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ORIGINAL ARTICLE

Male Fertility

Sperm fine-needle aspiration (FNA) mapping after failed microdissection testicular sperm extraction (TESE): location and patterns of found sperm

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We sought to evaluate the ability of fine-needle aspiration (FNA) mapping to find sperm and to guide sperm retrieval after failed microdissection testicular sperm extraction (micro-TESE) in nonobstructive azoospermic men. In this study of consecutive male infertility cases, interventions included testicular FNA mapping and subsequent sperm retrieval. Outcomes included the frequency and location of found sperm on FNA maps after failed micro-TESE and the salvage sperm retrieval success. Among 548 patients undergoing FNA mapping from 2010 to 2016, 82 men with previous micro-TESE procedures were identified. The mean time between micro-TESE and FNA mapping was 2.2 years. A total of 2825 (1424 on right and 1401 on left) sites were mapped. At least one site revealed mature sperm in 24 (29.3%) of 82 men with prior failed micro-TESE procedures. There was an equal likelihood of detecting sperm in either testis (6.1% right; 5.7% left; $P = 0.58$). Digital “heat maps” revealed differences in sperm findings within the testis with mature sperm more likely found in the testis periphery rather than centrally. Fifteen (62.5%) patients subsequently underwent sperm retrieval procedures guided by FNA maps. Sufficient sperm were retrieved in all cases, and in 10 (66.7%) of 15 cases, extra sperm were frozen for future use. In a significant proportion of failed micro-TESE procedures representing the largest study to date, sperm were detected by FNA mapping and could be reliably retrieved through FNA map-guided surgical sperm retrieval. When present, sperm were more likely to be found in the testis periphery rather than centrally with FNA mapping. *Asian Journal of Andrology* (2019) 21, 50–55; doi: 10.4103/aja.aja_68_18; published online: 31 August 2018

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INTRODUCTION

Azoospermia is the absence of spermatozoa in the semen from centrifuged semen samples.¹ Obstructive azoospermia is due to blockages within the reproductive tract, and nonobstructive azoospermia is generally due to testicular failure. With the advent of *in vitro* fertilization (IVF) in 1978² and intracytoplasmic sperm injection (ICSI) in 1992,³ the opportunities for men with both types of azoospermia to conceive have increased dramatically.

Nonobstructive azoospermia (NOA) underlies most cases of azoospermia. While all men with obstruction will have testicular sperm for IVF-ICSI, only about half of NOA cases will have mature testicular sperm.^{4,5} Furthermore, clinical parameters such as testicular size, hormone levels, and testicular biopsy patterns do not accurately predict whether a procedure will yield sperm.^{5,6} Further complicating sperm retrieval in NOA is the “focal” nature of spermatogenesis which makes failure to find sperm common in “blind” or randomly performed sperm retrievals.^{7,8}

To address the problem of focal sperm production in NOA, several strategies are employed. Multibiopsy testicular sperm

extraction (TESE) increases the chances of successful sperm retrieval by increasing biopsy number.⁶ Compared to a single tunical incision in a simple TESE procedure, multibiopsy TESE involves several tunical incisions (up to 15) with tissue extraction until sufficient sperm is obtained. Recent, larger series have reported sperm retrieval rates of 47%–48% using a multibiopsy approach.^{9,10} Another popular strategy is microdissection TESE (micro-TESE) which involves a large equatorial incision in the tunica albuginea followed by a thorough inspection of the testis parenchyma using operative microscopy and culminating in the biopsy of larger and more opaque seminiferous tubules that are more likely to contain sperm.¹¹ Based on two recent systematic reviews, sperm retrieval rates can be up to 1.5-fold higher with micro-TESE than with simple TESE.^{12,13}

A third strategy for finding sperm in NOA patients employs fine-needle aspiration (FNA) “mapping” which involves the systematic sampling of tissue needle “cores” throughout the testicle in three dimensions.¹⁴ After Papanicolaou staining, formal cytological analysis identifies sperm precursor cells (including spermatogonia, primary spermatocytes, and spermatids) as well as mature sperm and allows for the accurate assessment of complex histological patterns within NOA

