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Testicular ultrasound score: A new proposal for a scoring system to predict testicular function

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Abstract

Background: Testicular ultrasound (US) is routinely employed in the evaluation of reproductive and sexual function. However, its use for characteristics other than testicular volume is hampered by a lack of information on the prognostic value of its findings, which to date have only been incorporated in a score proposed by Lenz et al in 1993.

Objectives: We sought to explore whether testicular US examination can predict the quality of spermatogenesis and provide information on testicular endocrine function.

Materials and methods: We retrospectively reviewed 6210 testicular US examinations, finally selecting examinations from 2230 unique men. The following variables were considered: bitesticular volume and testicular asymmetry, parenchymal echotexture, echogenicity and presence of microlithiasis, solid lesions and varicocoele. Concurrent fasting hormonal data were available for 1160 men, while 979 had a semen sample available from the same day as the US examination.

Results: We derived a new US score, termed TU score, that can predict both impaired spermatogenesis (AUC 0.73, sensitivity 72%, specificity 61%, $P < .001$) and hypogonadism (AUC 0.71, sensitivity 71%, specificity 53%, $P < .001$) more accurately than the Lenz's score. In a multivariate analysis, a reduced sperm *composite index* (defined as total spermatozoa × total motility × normal forms) was independently predicted by bitesticular volume and by inhomogeneous echotexture, while hypogonadism was independently predicted also by reduced echogenicity and presence of microlithiasis.

Discussion and conclusions: We describe the testicular US characteristics that are independently associated with impaired spermatogenesis and hypogonadism and propose the TU score as a simple screening method for use in subjects referred for testicular US.

KEYWORDS

hypogonadism, infertility, spermatogenesis, testicular endocrine function, testicular ultrasound, testosterone

Pozza and Kanakis contributed equally to this work.

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1 | INTRODUCTION

Testicular US is routinely used in the assessment of male sexual and reproductive function, with both gray-scale and color-Doppler US imaging providing useful information in the assessment of testicular morphology and function.[1] It is the gold standard in assessing testicular volume (TV),[2] which tends to be overestimated by Prader's orchidometer.[3] A reduced TV, defined by most as being < 12 mL,[4] is associated with worse sperm parameters,[5] reduced fertility,[6] and hypogonadism.[7,8] Normal testicular echotexture comprises homogenously distributed medium-level echoes, termed *homogenous echotexture*.[4] Conversely, the presence of areas of altered echogenicity, mostly hypoechoic, which can be subtle, ill-defined focal or diffuse, has been termed testicular inhomogeneity (or *inhomogenous echotexture*).[9] Inhomogeneity has been associated with histological evidence of fibrosis, tubular sclerosis, and spermatogenic arrest,[10,11] and more recently, it has been proposed as an effective marker of male fertility.[12] Testicular echogenicity resembles the echogenicity of the normal thyroid gland and as a whole depends mostly on seminiferous tubule maturation and the testicular germ cell content, with the prepubertal testes being typically hypoechoic compared to adult.[4] As such, *reduced echogenicity* has been associated with reduced spermatogenesis and aberrant interstitial proliferation.[9]

Testicular microlithiasis (TML) is an incidental US finding of multiple 1-3 mm bright echogenic foci without acoustic shadowing[4] and is significant when at least 5 such foci are present in a single US scan. According to one classification, these foci can be described as limited, clusters or presenting a diffuse "starry-sky" appearance.[13] TML is caused by calcium deposits in the seminiferous tubules[14] and has been associated with testicular germ cell neoplasia in situ (GCNIS), especially when other risk factors are also present.[15,16] However, not all studies agree on its malignant significance.[17,18] TML has also been suggested to be associated with infertility [19] and the testicular dysgenesis syndrome.[20,21]

The volumetric predominance of one testis over the other, termed testicular asymmetry, has been proposed for the assessment of the functional ability of small testes, especially in patients with a history of cryptorchidism, and as a prognostic factor for varicocoele (VC) repair, with a cutoff for significance of ≥20%.[22] On US examination, non-palpable testicular solid lesions are usually associated with benign conditions such as focal Leydig cell hyperplasia or Leydig cell tumors,[23-25] whereas palpable solid lesions show a high probability of malignancy. Both types appear to be associated with cryptorchidism and Leydig cell tumors also with impaired spermatogenesis.[23] Lastly, color-Doppler imaging has been extensively employed in the detection and evaluation of testicular VC, which has been associated with ipsilateral hypotrophy and infertility, although this association is still under debate.[26,27]

The investigation and description of these testicular characteristics on US is hampered by the relative lack of literature evidence supporting the prognostic significance of its findings in relation to spermatogenesis and testicular endocrine function: to date, they have only been incorporated in a US score proposed by Lenz in 1993.[28] The aim of this study was to explore whether the information derived from testicular US in a large retrospective study could reliably predict sperm parameters and testicular endocrine function.

2 | MATERIALS AND METHODS

2.1 | Patients

We retrospectively studied 6210 testicular B-mode and color-Doppler US examinations performed in our unit between March 2005 and December 2013, corresponding to 3150 unique subjects referred to a tertiary-care andrological center (Section of Medical Pathophysiology, Food Science and Endocrinology, Department of Experimental Medicine, Rome, Italy). We excluded adolescents younger than 17 years [the age when maximum TV is reached [29]], subjects with genetic conditions known to be associated with testicular damage (eg, Klinefelter syndrome and congenital hypogonadotropic hypogonadism), subjects who had undergone testicular or pituitary surgery, and individuals treated with drugs active on the hypothalamic-pituitary-testicular axis. The final study cohort was composed of 2230 patients (Figure 1). Reasons for referral for testicular US were available for 1531 subjects (68.7% of the total) and are summarized in Table 1. Concurrent hormonal data from the Department's Endocrine Laboratory were available for 1160 subjects, while 979 had undergone a semen analysis, carried out by the local Seminology Unit, on the same day as the testicular US. The Local Review Board approved the study, and all patients provided written informed consent.

