AKADÉMIAI KIADÓ

Testicular ultrasound measurement of testicular volume and epididymis diameters: Prediction of semen quality in a prospective study

IMAGING

ORIGINAL ARTICLE

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ABSTRACT

Purpose: To identify the most predictive ultrasound parameters for the assessment of male infertility by a multiparametric study.

Materials and Methods: A total of 64 males were recruited in the study group and 14 men in the control group. At first, grey-scale and color and Power Doppler ultrasonographic imaging was used to analyze testicular morphological characteristics and detect intratesticular abnormalities. Various ultrasound parameters of B-mode US and strain-elastography were related to total sperm count (TSC).

Results: The prevalence of varicocele was not significant ($P = 0.33$), though presented a 2.53-fold odds ratio The strain ratios of both testes, the volume of the left testis and the size of the left appendix were most associated with the results of semen analysis according to a Variable Importance in the Projection (VIP) score of >1. The first latent variable from the PLS analysis explained a significant amount of variance in TSC, concentration, and motility parameters ($P < 0.0002$) and showed that bilateral strain ratio, the size of the testis, and the volume of left appendix were the most significant US predictors of the pathological semen and sperm cell features.

Conclusions: Our results showed that B-mode US with strain elastography is among more sensitive US approaches in evaluating male fertility.

KEYWORDS

strain elastography, multiparametic ultrasound, male infertility

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Introduction

An estimated 20% of Hungarian couples seek medical treatment for infertility after failure to achieve pregnancy within one year of trying to conceive. One in eight couples encountered problems when attempting to conceive the first child and one in six when attempting to conceive a subsequent child. In Hungary, the proportion of involuntarily childless couples is estimated at 24%, and one in four couples has infertility problems [1]. Three percent of women who are currently trying to conceive remain involuntarily childless, while 6% of women with previous deliveries are not able to have as many children as they wanted. A male-infertility-associated factor is found in 50% of involuntarily childless couples, usually

together with abnormal semen parameters. Therefore, all male patients belonging to infertile couples should undergo medical evaluations by a trained specialist in andrology [2].

A focused evaluation of the male patient must always be undertaken and should include a medical and reproductive history, physical examination, semen analysis with strict adherence to the World Health Organization (WHO) reference values for human semen characteristics. Other investigations (e.g., ultrasound, hormone level tests) may be required depending on the clinical features and semen parameters [1].

Ultrasound offers a fast, harmless, and cost-effective method of examination and is therefore the first-line modality for imaging the scrotum and extra- and intratesticular lesions. Indeed, ultrasound assessment of testicular abnormalities has been demonstrated to be closely related to the patient's symptoms and clinical picture (e.g., intratesticular mass of low echogenicity compared to normal testicular tissue or increased vascularization suggestive of inflammation) [3]. A high-frequency linear array transducer was used to achieve a high spatial resolution of the scrotal contents. Color Doppler or power Doppler measurements are also performed to detect, evaluate and differentiate intratesticular lesions. Furthermore, male infertility guidelines recommend scrotal ultrasound to distinguish between obstructive and non-obstructive azoospermia and may demonstrate inhomogeneous testicular echotexture and microlithiasis [1].

Since its first description in the 1990s [4], strain elastography (SE) has become a standard feature of most modern scanners. SE is a non-invasive, real-time method that allows for the measurement of tissue elasticity: the resistance of the tissue to deformation force and its return to the original status. The elasticity and deformation models can be described by Hooke's law, from which the Young's modulus of the tissue can be calculated. Alternatively, the strain ratio can be used as a semiquantitative measurement of elasticity. Here, the strain in a region of interest (ROI) containing the target lesion was measured relative to a normal reference area [5]. During the measurement, the elastographic response of the testicular tissue, measuring the elasticity (elastogram), was superimposed on the B-mode image.

Such measurements have already been used to assess testicular pathology. Specifically, testicular elasticity is linked to serum FSH levels and varicocele-related changes [6]. The strain ratio of the testis, especially on the left side, is elevated in varicocele well before atrophy of the testis. Consequently, SE could be useful for detecting damage caused by varicoceles [7]. Previous studies have also demonstrated a relationship between testicular stiffness and semen production. A negative correlation was found between semen volume, semen quality, and testicular stiffness [8].

Several studies found connections between the US parameters (volume, echogenicity) and the qualitative and quantitative features of semen quality. But the US parameters alone, without physical examination and hormone, were not able to identify testicular pathology. Furthermore, the traditional linear regression analysis employed in prior publications cannot unambiguously predict the importance of individual variables.

The non-parametric partial least squares (PLS) approach is able to distinguish a pattern of parameters that best predict the variable in question while also handling the problem of collinearity. Therefore, in this multiparametric study, we aimed to identify the most predictive ultrasound parameters for the assessment of male infertility.