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testes.¹⁵ Following FNA mapping, subsequent sperm retrieval which is “guided” by the map findings is performed. This two-step approach increases the chances of successful sperm retrieval by focusing TESE procedures on testicular regions known to have mature sperm.^{16,17} Importantly, sperm retrieval procedures performed in conjunction with FNA mapping do not rely on the visual cues of testicular tubule size and opacity that are critical for successful micro-TESE.^{18,19} Reported rates of finding sperm in NOA patients with FNA mapping range from 47% to 68% depending on the population studied.^{7,17,20}

Unfortunately, no prospective, randomized, controlled trials have compared the efficacy of various sperm retrieval approaches used in NOA. Because of the essential methodological differences between micro-TESE and the two-stage FNA-mapping approaches to sperm retrieval, we hypothesized that there might be cases in which FNA mapping detects sperm after previously failed micro-TESE. We evaluated a consecutive series of men who underwent FNA mapping after failed micro-TESE and analyzed the frequency of finding sperm, the location and distribution of found sperm, and the ability to subsequently retrieve sperm.

PATIENTS AND METHODS

Patient population

FNA-mapping procedures were performed in consecutive, infertile men with NOA. A medical history and physical examination was performed and testis volume was estimated using a standard Prader orchimeter (ASSI, Westbury, NY, USA). In addition, at least one semen analysis with a centrifuged pellet of ejaculate was examined to confirm true azoospermia. Endocrine tests included total testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Patients were offered a compound FNA-mapping procedure⁷ under the following conditions: (a) documented evidence of no mature sperm found on prior bilateral or unilateral (in cases of a solitary testicle) micro-TESE; (b) a period of at least 6 months elapsed between the micro-TESE and the planned FNA map; (c) any irreversible root cause of NOA was allowable (*e.g.*, genetic); (d) NOA due to reversible causes (*e.g.*, testosterone supplements) were excluded, and (e) patients with virtual azoospermia or cryptozoospermia were excluded. Complication rates after FNA mapping were also assessed. Anonymized data were collected retrospectively (exempt from patient consent), and all analyses were performed according to the Health Insurance Portability and Accountability Act (HIPAA, 1996) standards and with approval from the Sutter Health Institutional Review Board (San Francisco, CA, USA).

FNA-mapping technique

The FNA-mapping procedure was performed as previously described.¹⁴ Briefly, after local anesthesia was given, the testis was positioned with the epididymis directed posteriorly. The scrotal skin was stretched taut over the testis and wrapped behind it with a surgical sponge. The “testicular wrap” served as a convenient handle to manipulate the testis and also fixed the scrotal skin over the testis. Using a sterile marking pen, the planned aspiration sites were marked on the scrotal skin overlying the anterior testis, systematically placed in a grid-like manner at 18 sites about 5 mm apart. FNA was performed with a sharp-beveled, 23-gauge, 1-inch fine needle (Becton-Dickinson Co., Franklin Lakes, NJ, USA) along with a 10-ml syringe and Cameco syringe holder (Precision Dynamics Corp., San Fernando, CA, USA) using the established suction-cutting technique. Precise, gentle, in-and-out movements, varying from 5 mm to 25 mm, were used to aspirate tissue fragments which were then expelled onto a nonsterile microscope slide, smeared, and fixed in 95% ethyl alcohol. Pressure was applied to each

site for hemostasis. Subsequently, a routine Papanicolaou differential stain was performed along with a comprehensive cytological review by experienced cytopathologists (IC and KCP). Slides were examined for (a) specimen adequacy (at least 12 clusters of testis cells or at least 2000 well-dispersed testis cells) and (b) all testicular cell types, including the presence of sperm with tails.¹⁴

Sperm retrieval techniques

When sperm was found on FNA mapping, couples were counseled to proceed with sperm retrieval and IVF-ICSI. The chosen method of testis sperm retrieval was governed by the FNA-mapping findings and involved testicular sperm needle aspiration (TESA) if sperm were detected globally at all sites, TESE if >2 mapped sites showed sperm, or micro-TESE if <2 mapped sites showed sperm.⁸ A successful sperm retrieval was defined as having sufficient mature sperm for all eggs retrieved at IVF.¹⁶ The ability of the FNA mapping to guide sperm retrieval for IVF-ICSI after failed micro-TESE was assessed.

