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Prediction of Successful Sperm Retrieval in Patients with Nonobstructive Azoospermia

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ABSTRACT

Introduction: Our aim was to evaluate the predictive values of factors that indicate successful sperm retrieval in men with nonobstructive azoospermia.

Materials and Methods: We evaluated 85 infertile men with nonobstructive azoospermia who underwent multiple bilateral testicular biopsies. Factors including age, infertility period, surgical history, testicular volume, testicular consistency, serum follicle-stimulating hormone (FSH), serum inhibin B, serum luteinizing hormone, and serum total testosterone were assessed in relation to sperm retrieval results.

Results: Spermatozoa were retrieved in 18 biopsies (21.2%). Follicle-stimulating hormone, serum inhibin B, and testicular volume were associated with the results of sperm retrieval. Men with a higher testicular volume, a higher serum inhibin B, and a lower FSH had successful sperm retrieval. The cutoff points were determined as 9.5 mL for testicular volume, 9.9 IU/L for serum FSH, and 39.8 pg/mL for serum inhibin B. These 3 factors had strong correlations with each other. The sensitivities and specificities were 88.9% and 94% for testicular volume, 97% and 83.3% for FSH, and 72.2% and 95.5% for serum inhibin B, respectively. The positive predictive value for a combination of serum FSH and inhibin B was 100%.

Conclusion: Serum FSH and serum inhibin B are useful markers for evaluation of the presence of sperm in patients with nonobstructive azoospermia. Inhibin B has a high specificity when combined with serum FSH and their measurements can be helpful in all patients with nonobstructive azoospermia before decision making for sperm retrieval.

KEY WORDS: azoospermia, testicular biopsy, follicle-stimulating hormone, inhibin B

Introduction

Men with nonobstructive azoospermia (NOA) may be able to fertilize by intracytoplasmic sperm injection (ICSI) if sperm can be retrieved by testicular sperm extraction (TESE).[1,2] However, it has been reported that about half of the patients with NOA undergo unnecessary surgeries.[2] Each procedure in these patients must be accompanied by the partner preparation for oocyte retrieval, and on the other hand, the resultant irreversible traumatic injury and adhesion in testes arrest spermatogenesis for 6 to 8 weeks and brings about emotional and financial implications for the couple.[3]

For reducing complications of TESE, many researchers have tried to predict the success rate of sperm retrieval using hormonal or other

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markers.[4-12] However, previous studies for introducing certain predictor factors have been failed. To overcome this problem, we launched a study on 85 men with NOA and analyzed 9 preoperative factors to find a method to predict the success of TESE.

Materials and Methods

Between October 2002 and September 2004, infertile men with azoospermia in at least 2 semen analyses were studied. Patients with Klinefelter syndrome were excluded and 85 men who provided informed consent were selected.

The patients underwent a full clinical evaluation; history, physical examination, and measurement of serum levels of follicle-stimulating hormone (FSH; reference range, 1 IU/L to 10 IU/L), luteinizing hormone (LH; reference range, 1 IU/L to 9.5 IU/L), total testosterone (reference range, 2.4 ng/dL to 12 ng/dL), and inhibin B (enzyme-linked immunosorbent assay kits, Serotec, Oxford, UK).

Testicular volume was measured by orchidometer, and in some cases, by ultrasonography (a 7.5-MHz ultrasonic probe). Results of testis consistency assessment were scored as 1, very soft; 2, soft; 3, normal; 4, firm; and 5, very firm.

Multiple TESE was performed under local anesthesia. Through a small vertical incision in the median scrotal raphe, the skin, dartos muscle, and tunica vaginalis were opened to expose the tunica albuginea. The tunica albuginea was incised at the upper pole near the head of the epididymis. A sufficient volume of testis was excised and examined. If no sperm were seen in the specimen, subsequent samples were taken from other locations, in the midline of the testis and at the lower pole opposite the rete testis, and subsequently from the contralateral testis. The procedure was terminated when sperm were retrieved or all three samples from upper, middle, and lower sites per testis had been examined for the presence of testicular sperm.

Statistical analyses were performed by Student t test and chi-square test. In addition, the correlation between continuous variables was assessed by Pearson correlation test and the best cutoffs of factors that influence sperm retrieval were determined based on receiver operating characteristic (ROC) curves. All statistical analyses were performed with SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc, Chicago, Ill, USA). Continuous variables were shown as mean ± standard deviation. A P value of $< .05$ was considered significant.