Various ultrasound parameters of B-mode US and strain elastography are related to sperm features (motility and morphology) and semen quality (total sperm count, sperm concentration, total motility, and sperm morphology). The first aim is to choose one of the US parameters that best reflects semen abnormality. Second, we investigated the correlation between the strain ratio of the US parameter and semen abnormalities.

Materials and methods

Study population

Participants were recruited between December 2017 and June 2019 at the Andrology Unit of the Department of Urology and Department of Obstetrics and Gynecology, University of Szeged. The control group consisted of healthy volunteers from the university staff, their relatives and men from infertile couples, where normal TSC and semen quality were identified as higher than the WHO's lower reference limites. All subjects consented to undergo testicular ultrasound examination. Pathological gray-scale ultrasound (for details, see below) of the testis were excluded from the control group.

Patients in the study group were included if they presented abnormal semen parameters. Patients with present or history of testicular tumor, intratesticular cyst, acute orchitis/epididymitis, undescended testicles and hydrocele were excluded from the statistical analysis.

All procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 2000. The study protocol was approved by the Regional and Institutional Human Medical Biological Research Ethics Committees. Informed consent was obtained from all participants.

Altogether, 64 males were recruited in the study and 14 control groups, respectively. The average age was 37.1 years (range: 22–54) in the study group, and 36.3 years (25–42) in the control group ($P = 0.63$). In the study group, semen analysis revealed that 23.3% of patients had azoospermia, 9.5% had cryptozoospermia, 8.2% of infertile men had oligozoospermia, 12.3% had asthenozoospermia and 38.4% had oligo-asthenozoospermia (less common types: cryptozoospermia and hypospaermia 5.5%; hypospermia 1.4%, necrozoospermia1.4%). In the case of azoospermia, micro-TESE surgery was performed and the pathological Johnsen score correlated with the strain ratio in patients with non-obstructive azoospermia.

Sperm analysis

For all patients, physical examination and sperm analysis were performed. Semen was analyzed according to the Fifth Edition of the World Health Organization (WHO) Manual for the

examination of human semen. Total sperm count and sperm concentration were counted using phase-contrast optics at × 200 magnification, total sperm motility, progressive and non-progressive motility was measured in a Neubauer improved chamber (FertiCAD Kft., Budapest, Hungary)as described in the WHO laboratory manual for the examination of human semen. Sperm morphology was determined at × 1,000 magnification with oil immersion after Diff-Quik staining (Diff-Quik Staining Set, Medion Diagnostics AG, Düdingen, Switzerland). The head, neck, mid-piece, tail, and multiple morphological abnormalities were noted separately. In the case of abnormal semen parameters, a control sample was collected and analysed 3–4 weeks later. If the result of the control sperm analysis was abnormal, hormonal evaluation and ultrasound examination of the testes was performed. In certain cases, ultrasound examination of the prostate and seminal vesicles was also performed. When sperm concentration was less than 10 $\times 10^6$/ml karyotyping and below 5×10^6/mL screening for azoospermia factor microdeletion of the Y chromosome (AZFa /Sy84,86/; AZFb /Sy127,134/; AZFc /Sy254,255/ regions) were also performed.

Ultrasonography, imaging and measuring technique

Ultrasound examinations were performed on a GE Logiq E9 machine (General Electric Healthcare) with a 15 MHz linear probe. The examinations were performed by a radiologist with at least 5-year- ultrasound-experience. Grayscale, colour and Power Doppler ultrasonographic imaging were used to analyze testicular morphological characteristics and detect intratesticular abnormalities. Testicular volumes were calculated by multiplying the three longest diameters (L × T × AP) using a correction factor of 0.52. The largest diameter of the head of the epididymis was measured on both sides, the presence and length of the appendix testis were noted and the presence and grade of varicocele, according to Sarteschi-Ligouri, were described. We have chosen this classification while the Classification according to Sarteschi-Liguori is based on the degree of reflux, which is the most important factor in testicular damage. The appendix testis is a vestigial remnant of the Müllerian duct, so may be related to and may reflect indirectly the testicular status.

Next strain elastography was performed to measure the testicular tissue elasticity. For elastography, the same ultrasound equipment and linear transducer were used. The testicles were positioned on a rigid paper sheet to optimize strain elastosonographic imaging. The probe was adjusted perpendicular to the testis, displaying the largest possible cross-section and avoiding the mediastinum testis because of its different elasticity. Freehand technique was used. When the radiologist applied a slight pressure to the transducer, a compression bar appeared on the screen indicating the appropriate pressure in green. When this pressure is stable for at least 5–10 s, the radiologist should use this frame for subsequent analysis. In normal cases, a homogenous testis of normal size, without intratesticular lesions was detected by B-mode US. The testicular structure was also visualized using an elastogram, which is a color-coded picture of tissue elasticity. Tissue stiffness is encoded from red (soft) to blue (rigid), according to the standard settings of the US equipment. The color-coded image of the normal testis was homogeneous and surrounded by a relatively harder ring, representing the tunica albuginea of the testis with less relative strain (Fig. 1). During semi-quantitative analysis, the ROI was positioned at the midline of the testis. The strain ratio was calculated as the ratio of the stiffness of peritesticular fat to testicular parenchyma [8].