Analysis of FNA map findings

The overall rate of found sperm on FNA mapping after failed micro-TESE was calculated. Rates of found sperm in each testis were assessed and compared, as was the rate of finding sperm at each testis FNA site, using descriptive statistics.

To localize regions of detected sperm, we used the fact that FNA-mapping procedures were performed using a standardized, systematic template (**Figure 1**) and collated and analyzed data obtained from individual testis maps to establish whether there was a “sidedness” to find sperm in the testes and whether certain testis regions were more likely to contain sperm than others after failed micro-TESE. Similar to the analysis of FNA maps performed on nonmicrodissected men previously reported,⁷ we generated sperm spatial “heat maps” for each testis to describe the distribution of found sperm (Plotly, Montreal, Canada). Differences in sperm detection among various sampled regions of the testicle were compared using two-tailed unpaired *t*-test (GraphPad Software Inc., La Jolla, CA, USA).

As testicular histology is assessed at each FNA site, we analyzed the relationship between testis histologic pattern and found sperm at each FNA site using descriptive statistics. Histological patterns employed were those defined by Levin,²¹ and included Sertoli cell only or germ cell aplasia, early (primary spermatocyte) and late (spermatid) maturation arrest, hypospermatogenesis, and sclerosis.

RESULTS

Patient clinical characteristics

Among 548 patients undergoing FNA mapping from 2010 to 2016, 82 men with previous micro-TESE procedures were identified. Prior micro-TESE procedures were performed across 40 different centers worldwide; none were performed by study authors. The mean age of male patients was 34.3 (standard deviation [s.d.]: 5.1; range: 21–56) years and mean FSH and testosterone levels were 23 (s.d.: 20) mIU ml⁻¹ and 337 (s.d.: 140) ng ml⁻¹, respectively. Mean testicular volumes after micro-TESE and before FNA were 12.3 (s.d.: 4.3) ml for right and 11.9 (s.d.: 4.1) ml for left. The mean time from micro-TESE to FNA mapping was 2.2 (s.d.: 1.7) years.

Analysis of FNA map findings

A total of 2825 mapped sites (1424 on right and 1401 on left) were performed in 82 men after failed micro-TESE. At least one site showed mature sperm on FNA mapping in 29.3% (24/82) men with prior failed micro-TESE procedures. There was an equal likelihood of detecting sperm in either testis (6.1% [87/1424] on right sites; 5.7%

[80/1401] on left sites; $P = 0.58$). Complications after FNA mapping include a spermatic cord hematoma ($n = 1$) and painless gross hematospermia ($n = 3$), all resolving within 1 week.

Previously, we examined the testis location-specific frequencies of finding sperm among micro-TESE-naive patients with NOA and did not find significant differences across FNA sites within or between right or left testicles.⁷ In the same manner, sperm frequency maps were constructed for postmicro-TESE patients in this study (Figure 2). Digital spatial “heat maps” revealed differences in sperm findings within both testicles. A general observation was that the frequency of finding sperm in the central testis was lower than the peripheral testis in both testes. More specifically, sperm were more likely to be found in the lateral pole of the right testis (Figure 2a) and in the superior aspect of the left testis (Figure 2b) than elsewhere. Finally, the difference between the “peak” areas of sperm detection compared to the remaining FNA sites (“valleys”) in each testis was statistically significant on the left ($P = 0.03$) but not the right ($P = 0.08$) sides (Figure 3).