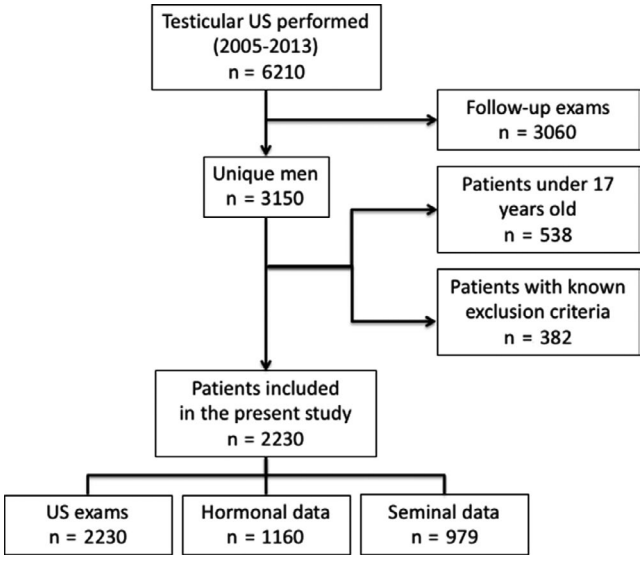

FIGURE 1 Flow diagram of patients included in the present study

2.2 | Hormonal evaluation

Morning baseline blood samples (07:45-09:30 hours) were obtained from all subjects by antecubital venous puncture after an overnight fast. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), sex hormone–binding globulin (SHBG), 17β-estradiol (E_2) and total testosterone (TTe) were double-measured with chemiluminescent microparticle immunoassay (CMIA, Architect System; Abbott Laboratories). Serum concentrations of inhibin B (INHB) were measured by enzyme-linked immunosorbent assay (ELISA) (GEN II; Beckman Coulter Laboratories). The laboratory reference ranges for adult men were as follows: 1.38-9.58 mIU/mL for FSH; 1.79-8.17 mIU/mL for LH; 12.0-38.20 nmol/L for TTe; 24-108 pg/mL for E2; 80-380 pg/mL for INHB and 11.1-78.2 nmol/L for SHBG. We calculated absolute and percentage values of free testosterone (cfTe) by using the Vermeulen equation,[30] based on the SHBG levels and assuming a fixed albumin concentration of 4.3 g/dL. We defined eugonadism as TTe > 12 nmol/L and LH < 8.17 mIU/mL (thus excluding subclinical hypogonadism). We also calculated the total testosterone/LH ratio (TTe/LH),[31] markers of Leydig cell function, and the inhibin B/FSH ratio (INHB/FSH),[32] marker of Sertoli cell function.

2.3 | Semen analysis

Semen samples were collected by masturbation directly into a sterile plastic container after 3-5 days of sexual abstinence and examined by optical microscopy (Leica DM5000B; Leica Microsystems, Wetzlar, Germany) according to World Health Organization (WHO) criteria.[33,34] The following variables were assessed: sperm concentration (n × 10^6/mL), total sperm number (n × 10^6/ejaculate), total motility (%), and morphology (% normal forms). Total motility was selected instead of progressive motility in order to circumvent the differences in motility assessment between semen analyses conducted in accordance with WHO 1999 semen analysis criteria and those conducted from 2010 onward (and hence in accordance with the current WHO 2010 recommendations).[35] This assumption was cross-validated by linear and logistic regression analyses employing data-splitting for the pre-2010 and post-2010 cohorts, estimating the models in each and comparing the resulting models in terms of respective *b*s and R^2 values. This comparison led to similar results in both groups in terms of predictors and their coefficients; therefore, the analyses were reconducted on the cohort as a whole and these results are reported herein. We employed a *composite index* based on the *Functioning Sperm Fraction* (FSF) in the existing literature,[36-38] defined as the total number of motile spermatozoa in the ejaculate with a normal morphology using a cutoff of 0.625 × 10^6/ejaculate, derived from the 5th centiles of the WHO 2010 reference parameters (39 × 10^6 spermatozoa × 40% motility × 4% normal forms).[35]

TABLE 1 Reason for testicular US referral (available for n = 1531)

Reason for referral	N (%)
Varicocoele	472 (30.8)
Infertility/Seminal alterations	348 (22.7)
Screening and prevention campaign	124 (8.1)
Scrotal pain	109 (7.1)
Suspected testicular lesions	101 (6.6)
Sexual dysfunctions	69 (4.5)
Sexually transmitted diseases	31 (2.0)
Hypogonadism	28 (1.8)
Cryptorchidism	26 (1.7)
Diabetes mellitus and obesity	23 (1.5)
Gynecomastia	12 (0.8)
Other	190 (12.4)

2.4 | Ultrasound

US examinations were performed using a Philips IU22 unit (Philips, Bothell, WA, USA) with a 7-15 MHz wideband linear transducer. The standardized protocol included axial and transverse examinations.[39] Two experienced sonographers performed all US examinations, stored the images and loops for subsequent analysis in our local image archiving system (eFilm Merge, Milwaukee, Wisconsin) and performed the revision in concert, blind to the written reports, while any dispute was adjudicated by a third author. All US examinations comprised the evaluation of the following parameters: left and right testicular volume, echotexture, echogenicity and presence of TML, solid lesions and left and/or right VC. TV was evaluated using the ellipsoid formula (height × width × length [cm] × 0.523) and expressed in mL as both left or right TV and bitesticular volume (BTV). After exploratory data analysis, testicular echotexture was categorized as either homogeneous or inhomogeneous and the results were compared to those obtained using Lenz's 5-category classification.[28] Overall testicular echogenicity was graded as normal or reduced.

TML was initially graded as absent or isolate, mild (at least five microcalcifications per US scan), moderate (more than 10 per US scan), or starry sky (homogenous presence of high-density microcalcifications in the testis).[40] However, exploratory data analysis revealed no significant differences between these groups in relation to the prediction of seminal and hormonal parameters, so only two groups were used for statistical analysis: absence or presence of TML.

We then evaluated the presence or absence of solid testicular lesions. Testicular asymmetry was evaluated according to the testicular atrophy index[41] and expressed as a percentage. A cutoff of 20% was adopted as distinctive of significant asymmetry, in accordance with the literature.[22] The presence of VC was assessed according to the Dubin-Solbiati scale[4] and is reported on 5-grades. Figure 2 shows typical US images of the evaluated features.

