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Relationship of Clinical and Ultrasonographic Grading of Varicocele with Semen Analysis Profile and Testicular Volume

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Abstract

Background: Varicoceles are a major cause of infertility. The purpose of this study was to determine the relationship of the clinical and ultrasonographic grades of varicocele with the semen analysis profile and testicular volume among men undergoing scrotal ultrasonography.

Methods: This cross-sectional analytical study involved 109 males undergoing scrotal ultrasonography for various indications in Shiraz, Iran, between January 2019 and January 2020. Varicoceles were graded with color Doppler ultrasonography (CDU) by an expert radiologist (Sarteschi's criteria) before an experienced urologist determined the clinical grade (Dubin and Amelar criteria) and requested further investigations. Next, the demographics, reasons for referral, testicular volumes, and semen analysis profiles across the different clinical/ultrasonographic grades were compared. Key statistical measures included Cohen's kappa coefficient, the Mann–Whitney and Kruskal-Wallis tests, and Spearman correlation. Data were analyzed using SPSS v. 21 with p-values <0.05 indicating statistical significance.

Results: Ultrasonographic grades 1 and 2 provided the highest correlation with subclinical cases, while ultrasonographic grades 3, 4, and 5 corresponded with clinical grades 1, 2, and 3, respectively. Further comparisons were made between subclinical and clinical cases, which were similar in terms of reason for referral, total testicular volume, testicular volume differential, and semen analysis profile. Notably, total testicular volumes below 30 *ml* were associated with oligoasthenoteratospermia.

Conclusion: The present study showed a relatively high correlation between varicocele grading based on clinical evaluation and CDU. However, the grades were similar in testicular volume parameters and semen analysis indices. Hence, decision-making should be guided by the infertility history, testicular atrophy, and abnormal semen analysis.

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Introduction

Varicoceles affect 15-20% of the general population and are diagnosed in 35-40% of men who attend infertility clinics, though only 15% of men with varicoceles are infertile (1, 2). This condition is characterized by dilation of the pampiniform plexus secondary to retrograde flow

in the spermatic veins. Varicoceles are predominantly identified in the left testicle, though the disease is believed to be bilateral in nature (3, 4).

Varicoceles can lead to decreased sperm quantity and quality, with a surgical repair being indicated for selected couples complaining of infertility (5). The semen analysis profile can elucidate the reproductive potential of individuals, though a unilateral reduction in testicular volume may not necessarily indicate testicular dysfunction secondary to a varicocele. Nonetheless, patients with unilateral left varicocele and ipsilateral testicular atrophy have been reported to have significantly worse semen analysis profile parameters compared to patients without atrophy (6).

Since long ago, the mainstay method of diagnosing varicoceles has been the physical examination (7). However, rapid developments in imaging techniques have made modalities like venography and color doppler ultrasonography (CDU) invaluable paraclinical tools for the physician, with the former representing the gold standard of diagnosis and the latter providing 97% sensitivity and 94% specificity (8, 9).

Radiologists have devised multiple systems for grading varicoceles, and although ultrasonography can diagnose varicoceles in their subclinical stages, studies with a focus on early diagnosis are limited. Furthermore, given the undeniable shift toward the use of paraclinical tools and considering the fact that patients may undergo scrotal ultrasonography for a wide variety of reasons, it is essential to evaluate different ultrasonographic findings. Hence, the purpose of the present study was to determine the relationship of the clinical and ultrasonographic grades of varicocele with the testicular volume and semen analysis profile among individuals undergoing scrotal ultrasonography.

Methods

Study design: This analytical cross-sectional study was conducted on 109 men scheduled for scrotal ultrasonography at the Motahari Clinic affiliated to Shiraz University of Medical Sciences (Shiraz, Iran) between January 2019 and January 2020. All men aged 15-65 who were referred for various reasons (pain, swelling, infertility, *etc*.) to our clinic were included for scrotal ultrasonography. After explaining the study protocol and obtaining written consent, the patients filled a data collection form including demographic characteristics and past medical history. Patients with a history of an operated inguinal hernia, testicular or varicocele sur-gery, diabetes, malignancy, transplantation, urinary tract infection, rheumatologic disease, or renal failure were excluded. All patients who used any medications that could affect the testicular size or semen analysis were also excluded. The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences (Code: IR.SUMS. MED.REC.1398.078).