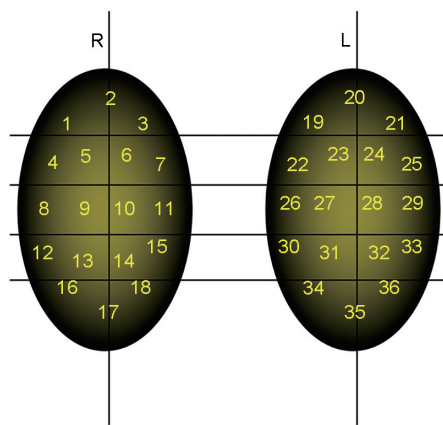


Figure 1: Standardized, compound (extended) FNA-mapping template used for all patients after failed micro-TESE procedures. Numbers represent the sequence of individual sites sampled by FNA. FNA: fine-needle aspiration; R: right testicle; L: left testicle; micro-TESE: microdissection testicular sperm extraction.

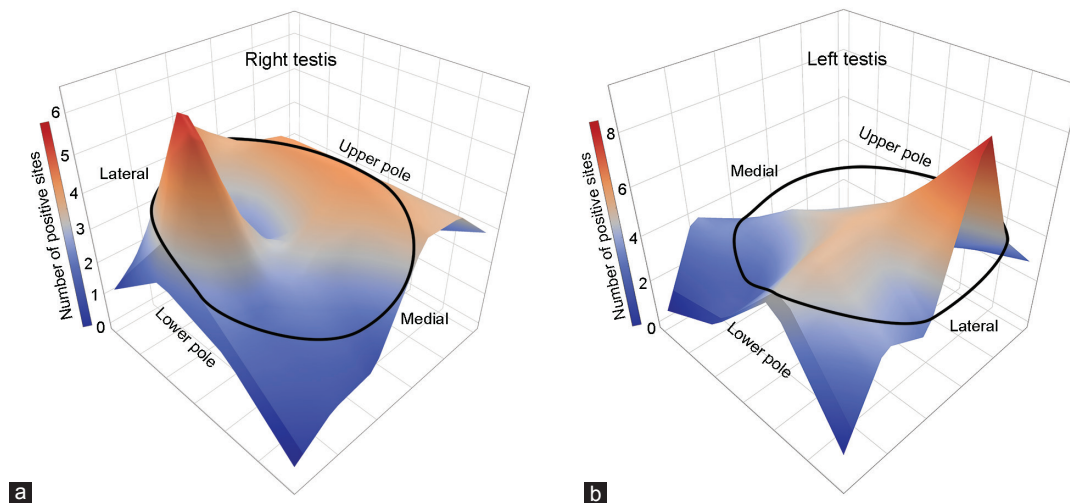


Figure 2: Digitally generated “sperm heat maps” of the (a) right and (b) left testicles showing the frequency of finding sperm by geographical location in each testis. The X-Y plane shows the relative location of found sperm in each testis FNA site. The vertical or Z-plane shows the relatively frequency of finding sperm at each FNA site. The higher the peak, the higher the frequency of found sperm at that site. Note that in each testicle, there is a dearth of found sperm in centrally located FNA sites compared with peripheral FNA sites. FNA: fine-needle aspiration.

Testicular histology was assessed at each FNA aspiration site, so it was possible to evaluate the histological patterns within each testis (Figure 4). In this analysis, we sought to identify correlations between testis histology and rates of sperm detection. Cumulatively, across all FNA sites, the Sertoli cell-only pattern was the most commonly observed finding (67.5% on right, 67.1% on left), followed by early maturation arrest (20.2%, 21.9%), late maturation arrest (6.6%, 4.9%), and complete spermatogenesis (5.7%, 6.1%). There were no significant differences in biopsy histology patterns between right and left testis sides. When FNA sites that most frequently showed sperm were compared to those that did not show sperm, there were also no significant shifts in associated histology frequencies (Figure 5).

