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The use of Doppler ultrasound as a potential fertility predictor in male goats

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Abstract: The aim of the current study is to correlate Doppler flowmetry with andrological features in male goats belonging to different age and fertility groups. Thirty native crossbreed bucks bred in Northeastern Brazil were subjected to B-mode, spectral and color Doppler ultrasound, as well as to andrological examinations. Color Doppler ultrasound was used to evaluate blood flow in the pampiniform plexus and testicular parenchyma. Semen was collected through the electroejaculation method in order to have its physical and morphological features evaluated. The animals were grouped based on age (young, mature, and old) and fertility level (high and low) before analysis. Data were subjected to Poisson regression analysis. The fertile bucks have shown higher plexus and parenchyma pixel responses ($P < 0.05$). Old animals had the highest values for plexus and parenchyma pixels, and for parenchyma score, and they were followed by mature and young animals, respectively. There was a significant correlation among testicular vascular flows, age, testicular morphometry, and fertility; however, there was no correlation between Doppler variables and sperm morphology. In conclusion, Doppler ultrasound had a potential effect on the assessment of testicular artery hemodynamics in male goats. Thus, it can be used as a complementary tool to indicate seminal quality, or even as a potential fertility predictor.

Key words: Reproduction, small ruminants, ultrasound, hemodynamics, breeding soundness examination

1. Introduction

The Doppler sonography is considered a relatively new tool in veterinary medicine. It provides realtime information about the vascular architecture and the hemodynamic aspects of blood vessels examined in several organs, including the testicles [1]. This technique is based on the Doppler effect, which may be defined as the physical principle according to which the frequency of the reflected sound waves changes when the reflecting object is moved in relation to a sound wave source [2].

A color map of the vasculature in tissues and organs is displayed in a two dimensional image (B mode) in the color Doppler [3,4]. The spectral Doppler analysis provides information about blood flow velocity and resistance. In addition, it has been used to feature the flowmetry in the testicular artery of rams and stallions [3,5].

The resistance index (RI) is a reliable indicator used in the clinical practice to identify infertile men [6]. The color Doppler, the RI, and the pulsatility index (PI) have been used as parameters to diagnose reproductive abnormalities in dogs [7,8]. The pulsed-wave Doppler sonography has been used to measure the testicular blood flow, as well as to correlate it with fertility in camelids [9].

Ultrasound imaging technology provides rapid, simple, and noninvasive access to the reproductive organs [10]. The B-mode ultrasonography has been used with some success to diagnose testicular degeneration in male goats [11]. In addition, it allows identifying the abnormalities before their clinical manifestation [12]. However, when it comes to color Doppler ultrasound, data available in the literature about goat reproduction remain scarce, mainly with regard to male animals [13-15]. Thus, the aim of the current study was to correlate Doppler flowmetry, testicular measurements, and semen features in male goats.

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2. Materials and methods

2.1. Experimental site and animals

The current study used 30 native crossbreed bucks, without defined breed, in the age group 4.28 ± 0.34 years (Range: 1 to 9 years), which presented a body condition scoring of 2.31 ± 0.09 (Range: 1 to 4). The animals were bred in Maranhão State, Northeastern Brazil (latitude: 3°44'26" N; longitude: 43°21'33" W; altitude: 93 m). The region presents mean annual temperature of 27.9°C, mean annual rainfall index of 1,613.2 mm^3, and it has a tropical climate of the Aw type (rainy summer season), based on Köppen's climate classification [16].

The animals were kept in a semiextensive system on native grass pasture with water ad libitum during the day and confined to a collective stall at night. The experiment was carried out in September (dry season); however, the experimental site is located in the equatorial region, which does not have a photoperiodic effect on animals, a fact that enables goats to reproduce throughout the year. The animals were subjected to two breeding soundness examinations (BSE) and semen collection once a week to standardize the sexual rest period and restore the sperm reserves. The animals were grouped by age into young (1–2 years; n = 10), mature (2.1–5 years; n = 8), and old (6 to 9 years; n = 12)], as well as by fertility level into high-fertility (motility ≥ 70% and vigor ≥ 3 in both examinations; n = 25) and low-fertility (motility < 70% and vigor < 3 in both examinations; n = 5), for the analyses.