Study measures: All 109 participants were asked about scrotal pain through a yes or no question. Married participants (n=44) were assessed for a history of infertility. All participants were referred for a semen analysis after abstaining for three days from sexual activity.

Imaging was performed by an experienced radiologist with ten years of experience in scrotal CDU. The testicular area was initially covered with a sheet before applying the prewarmed gel. Ultrasonography was carried out using a 5-12 MHz linear ultrasound probe (DC8 Expert, Mindray, China). First, the testicles were examined by ultrasound grayscale imaging to rule out any pathology other than varicocele. Also, the testicular dimensions (length, width, height) were measured, with the testicular volume (*ml*) being calculated using the below formula:

$$Testicular\ volume = length \times width \times height \times 0.7$$

The testicular volume differential (TVD) was subsequently determined as follows:

$$TVD\ (\%) = \frac{Left\ testicular\ volume - Right\ testicular\ volume}{Right\ testicular\ volume} \times 100$$

The total testicular volume (TTV) was calculated using the left and right testicular volumes. In line with some previous studies, TTVs below 30 *ml* and TVD percentages above 20% were considered abnormal, signifying testicular atrophy (10, 11).

Patients were then subjected to CDU in the standing position with and without the Valsalva maneuver to assess reflux in the inguinal canal and pampiniform plexus. The ultrasonographic grade was then recorded according to the Sarteschi criteria, which are presented in table 1 (5).

Subsequently, the study participants were referred to an experienced attending urologist for physical examination. The initial examination was performed in a warm and quiet room while the patient was standing, before switching the patient

Table 1. Sarteschi's criteria for grading varicocele based on color Doppler ultrasonography (5)

Grade	Features
1	Reflux in vessels in the inguinal canal is detected only during the Valsalva maneuver, while scrotal varicosity is not evident in the standard ultrasound study.
2	Small posterior varicosities extend to the superior pole of the testis. Their diameters increase and venous reflux is seen in the supratesticular region only during the Valsalva maneuver.
3	Vessels appear enlarged at the inferior pole of the testis when the patient is evaluated in the standing position; no enlargement is detected if the patient is examined in the supine position. Reflux is observed only during the Valsalva maneuver.
4	Vessels appear enlarged even when the patient is studied in the supine position; the dilatation is more marked in the upright position and during the Valsalva maneuver. Testicular hypotrophy is common at this stage.
5	Venous ectasia is evident even in the prone decubitus and supine positions. Reflux is observed at rest and does not increase during the Valsalva maneuver.

to the supine position and examining him with and without the Valsalva maneuver. Then, varicoceles were graded clinically according to the criteria of Dubin and Amelar (Grade 0/subclinical: impalpable varicocele but detected on ultrasonography; Grade 1: palpable during Valsalva; Grade 2: palpable at rest, but not visible; Grade 3: visible varicocele) (12). To minimize bias, the radiologist and urologist were each blinded to the grade given by the other physician.

Statistical analysis: Data were analyzed using SPSS *vs*. 21 (IBM, USA). Quantitative variables were expressed as mean±standard deviation (SD), whereas qualitative variables were reported as frequency and percentage. The statistical tests performed included the Chi-squared test and the independent t-test or their non-parametric tests when data was not normally distributed. Also, Cohen's kappa coefficient (κ) was used to assess the inter-rater reliability between the ultrasonographic and clinical grades of varicocele. To determine the relationship between patient age and the clinical/ultrasonographic grades of varicocele, the Kruskal-Wallis test was used. The independent t-test and Mann-Whitney U test were used where appropriate to compare the semen analysis indices and testicular volumes between patients with clinical (ultrasonographic grades 3-5) and subclinical (ultrasonographic grades 1-2) varicocele. Furthermore, Spearman correlation was performed to determine the semen analysis variables correlated with TTV and TVD as indices related to testicular function. Moreover, semen analysis indices were compared between two groups with TTV below and above 30 *ml* using Mann–Whitney U test. P-values <0.05 were considered statistically significant in all cases.

Results

Demographic data and the reason for referral: The study participants had a mean age of 28.7±8.5 years (range: 17-63 years). Among the 109 patients, 62 (58.9%) required scrotal ultrasonography due to pain, while 22 (20.2%) were referred due to infertility. The remaining participants sought medical attention due to signs like testicular swelling or were followed up based on a prior case of varicocele.