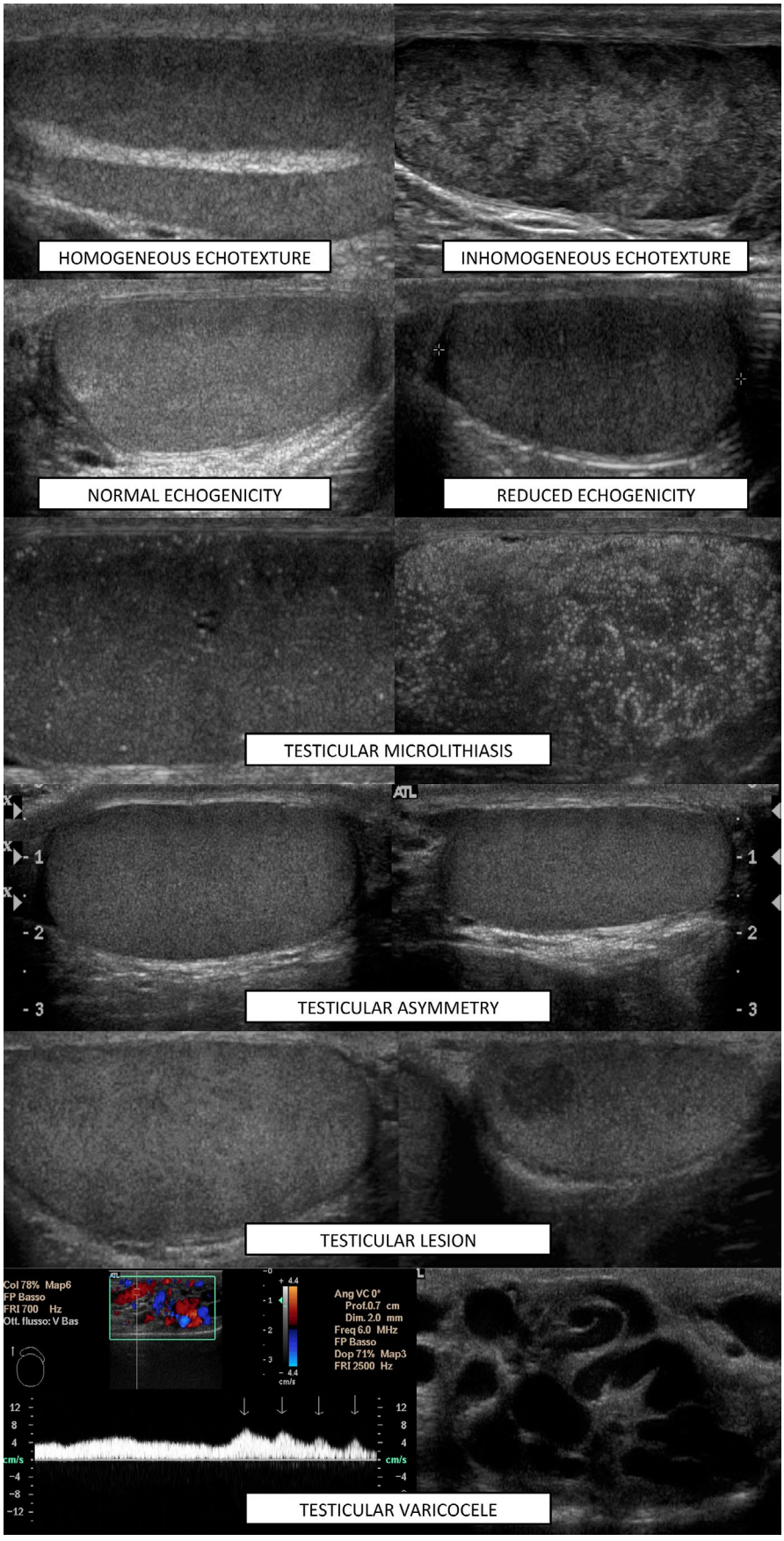

FIGURE 2 US features considered in developing the in-house score (TU score)

2.5 | Statistical analysis

Data are expressed as mean (M) and/or medians (m), as appropriate, and as standard deviations (SD), 95% confidence intervals (CI), and 25%-75% interquartile ranges (IQR). Data distribution was visually inspected by analysis of the respective histograms and normality plots. Student's *t* test, linear regression, and logistic regression were used for normally distributed data. Effect sizes are expressed as odds ratios and as Cohen's *d*s with descriptors, as originally suggested by Cohen and later expanded.[42] The linear regression analyses used Stein's formula for adjusted *R* squared (Adj R^2) to evaluate how well the model cross-validates across a different sample of data from the same population,[43] while for logistic regression analyses, we reported R^2 values according to Nagelkerke (R^2_N). A semi-quantitative scoring system called the TU score was created using the US characteristics deemed significant by logistic regression analyses. The scores were tuned through analysis of the receiver operating characteristic (ROC) curves for hormonal and semen parameters as follows: BTV (0 points if BTV ≥ 30 mL, +1 point if 24-29.9 mL, +2 points if 20-23.9 mL, +3 points if 14-19.9 mL, +4 points if < 14 mL), inhomogeneous echotexture (+1 point), reduced echogenicity (+1 point), presence of TML (+1 point) (Table 2). The score ranged from 0 to 7, with higher values indicating a more compromised condition. A cutoff of ≥ 2 was derived as a compromise between sensitivity and specificity, with preference given to the former. ROC curves' AUC were compared according to DeLong.[44] In the correlation analyses, we used Pearson's correlation coefficient (*r*) and Spearman's correlation coefficient (r_S) for normally and non-normally distributed data, respectively. The level of statistical significance was set to .05. All statistical computations were conducted with IBM SPSS Statistics for Windows, version 25 (IBM Corp.), and MedCalc, version 19.2.1 (MedCalc Software Ltd).

3 | RESULTS

Semen parameters, hormonal data, TVs, the Lenz's and the TU scoring systems, both applied on our cohort, are shown in Table 3, while the US findings are summarized in Table 4. The mean age of the participants was 32.5 ± 10.3 years.

3.1 | Ultrasound characteristics and testicular volumes

There was a significant correlation between the volumes of the two testicles (r = .763; P < .001), although the mean volume of the right testis (15.0 ± 5.4 mL) was significantly larger than the left (14.0 ± 5.0 mL) (P < .001). No correlation was found between age and BTV (P = .584). Patients with significant testicular asymmetry (26.1%) had a lower BTV than those without asymmetry (mean difference [MD] −2.6 mL; P < .001). Homogeneous testicular echotexture was found in 61.2% of patients and was associated with a larger BTV (MD 5.7 mL; P < .001). Accordingly, most of the patients had Lenz's score 1 or 2 (Tables 1 and 3), and the grade was inversely and significantly correlated with BTV (P < .001). With regard to testicular echogenicity, 88.3% presented a normoechoic testicular parenchyma, while the remainder showed reduced echogenicity (Table 4). Patients with normal echogenicity had a significantly larger BTV (MD 10.4 mL; P < .001).

TML was found in 87 patients, in 74% of whom it was mild. Patients with TML had a lower BTV than those without (MD −5.6 mL; P < .001). There was no significant correlation between the grade of TML and BTV. Solid testicular lesions were found in 3.5% of patients (Table 4), and these patients exhibited a lower BTV (MD −5.4 mL; P < .001) compared to those with absence of lesions.