Sperm retrieval success

Among 24 patients in whom FNA mapping detected sperm after failed micro-TESE procedures, 15 (62.5%) patients subsequently underwent sperm retrieval guided by the FNA maps. The mean baseline testosterone level among all men after micro-TESE and before FNA mapping was 310 (range: 36–500) ng dl⁻¹, with a mean FSH of 23 (range: 7–56) mIU ml⁻¹ and LH of 9.2 (range: 2.2–29) mIU ml⁻¹. Of note, 8 (53.3%) of 15 patients after micro-TESE were hypogonadal (serum total testosterone <300 ng dl⁻¹). Most men undergoing sperm retrieval (10/15, 66.7%) were “optimized” with medical therapy (anastrozole, clomiphene citrate, FSH, or human chorionic gonadotropin) or varicocele repair after FNA mapping and before sperm retrieval. The mean time from FNA map to sperm retrieval was 7.5 (range: 4–21) months.

The details of sperm retrieval procedure type and extent are summarized in Table 1. Sufficient sperm were retrieved in all cases ($n = 15$) to inject all oocytes at IVF. In addition, extra sperm were frozen in 10 (66.7%) of 15 cases. The mean number of sperm found was 113 (range: 25 to >200). Among female partners, the mean age at IVF-ICSI was 33 (range: 29–38) years. With complete follow-up on 9 (60.0%) of 15 couples pursuing IVF-ICSI, there were 6 pregnancies resulting in 4 births, 1 first-trimester miscarriage, and 1 ectopic pregnancy. Two couples did not conceive and 1 couple froze all embryos following preimplantation genetic testing.

DISCUSSION

Sperm retrieval in cases of NOA is fraught with complexity. Two large variables are that a significant proportion of NOA men will not have mature sperm, and if they do, sperm may be geographically localized within the testis.⁷ Further complicating the matter is the fact that NOA men considering sperm retrieval are 10-fold more likely to be hypogonadal than fertile men. This

Table 1: Sperm retrieval procedure and extent after failed microdissection (n=24 patients)

Descriptor	Results, n (%)
Patients for sperm retrieval with sperm found	15 (62.5)
Procedures performed (n=15)	
Micro-TESE	14 (93.3)
TESE	1 (6.6)
Bilateral procedures	2 (13.3)
Unilateral procedures	13 (86.7)
Right	7 (53.8)
Left	6 (46.2)
Seminiferous tubule findings (micro-TESE cases, n=14)	
Dilated tubules ²⁹	6 (42.9)
No dilated tubules	5 (35.7)
Equivocal	3 (21.4)
Successful sperm retrieval in patients with procedures	15 (100)

Micro-TESE: microdissection testis sperm extraction

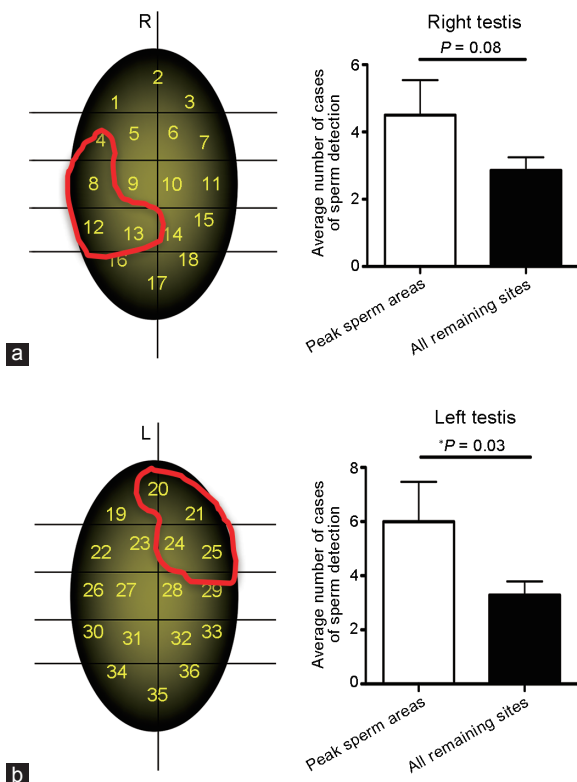


Figure 3: Peak area analysis of found sperm in the (a) right (R) and (b) left (L) testicles. The red lines encircle the areas of higher sperm detection in the schematic of each testicle. The associated bar charts compare the average sperm detection rates in the peak areas versus all of the remaining sites from the testicle. Statistically significant differences in sperm detection rates between peak areas and all other sites were demonstrated in the left but not the right testicles. * $P < 0.05$.