The experimental procedures adopted in the current study were approved by the Ethics Committee on Animal Use at Federal University of Maranhão (CEUA-UFMA), under Protocol no. 23115006493/2015-94.

2.2. Doppler flow examinations

An ultrasound device equipped with a 6.0 MHz linear transducer (Mindray, Model Z5Vet, Digital Ultrasonic Diagnostic Imaging System) was used in the current study. Scrotal trichotomy was performed to avoid interferences with image quality. The transducer was positioned longitudinally on the skin in the central region of both spermatic cords to view the pampiniform plexuses, and in the central region of the testis to view the testicular parenchyma. The device was set in two dimensional mode (B-mode) to allow locating the blood vessels. Then, the color Doppler mode was triggered to determine blood perfusion, wherein the blood flow direction was indicated through red or blue signals [17]. The blood flow was continuously monitored for 1 min.. The "cineloop" feature was used to choose the best image.

The spectral Doppler mode was adopted to monitor the Doppler velocimetry values of the pampiniform plexus. Data were obtained by positioning the cursor on the testicular artery, in the pampiniform plexus, as a way to get a sequence of spectral graphs showing distinct and symmetrical cardiac systole and diastole cycles. All the scanning procedures, in color and spectral Doppler modes, were performed in a constant configuration comprising cardiac cycle, gain, filter settings, and speed range definition.

The vascular perfusion was subjectively assessed by five appraisers who used the images showing the greatest blood perfusion extent in the pampiniform plexus and testicular parenchyma. Such images were captured in color Doppler mode. The maximum and minimum scores were discarded whereas the median scores were used.

The pampiniform plexus assessment scores ranged from 1 to 5 and were classified from extremely low (score 1) to extremely high (score 5) vascularity (Table 1). The testicular parenchyma scores ranged from 0 to 4; the values indicated the following, respectively: apparently null, low, intermediate, high, and very high vascularity (Table 2), which was similar to the one described for mares' uterus [18].

The subjective scores attributed to the vascular perfusion extent of the testicular parenchyma and pampiniform plexus were validated by objectively assessing the intensity of the colored pixels in the images, as described for mares and heifers [17–19]. The number of the colored pixels in the images was measured in the Adobe Photoshop CS5 software (Adobe Inc. California, CA, USA), which provided the vascularization, pampiniform plexus, and testicular parenchyma extents in a pixel scale [20].

2.3. Testicular measurement and semen examination

Scrotal circumference was measured with scrotal tape as recommended by Henry and Neves [21]. Semen was collected through electroejaculation method. After the samples were collected, the semen was assessed for its physical features (visual motility, vigor, sperm concentration, and mass movement) through conventional light microscopy method, using a slide heated at 37 °C. Vigor was ranged on a scale of 1 to 5 according to the movement of the spermatozoa under optical microscopy (100× amplification). Motility was expressed in percentage of mobile spermatozoa under optical microscopy (100× amplification). Semen was diluted in formaldehyde saline solution at the ratio of 1: 200, and cell count was used in five Neubauer chamber fields in order to calculate sperm concentration. Sperm counting was carried out based on conventional light microscopy at 400× magnification. Sperm morphology was assessed using a phase contrast microscope (1000× amplification) after buffered saline formaldehyde fixation. The abnormalities targeted were in the acrosome, head, middle piece, and tail. The defects were quantified in percentage of major and minor defects, as reviewed [22].

Table 1. Classification of scores (from 1 to 5) used to assess the images showing the pampiniform plexus of goats, obtained through color Doppler ultrasonography.

Score	Colored area rates	Visualization
1	0% – 20%	Extremely low vascularization
2	21% – 40%	Low vascularization
3	41% – 60%	Intermediate vascularization
4	61% – 80%	High vascularization
5	81% – 100%	Extremely high vascularization

Table 2. Classification of scores (from 1 to 5) used to assess the images obtained through color Doppler ultrasonography showing the testicular parenchyma of goats.

