertoli cell only and hypospermatogenesis were the predominant histologic patterns in Sp- and Sp+ subgroups, respectively (Table 2).

Differences in histopathology between initial biopsy and subsequent TESE were noted in 18 patients. Although initial biopsy had shown SCO pattern in 124 patients, 18 turned out to have mixed pattern hypospermatogenesis in subsequent evaluation during TESE. Almost all Sp+ patients had successful sperm retrieval irrespective of histopathological pattern. Furthermore, multiple sampling was associated with increased success rate, especially among Sp^+ patients and those with hypospermatogenesis (Table 3). Regardless of the result of prior biopsy, performing more than three biopsies, including contralateral testicular biopsy, did not improve the success rate in patients with SCO pattern or late maturation arrest. Statistical analysis showed that the outcome of prior biopsy and histopathology of the testis significantly affected the success rate ($P < .001$).

No clinically significant hematoma or testicular atrophy was reported during postoperative follow-up period. However, routine postoperative ultrasonography and testosterone measurement were not performed to detect hematoma and hypoandrogenism, respectively.

DISCUSSION

With the advent of TESE combined with ICSI, men previously considered infertile may father children. Isolated foci of active spermatogenesis may exist in the testes of men

Table 2. Frequency of different histopathological patterns in patients with positive and negative prior testicular biopsy.*

Histopathological pattern from TESE specimens	Patients with Sp^+	Patients with Sp^-	Total
Uniform hypospermatogenesis	63 (64.9%)	34 (35.1%)	97 (100%)
Early maturation arrest	27 (37.5%)	45 (62.5%)	72 (100%)
Late maturation arrest	4 (8.9%)	41 (91.1%)	45 (100%)
Sertoli cell only	12 (11.3%)	94 (88.7%)	106 (100%)
Hypospermatogenesis with mixed pattern	48 (39.7%)	73 (60.3%)	121 (100%)

*TESE indicates testicular sperm extraction; Sp^+, patients with positive prior biopsy; and Sp^-, patients with negative prior biopsy.

with NOA leading to successful sperm retrieval in these men. There is no consensus regarding the optimal number of biopsies for sperm retrieval in men with NOA. Some authors hypothesize that multifocal distribution of the spermatogenesis is present throughout the entire testis and accordingly, advocate a single testicular biopsy[15,16] whereas others find a patchy distribution of foci of active sperm production and recommend multiple samples from different sites.[17-21]

Hauser and colleagues in a study of 29 men with NOA noted that between 18% and 32% of spermatozoa would have been missed with single biopsy.[19] Comparing multiple and single sampling, Amer and associates revealed a significantly higher retrieval rate with multiple biopsies.[21] Nevertheless, multiple sampling has been postulated to be associated with increased risk of interruption of blood supply, fibrosis, or autoimmune response. Therefore, optimization of the number of biopsies and avoiding unnecessary multiple samplings may prevent the potential hazards of biopsy.[5,18]

Patchy distribution of foci with minimal spermatogenesis may be more prominent in specific histopathological patterns and accordingly performing multiple biopsies from different sites may be of greater importance in these histopathological patterns. Few investigators have thus far reported the optimal number of biopsies in NOA patients who undergo TESE. To the best of our knowledge, this is the first study which evaluates the optimal number of biopsies based on the standardized histopathological classification. In our study, multiple testicular sampling, including contralateral biopsy in men with hypospermatogenesis, was associated with increased success rate. This improvement was also evident in adverse histopathological patterns, including SCO pattern and germ cell maturation arrest. However, in case of SCO and late maturation arrest, further biopsies are associated with minimal increase in success rate, and

Table 3. Cumulative incidence of positive testicular sperm extraction according to the number of biopsies and the result of prior biopsy in different histopathological patterns.

Histopathological pattern		Number of biopsies				Contralateral biopsy
		1	2	3	4	
Uniform hypospermatogenesis	Sp^+	55 (87.3%)	60 (95.2%)	63 (100%)	-	-
	Sp^-	25 (73.5%)	31 (91.2%)	34 (100%)	-	-
Early maturation arrest	Sp^+	18 (66.7%)	22 (81.5%)	25 (92.6%)	27(100%)	-
	Sp^-	22 (48.9%)	25 (55.5%)	27 (60.0%)	27 (60.0%)	27(60.0%)
Late maturation arrest	Sp^+	2 (50%)	4(100%)	4 (100%)	4 (100%)	-
	Sp^-	9 (21.9%)	11 (26.7%)	12 (29.3%)	12 (29.3%)	12 (29.3%)
Sertoli cell only	Sp^+	6 (50.0%)	8 (66.7%)	10 (83.3%)	10 (83.3%)	10 (83.3%)
	Sp^-	16 (17.0%)	18 (19.1%)	20 (21.3%)	20 (21.3%)	20 (21.3%)
Hypospermatogenesis with mixed pattern	Sp^+	32 (66.7%)	36 (75.0%)	41 (85.4%)	46 (95.8%)	48 (100%)
	Sp^-	43 (58.9%)	46 (63.0%)	48 (65.7%)	50 (68.5%)	50 (68.5%)

Sp^+ indicates patients with positive prior biopsy; and Sp^-, patients with negative prior biopsy.

no improvement was noted when the number of biopsies increased from three to five. Our study is not capable of recommending a definite cut-off for optimal number of biopsies in each histopathology category. Nevertheless, the importance of performing multiple biopsies cannot be understated, especially in the case of mixed pattern and uniform hypospermatogenesis.