is important since sperm retrieval procedures carry the potential risk of worsening preexisting hypogonadism.^{19,22} For these reasons, several different strategies are used for sperm retrieval in NOA patients.⁸ Given the lack of randomized trials comparing various methods of sperm retrieval, no approach can claim to be the “gold standard,” and thus, comparisons between sperm retrieval techniques are needed to aid in clinical decision making for NOA patients.

In this study, we hypothesized that because of technical differences in sperm detection between two approaches to sperm retrieval in NOA men, there might also be differences in sperm retrieval success. Indeed, we observed that in a significant proportion of men who had failed micro-TESE procedures, we were able to (a) detect sperm by diagnostic FNA mapping and (b) subsequently reliably retrieve sperm through FNA map-guided surgical sperm retrieval. This demonstrates the ability of FNA mapping to “rescue” difficult NOA cases in which previous micro-TESE procedures failed.

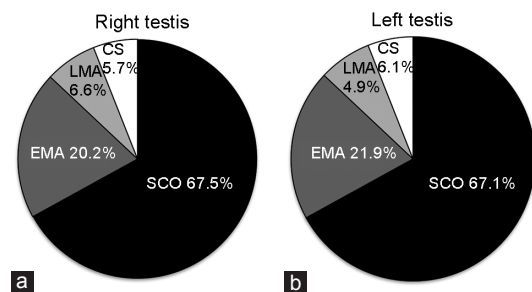


Figure 4: Charts showing the cumulative frequencies of the major testis histology patterns in the (a) right and (b) left testes based on FNA cytology. There were no significant differences in biopsy histology patterns between testis sides. SCO: Sertoli cell only; EMA: early maturation arrest; LMA: late maturation arrest; CS: complete spermatogenesis; FNA: fine-needle aspiration.

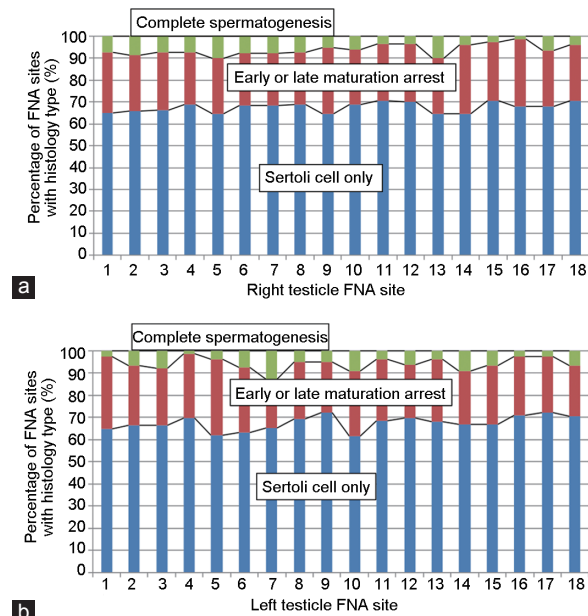


Figure 5: Charts showing the relative, site-specific frequencies for each of the major testis histology patterns in the (a) right and (b) left testes. Notably, when evaluated on a site-to-site basis, there were no shifts in histologic frequencies in sites that showed sperm compared to those that did not. FNA: fine-needle aspiration.

