Original Article

Testicular fine-needle aspiration versus testicular open biopsy: Comparable sperm retrieval rate in selected patients

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ABSTRACT

Background: Sperm recovery by testicular fine-needle aspiration (TESA) has resulted in variable sperm retrieval rate (SRR) and is generally considered inferior to open biopsy (testicular sperm extraction [TESE]).
Aims: To develop a predictive model for SRR by TESA and to identify factors associated with comparable SRR between TESA and TESE.
Settings and Design: Single-center controlled cross-sectional study on 450 infertile men with nonobstructive azoospermia.
Materials and Methods: Clinical, paraclinical, and histological information of patients were gathered. All patients underwent both TESA and TESE in a single operation. Predictors of SRR by TESA were identified, and the accuracy of TESA in predicting the outcome of TESE was determined.
Statistical Analysis Used: Categorical and continuous variables were compared using independent *t* test and —chi-square test. Logistic regression model was applied to develop a predictive model for SRR by TESA. Receiver Operating Characteristics (ROC) curve analysis was used to determine the accuracy of TESA in predicting TESE outcome.
Results: Sperm retrieval rate for TESA and TESE was 41.8 and 50.9%, respectively ($P = 0.04$). Age, duration of infertility, testis volume, luteinizing hormone, prolactin, and testosterone did not differ between patients with and without mature sperm in TESA samples. Serum follicular-stimulating hormone (FSH) < 15 IU/l (Exp (B) = 4.8, 95% CI: 1.4–18.5; $P = 0.001$) and histology of hypospermatogenesis (Exp (B) = 6.4, 95% CI: 2.1–27.4; $P < 0.001$) were predictors of SRR by TESA. In patients with FSH < 15 IU/l (57.4% versus 59.5%; Area under the curve (AUC) = 0.907) and testicular histology of hypospermatogenesis (68.0% versus 70.5%; AUC = 0.890), the SRR by TESA was predictive of SRR by TESE.
Conclusions: Serum FSH and testicular pathology were predictors of SRR by TESA. Patients with FSH < 15 IU/l and/or testicular pathology of hypospermatogenesis had comparable SRR by TESA versus TESE.

Key words: Follicular-stimulating factor, sperm recovery rate, testicular fine-needle aspiration, testicular open biopsy, testicular histology

INTRODUCTION

Testicular fine-needle aspiration (TESA), initially developed in 1992, is now an established method of sperm retrieval for assisted reproduction. Simplicity, cost-effectiveness, and less postoperative pain owing to its minimally invasive nature are considered the main advantages of TESA compared with traditional procedures such as testicular sperm extraction (TESE).[1,2] Moreover, TESA may ideally improve the chance of detecting active sites of spermatogenesis by reaching deeper testicular sites.[3] Given the unacceptably wide reported sperm retrieval rate (SRR) by this method which ranges from 11% to 54%,[4,5] TESA has failed to become the procedure of choice for sperm retrieval in many centers. Limited number of retrieved sperm by TESA in some studies has raised concerns about the convenience of TESA for cryopreservation.[4,6] Hauser *et al.*[7] reported that TESE was considerably superior to TESA in terms of SRR, the number of positive locations for sperm presence in the testis, and number of frozen straws.

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Access this article online

Quick Response Code:

Website: www.indianjurol.com

DOI: 10.4103/0970-1591.94954

Hormonal profile of azoospermic men, especially serum follicular-stimulating hormone (FSH), and testicular volume have been previously shown to be closely correlated with the outcome of TESA.[8,9] It was our assumption that the SRR by TESA could be comparable to that in TESE when patients are appropriately selected based on the hormonal profile, testicular size, and testicular histology. The specific aim of this study was to identify the factors that determine the SRR by TESA and to clarify the parameters associated with comparable SRR between TESA and TESE in a large cohort of infertile men with nonobstructive azoospermia (NOA).

