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

Diego Luiz dos Santos RIBEIRO1, Larissa Sarmento dos SANTOS2, Itamara Gomes de FRANÇA3, Héllyda Gomes PEREIRA4, Joaquim de Sousa LIMA4, Bento Douglas Brito MIRANDA4, Alcina Vieira de CARVALHO-NETA5, José Ribamar de Souza TORRES-JÚNIOR6*,

1Master in animal Science, Maranhão State University, São Luís/MA, Brazil
2Pathology Department, Maranhão State University, São Luís/MA, Brazil
3Biodiversity and Biotechnology Network of the Legal Amazon (BIONORTE), Maranhão State University, São Luís/MA, Brazil
4Federal University of Maranhão, Chapadinha/MA, Brazil
5Chemistry and Biology Department, Maranhão State University, São Luís/MA, Brazil
6Department of Oceanography and Limnology, Federal University of Maranhão, São Luís/MA, Brazil

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 real time 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.
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 mm³, 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].
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 (TI BCO 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 \( H_0 \) (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 tests 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 \( (P < 0.05; \text{Table 6}) \).

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
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) (r² = 0.48, P < 0.0001), as well as with the plexus score (PS) (r² = 0.39; P = 0.002). The pulsatility index (PI) showed correlation with PP (r² = 0.33, P= 0.009), PS (r² = 0.26, P = 0.045), PSV (r² = 0.62, P < 0.0001) and with end-diastolic velocity (EDV) (r² = –0.85, P < 0.0001).

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  |

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
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 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 |
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

| Table 6. Hemodynamic and andrological features (mean ± standard error) based on the age group of male goats. |
|----------------------------------------------------------|
| Variable                          | Age Group (years old) |       |       |       |
|-----------------------------------|-----------------------|-------|-------|-------|
|                                   | Young (1-2 years)     | Mature (2.1-5 years) | Old (6 to 9 years) | P-value |
| Plexus pixels                     | 6,881.58 ± 290.70a    | 10,432.06 ± 550.11b | 11,245.52 ± 554.8b | <0.0001 |
| Plexus score                      | 2.12 ± 0.08           | 2.92 ± 0.18          | 3.08 ± 0.16          | 0.15    |
| Parenchyma pixels                 | 456.57 ± 35.04c       | 634.25 ± 60.93b      | 832.33 ± 61.75c      | <0.0001 |
| Parenchyma score                  | 2.08 ± 0.072a         | 2.43 ± 0.122a        | 2.64 ± 0.112a        | 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 ± 0.24a          | 2.67 ± 0.49a         | 1.49 ± 0.19a         | 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    |
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
The authors acknowledge the financial support given by CAPES and would like to thank Maranhão Research and Scientific and Technological Development Foundation (FAPEMA - Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão) for granting the scholarship to the first author (Process BM-01333/13).

Conflict of interest
The authors declare no conflict of interest.

