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SEVERE TESTICULAR ATROPHY DOES NOT AFFECT THE SUCCESS OF MICRODISSECTION TESTICULAR SPERM EXTRACTION

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Abstract

Introduction and Objectives—Men with azoospermia and severe testicular atrophy may be counseled to avoid sperm retrieval due to perceived limited success. We evaluated the outcomes of microdissection testicular sperm extraction (micro-TESE) in men with severe testicular atrophy (volume ≤ 2 mL).

Methods—We reviewed the records of 1127 men with nonobstructive azoospermia who underwent micro-TESE followed by intracytoplasmic sperm injection. The men were classified into three groups based on average testicular volume (mL), ≤ 2 , >2 –10, >10 . Sperm retrieval, clinical pregnancy, and live birth rates were calculated. The clinical features evaluated included age, FSH level, history of cryptorchidism, Klinefelter syndrome, varicocele, and testicular histology from diagnostic biopsy.

Result(s)—Testicular sperm were successfully retrieved in 56% of the men. Sperm retrieval rates (SRR) in men with testicular volumes of ≤ 2 , >2 –10, and >10 mL was 55%, 56% and 55% respectively. Of those men who had sperm retrieved, clinical pregnancy and live birth rates were similar in the three groups (55.2%, 50.0%, and 47.0%; and 47.2%, 43.0% and 42.2% respectively). Of the 106 men with average testis volume ≤ 2 mL bilaterally, men who had sperm retrieved were younger (31.1 vs. 35.2 years), and were more likely to have a history of Klinefelter syndrome (82.2% vs 55.6%) compared to those in whom sperm was not found ($p < 0.05$). Men in this group had a higher prevalence of Klinefelter syndrome than men with testis >2 mL (72.6% vs 5.3%, $p < 0.0001$). Men younger than 30 years with Klinefelter syndrome had a higher SRR (81.8%) compared to men older than 30 without Klinefelter syndrome (33%, $p < 0.01$). There was no cut-point for age beyond which sperm could not be retrieved in men with small testes. On multivariable analysis, younger age was the only preoperative factor associated with successful sperm retrieval in men with small testes (< 2 mL).

Conclusion(s)—Testicular volume does not affect sperm retrieval rates at our center with micro-TESE. For men with the smallest volume testes, those younger men with Klinefelter

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syndrome had the highest sperm retrieval rates. Severe testicular atrophy should not be a contraindication for micro-TESE.

Keywords

Azoospermia; testis; biopsy; microorchidism; volume

INTRODUCTION

In patients with non-obstructive azoospermia (NOA), micro-TESE (testicular sperm extraction) has become a recognized and effective procedure in isolating sperm for intracytoplasmic sperm injection (ICSI). We have previously reported sperm retrieval rates with micro-TESE to be 50–60%.¹ Several factors have been evaluated and reported for prediction of sperm retrieval rates including but not limited to FSH^{2,3,4}, however no one single factor has been found to correlate with success. Men with NOA usually have smaller testicles than men with obstructive azoospermia⁵, except those with **early or late** maturation arrest histology⁶. Studies to date, however, have only shown poor correlations between testicular size and sperm retrieval rates,^{7,8} though no study has specifically evaluated the effectiveness of micro-TESE in men with severe testicular atrophy.

The primary aim of our study was to evaluate the outcomes of micro-TESE, including sperm retrieval, pregnancy rate, and live birth rate in patients with severe testicular atrophy (average testicular volume <2mL) and to identify preoperative clinical parameters that could help predict successful sperm retrieval in men with small testes.

PATIENTS AND METHODS

We retrospectively collected data from the records of 1127 men with NOA who underwent micro-TESE by a single urologist over a 12-year period between 1999 and 2011. Azoospermia was confirmed by analysis of two different specimens according to World Health Organization guidelines. An additional centrifuged semen sample was confirmed to be azoospermic on the day of the planned TESE. Testicular volume was measured by physical examination using a standardized orchidometer by a single surgeon⁹ and the average volume of the two testes was used for analysis. If only one testis was present then it was used in the calculation. The men were classified into three groups based on average testicular volume of <2mL, 2–10mL and >10mL. Clinical pregnancy in their female partners was defined by identification of gestational sac in which a fetal heartbeat could be seen on transvaginal ultrasound examination 6 weeks after embryo transfer. Confirmation of live birth was obtained by telephone interviews of couples who were identified with clinical pregnancy.