TABLE 2 TU score created from the different testicular US characteristics through analysis of the ROC curves for hormonal and semen parameters; the score ranges from 0 to 7

TU score	0	+1	+2	+3	+4
BTV (mL)	>30	24-29.9	20.0-23.9	14.0-19.9	<14.0
Echotexture	Normal	Inhomogeneous			
Microlithiasis	Absent	Present			
Echogenicity	Normal	Reduced			
Lenz's score (1993)					
1	"uniform pattern"				
2	"slightly irregular pattern"				
3	"irregular pattern with small echogenic points"				
4	"very irregular pattern" or "with coarse echogenic points"				
5	"irregular with demarcated areas raising suspicion of a tumor"				

Note: Lenz's original score is reported below; the score ranges from 1 to 5. For both scores, higher values indicate a more compromised condition.

Abbreviations: BTV, bitesticular volume; ROC, receiver operator characteristic; TU, testicular ultrasound; US, ultrasound.

TABLE 3 Semen parameters, hormonal data and testicular volumes for all patients; Lenz's and TU scores values

	Median [25%-75% IQ ranges]
Age (years)*	32.5 ± 10.3
Spermatozoa concentration (×10^6/mL)	25.0 [3.0-62.0]
Spermatozoa in ejaculate (×10^6)	67.5 [8.0-180.0]
Semen total motility (%)	40.0 [20.0-50.0]
Semen typical morphology (%)	20.0 [12.0-26.0]
Composite index (×10^6)	4.0 [0.04-20.1]
FSH (mIU/mL)	4.0 [2.6-7.3]
LH (mIU/mL)	3.1 [2.3-4.5]
Total testosterone (nmol/L)	18.8 [14.7-23.5]
SHBG (nmol/L)	34.7 [25.0-44.5]
Calculated free testosterone (ng/dL)	10.9 [8.3-14.1]
17β-estradiol (pg/mL)	26.0 [20.0-32.0]
Inhibin B (pg/mL)	81.0 [34.5-129.5]
TTe/LH ratio	6.0 [3.9-8.8]
INHB/FSH ratio	21.5 [4.8-45.5]
Right testicular volume (mL)*	15.0 ± 5.4
Left testicular volume (mL)*	14.0 ± 5.0
Bitesticular volume (mL)*	29.0 ± 9.7
Lenz's score	1.0 [1.0-2.0]
TU score	1 [0-3]

Note: Parameters marked by * show normal distribution and are presented as mean ± SD.

Abbreviations: FSH, follicle-stimulating hormone; INHB/FSH, inhibin B/follicle-stimulating hormone (ratio); LH, luteinizing hormone; SHBG, sex hormone–binding globulin; TTe/LH, total testosterone/luteinizing hormone (ratio); TU, testicular ultrasound.

A variable grade of VC was detected in 58.5% of patients, with the vast majority (97%) affecting the left side. Grade 2 VC was the most prevalent, followed by grade 3 (Table 4), with 8.9% of patients showing bilateral involvement. The patients with VC were younger than those unaffected (MD −2.5 years; $P < .001$), and the grade of VC was negatively correlated with age ($P < .001$).

3.2 | Relationship between ultrasound characteristics and semen parameters

The composite index (>0.625 × $10^{6/}$ejaculate as an expression of normal spermatogenesis) was found to be significantly lower in patients with reduced BTV ($P < .001$), TML ($P = .018$), inhomogenous echotexture ($P < .001$), reduced echogenicity ($P < .001$), presence of solid lesions ($P < .001$), and significant asymmetry ($P = .012$); these differences reflected small-to-medium effects. Table 5 describes the significant mean differences in semen parameters between groups with different US characteristics. We conducted a linear regression analysis to ascertain which US parameters were able to predict independently semen parameters; the significant results are summarized in Table 6 and reported below. BTV and echotexture were predictors of sperm concentration (×10^6/mL), total sperm number (×10^6), total sperm motility (%), percentage of normal forms (%), and the *composite index* (×$10^{6/}$ejaculate) ($P < .001$ in all cases). The findings were maintained even when adjusted for the presence of VC. A logistic regression analysis (Table 7) revealed that the only testicular US parameters able independently to predict a normal semen sample, and hence spermatogenesis, were the BTV and the presence of a normal echotexture. For every unit rise in BTV, the odds ratio (OR) for normal *composite index* was 1.089 ($P < .001$), while the presence of an inhomogeneous echotexture was associated with an OR of 0.454 ($P < .001$) (Table 7). The model was found to be a good fit of the data, with an overall predicted correct percentage of 74%. The ROC curve analysis of the TU score's ability to predict a reduced sperm *composite index* (Figure 3A) showed an area under the curve (AUC) of 0.73 (95% CI, 0.70-0.76, $P < .001$), significantly greater than the ROC obtained by applying Lenz's score (AUC = 0.66, 95% CI 0.63-0.69, $P < .001$ for the comparison), with a cutoff ≥ 2 points associated with a sensitivity of 72% and a specificity of 61%.

3.3 | Relationship between ultrasound characteristics and hormonal values

FSH and LH values (mUI/mL) were significantly higher among patients with reduced BTV ($P < .001$ for both), presence of TML ($P = .005$ and $P = .017$), presence of inhomogenous echotexture ($P < .001$ for both), reduced echogenicity ($P < .001$ for both), presence of solid lesions ($P < .001$ and $P = .002$), and significant asymmetry ($P = .001$ and $P = .005$). TTe values (ng/dL) were significantly lower in patients with reduced BTV ($P < .001$), presence of TML ($P = .001$), presence of inhomogenous echotexture ($P < .001$), and reduced echogenicity ($P < .001$) (Table 5). On the contrary, cfTe (ng/dL) values were not found to significantly differ according to ultrasound parameters (not shown). The TTe/LH, a marker of Leydig cell function, was lower in patients with reduced BTV (5.48 ± 5.38 vs 7.52 ± 3.70; $P < .001$), significant asymmetry (6.30 ± 4.21 vs 6.93 ± 4.62; $P = .036$), inhomogeneous echotexture (5.91 ± 5.17 vs 7.53 ± 3.66; $P < .001$), reduced echogenicity (5.35 ± 7.25 vs 7.01 ± 3.75; $P = .004$), TML (5.18 ± 5.48 vs 6.83 ± 4.45; $P = .043$), and presence of solid lesions (4.52 ± 3.39 vs 6.88 ± 4.54; $P < .001$). The INHB/FSH, a marker of Sertoli cell function, was significantly lower in patients with reduced BTV (12.92 ± 19.18 vs 46.57 ± 44.44; $P < .001$), significant asymmetry (22.9 ± 26.67 vs 38.66 ± 44.21; $P = .001$), inhomogeneous echotexture (46.12 ± 43.78 vs 20.71 ± 31.63; $P < .001$), reduced echogenicity (15.52 ± 27.7 vs 37.53 ± 41.59; $P < .001$), TML (14.39 ± 18.6 vs 34.95 ± 41.01; $P < .001$), solid lesions (12.3 ± 20.42 vs 35.1 ± 40.9; $P < .001$). Among hypogonadal patients, 43.8% had a primary and 56.2% a secondary (or mixed) hypogonadism (based on TTe and LH values). A linear regression analysis was conducted to determine