References
1. Carvalho CF, Chammas MC. Uso do ultra-som duplex Doppler no diagnóstico de shunt portossistêmico em gatos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2008; 60 (1): 109-112 (in Portuguese). doi: 10.1590/S0102-0935200800100016.
2. Vermillon RP. Basic physical principles. In: Snider, AR (editor). Echocardiography in Pediatric Heart Disease. 2nd ed. Missouri, USA: Mosby; 1997. pp. 1-10.
3. Pozor MA, Mcdonnell SM. Doppler ultrasound measures of testicular blood flow in stallions. Theriogenology 2002; 58 (2): 437-440. doi: 10.1016/S0093-691X(03)00227-9
4. Pozor MA, McDonnell SM. Color Doppler ultrasound evaluation of testicular blood flow in stallions. Theriogenology 2004; 61 (5): 799-810. doi: 10.1016/S0093-691X(03)00227-9
5. Batissaco L, Celeghini ECC, Pinaffi FLV, Oliveira BMM, Andrade AFC et al. Correlations between testicular hemodynamic and sperm characteristics in rams. Brazilian Journal of Veterinary Research and Animal Science 2013; 50 (5): 384-395.
6. Biagiotti G, Cavallini G, Modernini F, Vitali G, Gianaroli L. Spermatogenesis and spectral echo-colour Doppler traces from the main testicular artery. BJU International 2002; 90 (9): 903-908. doi: 10.1046/j.1464-410x.2002.03033.x
7. Günzel-Apel AR, Möhrke C, Nautrup CP. Colour-coded and pulsed Doppler sonography of the canine testes, epididymis and prostate gland: physiological and pathological findings. Reproduction in Domestic Animals 2001; 36: 236-240. doi: 10.1046/j.1439-0531.2001.00288.x
8. Gumbsch P, Gabler C, Holzmann A. Color-coded duplex sonography of the testes of dogs. Veterinary Record 2002; 151 (5): 140-144. doi: 10.1136/vr.151.5.140
9. Kutzler M, Tyson R, Grimes M, Timm K. Determination of testicular blood flow in camelds using vascular casting and color pulsed-wave Doppler ultrasonography. Veterinary Medicine International 2011; 1-7. doi: 10.4061/2011/638602
10. Pierson RA, Kastelic JP, Ginther OJ. Basic principles and techniques for transrectal ultrasonography in cattle and horses. Theriogenology 1988; 29 (1): 3-20. doi: 10.1016/0093-691X(88)90028-3
11. Cavalcante JMM, Brasil OO, Oliveira RV, Pessoa AW, Araújo AA et al. Ultrasonografia testicular em caprino com degeneração testicular associado a lesões escrotais: relato de caso. Revista Brasileira de Higiene e Sanidade Animal 2014; 08 (1): 54-72. (in Portuguese). doi: 10.5935/1981-2965.20140004
12. Ahmad N, Noakes DE, Subandrio AL. B-mode real time ultrasonographic imaging of the testis and epididymis of sheep and goats. Veterinary Record 1991; 25: 491-496. doi: 10.1136/vr.128.21.491
13. Samir H, Sasaki K, Ahmed E, Karen A, Nagaoka K et al. Effect of a single injection of gonadotropin-releasing hormone (GnRH) and human chionic gonadotropin (hCG) on testicular blood flow measured by color Doppler ultrasonography in male Shiba goats. Journal of Veterinary Medical Science 2015; 77: 549-556. doi: 10.1292/jvms.14-0633
14. Samir H, Nyametease P, Nagaoka K, Watanabe G. Effect of seasonality on testicular blood flow as determined by color Doppler ultrasonography and hormonal profiles in Shiba goats. Animal Reproduction Science 2018; 197: 185-192.
15. Samir H, El Sayed MA, Nagaoka K, Sasaki K, El-Maaty AMA et al. Passive immunization against inhibin increases testicular blood flow in male goats. Theriogenology 2020; 147: 85-91.
16. Alves, CA, Stefle JL, Sentelhas PC, de Moraes G, Leonardo J et al. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift 2013; 22 (6): 711-728.
17. Ginther OJ. Ultrasonic Imaging and Animal Reproduction: Color-Doppler Ultrasonography. 2007. Ginther OJ (ed). Cross Plains, USA: Equiservices Publishing, 258p.
18. Silva LA, Gastal EL, Beg MA, Ginther OJ. Changes in vascular perfusion of the endometrium in association with changes in location of the embryonic vesicle in mares. Biology of Reproduction 2005; 72 (3): 755-761. doi: 10.1095/biolreprod.104.036384
19. Araújo RR, Ginther OJ. Vascular perfusion of the reproductive organs in pony mares and heifers during sedation with detomidine and xylazine. American Journal of Veterinary Research 2009; 70: 141-148. doi: 10.2460/ajvr.70.1.141

20. Silva LA, Ginther OJ. Local effect of the conceptus on uterine vascular perfusion during early pregnancy in heifers. Reproduction 2010; 139: 453-463. doi: 10.1530/REP-09-0363