Results

Sperm was found in 18 out of 85 (21.2%) patients with NOA, and TESE failed in 67 (77.8%). Pathologic findings are summarized in Table 1.

The mean age of patients with and without retrieved sperm were not statistically different ($P = .745$). Furthermore, infertility period and past surgical history did not show any significant difference between the two groups of patients (Table 2).

Forty-seven patients underwent ultrasonography to determine testicular volume; the mean volume was 8.72 ± 5.92 mL in clinical measurement (by orchidometer) and 8.17 ± 5.40 mL on ultrasonography ($P = .019$). The mean testicular volume measured by orchidometer and ultrasonography was greater in the patients with retrieved sperm ($P < .001$), but testicular consistency score was not significantly different between the two groups of patients (Table 2).

TABLE 1. *Pathologic reports of specimens from TESE in patients with nonobstructive azoospermia*

Patients without retrieved sperm		
Histologic finding	**Number**	**Percent**
Maturation arrest	32	47.8
SCO	17	25.4
Mixed germ cell aplasia	8	11.9
Mixed germ cell aplasia with SCO	3	4.4
Maturation arrest with SCO	3	4.4
Atrophic testis	3	4.4
End stage testis	1	1.5
Total	**67**	**100**
Patients with retrieved sperm		
Histologic finding	**Number**	**Percent**
severe hypospermatogenesis	14	77.8
Germ cell hyperplasia	3	16.7
Maturation arrest	1	5.5
Total	**18**	**100**

TESE: testicular sperm extraction, SCO: Sertoli-cell-only syndrome

Table 2. *Clinical and paraclinical values in the patients with and without retrieved sperm*

	Patients with sperm	Patients without sperm	Confidence interval	*P* value
Age (year)	33.2 ± 6.1	34.5 ± 5.8	-4.43 to 1.79	.745
Infertility period (year)	7.6 ± 5.7	8.4 ± 6.2	-4.02 to 2.44	.629
Past surgical history				
No surgical history	13 (72.2%)	52 (77.6%)		.892
Scrotal or inguinal surgery	4 (22.2%)	12 (17.9%)		
Orchidectomy	1 (5.6%)	3 (4.5%)		
Testicular volume (mL)				
Right testis	17.56 ± 3.5	5.71 ± 2.4	10.37 to 13.32	< .001
Left testis	17.05 ± 4	5.65 ± 2.6	9.30 to 13.49	< .001
Mean of both testes	17.5 ± 3.52	5.68 ± 2.44	10.32 to 13.30	< .001
Testicular consistency scores				
Right testis	3.18 ± 0.54	3.05 ± 0.63	0.28 to .54	.524
Left testis	3.11 ± 0.58	2.87 ± 0.73	0.14 to 0.60	.219
Serum LH (IU/L)	8.04 ± 6.31	9.06 ± 53	-4.59 to 2.54	.563
Serum total testosterone (ng/dL)	5.81 ± 1.24	6.103 ± 3.91	-2.09 to 1.64	.810
Serum FSH (IU/L)	5.83 ± 3.51	20.82 ± 5.85	-12.10 to -17.87	< .001
Serum inhibin B (pg/mL)	48.77 ± 15.64	31.4 ± 5.91	-12.70 to -22.03	< .001

Serum levels of LH and testosterone were not significantly different, but the mean serum level of FSH was lower in the patients with retrieved sperm (5.83 ± 3.51 IU/L versus 20.82 ± 5.85 IU/L; $P < .001$) and the mean serum level of inhibin B was higher in this group of patients (48.77 ± 15.24 pg/mL versus 31.4 ± 5.91 pg/mL; $P < .001$) (Table 2).

The cutoff points were determined using ROC curves (Figures 1 and 2) that were 9.5 mL for testicular volume, 9.9 IU/L for serum FSH, and 39.8 pg/mL for serum inhibin B (areas under the curve 0.95, 0.96, and 0.84, respectively). These 3 variables had strong correlations with each other (FSH and inhibin B, r = -0.47, $P < .001$; FSH and testicular volume, r = -0.64, $P < .001$; inhibin B and testicular volume, r = 0.59, $P < .001$). The sensitivities and specificities were 88.9% and 94% for testicular volume, 97% and 83.3% for FSH, and 72.2% and 95.5% for serum inhibin B, respectively (Table 3).