TABLE 4 Testicular ultrasound characteristics of the entire study cohort

	N 2230	%		N 2230	%
BTV (mL)			Solid lesions		
Normal (≥24 mL)	1570	70.4	Absent	2152	96.5
Reduced (<24 mL)	660	29.6	Present	78	3.5
Asymmetry			Left Varicocoele[a]	1266	56.8
<20% (not significant)	1648	73.9	1	232	10.4
≥20% (significant)	582	26.1	2	476	21.3
Echotexture			3	402	18
Homogeneous	1365	61.2	4	149	6.7
Inhomogeneous	865	38.8	5	7	0.3
Microlithiasis	85	3.9	Right Varicocoele[a]	237	10.6
Absent	2143	96.1	1	136	6.1
Mild	86	3.9	2	85	3.8
Echogenicity			3	14	0.6
Normoechoic	1969	88.3	4	1	0
Hypoechoic	261	11.7	5	1	0

Abbreviation: BTV, bitesticular volume.

[a]Varicocoele was graded according to the Dubin-Solbiati scale.[4]

which US parameters were able to predict independently hormonal values; the significant results are summarized in Table 6. FSH values were predicted negatively by BTV ($P < .001$) and positively by inhomogeneous echotexture ($P < .001$), reduced echogenicity ($P = .012$), presence of TML ($P = .005$), and presence of solid lesions ($P = .001$). LH values were predicted negatively by BTV ($P < .001$) and positively by reduced echogenicity ($P < .001$), presence of TML ($P = .043$), and presence of solid lesions ($P = .026$). TTe values were predicted positively by BTV ($P = .002$) and negatively by presence of inhomogeneous echotexture ($P < .001$) and TML ($P = .005$). Inhibin B values were predicted positively by BTV ($P < .001$) and negatively by presence of inhomogeneous echotexture ($P < .001$). cfTe values were not predicted by any ultrasound characteristic alone ($P = .847$ for the model, not shown). The findings were maintained even when adjusted for the presence of VC.

A logistic regression analysis was also conducted to analyze which US parameters were able to predict independently eugonadism, as defined above. BTV was positively associated with normal TTe and LH values (OR 1.060 per mL; $P < .001$), while the following characteristics were associated with an increased risk of hypogonadism: presence of inhomogeneous echotexture (OR 0.538; $P = .004$), reduced echogenicity (OR 0.498; $P = .002$), and presence of TML (OR 0.424; $P = .013$) (Table 7). The model was found to be a good fit of the data, with an overall predicted correct percentage of 86.5%. The ROC curve analysis of the TU score's ability to predict hypogonadism showed an AUC of 0.71 (95% CI 0.69-0.73, $P < .001$), significantly greater than the ROC obtained by applying Lenz's score (AUC = 0.68, 95% CI 0.65-0.69, $P = .008$ for the comparison) with a cutoff ≥ 2 points associated with a sensitivity of 71% and a specificity of 53% (Figure 3B).

3.3.1 | Odds ratios

We proceeded to determine the OR for normal gonadal function in our cohort of patients, to calculate the risk of a patient whose testicular US is available for reduced spermatogenesis and hypogonadism. As a reference, the prevalence of reduced *composite index* in our cohort of patients with a BTV of at least 24 mL and a homogenous echotexture was 18.3%, compared with an overall prevalence of 37.7%. As for hypogonadism, the prevalence among patients with a BTV of at least 24 mL, a homogenous echotexture, a normal echogenicity and absence of TML was 8.6%, compared with an overall prevalence of 17.6%.

3.3.2 | ORs for normal composite index

The two parameters independently predicting a normal *composite index* were BTV and echotexture (Table 8). Assuming as reference an individual with a BTV of 24 mL and a homogenous echotexture, we can derive a baseline OR for a normal *composite index* of 26.14. By comparison, a patient presenting with a BTV of 16 mL and a homogenous echotexture will have a baseline OR of 17.42, while a patient with a BTV of 20 mL and a inhomogenous echotexture will have a baseline OR of 9.9. The first patient will present an OR for a normal *composite index* compared with our reference of 0.67 and as such a 33% higher risk of having a reduced semen quality, while the second patient will present an OR for a normal *composite index* of 0.38, with a 62% higher risk of presenting a reduced semen quality. Table 8 provides constant (α) values to be used. Constant (α) and Reference OR for BTV values are already set (respectively, 1.089 and 26.14), while

TABLE 5 Semen parameters and hormonal data showing a significant mean difference with testicular US characteristics