Microdissection TESE

The procedure has been described previously¹⁰. Briefly, a midline incision was made in the scrotum, and the scrotal content was pushed out preferentially from the side with the larger testis. The tunica vaginalis was opened, the testis uncovered, and 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 12 to 18× magnification. The examination included as much of the testicular parenchyma as possible. In some cases, it was necessary to use a right angle clamp, opening up the tunica albuginea to adequately expose the testicular parenchyma for dissection. Small samples (1–3 mg) of the larger, more opaque¹¹ tubules were dissected. Each sample was dropped into a suspension, passed through a 24ga angiocatheter and examined immediately by a skilled embryologist for the presence of the testicular spermatozoa by placing a small droplet of dispersed tissue suspension on a glass slide under a phase-contrast microscope at 200× magnification. 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. After the TESE procedure, the best testicular samples were pooled in 5 mL tubal fluid medium and the suspension was subjected to centrifugation at 1800g and examined carefully for the presence of even a single spermatozoon. The procedure was terminated when spermatozoa were retrieved or when further dissection was thought likely to jeopardize the testicular blood supply. If no spermatozoa were seen, then the sedimented testicular tissue was digested¹⁰ and analyzed.

Statistical Analysis

Data were analyzed using Stata version 9 (Stata Corp., College Station, TX). Fisher's exact test (two tailed) was used to compare testicular volumes for the outcome of sperm retrieval, pregnancy, live birth, and other categorical outcomes such as cryptorchidism. Student's t test (unpaired) was used to compare continuous data such as FSH level, male and female ages. Fisher's exact test, Chi-square analysis and Student's t test was used to calculate the p-values between the ≤ 2 mL and >10 mL groups for all dichotomous and continuous variables, respectively. P values of less than 0.05 were determined to be statistically significant.

RESULTS

A total of 106 men (9.4%) had ≤ 2 mL testis volume, 573 (50.8%) had testis volume between 2 mL and 10 mL, and 448 (39.8%) had testis volumes >10 mL. Sperm retrieval was successful in 631 (56%) men. The average testicular volume in men that failed to retrieve sperm (9.1 mL $\pm$ 4.85 mL) was similar to men that had successful sperm retrieval (9.1 mL $\pm$ 5.6 mL, $p=0.9$). Sperm retrieval rates were similar among men with different testicular sizes (54.7%, 56.2% and 55.1%, $p=0.53$ for sizes of <2 mL, between 2 and 10 mL, and >10 mL respectively). Similarly, of the men in whom sperm was retrieved, there were no significant differences seen in the rates of pregnancy in their female partners (55.2% 50.0% and 47.0%, $p=0.39$). In the women who achieved clinical pregnancy, there was no difference in the rates of live birth with 87.5% 83.2% and 82.8% ($p=0.30$) as well (Figure 1).

Men with testicular volumes ≤ 2 mL had higher ($p<0.001$) FSH levels (39.9 $\pm$ 17.6 IU/mL) than men with larger (>10 mL) testes (18.3 $\pm$ 12.3 IU/mL) (Table 1), and had a higher proportion of Klinefelter syndrome than men with testes >2 mL (72.6% versus 5.3%, $p<0.0001$). Men without Klinefelter and testicular volumes ≤ 2 mL ($n=33$) had a SRR of 34.4%.

The histology patterns differed among the groups, with Sertoli-cell only histology seen more frequently in men with testicles $\leq 2\text{mL}$ than men with testes $>10\text{mL}$ (72% versus 50%, $p=0.001$), conversely maturation arrest and hypospermatogenesis histology were found less frequently (6.0% vs 25.4% $P<0.0001$ and 7.0% vs 23.5% $P<0.0001$, respectively).

DISCUSSION

It has been previously assumed that patients with significantly reduced testicular sizes would have worse testicular function, and therefore a decreased chance of sperm retrieval with micro-TESE.

In this study, of 106 men with extremely small testes ($\leq 2\text{mL}$), we found that men that had sperm retrieved were younger (31.1 vs 35.3 years, $p=0.0006$) and were likely to have a diagnosis of Klinefelter syndrome (KS) (81.0% vs 58.8%, $p=0.01$) (Table 2). The average testis size for both successful and failed sperm retrieval had a large deviation owing to there being sperm recovered at all testicular sizes, and the heterogeneity of testicular sizes in our population, ranging from 1mL to 25mL. There was no cut-point for testicular volume in which sperm was not found.

In men with severe atrophy, sperm were retrieved successfully in 81.8% of young men aged <30 years with KS, whereas sperm was found only in 33.3% of men over the age of 30 and without KS. It remains unclear why men with Klinefelter syndrome can have isolated areas of intact spermatogenesis. A possible theory is that rare non-disjunction events may select for XY clones in developing germ cells, which are then able to proceed through meiosis to form normal haploid spermatogonia¹². We have previously shown a similar correlation of decreasing sperm retrieval with increasing age in men with KS¹¹. It is possible that with aging, men with NOA lose some focal areas of spermatogenesis and may have less spermatozoa maturation, decreasing the chance of finding sperm.