Statistical analysis

For the statistical analysis of group differences two-tailed independent sample *t*-tests were used and Bonferroni

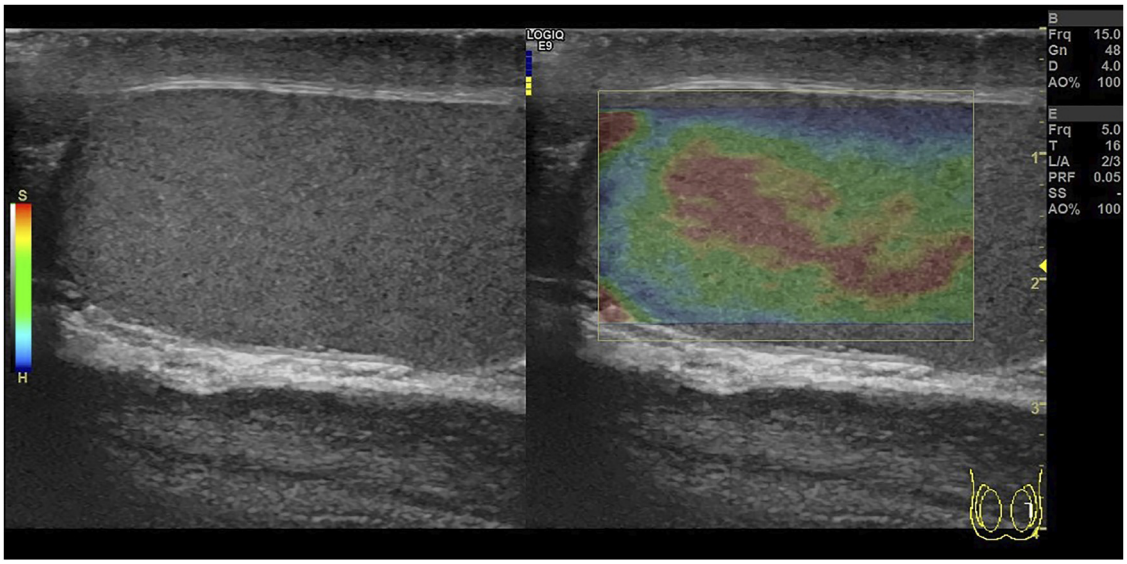

Fig. 1. Strain elastography (color coded picture – elastogram) and B-mode images of the normal testis. The central parenchyma is green color with blue edges ('boundary' effect), which is surrounded by red bands

correction was performed to correct for multiple comparisons. Mann-Whitney U-test was used to detect differences between the strain ratios of the left and right testis.

For a subset of patients (n = 38), we calculated the Spearman-Rank correlation between levels of TSH, prolactin, testosterone, SHBG, FSH, and LH and ultrasonography parameters and repeated the same for functional measures.

To determine if strain ratios differed between diagnoses (according to semen analysis), we employed the Kruskal-Wallis test. Mann-Whitney U-tests were used as post-hoc tests and multiple comparisons were corrected using the Bonferroni correction.

For the partial least squares regression, we chose 10 measures as explanatory or descriptor variables: participant age, volumes, strain ratios of the bilateral testes and epididymis and the presence and size of the appendix testis on both sides. Because some of the variables exhibit a non-normal probability distribution and especially in the case of the bilateral parameters, co-linearity, we opted for a partial least squares regression (PLS) approach. The PLS method is based on a linear decomposition of the predictor and response matrix and is optimized so that the covariance between the predictor and response component matrix is maximal and the residual terms are minimal [9]. We investigated these parameters: total sperm count (TSC), sperm quality parameters (measures of motility, ratio of cells with abnormal morphology and specific measures of morphology, such as alterations of the head, neck, midpiece, and tail), this is the first principal component of these measures as dependent variables. We tested latent variables in the PLS for significance using a permutation-based approach: the dependent variable matrix was shuffled 5,000 times and for each permutation, the covariance matrix of the predictor and response factors were computed; thus we were able to obtain a null distribution for the eigenvalue corresponding to the tested latent variable. We calculated the exact P-values according to Phipson and Smyth [10]. To correct for multiple hypothesis testing, the P-values obtained were corrected according to the Benjamini-Hochberg method of false discovery rate control [11]. We also calculated Variable Importance for Projection (VIP) scores for the explanatory variables, which quantify the extent to which a given variable participates in explaining the response variable [12]. Statistical analyses were conducted using the Statistics and Machine Learning Toolbox in MATLAB (version R2014B; MathWorks, Inc.).