Score	Colored area rates	Visualization
0	0% – 20%	Apparently null vascularization
1	21% – 40%	Low vascularization
2	41% – 60%	Intermediate vascularization
3	61% – 80%	High vascularization
4	81% – 100%	Extremely high vascularization

2.4. Statistical analysis

Data were analyzed in the statistical analysis system for Windows SAS™ software (SAS Institute Inc., North Carolina, USA) [23]. The Proc Univariate application was used to test the data for residue normality, whereas the Shapiro–Wilk test was used to check the homogeneity of the variances. All the variables were considered as not normally distributed (nonparametric). Data transformation was not necessary for any response variables. Poisson distribution was assumed in the experiment [24]. Data were subjected to Poisson regression analysis with the model adjusted for Poisson distribution, based on GLIMMIX procedure by SAS™ (SAS Institute Inc., USA).

The relationship between the variables was studied through the principal component analysis (PCA) method, using the Statistica 7.1 software (TIBCO Software Inc., California, USA) [25], producing a two axis graph to illustrate the importance of the main components in the total variation. Also, the Spearman correlation coefficients between the variables were set [26]. Based on the coefficient of determination (r^2), the correlations were classified as moderate (r^2 = 0.50 – 0.69), high (r^2 = 0.70 – 0.89), and very high (r^2 = 0.90 – 1.00) [27].

The significance level to reject H0 (null hypothesis) was 5%, i.e. a significance level lower than 0.05 indicated the effect of the classificatory variables and of the interactions between them.

3. Results

Data about normal genital structures and visually symmetrical testes of the investigated bucks were included in the study. The scrotal circumference showed mean value 28.08 ± 0.38 cm, (Range: 24 to 35.5 cm). Mean ejaculate volume presented minimum and maximum variations from 0.20 to 2.50, and a mean value of 0.63 ± 0.41. Mean individual sperm motility was 72.17 ± 2.60% (Range: 20% to 95%), and the mean sperm vigor was 2.67 ± 0.17 (Range: 0 to 5). Mean sperm concentration was 1.93 ± 1.38 billion, and the values ranged from 0.20 to 6.60 billion sperm cells per milliliter (mL) of semen. The semen morphology assessment found 4.28 ± 0.16% minor defects (Range: 2% to 9%) and 1.85 ± 0.12% major defects (Range: 0.50% to 5.50%). The total abnormal sperm was 6.13 ± 0.19% (Range: 3% to 10%).

The hemodynamic features did not show a difference between the right and left testes (Table 3). The same result was seen in the two semen collections (Table 4). The comparison between the semen samples showed that only the volume (VOL) and total minor defects (TMiD) significantly differed among the andrological variables (Table 4).

Based on Table 5, fertility has influenced plexus and parenchyma pixel responses ($P < 0.05$). However, there were not significant changes in the other measured variables ($P > 0.05$). With respect to age-based classification, old animals had the highest values for variables, such as plexus and parenchyma pixels and parenchyma score. They were followed by mature and young animals, respectively ($P < 0.05$; Table 6).

Figure shows the Principal Component Analysis (PCA). There was a high relationship between the Doppler variables, as they formed acute angles among themselves, and are also positively related to age and scrotal circumference. There is a clearly inverse relationship between end-diastolic velocity (EDV) and the other Doppler variables, forming an angle close to 180° in the PCA graph. As a physiological principle, at the end of diastole, the lowest flow velocity and the lowest pressure in the arteries are registered, being called minimum pressure.

The acute angles between the vectors of vigor, motility, mass movement, and sperm concentrations demonstrate that these variables are related to each other (Figure). Thus, the contrast between the Doppler/age variables and semen variables was evident.

In Component 1, the variables motility and vigor stood out as they had longer vectors and were closer to the axis Component 1. As for Component 2, the variables that contributed most were Parenchyma Pixels (PaP) and Age.

In order to understand the importance of each variable in the construction of the principal components, the

Table 3. Hemodynamic features (mean ± standard error) of the right and left testes of male goats.