		Mean difference (95% CI)	*P*	*d*[a]
Spermatozoa/mL ± SD (×10^6)				
BTV	58.6 ± 65.1 vs 18.2 ± 28.5	40.42 (34.3, 46.54)	<.001	0.62
TML	28.1 ± 33.8 vs 44.3 ± 58.7	−16.18 (5.1, 27.25)	.005	0.28
Echotexture	55.94 ± 58.39 vs 29.85 ± 54.07	26.09 (18.81, 33.37)	<.001	0.45
Echogenicity	46.71 ± 57.54 vs 26.18 ± 56.6	20.54 (10.29, 30.78)	<.001	0.36
Solid lesions	21.52 ± 33.85 vs 44.8 ± 58.65	−23.28 (12.64, 33.92)	<.001	0.40
Asymmetry	33 ± 35.83 vs 47.4 ± 63.58	−14.39 (−20.97, −7.81)	<.001	0.23
Total motility ± SD (%)				
BTV	39.7 ± 16.5 vs 25.7 ± 17.6	13.96 (11.4, 16.51)	<.001	0.84
Echotexture	39.6 ± 16.5 vs 29.7 ± 18.5	9.92 (7.41, 12.42)	<.001	0.60
Echogenicity	36.7 ± 17.5 vs 26.1 ± 19.1	10.61 (6.7, 14.53)	<.001	0.61
Solid lesions	27.9 ± 19.1 vs 35.7 ± 17.9	−7.72 (0.94, 14.5)	.027	0.43
Normal forms ± SD (%)				
BTV	21 ± 9.9 vs 15.5 ± 10.9	5.51 (3.91, 7.12)	<0.001	0.56
Echotexture	21.2 ± 9.9 vs 16.9 ± 10.9	4.27 (2.79, 5.75)	<0.001	0.43
Echogenicity	19.9 ± 10.3 vs 15.5 ± 11.4	4.39 (2.06, 6.72)	<0.001	0.43
Composite Index ± SD (×10^6/ejaculate)				
BTV	22.2 ± 34 vs 5.86 ± 13.4	16.35 (13.23, 19.47)	<.001	0.48
TML	10 ± 16.2 vs 16.4 ± 29.7	−6.48 (1.13, 11.83)	.018	0.22
Echotexture	21.7 ± 33.9 vs 9.9 ± 21.2	11.81 (8.18, 15.45)	<.001	0.35
Echogenicity	17.7 ± 30.7 vs 7.7 ± 16.5	9.95 (6.46, 13.44)	<.001	0.35
Solid lesions	6.5 ± 12.9 vs 16.7 ± 29.8	−10.13 (5.88, 14.38)	<.001	0.34
Asymmetry	12.7 ± 21.5 vs 17.4 vs 31.5	−4.63 (−8.23, −1.03)	.012	0.15
FSH ± SD (mIU/mL)				
BTV	4.05 ± 3.28 vs 10.87 ± 11.47	−6.81 (−7.96, −5.67)	<.001	2.07
TML	13.05 ± 16.63 vs 6.31 ± 7.38	6.74 (−11.3, −2.18)	.005	0.91
Echotexture	4.15 ± 3.53 vs 9.3 ± 10.62	−5.15 (−6.11, −4.19)	<.001	1.46
Echogenicity	5.55 ± 6.46 vs 12.35 ± 12.82	−6.8 (−8.77, −4.83)	<.001	1.05
Solid lesions	14.04 ± 13.52 vs 6.23 ± 7.62	7.82 (−11.5, −4.13)	<.001	1.03
Asymmetry	8.19 ± 9.94 vs 6.07 ± 7.41	2.13 (0.87, 3.38)	.001	0.29
LH ± SD (mIU/mL)				
BTV	3.14 ± 1.67 vs 5.23 ± 4.9	−2.08 (−2.57, −1.59)	<.001	1.25
TML	6.06 ± 6.64 vs 3.82 ± 3.16	2.23 (−4.06, −0.41)	.017	0.71
Echotexture	3.2 ± 1.63 vs 4.71 ± 4.52	−1.51 (−1.92, −1.1)	<.001	0.93
Echogenicity	3.53 ± 2.52 vs 6.04 ± 5.95	−2.51 (−3.4, −1.61)	<.001	1.54
Solid lesions	6.2 ± 5.54 vs 3.81 ± 3.25	2.39 (−3.88, −0.89)	.002	0.74
Asymmetry	4.53 ± 4.79 vs 3.71 ± 2.72	0.82 (0.25, 1.39)	.005	0.30
Total Testosterone ± SD (ng/dL)				
BTV	579.9 ± 192.8 vs 527.4 ± 191.9	52.5 (28.9, 76.2)	<.001	0.27
TML	454.1 ± 210.1 vs 565.2 ± 191.8	−111.1 (50.32, 171.87)	.001	0.58
Echotexture	592 ± 188.6 vs 525.6 ± 194.1	66.4 (43.6, 89.27)	<.001	0.35
Echogenicity	572 ± 189.7 vs 497.9 ± 204.6	74.11 (41.13, 107.08)	<.001	0.39
Inhibin B ± SD (pg/mL)				
BTV	116.8 ± 74.5 vs 45 ± 49.5	71.9 (62.4, 81.3)	<.001	0.97
TML	56 ± 51.1 vs 91.6 ± 75.4	−35.57 (14.97, 56.16)	.001	0.47

(Continues)

TABLE 5 (Continued)

		Mean difference (95% CI)	*P*	*d*[a]
Echotexture	114.4 ± 78.2 vs 63 ± 60.3	51.44 (40.83, 62.1)	<.001	0.66
Echogenicity	98.4 ± 75.5 vs 46.1 ± 52.9	52.29 (40.31, 64.27)	<.001	0.69
Solid lesions	40.4 ± 49.1 vs 92.4 ± 75	−51.96 (32.14, 71.78)	<.001	0.69
Asymmetry	74.4 ± 80.5 vs 96.5 ± 71.5	−22.1 (−35.26, −8.93)	.001	0.31

Note: Group comparisons were conducted as follows: BTV: ≥24 mL vs < 24 mL, TML: present (any grade) vs absent, Echotexture: homogenous vs inhomogeneous, Echogenicity: normoechoic vs hypoechoic, Solid lesions: present vs absent, Asymmetry: present vs absent.

Abbreviations: BTV, bitesticular volume; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TML, testicular microlithiasis; TTe, total testosterone; US, ultrasound.

[a] Cohen's *d*.

echotexture α values should be chosen and inserted in the *formula* accordingly.

3.3.3 | ORs for eugonadism

Four different ultrasound parameters are able to predict eugonadism: BTV, echotexture, echogenicity, and presence of TML (Table 8). Assuming our reference standard to have a BTV of 24 mL, a homogenous echotexture, normal echogenicity, and absence of TML, he will possess a baseline OR of 25.44. By comparison, a patient with a BTV of 30 mL, homogenous echotexture, normal echogenicity, and presence of TML will have a baseline OR of 13.48, while a patient with a BTV of 20 mL, inhomogenous echotexture, reduced echogenicity, and absence of TML will have a baseline OR of 5.68. The first patient will present an OR of 0.53 and as such a 47% higher risk of hypogonadism compared with our reference, while the second patients will have an OR of 0.22 and as such a 78% higher risk of hypogonadism. Table 8 provides constant (α) values to be used. Constant (α) and Reference OR for bitesticular volume values are already set (respectively, 1.060 and 25.44), while echotexture, echogenicity, and microlithiasis α values should be chosen and inserted in the *formula* accordingly.

$$OR_{Eugonadism} = \frac{[1.060 * BTV\,(mL)] * Constant\,(\alpha)}{25.44}.$$

4 | DISCUSSION

To our knowledge, this is the first comprehensive study to incorporate and correlate the various testicular US, endocrine, and sperm parameters data from a large number of patients referred to a tertiary-care andrological center, encompassing the entire spectrum of gonadal function (from normal to severely compromised). Aim of our work was to assess whether, and to what extent, testicular US is informative for normal sperm parameters (as a surrogate fertility marker) and eugonadal state, and which US parameters are most informative.