Results

The Kruskal-Wallis test detected significant differences in strain ratios between the different laboratory diagnoses (right: $P < 0.0029$; left: $P < 0.0003$). The right-sided strain ratio was higher in patients with azoospermia than in those with asthenozoospermia ($P < 0.022$) and oligo-asthenozoospermia ($P < 0.012$). The left-side strain ratio was higher in patients with azoospermia than in those with oligozoospermia ($P < 0.033$), asthenozoospermia ($P < 0.002$), and oligo-asthenozoospermia ($P < 0.006$). The P-values were corrected for multiple comparisons (Fig. 2).

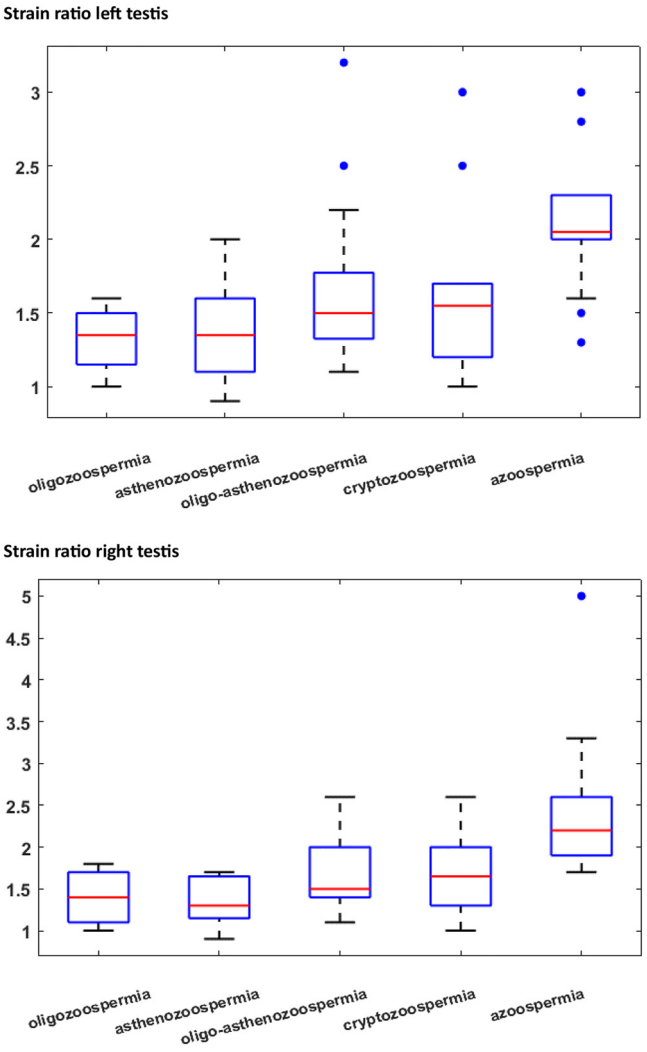

Fig. 2. Distribution of the strain ratio in the left and right testis in the patient group sorted by diagnosis based on semen analysis. Boxplots denote the interquartile range and whiskers denote 10 and 90 percentiles. Outliers are depicted as individual dots

The prevalence of varicocele was 29.6% in the study group and 14.3% in the control group, Surprisingly, in our study no statistical difference was observed between patients with and without varicocele regarding age (P = 0.16); testicular volumes (P = 0.77 for the right, P = 0.25 for the left testis); sperm concentration (P = 0.74); total sperm number (P = 0.88); total motility (P = 0.78), progressive motility (P = 0.66) and normal morphology (P = 0.93). No significant difference was detected between the strain ratios of the two testes in patients with varicocele (P = 0.39), without varicocele (P = 0.41), or in the entire study population (P = 0.28). The number of cases in this study is low, so presumably this is the reason why we did not find a correlation between varicocele and the other study data. The testicular volumes, diameters of the epididymis head on both sides and sperm parameters were statistically different between the study and control groups. The main data of the participants are summarized in Table 1.

Table 1. Main attributes of the study and control group

	Study group (n = 64)	Control group (n = 14)	P value	Corrected P-value threshold
Age (years) (mean, range)	37.1 (22–54)	36.3 (25–42)	0.63	–
Sperm concentration (million/ml) (mean ± SD, median)	1.5 ± 3.5 (0)	57.5 ± 29.4 (47)	<0.0001*	0.007
Total sperm count (million/ejaculate) (mean ± SD, median)	6.3 ± 15.5 (0)	242.2 ± 160.9 (220)	<0.0001*	0.007
Total sperm motility (%) (mean ± SD, median)	28.6 ± 27.4 (24.5)	73.6 ± 8.5 (70.5)	<0.0001*	0.007
Progressive motility (%) (mean ± SD, median)	14.3 ± 15.7 (10)	53.4 ± 16.4 (50)	<0.0001*	0.007
Non-progessive motility (%) (mean ± SD, median)	14.3 ± 14.0 (14)	21.6 ± 12.7 (19.5)	0.08	0.007
Normal morphology (%) (mean ± SD, median)	2.0 ± 2.4 (1.5)	19.7 ± 8.2 (21)	<0.0001*	0.007
Presence of microlithiasis (%)	17.2	0	0.2	–
Presence of varicocele grade II-III (%)	29.6	14.28	0.33	–
Presence of appendix testis (%)	14	0	0.2	–
Right testis volume (ml) (mean ± SD)	11.9 ± 6.0	19.1 ± 4.2	<0.0001*	0.01
Left testis volume (ml) (mean ± SD)	11.1 ± 5.3	17.7 ± 4.6	<0.0001*	0.01
Right epididymis head diameter (mm) (mean ± SD)	9.7 ± 2.3	12.1 ± 2.6	0.001*	0.01
Left epididymis head diameter (mm) (mean ± SD)	8.9 ± 2.4	11.4 ± 3.5	0.002*	0.01