Correlation between clinical and ultrasonographic grading: First, correlation between the grades given by the urologist and radiologist was examined. The frequency of different grades based on clinical evaluation and ultrasonography of the left testicle indicated a relatively high correlation between the two grading systems. In roughly three-quarters of cases, the grading of left testicular varicoceles was consistent between the ultrasonographic and clinical grading systems (Kappa= 0.74, p<0.001) (Table 2). Similarly, an acceptable and significant correlation was found between the two systems in grading right testicular varicoceles (Kappa=0.68; p<0.001) (Table 3). Overall, it was found that ultrasonographic grades 1 and 2 provided the highest compatibility and correlation with subclinical cases, while ultrasonographic grades 3, 4, and 5 corresponded with clinical grades 1, 2, and 3, respectively.

Testicular volume and patient age according to varicocele grade: Our results showed no significant relationships between the testicular volume and

Table 2. Correlation between ultrasonographic and clinical grades of left-sided varicoceles

Clinical grade		Ultrasonographic grade				Total frequency (row)
		1-2	3	4	5	
0	Frequency	16	2	1	0	19
	Percentage relative to clinical grade	84.2%	10.5%	5.3%	0.0%	
	Percentage relative to ultrasonographic grade	84.2%	8%	2.3%	0.0%	
1	Frequency	3	20	5	0	28
	Percentage relative to clinical grade	10.7%	71.4%	17.9%	0.0%	
	Percentage relative to ultrasonographic grade	15.8%	80%	11.6%	0.0%	
2	Frequency	0	2	36	5	43
	Percentage relative to clinical grade	0.0%	4.7%	83.7%	11.6%	
	Percentage relative to ultrasonographic grade	0.0%	8%	83.7%	22.7%	
3	Frequency	0	1	1	17	19
	Percentage relative to clinical grade	0.0%	5.3%	5.3%	89.5%	
	Percentage relative to ultrasonographic grade	0.0%	4.0%	2.3%	77.3%	
Total frequency (column)		19	25	43	22	109
Kappa		0.74				p<0.001

Table 3. Correlation between ultrasonographic and clinical grades of right-sided varicoceles

Clinical grade		Ultrasonographic grade				Total (row)
		1-2	3	4	5	
0	Frequency	51	4	0	0	55
	Percentage relative to clinical grade	92.7%	7.3%	0.0%	0.0%	
	Percentage relative to ultrasonographic grade	82.3%	13.3%	0.0%	0.0%	
1	Frequency	10	21	1	0	32
	Percentage relative to clinical grade	31.3%	65.6%	3.1%	0.0%	
	Percentage relative to ultrasonographic grade	16.1%	70.0%	7.1%	0.0%	
2	Frequency	1	5	13	0	19
	Percentage relative to clinical grade	5.3%	26.3%	68.4%	0.0%	
	Percentage relative to ultrasonographic grade	1.6%	16.7%	92.9%	0.0%	
3	Frequency	0	0	0	3	3
	Percentage relative to clinical grade	0.0%	0.0%	0.0%	100.0%	
	Percentage relative to ultrasonographic grade	0.0%	0.0%	0.0%	100.0%	
Total (column)						
	Frequency	62	30	14	3	109
Kappa		0.68				p<0.001

the ultrasonographic varicocele grade of both the left and right testis (p=0.171 and 0.099, respectively). This finding was repeated when comparing testicular volume against the clinical varicocele grade for both the left and right testis (p= 0.245 and 0.136, respectively). Furthermore, patient age had no meaningful relationship with neither the ultrasonographic grade of the left and right testes (p=0.126 and 0.08, respectively) nor the clinical grade of the left and right testes (p= 0.226 and 0.965, respectively).

Pain and infertility in clinical and subclinical varicocele: Out of 109 patients, 90 had clinical varicocele, corresponding with ultrasonographic grades

3-5, while 19 had subclinical varicocele, corresponding with ultrasonographic grades 1-2. Hence, the subsequent investigations were done by comparing these two groups of patients. Notably, there was no significant difference between the clinical and subclinical groups in terms of pain (p=0.922) and infertility (p=1.000) as reasons for referral to the radiology clinic.