Knowledge of the many clinical information ultrasonography can provide in testicular disease workup is gaining momentum, as US scan is now becoming a readily available tool in the andrologist's office. The increasing number of surveillance programs assessing men's health also contributed to an expansion of testicular US scans. Thus, there is the need to simplify and standardize the diagnostic workup to avoid unnecessary laboratory requests.[45] Lenz et al [28,46,47] developed a US score as a possible screening method for testicular function based on testicular biopsies performed on patients affected by contralateral testicular tumors. Their score considered five possibilities, ranging from 1 (normal testis) to 5 (testis affected by a solid lesion). They found that a higher score was correlated with a lower sperm count, negatively correlated with US testicular volume and not correlated with tubular membrane thickness or the estimated number of Leydig cells. These findings supported the theory that irregular echotexture reflects testicular damage and that there is a correlation between testicular function, US volume and echotexture.

With this in mind, the TU score was developed on the assumption that testicular US can provide not only morphological but also functional information and that each feature observed during an US examination might offer a different piece of information and have its own weight in relation to testicular health and should therefore be considered separately. TML may be found in otherwise homogeneous and normally functioning testes; solid lesions can develop in testes with normal volume and function; reduction in gonadal function can be found in slightly inhomogeneous testes. The TU score is accurate and comprehensive, considers the most important features that should always be described in an US report, and has proved significantly more accurate than Lenz's score in predicting both impaired spermatogenesis and hypogonadism. The high statistical significance of linear and logistic regression models performed confirms the relevance of US-assessed BTV, positively correlated with fertility status, testosterone levels, and TTe/LH ratio,[48] but also underscores the importance of echotexture and echogenicity, which should always be considered. Reduced BTV and inhomogeneous echotexture appear to be risk factors for reduced *composite index*, as a surrogate fertility marker, while reduced BTV, inhomogeneous echotexture, reduced echogenicity, and the presence of TML appear to be all risk factors for hypogonadism. This is consistent with the current awareness that inhomogeneous echotexture is indicative

TABLE 6 Linear model of US predictors of seminal parameters and hormones

	β	*b*	*P*
Semen analysis			
Sperm concentration			
BTV (mL)	0.374	2.232 (1.853, 2.611)	<.001
Inhomogeneous echotexture	−0.135	−15.642 (−23.233, −8.050)	<.001
Total sperm motility			
BTV (mL)	0.359	0.726 (0.592, 0.860)	<.001
Inhomogeneous echotexture	−0.203	−7.383 (−9.920, −4.847)	<.001
Normal sperm morphology			
BTV (mL)	0.263	0.311 (0.229, 0.394)	<.001
Inhomogeneous echotexture	−0.156	−3.312 (−4.873, −1.752)	<.001
Composite index			
BTV (mL)	0.337	1.016 (0.821, 1.212)	<.001
Inhomogeneous echotexture	−0.124	−7.275 (−11.191, −3.359)	<.001
Hormonal data			
FSH			
BTV (mL)	−0.401	−0.327 (−0.372, −0.282)	<.001
Inhomogeneous echotexture	0.131	2.149 (1.213, 3.085)	<.001
Reduced echogenicity	0.074	1.659 (0.366, 2.951)	.012
TML	0.076	2.837 (0.852, 4.822)	.005
Solid lesions	0.088	3.257 (1.260, 5.255)	.001
LH			
BTV (mL)	−0.302	−0.104 (−0.124, −0.084)	<.001
Reduced echogenicity	0.107	1.000 (0.421, 1.578)	<.001
TML	0.058	0.927 (0.029, 1.825)	.043
Solid lesions	0.065	1.017 (0.122, 1.912)	.026
Total testosterone			
BTV (mL)	0.102	0.068 (0.026, 0.110)	.002
Inhomogeneous echotexture	−0.124	−1.673 (−2.556, −0.790)	<.001
TML	−0.088	−2.818 (−4.777, −0.860)	.005
Inhibin B			
BTV (mL)	0.469	3.415 (2.912, 3.919)	<.001
Inhomogeneous echotexture	−0.175	−26.274 (−36.922, −15.627)	<.001

Note: Only the variables included in the models are reported in the table. 95% confidence intervals reported in parentheses.

Abbreviations: BTV, bitesticular volume; FSH, follicle-stimulating hormone; LH, luteinizing hormone TML, testicular microlithiasis; US, ultrasound; VC, varicocoele.

of testicular fibrosis, tubular sclerosis, and spermatogenic arrest,[11] while reduced echogenicity has also been associated with interstitial proliferation,[9,49] possibly involving Leydig cell function, although this finding needs to be further confirmed by future studies. The role of TML in predicting hypogonadism is less clear; hypogonadism could reflect other congenital or acquired risk factors, as it is associated with conditions such as cryptorchidism or testicular dysgenesis syndrome.[50]

Taking into account our results, derived from a large cohort of subjects ranging from potentially "healthy" individuals—recruited during prevention campaigns—to testicular cancer patients, we calculated the individual TU score and recommend that patients scoring ≥ 2 should undergo further evaluation. Although the decision to request a semen analysis and/or hormonal evaluation in the clinical setting is based on clinical history, presenting complaint, and physical examination, the rationale of creating an US score is to provide the less experienced sonographer, or the non-andrologists, with a more objective tool to triage patients toward the need for further investigations or follow-up. Similarly, given the score's good ability to predict testicular function, it could be used, in the setting of large screening programs, to select those asymptomatic patients who can avoid further analyses, saving time and money. Finally, we derived a

TABLE 7 Logistic regression analysis models for impaired spermatogenesis and hypogonadism

	Variables in the equation	β	P	OR	95% CI Lower bound	95% CI Upper bound
Normal composite index	BTV (mL)	0.085	<.001	1.089	1.068	1.110
	Inhomogeneous echotexture	−0.789	<.001	0.454	0.328	0.628
Eugonadism	BTV (mL)	0.059	<.001	1.060	1.038	1.083
	Inhomogeneous echotexture	−0.619	.004	0.538	0.354	0.819
	Reduced echogenicity	−0.697	.002	0.498	0.318	0.780
	TML	−0.857	.013	0.424	0.216	0.834

Note: Normal composite index: R^2_N = .236, model χ^2 172.927, P < .001. Eugonadism: R^2_N = .191, model χ^2 127,417, P < .001.
Abbreviations: BTV, bitesticular volume; TML, testicular microlithiasis.