Variable	Side		P-value
	Right	Left	
Plexus pixels	9790.22 ± 437.08	9357.68 ± 383.56	0.59
Plexus score	2.76 ± 0.12	2.68 ± 0.10	0.82
Parenchyma pixels	717.05 ± 53.82	591.47 ± 32.97	0.25
Parenchyma score	2.45 ± 0.08	2.37 ± 0.07	0.85
Peak systolic velocity (PSV)	16.81 ± 0.36	16.80 ± 0.38	0.89
End-diastolic velocity (SDV)	7.78 ± 0.26	8.01 ± 0.29	0.64
Pulsatility index (PI)	0.83 ± 0.04	0.80 ± 0.04	0.52
Resistance index (RI)	0.53 ± 0.02	0.50 ± 0.02	0.45

Table 4. Hemodynamic and andrological features (mean ± standard error) of male goats.

Variable	Semen Collection		P-value
	1	2	
Plexus pixels	9504.15 ± 538.39	9643.75 ± 531.78	0.74
Plexus score	2.75 ± 0.14	2.69 ± 0.14	0.72
Parenchyma pixels	609.22 ± 48.76	609.22 ± 48.76	0.14
Parenchyma score	2.35 ± 0.10	2.47 ± 0.08	0.21
Peak systolic velocity (PSV)	16.81 ± 0.36	17.03 ± 0.44	0.64
End-diastolic velocity (SDV)	7.77 ± 0.36	8.03 ± 0.34	0.60
Pulsatility index (PI)	0.82 ± 0.06	0.81 ± 0.06	0.85
Resistance index (RI)	0.52 ± 0.03	0.52 ± 0.03	0.99
Motility (%)	72.17 ± 4.06	72.17 ± 3.34	0.50
Vigor (score from 1 to 5)	2.80 ± 0.28	2.53 ± 0.20	0.35
Mass movement (score from 1 to 5)	2.62 ± 0.31	2.57 ± 0.26	0.69
Total major defects (%)	1.88 ± 0.13	1.82 ± 0.20	0.28
Total minor defects (%)	4.68 ± 0.17	3.88 ± 0.25	0.001

correlation between the original variables was calculated. Overall, the correlations between the Doppler variables, "age" (4.28 ± 0.34 years) and "scrotal circumference" (28.08 ± 0.38 cm) were moderate; the r2 values ranged from 0.68 to 0.36 whereas the P-values ranged from < 0.005 to < 0.0001.

Peak systolic velocity (PSV) showed a significant correlation with the number of plexus pixels (PP) (r^2 = 0.48, P < 0.0001), as well as with the plexus score (PS) (r^2 = 0.39; P = 0.002). The pulsatility index (PI) showed correlation with PP (r^2 = 0.33, P= 0.009), PS (r^2 = 0.26, P = 0.045), PSV (r^2 = 0.62, P < 0.0001) and with end-diastolic velocity (EDV) (r^2 = –0.85, P < 0.0001).

Significant values between age (AGE) and PSV (r^2 = 0.37; P = 0.003), as well as between PI (r^2 = 0.41; P = 0.001) and resistance index (RI) (r^2 = 0.36; P = 0.005) were seen after correlating the hemodynamic and andrological variables. The scrotal circumference showed a positive correlation with PSV (r^2 = 0.43; P = 0.000), PI (r^2 = 0.47; P = 0.000) and with RI (r^2 = 0.49; P < 0.000).

4. Discussion

This is the first study to correlate Doppler-sonography of testicular blood flow with andrological traits according to age and fertility groups in male goats. The adopted Doppler parameters herein were not influenced by the position of testis or by the examination day. On the other hand, previous studies have found significant difference in testicular thickness between the right and left testes in mature rams [28], stallions [29], and dogs [30]. When

Table 5. Hemodynamic and andrological features (mean ± standard error) based on the fertility group of male goats.