TTV and TVD in clinical and subclinical varicocele: According to our results, the TTV and TVD ranged between 9-67 *ml* and 0-67.5%, respectively. Two of 19 patients (10.5%) with subclinical varicocele and 16 out of 90 patients (17.8%) with clinical varicocele had TTV values of less than 30 *ml*; no significant difference was found between the two groups in this parameter (p=0.573). Regarding TVD, 3 out of 19 patients (15.8%) with subclinical varicocele and 21 out of 90 patients (23%) with clinical varicocele had TVD values of above 20%, demonstrating an insignificant difference (p=0.329).

Semen analysis and testicular volume in patients with clinical and subclinical varicocele: The results of the semen analysis and the testicular volume parameters for patients with clinical and subclinical varicocele are compared in table 4. No significant differences were found between the mentioned groups in the studied parameters.

Correlation of semen analysis parameters with the TTV and TVD indices: Given the lack of a significant difference between the high and low ultrasonographic grades of varicocele in terms of the TTV and TVD indices, the relationship of these important ultrasonographic indices with semen analysis parameters was examined. Statistical analysis revealed that the TVD index was not correlated with any of the semen analysis indices, though the TTV index had positive and significant relationships with sperm count, morphology, and motility (Table 5).

Comparison of semen analysis indices between two groups of normal and abnormal TTV: Considering the observed correlation between TTV and some components of the semen analysis profile, a further investigation was performed in which the semen analysis indices were compared between

Table 4. Comparison of semen analysis indices and testicular volume between patients with clinical (ultrasonographic grades 3-5) and subclinical (ultrasonographic grades 1-2) varicoceles

	Varicocele status	N	Mean	SD	p-value
Semen analysis					
Sperm count (×10^6 *cells/ml*)	Subclinical	19	68.48	49.48	0.200
	Clinical	90	54.20	47.43	
Semen volume (*ml*)	Subclinical	19	3.49	2.00	0.211
	Clinical	89	4.48	6.28	
Morphology (% normal)	Subclinical	19	45.89	14.57	0.753
	Clinical	89	45.52	16.61	
Grade 4 motility (%)	Subclinical	19	38.94	21.76	0.239
	Clinical	90	32.27	22.90	
Grade 3 motility (%)	Subclinical	18	4.44	8.38	0.236
	Clinical	90	8.57	14.87	
Progressive motility (%)	Subclinical	19	43.15	18.72	0.650
	Clinical	90	40.84	20.47	
Grade 2 motility (%)	Subclinical	19	6.73	4.72	0.058
	Clinical	90	4.75	4.25	
Grade 1 motility (%)	Subclinical	19	8.94	16.18	0.389
	Clinical	90	4.90	2.748	
Grade 0 motility (%)	Subclinical	19	43.78	19.60	0.384
	Clinical	90	48.86	19.66	
Testicular volume (*ml*)					
Left *	Subclinical	19	19.47	5.21	0.700
	Clinical	90	18.95	5.41	
Right	Subclinical	19	21.16	6.160	0.820
	Clinical	90	20.19	5.74	

* Independent t-test (Mann-Whitney U test was used in all other cases)

Table 5. Correlation between semen analysis variables and two indicators of TTV and TVD

	Statistic	TTV	TVD
Sperm count	R	0.425 *	-0.047
	P	<0.001	0.631
Semen volume	R	0.042	-0.047
	P	0.669	0.632
Grade 4 motility	R	0.283 *	-0.016
	P	0.003	0.869
Grade 3 motility	R	-0.059	-0.040
	P	0.544	0.679
Progressive motility	R	0.274 *	-0.040
	P	0.004	0.679
Grade 2 motility	R	-0.048	-0.020
	P	0.619	0.835
Grade 1 motility	R	0.097	-0.081
	P	0.318	0.402
Grade 0 motility	R	-0.324 *	0.078
	P	0.001	0.421
Morphology	R	0.272 *	-0.077
	P	0.004	0.427

* Statistical significance (p<0.01); R, Spearman correlation coefficient; P, p-value

patients with normal (≥30 *ml*) and abnormal (<30 *ml*) TTV values. The sperm count was significantly lower among patients with abnormal TTV than the normal counterparts (p=0.003). A similar result was obtained for Grade 4 motility (p=0.027). Furthermore, patients with abnormal TTV had significantly higher rates of both immobile and abnormal sperm (p=0.016 and 0.007, respectively) (Table 6).