Table: Mann-Whitney test was used for the analysis of sperm parameters; Two-tailed independent samples T-tests were used for other parameters; Corrections for multiple comparisons were carried out according to Bonferroni. SD: standard deviation.
*: statistically significant correlation, when P-value is equal or lower than the corrected P-value threshold ($P < 0.001$).

Reduced testicular volumes were found in the study group (left: $P < 0.0003$, right: $P < 0.0004$) and reduced epididymal diameters (left: $P < 0.006$, right: $P < 0.01$). After correction for multiple comparisons, higher strain ratios were found for both testis, which was more pronounced at the left side (1.687 ± 0.539 vs. 1.114 ± 0.156, $P < 0.001$ for the right testis; 1.800 ± 0.657 vs. 1.193 ± 0.220, $P < 0.006$ for left testis) compared to the control patients.

Significant correlations were found among sperm parameters and testicular morphology, as presented in Fig. 3. The left testicular volume, testicular elasticity and size of the left testicular appendix were the most predictive of problems TSC and quantitative sperm parameteres. To eliminate the effect of cross correlations, PLS regression was used as a multivariate method for further calculations. According to the VIP scores, the strain ratios of both testes, volume of the left testis, and size of the left appendix were identified as the most important factors associated with the results of semen analysis. In this study, we focused on the testicular strain ratios. The optimal model order for the PLS regression

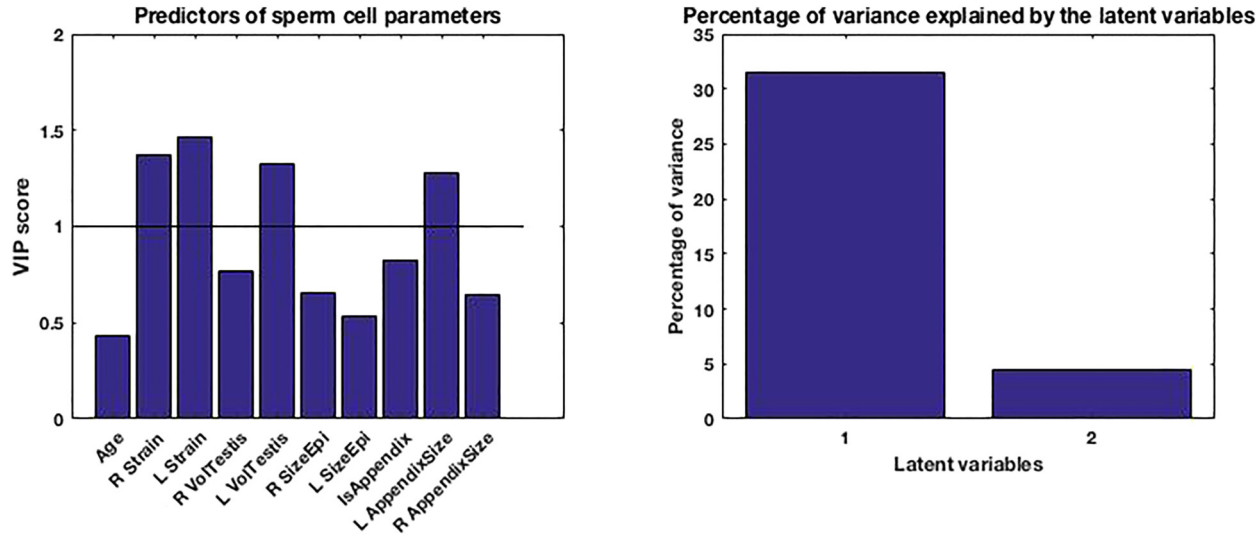

Fig. 3. Cross-correlation between sperm parameters. The figure depicts the cross-correlation matrix of sperm cell parameters. Circle size and color shows the strength and direction of the correlation. The color bar depicts Spearman's rank correlation coefficients