21. Henry M, Neves JP. Manual para exame andrológico e avaliação de sêmen animal. 1998. 2nd ed. Belo Horizonte: Colégio Brasileiro de Reprodução Animal, p. 49. (in Portuguese)

22. Franken DR, Oehninger S. Semen analysis and sperm function testing. Asian Journal of Andrology 2012; 14 (1): 6-13. doi: 10.1038/aja.2011.58

23. Statistical Analysis System - SAS. System for Microsoft Windows: release 8.2. Cary: 2001. CD-ROM.

24. McElduff F, Cortina-Borja M, Chan SK, Wade A. When t-tests or Wilcoxon-Mann-Whitney tests won’t do. Advances in Physiology Education 2010; 34 (3): 128-133. doi: 10.1152/advan.00017.2010.

25. Statsoft, INC. STATISTICA (data analysis software system). 2007. Version 7, 1984-2004.

26. Sampaio IBM. Estatística aplicada à experimentação animal. 2002. 2nd ed. Belo Horizonte: Fundação de Estudo e Pesquisa em Medicina Veterinária e Zootecnia, p. 265. (in Portuguese).

27. Mukaka MM. Statistics Corner: A guide to appropriate use of correlation coefficient in medical research. Malawi Medical Journal 2012; 24: 69-71. PMCID: PMC3576830

28. Hedia M, El-Belely M, Ismail S, Abo-El-Maayt A. Evaluation of testicular blood flow and ultrasonographic measurements in rams with emphasis on laterality. Journal of Advanced Veterinary Research 2020; 10 (1): 17-20.

29. Kavak A, Lundeheim N, Aidnik M, Einarroson S. Testicular measurements and daily sperm output of Tori and Estonian breed stallions. Reproduction Domestic Animal 2003; 38: 167-169.

30. Souza MB, Barbosa CC, Pinto JN, Uchoa DC, Campello CC et al. Comparison of testicular volume between French Bulldog and Brazilian Terrier dogs. In: Proc. International Symposium on Canine and Feline Reproduction; Whistler, Canada; 2012. p. 2.

31. Gloria A, Carluccio A, Wegher L, Robbe D, Valorz C et al. Pulse wave Doppler ultrasound of testicular arteries and their relationship with semen characteristics in healthy bulls. Journal of Animal Science and Biotechnology 2018; 9 (1): 14. doi: 10.1186/s40104-017-0229-6

32. Vale WG. Avances biotecnológicos em reprodução de búfalos. Tecnologia em Marcha 2011; 24 (5): 89-90.

33. Henry M, Brito MF, Neves BP, Auler PA, Almeida J et al. Peculiarities of the buffalo species for andrological evaluation—results of four years of study and weekly semen collection schedule. Animal Reproduction 2018; 14 (Supplement 1); 1225-1233. doi: 10.21451/1984-3143-AR0005

34. Bergh A, Damber JE. Vascular controls in testicular physiology. In: de Kretser, DM (editor). Molecular Biology of the Male Reproductive System. New York: Academic Press 1993. pp. 439-468.

35. Kay GW, Grobbelaar JA, Hattingh J. Effect of surgical restriction of growth of the testicular artery on testis size and histology in bulls. Journal of Reproduction and Fertility 1992; 96: 549-553. doi: 10.1530/jrf.0.0960549

36. Bergh A, Collin O, Lissbrant E. Effects of acute graded reductions in testicular blood flow on testicular morphology in the adult rat. Biology of Reproduction 2001; 64: 13-20.

37. Ahmadi B, Lau CP, Giffin J, Santos N, Hahnel A et al. Suitability of epididymal and testicular ultrasonography and computerized image analysis for assessment of current and future semen quality in the ram. Experimental Biology and Medicine 2012; 237: 186-193.