Although several studies have reported conflicting evidence that testicular volume can play a role in affecting sperm retrieval rates, no study to our knowledge has directly evaluated micro-TESE in men with small testes. Marconi *et al* described men with testicular volumes less than 8mL and FSH levels $>12.4\text{IU}$ as having a “low chance” (approximately 30%) for testicular sperm retrieval¹³ with micro-TESE. One small study of 106 men by Bromage *et al* reported that testes with volume less than 4mL or with FSH levels greater than 10IU had poorer sperm retrieval outcomes with percutaneous epididymal sperm aspiration (PESA) or TESE (29%), though micro-TESE itself was not directly evaluated¹⁴. Similarly, another study of 85 men with NOA by Ziaee *et al* in 2006 reported that men with successful sperm retrieval had on average larger testes of 17.5mL, compared with patients who had unsuccessful retrieval at an average testicular volume of 5.7mL¹⁵, although they used multiple testicular biopsies rather than micro-TESE. These studies lend evidence that smaller testicle size has decreased chances of SRR in methodologies other than micro-TESE, highlighting the value of microdissection for locating areas of sperm production.

We have published the nomogram for sperm retrieval with micro-TESE¹⁶. Using this, a 40 year-old male with 2mL testis volume and KS has an estimated 70–80% chance of sperm

retrieval whereas if he did not have KS, the chance of SR decreases to less than 50%¹⁶. KS remains a good predictor of successful sperm retrieval with micro-TESE. It is possible that KS as an etiology for NOA is relatively amenable to micro-TESE for sperm retrieval, whereas comparable men without KS having other etiologies of NOA (or idiopathic NOA) are less likely to have sperm retrieved.

The present study found that sperm retrieval, pregnancy rate, and live birth rates are comparable in men with severe testicular atrophy as for men with larger testes. Though not statistically significantly different, there was a trend toward higher pregnancy and live birth rates in men with severely atrophic testes (Figure 1). Even men with the smallest testicular volume had sperm found. On a multivariable analysis, only younger age affected the chance of successful sperm retrieval in men with small testes. This is important for patient counselling as those urologists attempting a micro-TESE in men with small testes can select patients based on these characteristics.

Micro-TESE currently gives the best rate of sperm retrieval in men with NOA. This study can be used to counsel men on their chances of success. Though we found an isolated population of men with severely atrophic testicles who had a significantly higher chance of success, we would not discourage other men from utilizing this technique, as sperm retrieval is still a possibility with micro-TESE and may be their best medical option for fertility.

This study has the limitation of being retrospective and a single-surgeon series, but is the largest series reported to date describing men with severely atrophic testes who underwent micro-TESE.

CONCLUSION

Small testicles should not be a contraindication for sperm retrieval in men with non-obstructive azoospermia. For men with small testes, younger age, and a diagnosis of Klinefelter syndrome enhance the chance of sperm retrieval.

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Table 1

	Testicular Vol (ml)			p Value
	2 or Less	Greater Than 2-Less Than 10	10 or Greater	
No. pts	106	573	448	–
Mean $\pm$ SD male age	33.1 $\pm$ 6.4	35.8 $\pm$ 7.4	35.2 $\pm$ 7.8	<0.001
Mean $\pm$ SD female age	29.8 $\pm$ 5.3	31.8 $\pm$ 5.1	31.7 $\pm$ 5.3	<0.001
Mean $\pm$ SD FSH (IU/ml)	39.9 $\pm$ 17.6	34.8 $\pm$ 16.4	18.3 $\pm$ 12.3	<0.0001
No. histopathology	100	542	422	–
% Atrophy/sclerosis	11	5	1.4	<0.0001
% Sertoli-cell only	76	62.2	50	0.001
% Maturation arrest	6	16.1	25.4	<0.0001
% Hypospermatogenesis	7	16.8	23.5	<0.0001
% KS	70	8.1	1.5	<0.0001
% Micro Y deletion	1.8	3.1	5.5	Not significant
% Varicocele	10.9	22.2	27.3	0.0003
% Cryptorchidism	6.4	15.3	14.6	0.025
% Chemotherapy history	4.5	5.9	6.6	Not significant

Table 2

Men with testicular volume 2 ml or less

	Sperm	No Sperm	p Value
Mean $\pm$ SD male age	31.1 $\pm$ 6.2	35.3 $\pm$ 6.0	0.0006
Mean $\pm$ SD FSH (IU/ml)	40.3 $\pm$ 18.5	39.5 $\pm$ 16.6	Not significant
No. Sertoli-cell only (%)	80.9	77.9	Not significant
No. maturation arrest (%)	9.2	15.9	Not significant
No. hypospermatogenesis (%)	10.3	7.0	Not significant
No. Y chromosome microdeletions (%)	0.0	3.9	Not significant
No. varicocele (%)	10.3	11.8	Not significant
No. cryptorchidism (%)	5.2	7.8	Not significant
No. KS (%)	81.0	58.8	0.01
No. Ca history (%)	1.7	7.8	Not significant