Discussion

The present study was conducted on 109 males undergoing scrotal ultrasonography for various reasons to determine the relationship of varicocele grading via ultrasonography and clinical examination with testicular volume and semen analysis indices. The study sample included all men undergoing scrotal ultrasonography for various reasons (pain, swelling, infertility, follow-up, *etc*.) as our purpose was to assess the value of the grading systems for the general population of men with testicular symptoms rather than limiting it to those who are infertile. Our results are significant as they confirmed the correlation between clinical evaluation and ultrasonographic grading while providing novel evidence on the relationship of the ultrasonographic/clinical grades with the testicular volume and semen analysis profile.

Diagnostically, disease grading is an indicator that helps to understand the patient's condition better. It also may help in selecting the appropri-

Table 6. Comparison of semen analysis indices between two groups of total testicular volume (TTV) below and above 30 *ml*

	TTV (*ml*)	N	Mean	SD	p-value *
Sperm count (×10⁴ *cells/ml*)	<30	18	30.10	25.15	**0.003**
	>30	91	61.95	49.61	
Semen volume (*ml*)	<30	17	3.47	1.63	0.456
	>30	91	4.46	6.23	
Grade 4 motility (%)	<30	18	22.00	21.02	**0.027**
	>30	91	35.70	22.50	
Grade 3 motility (%)	<30	17	12.05	17.44	0.144
	>30	91	7.10	13.30	
Progressive motility (%)	<30	18	33.38	18.69	0.071
	>30	91	42.80	20.11	
Grade 2 motility (%)	<30	18	3.72	2.19	0.160
	>30	91	5.37	4.65	
Grade 1 motility (%)	<30	18	4.55	3.71	0.162
	>30	91	5.81	7.73	
Grade 0 motility (%)	<30	18	58.11	17.83	**0.016**
	>30	91	45.97	19.47	
Morphology (%)	<30	18	35.89	15.78	**0.007**
	>30	91	47.51	15.68	

* Mann–Whitney U test. Bold p-values represent statistical significance (p<0.01)

ate definitive diagnostic method, treatment, and follow-up. Our findings show the correlation in grading between the physical examination and ultrasonography, showing that a common language may be established between urologists and radiologists, which may facilitate better diagnosis and management of varicoceles. Nonetheless, significant differences remain between the two methods, reminding us of the need for accurate methods like an ultrasound for reaching the definitive diagnosis. These results reaffirm those of our previous study (13). In a related study, Jedrzejewski et al. (2019) compared the CDU findings between the normal and affected testis in adolescents with unilateral left-sided varicoceles. Decreased tissue perfusion was reported on the affected side according to all CDU parameters, with the difference reaching statistical significance for the mean velocity and resistance indices and changes being particularly prominent in grade 3 varicoceles (14).

The novel aspect of our study was comparing clinical and ultrasonographic grades of varicocele in terms of a number of essential parameters among males scheduled for scrotal ultrasonography. First, no significant differences in testicular volume and patient age were found between the various ultrasonographic and clinical grades of varicoceles. Then, considering the identified correlation between ultrasonographic grades 1-2 and subclinical disease and between ultrasonographic grades 3-5 and clinical disease, these two groups were compared in our subsequent analysis. Our findings indicated that among men undergoing scrotal ultrasonography for various reasons, patients with subclinical and clinical varicocele had no significant differences in terms of the reasons for referral (pain and infertility), TTV, TVD, and semen analysis profile. It is important to note that while varicoceles are a major cause of infertility and are diagnosed in 40% of infertile men, only 15-20% of men with varicocele are infertile (2, 15). Furthermore, varicoceles are present in about 15% of normal male population, and this figure is expected to rise if subclinical cases are included as well. Recent studies indicate that infertile men with varicocele have decreased sperm count, motility, and normal morphology (16, 17). Furthermore, surgical treatment can improve the semen analysis profile among such patients (18, 19), with some evidence even indicating an improvement in forward progressive sperm motility after surgical treatment of subclinical varicocele (20). However, such results were not replicated among our study population as it included all men referring for scrotal ultrasonography for a variety of reasons. In fact, 60% of our patients were referred due to scrotal pain, which only occurs in 10% of varicocele patients (21). Moreover, just 15% of our study population underwent testicular ultrasonography due to infertility, and oligoasthenoteratospermia was detected in only about 16% of our patients. It should also be taken in to account that 45-65% of men with clinical grades 1-3 varicocele have normal semen parameters (22). Hence, despite the well-established detrimental effects of varicoceles on semen quality and sperm function among infertile men (17), no significant differences were observed in these parameters among our study population of mostly fertile males with different ultrasonographic and clinical grades of varicocele.