The fact that certain sperm retrieval procedures can find sperm when other approaches fail is not a new observation. We described the ability of FNA mapping to detect sperm in cases in which testis biopsies failed.⁷ Several authors have also noted the ability of micro-TESE to rescue cases after simple TESE procedures were unsuccessful.^{23–25} In these small series, the sperm retrieval rates of “salvage” micro-TESE procedures in NOA patients performed after failed biopsy or simple TESE procedures ranged from 45% to 60%. There is also an emerging literature that addresses the ability of “salvage” micro-TESE procedures to find sperm in cases of previously failed micro-TESE procedures. A report by Talas *et al.*²⁶ noted the finding at least 1 sperm in 3 of 5 cases of previously failed micro-TESE procedures. A sperm retrieval success rate of 10% was found using salvage micro-TESE at Weill Cornell Medical Center after prior failed micro-TESE procedures.²⁷ Finally, in the largest published series to date, among 48 cases of failed micro-TESE procedures, a salvage procedure found sperm in 6 (12.5%) overall and in 21% of cases receiving adjuvant hormonal therapy.²⁸ Based on this literature, FNA mapping compares favorably to “salvage” micro-TESE procedures regarding the ability to detect and retrieve sperm after prior failed micro-TESE procedures.

There are other observations from this research that merit emphasis. From the heat maps created from FNA-mapped testicles, the location of found sperm after failed micro-TESE appears to cluster in the organ periphery and much less so centrally. This is true for both the right and left testes. This suggests that micro-TESE procedures might adequately sample the central testis for sperm but may lack the same precision in the testis periphery. This, in turn, may relate to varying levels of aggressiveness used by different clinicians to retrieve seminiferous tubules during micro-TESE procedures.²⁷ Or, it may simply be easier to inspect the central testis than the peripheral testicle during micro-TESE procedures. Either way, these data serve as guidance to micro-dissectionists performing difficult sperm retrieval cases.

It is also notable that, in the vast majority of FNA map-guided salvage sperm retrieval procedures performed after failed micro-TESE, unilateral procedures were sufficient to procure sperm for IVF-ICSI, and surplus sperm was frozen in the majority of cases. By reducing both the extent (unilateral instead of bilateral) and need (by freezing sperm for future use) of micro-TESE procedures, this approach is “testis sparing” in nature. It also points out the value of “knowing before you go” that FNA mapping provides when contemplating sperm retrieval in difficult NOA cases. Finally, the fact that no dilated or only marginally dilated seminiferous tubules were observed in over half of the sperm retrieval cases after FNA mapping suggests that the finding of enlarged or dilated seminiferous tubules during micro-TESE, although helpful, is neither necessary nor sufficient for successful sperm retrieval in NOA.

Several limitations of this study can also be appreciated. It is neither randomized nor prospective in nature, thus claiming that FNA mapping and map-directed sperm retrieval is the new “gold standard” is not possible. In addition, prior micro-TESE procedures were performed by many clinicians and likely involved large variations in procedural performance, making it difficult to control for surgeon bias. Based on the limited number of patients proceeding to sperm retrieval, conclusions regarding the quality of retrieved testicular sperm are not possible. However, we believe that this proof of concept study, constituting the largest case series to date of NOA patients who have failed micro-TESE procedures, confirms the hypothesis that FNA mapping is a sperm retrieval approach that is conceptually distinct and different from, and a viable alternative to, micro-TESE procedures in these cases.

CONCLUSION

We report that sperm FNA “mapping” can find sperm in a substantial proportion of failed micro-TESE cases. Digital “heat maps” created with the FNA-mapping technique revealed that sperm was more likely to be found in the testis periphery rather than centrally after failed micro-TESE. In addition, the visualization of enlarged and opaque tubules was not necessary for finding sperm using FNA map-guided sperm retrieval. It is our hope that these data could provide information and guidance for urologic microsurgeons performing difficult sperm retrieval cases.

AUTHOR CONTRIBUTIONS

SJ collected the study data, performed data analysis, constructed tables and figures, and wrote and revised the manuscript. HKY and NT collected the study data, performed data analysis, constructed tables and figures, and reviewed the manuscript. IC and KCP collected and analyzed data and reviewed the manuscript. JWAR and PJT conceived the study concept and design, oversaw data collection and analysis, and wrote and revised the manuscript. PJT was responsible for organizing author input and submitting manuscript revisions. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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