MATERIALS AND METHODS

Study population

This study was a single-center controlled cross-sectional study. A total of 450 infertile men with presumptive diagnosis of NOA referred to Vali-e-Asr Infertility Center, Tehran, Iran, for testicular biopsy between January 2004 and September 2010 were considered as potential participants. All patients underwent routine infertility evaluation including medical and reproductive history, thorough physical examination, semen analysis, hormonal profile including FSH, luteinizing hormone (LH), prolactin, testosterone, and serum inhibin B (Oxford Bio-Innovation UK kit); and scrotal ultrasonography. Chromosomal karyotyping and evaluation for Y chromosome microdeletions were also performed. Inclusion criteria were as follows: (a) presence of azoospermia according to the definition by World Health Organization (1999) in two consecutive semen analyses during the last 12 months at the interval of at least 3 months and (b) nonobstructive etiology of azoospermia proven by testicular histology. Exclusion criteria were as follows: (a) karyotype abnormality; (b) Y chromosome microdeletions; and (c) testicular volume less than 5 ml (to prevent subsequent defect in androgen synthesis). A study staff explained the steps of the project to candidates and then asked them to sign the informed consent form. Institutional Review Board of Tehran University of Medical Sciences approved the study protocol. Forty-five men did not meet the inclusion/exclusion criteria and 20 men did not accept to participate. Therefore, a total of 385 patients were enrolled in the study.

Procedures

Study participants underwent both TESA and TESE in a single operation by the same surgeon. Testicular sperm aspiration was primarily attempted in all participants to prevent the possible detrimental effect of TESE on the quality of sampling. To perform TESA, the area around the spermatic cord was locally anesthetized by injecting 5 ml of 2% lidocaine. The aspiration was then performed in the center as well as in the upper and lower poles of each testis using a 23-G needle with a 20-ml syringe attached to it. A constant negative pressure was applied to the syringe when the needle reached the center of the testis and aspiration was done with gentle back and forth movements of the needle at different angles in each puncture location. The aspirated tissue from each location was placed on a separate slide, air-dried, and stained with May-Grünwald Giemsa.

TESE was subsequently performed on both testes. Tunica albugina and epididymis were exposed through a 5- to 15-mm incision in scrotal skin and tunica vaginalis. Three different incisions were made in tunica albugina near the sites of needle insertion, and a sample of roughly $5 \times 3 \times 2$ mm was excised from each site. The specimens were placed in separate tubes containing Hamm's F-10 medium and were immediately transferred to pathology laboratory. Tunica albugina were closed with continuous suture using Vicryl 4/0. Patients were closely observed for 2 hours and after prescribing antibiotic and pain killer, a follow-up visit was scheduled for the next week.

In pathology laboratory, the same pathologist who was blinded to the patients' characteristics carefully examined the samples obtained by TESA and TESE for detection of mature sperm and their histological pattern.

Data analysis

Data were analyzed using SPSS, version 16. Gathered information was compared between patients divided into two groups according to the presence of mature sperm in TESA sample using independent sample *t* test and —chi-square test for continuous and categorical parameters; respectively. Logistic regression analysis was then applied to determine the independent predictors of mature sperm extraction by TESA. Being stratified with respect to the independent predictors of TESA outcome identified by regression model, Receiver operating characteristics (ROC) curve analysis was applied to determine the accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of TESA in predicting the outcome of TESE. Data were presented as mean ± SD, and $P < 0.05$ was considered statistically significant.

RESULTS

Patients' characteristics and sperm retrieval rate by TESA

The mean age of the participants and the mean duration of infertility were 33 ± 7.6 (range 22–65) years and 9.3 ± 6.3 (range 1–25) years, respectively. Other characteristics of patients are summarized in Table 1. Fine-needle aspiration detected mature sperm in 161 of 385 (41.8%) patients. The comparison between clinical features, hormonal profile, and testicular histology of patients with respect to the outcome of TESA is presented in Table 2. Accordingly, there was no significant difference between two groups regarding the age, duration of infertility, testis volume, LH, prolactin, and testosterone. Serum FSH was significantly lower in positive TESA group compared with the other group with no mature sperm recovery ($P < 0.001$). The presence of mature sperm in TESA was also associated with statistically significantly

Table 1: Characteristics of study population

Testicular volume (ml)	14.8 ± 5.8 (5–25)*
Hormonal profile	
FSH (IU/l)	21.7 ± 15.5 (0.9–68)
LH (IU/l)	12.7 ± 7 (0.7–32)
Testosterone (ng/ml)	4.5 ± 1.2 (2.4–7.3)
Prolactin (ng/ml)	8.3 ± 4.9 (1.3–19)
Inhibin B (pg/ml)	146.3 ± 37 (29–347)
Testicular pathology	
Hypospermatogenesis	119 (31.0)†
Maturation arrest	141 (36.6)
SCOS	125 (32.4)

*Mean ± SD (range), †Number (%), FSH: Serum follicular-stimulating hormone, LH: luteinizing hormone, SCOS: Sertoli-cell-only syndrome