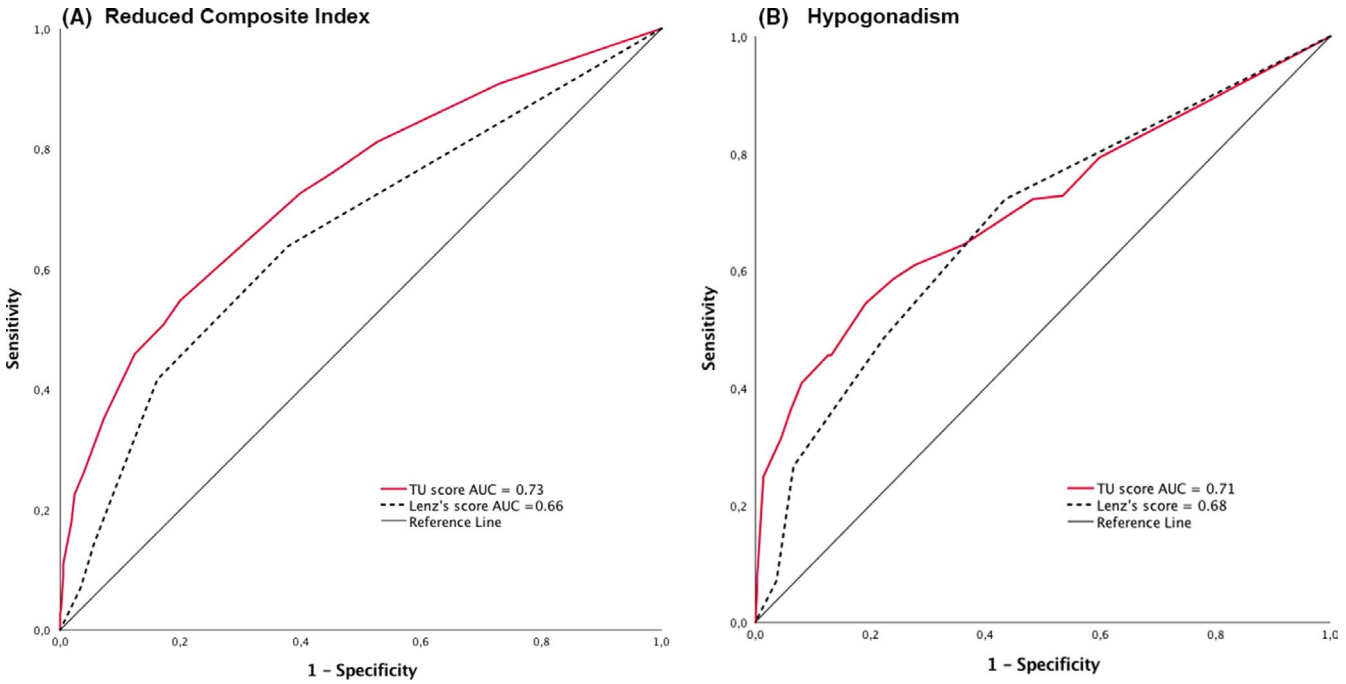

FIGURE 3 Left (a): ROC curve analysis of the TU score's ability to predict a reduced composite index; right (b): ROC curve analysis of the TU score's ability to predict hypogonadism, defined as TTe < 12 nmol/L

formula to calculate specific ORs for a normal composite index and eugonadism.

The strengths of this study are its high statistical power, deriving from a large cohort of patients, and the availability of hormonal data, semen analyses, and US examinations, all performed at a single tertiary center. It is limited by the lack of complete data on the various reasons why patients were originally referred to our center, and on the risk factors shown to be associated with reduced fertility. However, we provided US reasons of referral for approximately two thirds of our cohort and have excluded all patients with known genetic conditions associated with altered testicular function, patients who had undergone testicular or pituitary surgery and those on therapy with drugs active on the hypothalamic-pituitary-testicular axis. Another drawback was the need to consider total sperm motility instead of progressive motility, as patients were enrolled both before and after publication of the WHO 2010 semen analysis criteria. Moreover, we did not evaluate the predictive value of VC in terms of spermatogenesis or Leydig cell function: yet, the present study was not designed to assess its specific role, already defined, in determining testicular impairment, but rather aimed at describing the individual predictive contribution of each US parameter. Anyhow, our findings were maintained even when adjusted for the presence of VC. Further studies could explore the informative role of other testicular imaging methods, such as parenchymal Doppler and contrast-enhanced US, elastosonography and magnetic resonance imaging, which has already proven valuable in characterizing small solid lesions,[51,52] and whether they possess different predictive values for different clinical conditions.

In conclusion, our study is the first to comprehensively describe the testicular US parameters independently associated with both impaired spermatogenesis and hypogonadism in a large cohort of patients. In

TABLE 8 Constant (α) and Reference OR values to be used to calculate odds for normal composite index and eugonadism according to US parameters observed (see the text). Constant (α) and Reference OR for bitesticular volume values are already set (in bold), while echotexture, echogenicity, and microlithiasis α values should be chosen and inserted in the *formula* accordingly

		Reference OR (24 mL)
Normal composite index		
Bitesticular volume	**1.089 (per mL)**	**26.14**
	Constant (α)	
+ homogeneous echotexture	1.000	
+ inhomogeneous echotexture	0.454	
Eugonadism		
Bitesticular volume	**1.060 (per mL)**	**25.44**
	Constant (α)	
+ homogeneous echotexture	1.000	
+ normal echogenicity	1.000	
+ absence of microlithiasis	1.000	
+ inhomogeneous echotexture (IE)	0.538	
+ reduced echogenicity (RE)	0.498	
+ presence of microlithiasis (TML)	0.424	
+ IE + RE	0.268	
+ IE + TML	0.228	
+ RE + TML	0.211	
+ IE + RE +TML	0.114	

Abbreviations: OR, odds ratio; US, ultrasound.

addition, we propose the new TU score as a simple and informative screening tool, which could be applied during routine testicular US examination in order to identify patients who require further evaluation.

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DISCLOSURES

The authors have nothing to disclose.

AUTHORS' CONTRIBUTIONS

AMI, CP, GK, DG guaranteed the integrity of entire study. CP, GK, DG, FC analyzed and interpreted the data. MT, MM, AL, FS, MGT contributed to literature research. AMI, CP, DG, AL, AA, DP, MT, RP, FS acquired data. FC performed statistical analysis. AMI, CP, GK, DG, FC, MT edited the manuscript. All authors were involved in study concept/study design or data acquisition, manuscript drafting or manuscript revision for important intellectual content, and manuscript final version approval.

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