included two latent variables, but the second latent variable was not deemed significant ($P < 0.102$) as per the permutation test for any response variable. Thus, we excluded it from further analysis. The first latent variable explained a significant amount of variance in TSC and concentration, as well as in progressive motility and morphological parameters ($P < 0.0002$). The VIP scores and percentages of variance for the two latent variables are shown in Fig. 3. The first latent variable from the PLS analysis explained a significant amount of variance in TSC, sperm concentration, and motility parameters ($P < 0.0002$), and showed that bilateral strain ratios, the volume of the left testis, and the size of the left appendix were most associated with semen parameters based on a Variable Importance in the Projection (VIP) score of >1 (left strain ratio: VIP = 1.37, right strain ratio: VIP = 1.46, left testicular volume: VIP = 1.32, left appendix size: VIP = 1.28). Correlation analysis confirmed this finding, showing that elevated strain ratios on both sides resulted in reduced TSC (right: $R = -0.45$, $P < 0.0001$; left: $R = -0.46$, $P < 0.0001$), sperm concentration (right: $R = -0.46$, $P < 0.0001$; left: $R = -0.48$, $P < 0.0001$), total (right: $R = -0.39$, $P < 0.001$), progressive motility (right: $R = -0.4$, $P < 0.001$), non-motile TSC (left: $R = -0.37$, $P < 0.003$), and percentage of normal morphology (right: $R = -0.37$, $P < 0.003$). Correlations between sperm parameters and strain ratios of the testes are shown in Table 2, and the correlation coefficients between the testicular strain ratios and sperm parameters are presented in Fig. 4.

After correcting for multiple hypothesis testing in the same way as described in the PLS section, the volumes of the bilateral testes correlated negatively with FSH levels ($R = -0.56$, $P < 0.007$ and $R = -0.67$, $P < 0.0003$). Sperm cell concentration and TSC decreased with increasing FSH levels ($R = -0.52$, $P < 0.029$ and $R = -0.58$, $P < 0.01$) (not shown in the tables).

Discussion

Testicular ultrasound examination is important in the investigation of male infertility because of its sensitivity and cost-effectiveness. Ultrasound examination is widely used for the differential diagnosis of intratesticular lesions, especially if they are non-palpable. Multiparametric ultrasound can also help to differentiate between malignant and benign intratesticular lesions [13]. Accessibility and non-invasiveness makes US an excellent tool for evaluating male infertility; however, it is crucial to know which US parameters are the best predictors for semen analysis. To our knowledge, this is the first study to attempt to identify the most characteristic ultrasound parameters of male infertility using an exploratory multivariate approach.

Age is known to be associated with changes in the testicular parenchyma, which contribute to the elasticity of the testis, as well as increased tubular sclerosis, Leydig-cell hyperplasia and focal monoclonal inflammation. Tubular ectasia of the rete testis and capsular smooth muscle hyperplasia may also occur with increasing age [14]. Peritubular fibrosis restricts seminiferous tubules and may cause

Table 2. Correlations between the testicular strain ratios and sperm parameters

		Sperm cell concentration	Total sperm count	Total motility	Progressive motility	Non-progressive motility	Non-motile sperm cell number	Percent of normal morphology	Percent of abnormal morphology	Percent of head abnormality	Percent of neck and midpiece abnormality	Percent of tail abnormality	Percent of combined abnormality
Right testis strain ratio	Spearman's rho	−0.451	−0.462	−0.395	−0.404	−0.340	−0.259	−0.370	−0.131	−0.157	−0.295	−0.153	−0.331
	P-value	0.0001*	0.0001*	0.001*	0.0009*	0.0058	0.038	0.0025*	0.2991	0.2151	0.0177	0.2264	0.0075
	corrected *P*-value	0.0038											
	threshold												
Left testis strain ratio	Spearman's rho	−0.456	−0.479	−0.296	−0.315	−0.238	−0.371	−0.285	−0.230	−0.132	−0.274	−0.193	−0.349
	P-value	0.0001*	<0.0001*	0.0174	0.0111	0.0576	0.0025*	0.0222	0.0665	0.2979	0.0282	0.1262	0.0047
	corrected *P*-value	0.0038											
	threshold												

Table: Correlations between the testicular strain ratios and sperm parameters in pathozoospermic men ($n = 64$) Strain ratio was calculated as the ratio of the stiffness of the peritesticular fat and testicular parenchyma [8]. Semen was analyzed, Partial least squares regression was used to describe the covariance between strain ratios of the testis and sperm parameters. Spearman's rho coefficient was calculated. Corrections for multiple comparisons were carried out according to Bonferroni.

*: statistically significant correlation, when *P*-value is equal or lower than the corrected *P*-value threshold ($P < 0.001$).