Table 2: Clinical information, hormonal profile, and testicular histology of patients with and without mature sperm in samples obtained by TESA

	Mature sperm recovered by TESA		*P* value
	Positive (n = 161)	Negative (n = 224)	
Age (year)	32 ± 6.1*	34 ± 5.7	0.4
Duration of infertility (year)	8.8 ± 2.8	9.2 ± 7.4	0.3
Testis volume (ml)	16 ± 4.6	14 ± 5.4	0. 2
LH (IU/l)	10.7 ± 3.2	12.6 ± 2.8	0.2
FSH (IU/l)	13 ± 4.7	23.2 ± 6.1	<0.001‡
Prolactin (IU/l)	8.1 ± 2.3	8.4 ± 3.1	0.7
Testosterone (IU/l)	5.4 ± 2.1	4.2 ± 2.3	0.4
Inhibin B (pg/ml)	121.3 ± 44.2	164.6 ± 38.7	<0.001‡
Testicular histology			
Hypospermatogenesis	81 (50.3%)†	38 (16.9%)	<0.001‡
Maturation arrest	48 (29.8%)	93 (41.5%)	
Sertoli-cell-only syndrome	32 (19.9%)	93 (41.6%)	

*Mean ± SD. †Number (%). ‡*P* < 0.05 was statistically significant, FSH: Serum follicular-stimulating hormone, LH: Luteinizing hormone,

Table 3: Logistic regression analysis to determine independent predictors of mature sperm retrieval by TESA

	Wald	Exp(B) (95% CI)	*P* value
Age (year)		1	
<33			
≥33	2.1	0.8 (0.1–1.9)	0.6
Duration of infertility (year)			
≥9		1	
<9	3.2	1.2 (0.2–4.5)	0.4
Testicular volume (ml)			
<11		1	
≥11	7.3	2.5 (0.7–10.3)	0.1
LH (IU/l)			
≥12		1	
<12	4.2	1.6 (0.1–4.4)	0.2
FSH (IU/l)			
≥20		1	
≥15 and < 20	5.2	2.3 (0.6–8.2)	0.3
<15	12.4	4.8 (1.4–18.5)	0.001*
Prolactin (IU/l)			
≥8		1	
<8	4.4	1.7 (0.3–2.1)	0.3
Testosterone (IU/l)			
<5		1	
≥5	3.8	1.5 (0.2–2.2)	0.4
Inhibin B			
≥140		1	
<140	7.5	3.1 (0.9–9.8)	0.09
Testicular histology			
SCOS		1	
Maturation arrest	6.2	1.9 (0.7–4.1)	0.1
Hypospermatogenesis	12.7	6.4 (2.1–27.4)	<0.001*

**P* < 0.05 was statistically significant, TESA: Testicular fine-needle aspiration, TESE: Testicular sperm extraction, FSH: Serum follicular-stimulating hormone, SCOS: Sertoli-cell-only syndrome

lower levels of serum inhibin B ($P < 0.001$). Regarding the testicular histology, more than half of specimens in the positive TESA group showed hypospermatogenesis, while only 38 of 224 (16.9%) specimens in the other group had hypospermatogenesis ($P < 0.001$).

Predictive model for sperm retrieval by TESA

The results of logistic regression analysis to identify the predictors of mature sperm detection by TESA are demonstrated in Table 3. Correspondingly, serum inhibin B failed to show significant predictive value. However, the odds of detecting mature sperm in patients with serum FSH < 15 IU/L were 4.8 times higher than those with FSH ≥ 20 IU/l ($P < 0.001$). The odds of detecting mature sperm in TESA were also 6.4 times higher in patients with testicular histology of hypospermatogenesis compared with those with Sertoli-cell-only syndrome (SCOS) ($P < 0.001$).

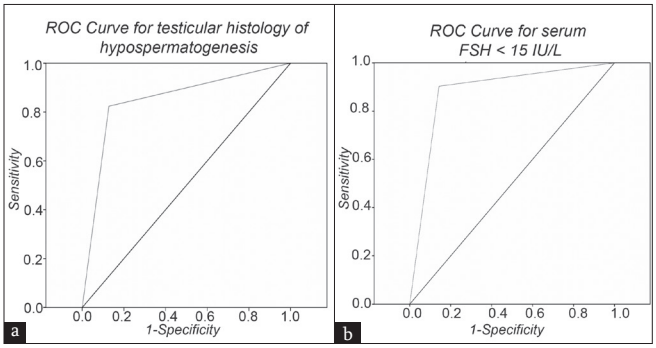

Figure 1: ROC curve analysis of SSA by TESA versus TESE in patients with (a) testicular histology of hypospermatogenesis and (b) serum FSH level < 15 IU/l