The ultrasound examination is a method that can provide a quantitative evaluation of varicoceles using a number of indices. Semiz et al. (2014) investigated the relationship between semen analysis parameters and intraparenchymal testicular spectral Doppler indices in patients with clinical varicocele (23). However, no significant correlation was observed between three Doppler parameters of the testicular arteries (end-diastolic velocity [EDV], resistivity index [RI], and pulsatility index [PI]) with semen analysis parameters such as number, motility, volume, and morphology of sperm. On the other hand, the peak systolic velocity (PSV) index showed a significant relationship with sperm count. In our study, ultrasonographic grades 1-2 of varicocele did not significantly differ from ultrasonographic grades 3-5 in terms of indices related to the testicular volume and semen analysis. As described previously, variations between studies can be explained by differences in study populations, with only a minority of our patients complaining of infertility. According to our findings, the ultrasonographic grade is of little value in isolation among men undergoing scrotal ultrasonography for various reasons.

Given the fact that differences in the study measures between the various ultrasonographic grades could not be identified in this research, the effect of testicular volume (as another ultrasonographic parameter) on the semen analysis profile was also evaluated. According to previous studies, TTV values below 30 *ml* are associated with decreased sperm production (11). Varicoceles appear to give rise to a progressive disease, with increased testicular atrophy prevalence having been reported as

children with varicoceles progress through puberty (24). In the present study, it was found that TTV parameter had a significant relationship with semen analysis parameters, while TVD failed to show a meaningful relationship in this regard. In fact, testicular atrophy (TTV <30 *ml*) was associated with a drop in both the quantity and quality of spermatozoa. Our results are in alignment with those of Kurtz et al. (2015), who investigated the association between TTV/TVD and semen analysis parameters and reported a direct significant relationship between TTV and total sperm motility (14). The research of Oliva and Multigner (2018) described low sperm production and motility in patients with grade 3 varicocele, as well as a high proportion of sperm with abnormal morphology (25). Notably, Sakamoto et al. (2008) confirmed improvements in semen analysis parameters (sperm count and motility) and left testicular volume following surgical repair of varicocele (26). In our study, the semen analysis yielded acceptable results for determining the progression of varicocele disease, indicating that this highly available test can provide useful and comparable clinical data to the ultrasound study in centers that lack radiology facilities. In line with our findings, Krishna et al. reported that variables such as testicular volume, sperm count, and sperm motility are useful when following patients treated surgically for varicocele. These researchers found significant differences in sperm motility and concentration between different clinical grades of varicocele and asserted that the testicular volume has a good correlation with the severity of oligospermia (27). Overall, it can be said that the semen analysis and testicular volume results are essential in guiding the decision-making process when managing and following up patients with varicoceles.

The present study had some limitations. One was the fact that the sample size was limited in that only 19 out of 119 patients were categorized as having subclinical varicocele. Hence, it is likely that a larger sample would have yielded more significant differences in the investigated variables between the study groups. Another limitation was that pain was not measured quantitatively using a visual analog scale, though this did not affect our primary outcomes. Finally, there was no point of comparison against a group of normal individuals in this study and no particular complaints, which can be an interesting subject for future research.

Conclusion

The present study showed a relatively high correlation between grading based on clinical evaluation and scrotal CDU. However, given that the grades were similar in terms of testicular volume parameters and semen analysis indices, the clinical or ultrasonographic varicocele grade alone does not provide significant clinical information about men undergoing scrotal sonography based on any indication. Rather, decision-making should be guided according to infertility history, testicular atrophy, and abnormal semen analysis.

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Conflict of Interest

None declared by all authors. Availability of data and material.

Available upon reasonable request.

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