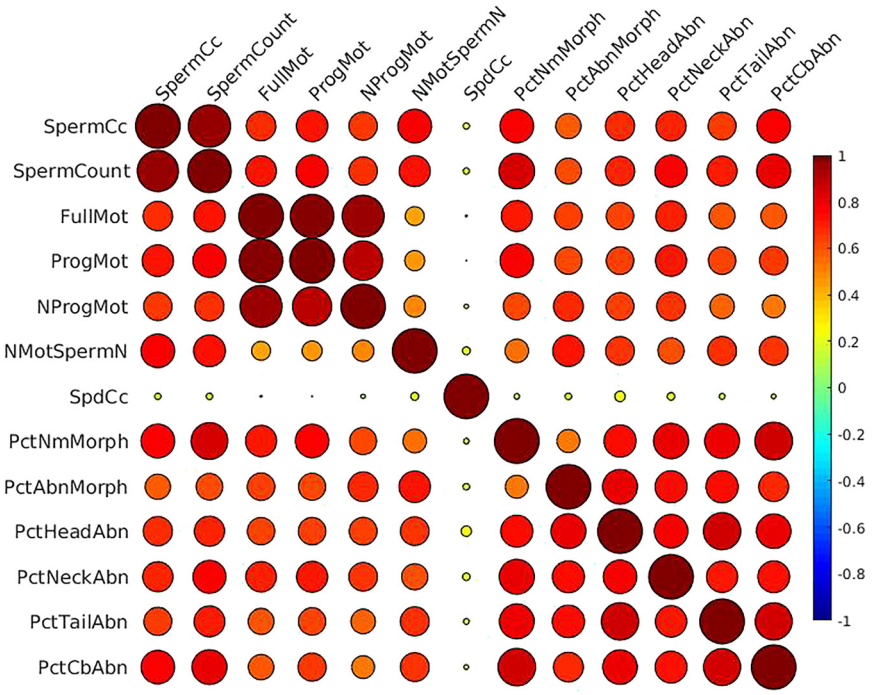

Fig. 4. Results of the partial least squares analysis. The bar plots on the right depict the amount of variance explained by the first two latent variables estimated by the PLS analysis. Only the first latent variable was significant (see Results section for details). Bar plots on the left depict the Variable Importance Projection (VIP) score of the sperm cell parameters which quantifies their contribution to the model. The horizontal line is set at VIP = 1, which was determined empirically, based on previous literature, as the threshold of significance for variable contribution

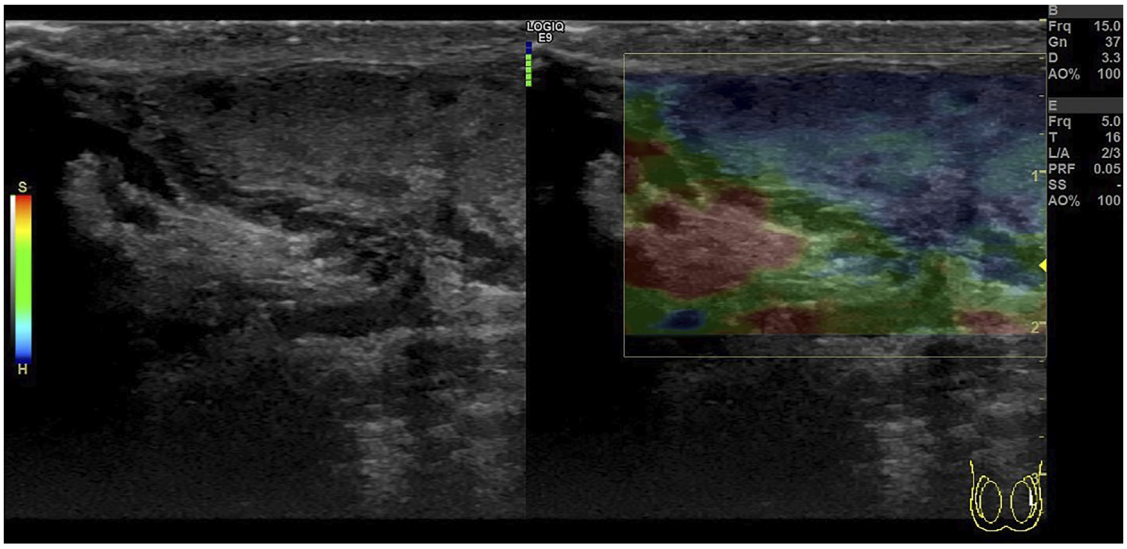

Fig. 5. Elastogram of testis of a patient (29-year old) suffering from non-obstructive azoospermia. The central parenchyma has inhomogeneous, decreased echogenicity in the B-mode image, and blue (rigid) in the elastogram lack of 'boundary' effect

a decline in spermatogenesis [15]. These pathological changes may lead to more rigidity, than in a normal testis, which can be detected with strain elastography [16] (Fig. 5).

Our multivariate exploratory analysis revealed that elasticity of the testes and the volume of the left testis and appendix were the most significant US predictors of pathological semen and sperm cell features. As well-known from the previous literature, correlation was found between the serum FSH levels and size of the testes and in turn significant correlation between the semen and sperm cell features and FSH levels.

Our results are consistent with other studies. The smaller testicular volume is associated with decreased sperm motility and count and the high FSH and LH levels affecting negatively the male fertility and have been related to smaller testicular volume [1, 17].