Variable	Fertility Group		P value
	High fertility	Low fertility	
Plexus pixels	9898.52 ± 427.27	7951.10 ± 467.13	<0.0001
Plexus score	2.84 ± 0.11	2.10 ± 0.09	0.20
Parenchyma pixels	659.55 ± 43.17	627.80 ± 69.74	0.0007
Parenchyma score	2.41 ± 0.07	2.40 ± 0.12	0.98
Peak systolic velocity (PSV)	16.74 ± 0.31	17.19 ± 1.13	0.75
End-diastolic velocity (SDV)	7.96 ± 0.27	7.57 ± 0.63	0.68
Pulsatility index (PI)	0.81 ± 0.04	0.88 ± 0.14	0.82
Resistance index (RI)	0.51 ± 0.02	0.54 ± 0.06	0.91
Age (years)	4.42 ± 0.39	3.60 ± 0.62	0.25
Body condition score	2.24 ± 0.10	2.70 ± 0.13	0.39
Scrotal circumference (cm)	28.07 ± 0.43	28.13 ± 0.77	0.97
Motility (%)	78.20 ± 2.10	42.00 ± 5.01	<0.0001
Vigor (score from 1 to 5)	3.0 ± 0.17	1.05 ± 0.13	0.002
Mass movement (score from 1 to 5)	2.99 ± 0.20	0.65 ± 0.18	0.0003
Volume	0.67 ± 0.06	0.45 ± 0.05	0.43
Sperm cell concentration/mL ($x10^9$)	2.16 ± 0.20	0.77 ± 0.13	0.007
Total major defects (%)	1.82 ± 0.13	2.00 ± 0.30	0.70
Total minor defects (%)	4.30 ± 0.18	4.20 ± 0.40	0.89

considering the examination day, similar results were found by Gloria et al. [31] in healthy bulls, which shows the accuracy of Doppler examinations performed in different collection days. According to Vale [32] and Henry et al. [33], variation in seminal volume, sperm concentration, and minor abnormalities can be observed in both young and adult ruminants. It is well known that most of the time, when variations in some seminal parameters are detected, a normalization of these values occur in subsequent seminal collection attempts (1-week interval), rejecting the diagnosis of reproductive disease [33].

It was observed in this study that animals with high fertility had higher values of pixels of the plexus and parenchyma, besides, obviously, greater motility, vigor, mass movement, and sperm concentration than animals with low fertility ($P < 0.05$). Testicular blood flow is the main pathway used to transport nutrients, regulatory hormones, and secretory products to and from animals' testes; thus, it has direct influence on sperm production [34]. Reduced blood flow caused by surgical restriction in bulls leads to spermatogenesis deterioration [35]. Studies have reported that early spermatogenesis stages are sensitive to moderate blood flow reduction [36], which can be observed through pixel intensity.

Parenchyma pixel intensity was also previously associated with seminal quality, mainly with sperm motility and vigor in other species, similar to the current study. Ahmadi et al. [37] reported association between testicular parenchymal heterogeneity and semen quality in a small study conducted with rams. They found an inverse correlation between pixel intensity and percentage of sperms presenting normal morphology and progressive motility in samples collected 60 days after the ultrasound examination. Moxon et al. [38] reported reduced sperm production in dogs presenting decreased testicular parenchyma pixel intensity.

It became evident that the plexus vascularization is highly related to the parenchyma vascularization ($r = 0.40$; $P = 0.001$). This relation may be anatomically explained because the testicular artery originates from the dorsal aorta [39], extends beyond the pampiniform plexus and reaches the testes, where it branches from the parenchymal surface to the epididymis [40]. On the other hand, the testicular veins originate from the pampiniform plexus, which is formed by the union of small testicular parenchyma veins [41] located in the testis capsule, forming the superficial vascular wall [42,43].

Results in the current study have shown that older bucks presented a higher number of plexus and parenchyma

Table 6. Hemodynamic and andrological features (mean ± standard error) based on the age group of male goats.