Sperm recovery rate by TESA versus TESE with respect to FSH and testicular histology

Sperm extraction rate by TESE was statistically significantly higher than SRR by TESA (50.9% versus 41.8%; $P = 0.04$). In addition, open biopsy resulted in considerably higher SRR compared with TESA in patients with serum FSH ≥ 15 IU/l (32.7% versus 45.9%; $P = 0.01$). In subgroup of patients with FSH < 15 IU/l, however, the SRR by TESA was accurately predictive of SRR by TESE (57.4% versus 59.5%; $P = 0.8$; area under ROC curve = 0.907) [Figure 1, Tables 4 and 5]. Regarding testicular histology, SRR by TESA in hypospermatogenesis group was accurately comparable with SRR by TESE (68.0% versus 70.5%; $P = 0.6$; area under ROC curve = 0.890) [Figure 1, Tables 4 and 5]. The SRR by TESA, however, was significantly lower compared with SRR by TESE in maturation arrest and SCOS groups (34.0% versus 45.3%; $P = 0.02$ and 25.6% versus 38.4%; $P = 0.01$, respectively).

DISCUSSION

Despite its simplicity, cost-effectiveness, and minimal invasiveness of the procedure, the lower SRR, compared with the standard method of TESE, has always been considered the main limitation for widespread application of TESA in reproductive medicine.[1] This study provided a regression-based analytic model to identify the predictive factors of mature sperm retrieval by TESA. It was shown here that testicular pathology of hypospermatogenesis and serum FSH level ≤15 IU/l were two independent predictors of mature sperm retrieval by TESA. These two factors were also associated with comparable SRR between TESA and TESE. Although serum inhibin B was conversely associated with the presence of mature sperm in TESA, it failed to show any significant predictive value in this report.

Testicular fine-needle aspiration of mature sperm features superior capability in detecting active sites of spermatogenesis in deeper parts of testicular tissue. There are, however, apparent controversies in the literature regarding the SRR by TESA in comparison to conventional methods such as TESE. Friedler *et al.* conducted the first well-designed study regarding the SRR by TESA versus TESE in a cohort of 37 men with NOA. They found that mature sperm was present in only 4 of 37 (11%) patient samples obtained by TESA, while TESE yielded spermatozoa in 16 cases (43%).[4] Some studies subsequently reported similar findings.[10-12] More recently, Hauser *et al.*[7] compared the SRR between TESA and TESE in 87 men patients with NOA and concluded that TESA was considerably inferior to TESE with regard to sperm retrieval for assisted reproduction (24.1% versus 62.1%). Nevertheless, some observational studies have shown comparable SRR by these two methods. Lewin *et al.* performed 111 TESA cycles with a mean of 15 punctures for aspiration in each testis on 85 azoospermic men and detected mature testicular spermatozoa in 65 (58.5%) cycles from 50 (58.8%) patients.[3] A larger study from the same group, included 236 TESA on 152 patients, reported a SRR of 53.8%.[1] Another large-scale study reported mature sperm in 63% of TESA procedures using a 21-G needle.[1] To our knowledge, this study is the largest controlled study comparing SRR by TESA and TESE in infertile men with NOA. It was found here that TESA, on the whole, resulted in significantly lower SRR compared with TESE. The SRR, however, was comparable between TESA and TESE in subgroups of patients with serum FSH < 15 IU/l and/or those with testicular histology of hypospermatogenesis.

FSH has been shown to play a crucial role in promoting quantitative spermatogenesis. It has been demonstrated that inactivation of FSH receptors or FSH-beta subunit would result in substantial reduction in quality and quality of sperm production. This hormone is believed to indirectly stimulate mitotic and meiotic DNA synthesis in spermatogonia and preleptotene spermatocytes through its action on Sertoli

Table 4: Mature sperm extraction rate in patients underwent TESA versus TESE with respect to testicular histology and serum FSH level