Discussion

Retrieval of testicular sperm for ICSI is considered as a useful approach in patients with NOA.[13,14] However, depending on the techniques, sperm retrieval chance for each biopsy is 20% to 50% in these patients.[15] Partner preparation problems, emotional stress, high costs, drug reactions, damage to the testes due to each additional unnecessary biopsy, and the likelihood of negative findings in TESE have motivated many researchers to find noninvasive techniques to predict sperm retrieval chance.

In our study, the chance of sperm retrieval was 21.2% that was lower than the findings of other

Table 3. *Predictive values of tests for successful sperm retrieval in men with nonobstructive azoospermia**

Test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
FSH	97	83.3	88.2	95.5
Inhibin B	72.2	95.5	81.3	92.8
Testicular volume	88.9	94	80	96.9
FSH + inhibin B	55.6	100	100	89.3
FSH + inhibin B + testicular volume	50	100	100	88.2

*values are percents.

studies.[15-16] This is proportionally due to the more effective techniques used in these studies such as microdissection TESE and testicular fine-needle aspiration. Tsujimura and colleagues[2] have compared multiple TESE and microdissection TESE in 37 and 56 patients and found sperm retrieval rates of 35.1% and 42.9%, respectively.

We demonstrated that testis volume measured either by ultrasonography or by orchidometry was greater in patients with successful sperm retrieval. In addition, a higher serum level of inhibin B and a lower serum level of FSH were found in these patients. The relationship between testicular consistency and sperm retrieval was not significant in our study. To our knowledge, this parameter has not been examined in any studies.

In the study by Tsujimura and colleagues,[2] serum levels of FSH, total testosterone, and inhibin B were the most influential preoperative factors. We found no association between total serum testosterone and sperm retrieval success. This is probably because in our study, the most common pathologic finding in patients without retrieved sperm was maturation arrest or Sertoli-cell-only syndrome, while interstitial and leydig cells were not affected. Our findings reiterate those of Foresta and coworkers'.[9]

In agreement with the results of other studies,[2,4,5] the difference of FSH level between the patients with or without successful sperm retrieval was significant in our study. However, serum level of FSH is correlated with the presence of germ cells and not with spermatogenesis. We expect normal serum FSH levels in maturation arrest or Sertoli-cell-only syndrome. Thus, serum FSH is dependent to the pathologic etiology of azoospermia and cannot be a good predictive factor.

Inhibin B is a heterodimeric glycoprotein secreted from the testis as a product of Sertoli cells. A strong inverse correlation exists between inhibin B and FSH levels in men with normal and disturbed spermatogenesis.[8] Measurement of inhibin B has raised many challenges between authors; Vernaeve and colleagues[4] have not found any significant predictive value for inhibin B, while many other authors have emphasized that inhibin B can predict sperm retrieval.[2,5,7,9,11,12] We found a significant difference in serum inhibin B between the two groups.

Testicular volume was another influential factor on successful sperm retrieval. Clinically, it is correlated with spermatogenesis, but topographical variations in testicular pathology independent of testicular volume can occur.[2] Thus, testicular volume may not be a good predictive factor of successful sperm retrieval for ICSI.

Serum FSH in combination with inhibin B has been reported to have a marked sensitivity and specificity in the retrospective studies by Bohring and coworkers[6] and von Eckardstein and colleagues.[15] Based on our findings, a serum FSH $\leq$ 9.9 IU/L and a serum level of inhibin B $\geq$ 39.8 pg/mL can predict the possibility of successful sperm retrieval with 100% specificity and positive predictive value that is enough for a clinical decision making and avoids unnecessary biopsy. However, in other individuals with a testicular volume < 9.5 mL, a serum level of FSH > 9.9 IU/L, and a serum level of inhibin B $\leq$ 39.8 pg/mL, testis biopsy is indicated and these factors are not able to predict absence of sperm due to multifocal spermatogenesis in testes. Thus, we have found a simple way for this prediction without including many factors. However, the limitation of our relatively small sample warrants further investigation of these findings.

Conclusion

Serum levels of FSH and inhibin B are useful markers for evaluation of sperm detection in patients with nonobstructive azoospermia. Inhibin B has a high specificity when combined with serum FSH and we recommend these measurements in all patients with nonobstructive azoospermia who are candidates for ICSI. Further study to find more sensitive markers is warranted.

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