Variable	Age Group (years old)			P-value
	Young (1-2 years)	Mature (2.1-5 years)	Old (6 to 9 years)	
Plexus pixels	6,881.58 ± 290.70^{c}	10,432.06 ± 550.11^{b}	11,245.52 ± 554.8^{a}	<0.0001
Plexus score	2.12 ± 0.08	2.92 ± 0.18	3.08 ± 0.16	0.15
Parenchyma pixels	456.57 ± 35.04^{c}	634.25 ± 60.93^{b}	832.33 ± 61.75^{a}	<0.0001
Parenchyma score	2.08 ± 0.07^{b}	2.43 ± 0.12^{a}	2.64 ± 0.11^{ab}	0.49
Peak systolic velocity (PSV)	15.48 ± 0.55	18.26±0.74	16.95±0.25	0.14
End-diastolic velocity (SDV)	8.80 ± 0.40	6.93 ± 0.41	7.80 ± 0.38	0.15
Pulsatility index (PI)	0.59 ± 0.06	1.04 ± 0.06	0.86 ± 0.06	0.32
Resistance index (RI)	0.42 ± 0.03	0.61 ± 0.02	0.53 ± 0.03	0.70
Body condition score	2.55 ± 0.11	2.62 ± 0.18	1.92 ± 0.14	0.26
Scrotal circumference (cm)	26.93 ± 0.52	29.25 ± 0.77	28.24 ± 0.61	0.42
Motility (%)	75.25 ± 4.17	69.69 ± 6.15	71.25 ± 3.80	0.13
Vigor (score from 1 to 5)	2.92 ± 0.29	2.69 ± 0.34	2.44 ± 0.28	0.62
Mass movement (score from 1 to 5)	2.55 ± 0.37	2.84 ± 0.40	2.46 ± 0.30	0.75
Volume	0.57 ± 0.04	0.72 ± 0.12	0.62 ± 0.10	0.85
Sperm cell concentration/mL (x10^{9})	1.87 ± 0.24^{ab}	2.67 ± 0.49^{a}	1.49 ± 0.19^{b}	0.04
Total major defects (%)	1.65 ± 0.14	1.81 ± 0.20	2.04 ± 0.23	0.63
Total minor defects (%)	3.85 ± 0.25	4.91 ± 0.40	4.23 ± 0.18	0.32

pixels and a higher parenchyma score. Several reports have shown that blood flow in human testes increases as men get older [44,45]. Pozor and McDonnell [4] have reported the aging effect on blood flow of adult stallions. It happens because blood flow in the testicular artery is linked to spermatogenesis rate [35]. Vascular score and the number of plexus and parenchyma pixels indicate testicular vascularization. Paltiel et al. [46] have evaluated testicular vascularization in healthy boys (3 to 17.5 years old) and found increased testicular blood vascularization as they got older; this increase occurs due to puberty and reproductive functionality acquisition. Previous studies have shown that testicular parenchyma pixel intensity is histologically associated with seminiferous tubule height, tubule-to-lumen ratio, and lumen size [47,48].

The high peripheral resistance in testicular arteries prevents diastolic flow at rest, whereas the low peripheral resistance enables significantly high diastolic flow. The positive correlations among resistance index, pulsatility index, and systolic pulse due to ramifications of the testicular artery, after it crosses the pampiniform plexus, as well as capillarization on the parenchyma surface towards the epididymis [40]. It reduces the artery size and increases the vascular resistance; consequently, the blood velocity increases to keep the same flow throughout the branches.

The low correlation between PSV and EDV (r^2 = 0.214, P = 0.101) happens because they are independently influenced by vascular bed resistance values, and it shows that these two variables are not related to each other [49]. This finding may also result from the difficulty for the operator in following the entire length of the blood vessel with reliable angle correctness due to the convoluted course of the blood vessels over the testicular vascular cone. Such difficulty may generate less accurate measurements and lead to variations between parameters [6,50]. These low correlations also take place in prepubertal individuals showing flowing waves only during systole, with no diastolic flow, which reflects the nonfunctional stage of the testes [49].

The resistance index in the current study correlated negatively with the EDV (P = –0.852). The same result was found by Batissaco et al. [5] in an experiment conducted with sheep. According to Wood et al. [49], high resistance rates were observed in the blood vessels supplying high-resistance vascular beds and requiring intermittent blood supply.

Biagiotti et al. [6] conducted studies in men and found that varicocele patients showed the highest systolic pulse and resistance index values. The authors concluded that the peak systolic velocity and the resistance index may be