38. Moxon R, Bright L, Pritchard B, Bowen IM, de Souza MB, et al. Digital image analysis of testicular and prostatic ultrasonographic echogenicity and heterogeneity in dogs and the relation to semen quality. Animal Reproduction Science 2015; 160: 112-119.

39. Dogra VS, Bhatt S, Rubens DJ. Sonographic evaluation of testicular torsion. Ultrasound Clinics 2006; 1: 55-66. doi: 10.1016/j.uclt.2005.09.006

40. Budras KD, Mccarthy PH, Fricke W, Richter R. Anatomy of the Dog. 5th ed. London, UK: Manson; 2007. pp. 68-70.

41. Nickel R, Schummer A, Seiferle E. The Visceral of the Domestic Mammals. 2nd ed. Berlin: Verlag Paul Parey; 1979. p. 401.

42. Smith JA. Biopsy and the testicular artery of the horse. Equine Veterinary Journal 1974; 6: 81-83. doi: 10.1111/j.2042-3306.1974.tb03934.x

43. Schummer AUB, Vollmer H. Harn- und Geschlechtsapparat. In: Nickel, R., A. Schummer u. E. Seiferle (Editors). Lehrbuch der Anatomie der Haustiere, Band II: Eingeweide. Berlin-Wien: Blackwell Wissenschafts-Verlag; 1995. pp. 300-420.

44. Middleton WD, Thorne DA, Melson GL. Color Doppler ultrasound of the normal testis. American Journal of Roentgenology 1989; 152: 293-297. doi: 10.2214/ajr.152.2.293

45. Ohdan RH. Scrotal ultrasound. European Journal of Radiology 2002; 12 (1): 19-34. doi: 10.1007/s00330-001-1224-y

46. Paltiel H J, Rupich RC, Babcock DS. Maturational changes in testicular blood flow on testicular morphology in the adult rat. Journal of Reproduction and Fertility 1992; 96: 549-553. doi: 10.1530/jrf.0.0960549

47. Evans AC, Pierson RA, Garcia A, McDougall LM, Hrudka F et al. Comparison of testicular volume between French Bulldog and Brazilian Terrier dogs. In: Proc. International Symposium on Canine and Feline Reproduction; Whistler, Canada; 2012. p. 2.

48. Giffin JL, Franks SE, Rodriguez-Sosa JR, Hahnel A, Bartleewski PM. A study of morphological and haemodynamic determinants of testicular echotexture characteristics in the ram. Experimental Biology and Medicine 2009; 234: 794-801. doi: 10.3181/0812-RM-364
49. Wood MM, Romine LE, Lee YK, Richman KM, O’Boyle MK et al. Spectral Doppler signature waveforms in ultrasonography: a review of normal and abnormal waveforms. Ultrasound Quarterly 2010; 26 (2): 83-99. doi: 10.1097/RUQ.0b013e3181dcf67.

50. Pinggera GM, Mitterberger M, Bartsch G, Strasser H, Gradi J et al. Assessment of the intratesticular resistive index by colour Doppler ultrasonography measurements as a predictor of spermatogenesis. BJU International 2008; 101: 722-726. doi: 10.1111/j.1464-410X.2007.07343.x

51. Tarhan S, Gümüş SB, Gundu ZI, Ayyıldız V, Göktan C. Effect of varicocele on testicular artery blood flow in men color Doppler investigation. Scandinavian Journal of Urology 2003; 37 (1): 38-42. doi: 10.1080/00365590310008677

52. Zelli R, Troisi A, Elad Ngonput A, Cardinali L, Polisca A. Evaluation of testicular artery blood flow by Doppler ultrasonography as a predictor of spermatogenesis in the dog. Research in Veterinary Science 2013; 95: 632-637. doi: 10.1016/j.rvsc.2013.04.023

53. Schurich M, Aigner F, Frauscher F, Pallwein L. The role of ultrasound in assessment of male fertility. European Journal of Obstetrics & Gynecology and Reproductive Biology 2009; 144: 192-198. doi: 10.1016/j.ejogrb.2009.02.034

54. Bumin A, Kaya M, Kaya Ü, Kibar M, Alkan Z. Gray-scale, colour and power Doppler sonography of scrotal disorders in dogs. Revue de Médecine Vétérinaire 2007; 158: 128-133.