In line with previous studies we tried to find the role of strain elastrography in US examination during the investigation

of male infertility [8, 18]. The results demonstrate two things: firstly, the left testis is more important for fertility than the right. Secondly, a singular US parameter is not sufficient for accurate predictions. However, in the hands of an experienced radiologist, strain elastography can clarify the expected result without prolonged examination times.

We have shown that the size of the left testis and appendix are determining, characterizing the male fertility more than the right. There is a natural difference between left and right testicular volume in the healthy population. This is because in humans, the testis on the right seems to develop faster than on the left. In females, this phenomenon is the opposite, with the ovaries developing first on the left [19]. A study shows a positive Testicular Asymmetry Index (TAI) for the entire population and for all Tanner stages excluding TSG1, meaning there is a natural difference between left and right testicular volume in a healthy adolescent population, the left testis being smaller. Small testicular volumes and high testicular volume differences are associated with poor semen analysis outcomes. Large testicular volume differences are expected to be seen in patients with unilateral inguinoscrotal disorders [20].

Ultrasound examination, in addition to knowledge of urological anamnestic data and hormone levels, is useful for diagnosing the type of male infertility. Strain elastography may provide additional information on scrotal and testicular conditions [21, 22]. Strain elastography-supplemented ultrasonography is better correlated with the semen parameters than the B-mode US examination. The strain elastography-supplemented US examination could help the urologist to choose the most accurate treatment for the patients. Male infertility is a complex, multi-factorial disease, but an appropriate ultrasound examination can help to establish and confirm the diagnosis. The diagnosis and differentiation between obstructive and non-obstructive azoospermia is based on several factors. The physical examination (enlarged and dilated epididymis) and patient history (previous inguinal surgery) are primary. In the case of obstructive azoospermia the levels of hormones including FSH and inhibin B is normal range [1]. This differentiation may be helped and confirmed by strain elastography, the elastogram showing lower testicular rigidity, which could help in the prediction of the alterations in the semen parameters and the success of micro-TESE (microsurgical testicular sperm extraction) operation the success of fertilization.

Our research showed an interesting correlation between the semen parameters and length of the appendix testis. The appendix testis is a vestigial remnant of the Müller's duct and expresses androgen and oestrogen receptors. The regression of the Müllerian duct in male fetus is caused by anti Müllerian hormone (AMH). A correlation was suggested between the level of AMH, cryptorchidism and length of appendix testis, ut researchers have not found significantly correlation, but however, elevated level of AMH with high expression of oestrogen receptors in the appendix testis, may play an important role in cryptorchidism in some cases [23]. The appendix testis correlates not only with androgen and oestrogen receptors, but also the level of insulin-like 3 hormone produced by the testicular Leydig cells [24]. These studies were conducted in childhood, but the cryptorchidism in children may play an important role in development of male infertility, conceivable that, the role of appendix testis may be more important than previously thought.

The main limitation of our study is the low number of participants and the few azoospermic males (both obstructive and non-obstructive azoospermic patients group) in our sample. The distinction between obstructive and non-obstructive azoospermia is important, in which medical history (cryptorchidism), levels of hormones (LH, FSH and testosterone) may help. Sonographic features that may be present with obstructive azoospermia include normal testicular size and echogenicity (testicular echogenicity is same as the thyroid gland in euthyreosis), rete testis ectasia or spermatocele. The smaller testicular volume, the low echogenicity, and the presence of varicocele are some of the characteristic of the non-obstructive azoospermia. Presumably also the low number of participants is the reason why no correlation with the grade of varicocele was found. In future work, investigating the connection between strain elastography and histopathological patterns of testicular biopsy in males suffering from non-obstructive azoospermia might provide further important insights into male infertility.

Conclusion

Ultrasound imaging technics develop rapidly and in the case of scrotal imaging the availability, spatial resolution and the non-ionising radiation based imaging offers a unique opportunity to investigate male infertility. The research is limited due to the small number of patients, but radiologists may focus on the left testicular elastogram, echogenicity and the presence of the appendix testis because of they have the most predictive value regarding our study findings. In our results the size of the left testis and appendix are determining, characterizing the male fertility more than the right. There is a natural difference between left and right testicular volume in the healthy population. This is because, in humans, the testis on the right seems to develop faster than on the left.

However, further research is needed with a higher number of patients and other elastographic methods (shear wave elastography) to confirm this.

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Ethical statement: All procedures performed in studies involving human participants were in accordance with the

ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Regional Committee on Research Ethics of the University of Szeged 56/2018 SZTE.

ABBREVIATIONS

LH	Luteinizing hormone
FSH	Follicle-stimulating hormone
micro-TESE	microsurgical testicular sperm extraction
ROI	Region of Interest
PLS	Partial least squares
SE	strain elastography
SHBG	Sex hormone-binding globulin
TAI	Testicular Asymmetry Index
TSC	total sperm count
US	Ultrasonography
VIP	Variable Importance for Projection
WHO	World Health Organization
AMH	Anti-Müllerian hormone

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