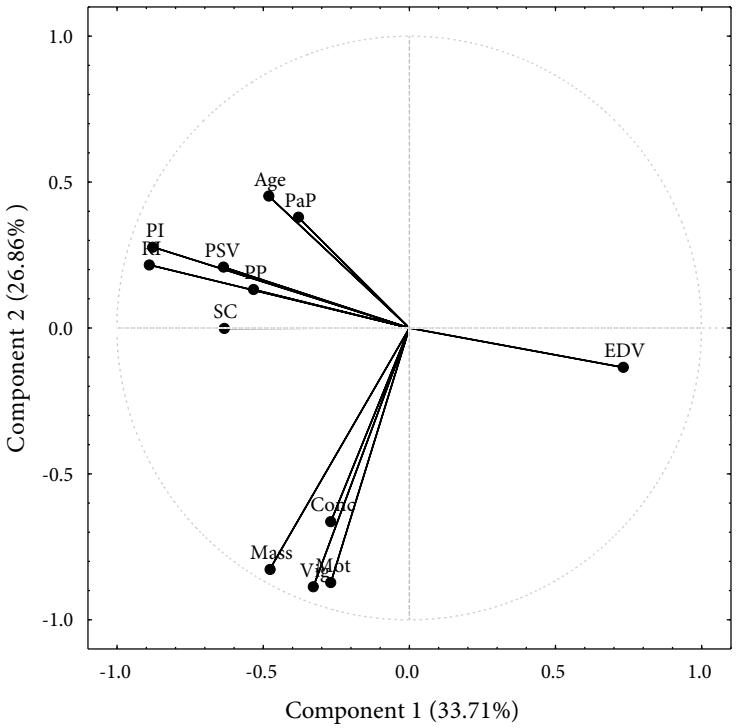

Figure. Biplot of principal component analysis (PCA) of the relationship between variables obtained by Doppler ultrasound, testicular morphometric, and sperm variables of male goats. PP = plexus pixels; PaP= parenchyma pixels; PI = pulsatility index; RI = resistance index; PSV = peak systolic velocity; EDV = end-diastolic velocity; SC = scrotal circumference; Mot = sperm motility; Vig = sperm vigor; Conc = sperm concentration; Mass = mass movement.

used as reliability indicators in the routine assessment of men suffering from infertility or of individuals showing genital anomalies resulting from dispermic fertilization. In addition, significant correlations were found between semen analysis and ultrasound imaging performed in men with varicocele [51].

The high correlation between resistance and pulsatility indices (P = 0.978) is observed due to the fact that the testicular artery presents low pulsatility and resistance and flows with large and continuous systolic peaks. It also presents high-velocity flow during diastole, which is typical of parenchymal organs that continuously demand blood [1]. The testicular tissue requires adequate vascular perfusion to perform spermatogenesis. Accordingly, recent reports have demonstrated that Doppler indices (PI and RI) are adequate spermatogenesis indicators in different species [50,52]. However, increased RI and PI indicate decreasing distal tissue perfusion, which is associated with testicular changes, such as Orchitis, epididymitis, cryptorchidism, and testicular tumors in men [53], dogs [54], and stallions [55].

The positive correlation between the scrotal circumference and the peak systolic velocity, as well as between the scrotal circumference and the pulsatility and resistance indices, reflects the blood flow increase in the testis during sexual development, and consequently, the increased sperm functionality and production [56]. Several authors found a high correlation between scrotal circumference and sperm quality and production in bucks [57], rams [58], bulls, [59,60], pigs, [61] and dogs [62].

The Doppler-analyzed variables showed significant correlations with aging and testicular measurements, but not with the spermogram. The goat semen has specific physical and biochemical features, which may vary depending on factors such as breed, individual, age, time of the year, collection method, feeding [63], collection frequency, social hierarchy, libido [64], and climate [64,65,66].

Studies conducted by Semiz et al. [67] found that the spectral Doppler analysis is a noninvasive method which is able to provide valuable information for the diagnosis of hemodynamic changes and the testicular microcirculation status. Additionally, the Doppler velocimetric values of the testicular artery can be used as a complementary tool to indicate seminal quality or even for the prediction of fertility potential.

The current study provides important information about the validation of color and spectral Doppler techniques applied to testicular evaluation in goats, besides establishing reference values for healthy animals. In addition, it is important to evaluate the applicability of these imaging techniques to the diagnosis of testicular abnormalities in goats, as previously described for dogs [68] and stallions [55].

5. Conclusion

The present study has shown significant correlations between testicular plexus and parenchymal vascular flows, as well as among Doppler flowmetric variables and animals' age, testicular morphometry, and fertility. However, there was no correlation between Doppler variables and sperm morphology in male goats.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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