55. Pozor MA, Nolin M, Roser J, Runyon S, Macperson ML et al. Doppler index of vascular impedance as indicator of testicular dysfunction in stallions. Journal of Equine Veterinary Science 2014; 34: 38-39. doi: 10.1016/j.jevs.2013.10.021

56. Brito LFC, Silva AEDF, Rodrigues LH, Vieira FV, Cardoso RCS, Silva LDM. Relação entre perímetro escrotal e concentração espermática em cães, clinicamente normais, da raça Pastor Alemão. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2002; 54 (5): 549-550. (in Portuguese). doi: 10.1590/S0102-09352002000500017

57. Al-Ghalban AM, Tabbaa MJ, Kridli RT. Factors affecting semen characteristics and scrotal circumference in Damascus bucks. Small Ruminant Research 2004; 53: 141-149. doi: 10.1016/j.smallrumres.2003.10.003

58. Mickelsen WD, Paisley LG, Damen JJ. The effect of scrotal circumference, sperm motility and morphology in the ram on conception rates and lambing percentage in the ewe. Theriogenology 1981; 16 (1): 53-59. doi: 10.1016/0093-691x(81)90113-8

59. Torres-Júnior JRS, Henry M. Sexual development of Guzerat (Bos taurus indicus) bulls raised in a tropical region. Animal Reproduction 2005; 2 (2): 114-121.

60. Menon AG, Barkema HW, Wilde R, Kastelic JP, Thundathil JC. Associations between sperm abnormalities, breed, age, and scrotal circumference in beef bulls. Canadian Journal of Veterinary Research 2011; 75 (4): 241-247. PMCID: PMC3187629

61. Huang YT, Johnson RK. Effect of selection for size of testes in boars on semen and testis traits. Journal of Animal Science 1996; 74: 750-760. doi: 10.2527/1996.744750x

62. Cortez AA, Aquino-Cortez A, Silva AR, Cardoso RCS, Silva LDM. Relação entre perímetro escrotal e concentração espermática em cães, clinicamente normais, da raça Pastor Alemão. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 2002; 54 (5): 549-550. (in Portuguese). doi: 10.1590/S0102-09352002000500017

63. Corteel JM. Production, storage and insemination of goat semen. In: Symposium of Reproduction in Sheep and Goats. 1977, Madison – University of Wisconsin. Anais… 41-57.

64. Mies Filho A. Reprodução os Animais. 6th ed. Porto Alegre: Sulina; 1987. p. 423. (in Portuguese).

65. Silva AEDF, Dode MAN, Porto JA, Abreu UGP. Estacionalidade na atividade sexual de machos bovinos Nelore e mestiços Fleckvieh e Chianina x Nelore: características biométricas testiculares. Pesquisa Agropecuária Brasileira 1991; 26 (10): 1745-1750. (in Portuguese).

66. Fatet A, Pellicer-Rubio MT, Leboeuf B. Reproductive cycle of goats. Animal Reproduction Science 2011; 124: 211-219. doi: 10.1016/j.anireprosci.2010.08.029

67. Semisz I, Tokgöz O, Tokgoz H, Voyvoda N, Serifoglu I et al. The investigation of correlation between semen analysis parameters and intraparenchymal testicular spectral Doppler indices in patients with clinical varicocele. Ultrasound Quarterly 2014; 30 (1): 33-40. doi: 10.1097/RUQ.0000000000000055

68. Souza MB, England GC, Mota Filho AC, Ackermann CL, Sousa CV et al. Semen quality, testicular B-mode and Doppler ultrasound, and serum testosterone concentrations in dogs with established infertility. Theriogenology 2015; 84 (5): 805-810. doi: 10.1016/j.theriogenology.2015.05.015