Testicular histology	Serum FSH level (IU/l)	Mature sperm extraction rate		*P* value
		TESA	TESE	
Hyposper-matogenesis	≥20	19/31 (61.3)	20/31 (64.5)	0.4
	≥15 and <20	27/43 (62.8)	29/43 (67.4)	0.6
	<15	35/45 (77.7)	35/45 (77.7)	1
Maturation arrest	≥20	9/44 (20.4)	18/44 (38.6)	<0.001*
	≥15 and <20	11/41 (26.8)	17/41 (41.4)	0.01*
	<15	28/56 (50.0)	29/56 (51.7)	0.9
SCOS	≥20	6/50 (0.0)	12/50 (23.0)	0.01*
	≥15 and <20	8/35 (0.0)	16/35 (33.4)	0.01*
	<15	18/40 (45.0)	20/40 (50.0)	0.4

*$P < 0.05$ was statistically significant, TESA: Testicular fine-needle aspiration, TESE: Testicular sperm extraction, FSH: Serum follicular-stimulating hormone, SCOS: Sertoli-cell-only syndrome

Table 5: Accuracy of TESA in obtaining mature sperm in selected groups of patients compared with standard method of TESE

	Sensitivity	Specificity	Accuracy	Positive predictive value	Negative predictive value
FSH < 15 IU/l	91.6	91.3	90.7	93.9	87.9
Pathology of hypospermatogenesis	90.5	85.1	89.0	93.8	78.9

cells. Dajani *et al*.[9] on their study on 1000 infertile men reported a strong correlation between serum FSH and the presence of mature sperm in TESA. They showed that FSH <10 IU/l was associated with highest percentage of sperm mature retrieval by TESA. Mourad *et al*.[8] also showed that FSH > 19 IU/l was accompanied by very low probability of obtaining sperm by TESA and using this cut-off point value would result in 40% reduction in the number of TESA cycles. It was similarly shown here that the serum FSH level is a valuable predictor of obtaining mature sperm by TESA.

The predictive role of testicular histology on sperm extraction by TESE has been established in several studies.[13-15] Nonetheless, the reports on the association between testicular histology and the presence of mature sperm by TESA have been scarce. Khadra *et al*.[5] reported that the pathologic diagnosis of hypospermatogenesis was associated with significantly higher sperm extraction rate compared with maturation arrest and SCOS groups. Hauser *et al*.[7] also found higher SRR in hypospermatogenesis by both TESE and TESA compared with other testicular histologies. They did not report, however, a significant difference in SRR between these two methods with respect to testicular histology. Using a regression analysis model, this study provided strong support for the latter reports regarding the association of hypospermatogenesis with high SRR by TESA and also introduced the testicular histology as the predictive factor of sperm retrieval by TESA.

Serum inhibin B is a glycoprotein produced almost exclusively by the Sertoli cells and has been proposed to selectively suppress FSH secretion in gonadotropes by inhibiting transcription of the gene encoding the β-subunit of FSH. Nevertheless, its role in predicting the successful retrieval of sperm by different methods of sperm extraction has not been established so far. Our previous study showed that serum inhibin B could positively affect the probability of mature sperm extraction by TESE.[16] The available reports in the literature, however, do not support the association between serum inhibin B and mature sperm retrieval by TESA. Halder *et al*.[17] reported that inhibin B was less reliable in predicting sperm retrieval by TESA compared with FSH. In a controlled study on 51 azoospermic men, Goulis *et al*.[18] also found that serum inhibin B, with or without anti-Müllerian hormone, was not superior to FSH as predictor of presence of mature sperm in TESA and was not recommended for this purpose. In line with the latter studies, it was shown here that serum inhibin B had no predictive role in obtaining mature sperm by TESA.

The findings of this study should be considered in the context of some limitations. The lack of data on the intracytoplasmic sperm injection and clinical pregnancy rate was the main drawback of this study. This comparison could provide additional clue for more accurate comparison between TESA and TESE in men with NOA. The information on the number of frozen straws per subject was also not presented in this study. This could provide useful evidence for the suitability of TESA for recovery of supernumerary spermatozoa for cryopreservation.

In summary, this controlled study showed that testicular histology of hypospermatogenesis and FSH <15 IU/l were predictors of successful sperm retrieval by TESA as well as best indicators of comparable SRR between TESA and TESE. Future large-scale controlled studies comparing the pregnancy rate and the number of frozen straws per subject between TESA and TESE in subgroups of men with NOA are absolutely needed to draw a definite conclusion in this regard.

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How to cite this article: Nowroozi MR, Ahmadi H, Ayati M, Jamshidian H, Sirous A. Testicular fine-needle aspiration versus testicular open biopsy: Comparable sperm retrieval rate in selected patients. Indian J Urol 2012;28:37-42.
Source of Support: Nil, **Conflict of Interest:** None declared.