Hypospermatogenesis with mixed pattern is likely to show patchy distribution of spermatogenesis. This term has recently been defined in a more concise way in the literature and has changed prior methods of interpretation of histopathology sections in which the predominant pattern was reported.[22] In the current histopathological classification, the term SCO syndrome corresponds to the cases in which all the tubules show absence of germ cells and small foci of spermatogenesis precludes the diagnosis of SCO.[22]

Both prior successful TESE and biopsy have been associated with high success rate of subsequent TESE.[23] A retrospective study showed that in men with prior successful TESE, the second and third attempt are associated with 74.7% and 82.3% sperm retrieval rate, respectively.[1] In the present study, sperm was retrieved in 152 of 154 patients with prior successful biopsy (Sp^+), and the success rate among patients with prior negative biopsy (Sp^-) varied from 21.3% to 100% depending on the histopathological pattern. The likelihood of Sp^+ biopsy was higher in hypospermatogenesis. Furthermore, 18 patients with Sp^+ biopsies with SCO pattern turned out to be hypospermatogenesis with mixed pattern in the subsequent TESE.

The term SCO syndrome corresponds to the pattern wherein no germ cells are found. Therefore, the sperm retrieval rate is expected to be extremely lower in SCO pattern than what is present in the literature. This overestimation may be related to the absence of germ cells in the biopsied specimen, which leads to the diagnosis of SCO pattern, whereas there may be other undetected foci of seminiferous tubules showing normal or decreased maturation, which corresponds to hypospermatogenesis with mixed pattern and is associated with higher success rate.

Due to inconsistency between different parts of testicular tissue and considering that only a small specimen from a single focus of the testis will be available for histopathological evaluation, accurate diagnosis may be difficult in NOA. Furthermore, a prior sperm extraction procedure or diagnostic biopsy is required to provide information concerning testicular histology. Histopathology is not applicable as a prognostic factor for many patients who undergo sperm extraction as the majority of patients undergo TESE without performing diagnostic biopsy. A considerable number of patients presented in this study were referred to our institution with prior diagnostic biopsies. However, this is not a reasonable practice, especially when it is not associated with cryopreservation. Despite the inaccuracy of histopathological diagnosis, it may be helpful in planning multiple sampling in selected cases when it is available prior to TESE procedure. In the case of hypospermatogenesis, either uniform or mixed pattern, the probability of the presence of spermatozoa is relatively high and performing further biopsies may improve the outcome.

Fine needle aspiration biopsy is a simple and less invasive biopsy technique to retrieve spermatozoa. Lewin and colleagues showed 60% retrieval rate using this technique.[24] Another report has also mentioned 47% success rate with no complication.[25] Despite high success rate in some studies, several studies have shown that open surgery is successful more often than aspiration biopsy.[26,27] Nevertheless, it should be considered that prior history of successful sperm retrieval and favorable histopathology, if known pre-operatively, can provide an opportunity to apply aspiration biopsy prior to open surgery. In these conditions, there would be a high probability to retrieve spermatozoa using a less invasive aspiration technique.

Microdissection TESE was developed as an alternative to conventional TESE to minimize the testicular tissue loss and enhance retrieval success rate. Some investigators have shown that microdissection TESE increases sperm retrieval rate.[28] Sperm retrieval success rate in TESE varies between 16.7% and 62% in different studies[1-4,29,30] while ranges between 43% and 63% in microdissection TESE.[2-4,31] Therefore, no robust data confirm the superiority of microdissection TESE, and various studies have shown controversial data. Some authors have shown that microdissection TESE is associated with higher success rate in cases of SCO pattern[2,32] while others found a significantly higher sperm re-

trieval rate in cases of hypospermatogenesis.[33] However, as mentioned previously, many patients with mixed pattern hypospermatogenesis may be considered as having SCO syndrome or maturation arrest. This misinterpretation overestimates the success rate of TESE for NOA patients with either SCO syndrome or maturation arrest.

Conventional TESE has been replaced by microdissection TESE in many centers, including our institution. Nevertheless, it should be considered that this technique is more intricate and requires a significant learning curve.[34] This technique requires magnification equipment and is less accessible to all centers. Furthermore, operation time is significantly longer in microdissection TESE. Therefore, TESE with multiple sampling may still be an acceptable option for selected patients with NOA.

CONCLUSION

Although less testicular tissue is extracted with microdissection TESE, which makes it more appropriate for small volume testes, conventional TESE can be an alternative in selected cases. Two different approaches have been considered to perform conventional TESE, including sampling a larger testicular tissue through a single incision and multiple biopsies through different small incisions in tunica albuginea. We do not recommend less than 3 samples when the latter approach is applied. Further biopsies are also reasonable when the histopathology is known to be either mixed pattern or uniform hypospermatogenesis prior to procedure.

CONFLICT OF INTEREST

None declared.

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