Correlations between testicular hemodynamic and sperm characteristics in rams

Correlações entre a hemodinâmica testicular e as características espermáticas em carneiros

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Abstract

The testicular artery is responsible for the blood supply that reaches the testis and has great importance in heat radiation. Vascular changes in the testis may lead to damage in sperm production, reflected in sperm motility and morphology. The aim of the present study was to evaluate correlations between testicular vascularity and sperm characteristic. Eight adult Santa Ines rams showing different reproductive status were used. The testicular vascularity and sperm characteristics were evaluated fortnightly during 90 days. Color Doppler ultrasonography was used to evaluate the testicular hemodynamic. Resistance index (RI) and pulsatility index (PI) of the testicular artery were evaluated by spectral-Doppler mode. The color-Doppler mode was used to evaluate the blood flow of the pampiniform plexus and testicular parenchyma. The semen analyses assessed were volume, concentration, motility, and morphology. The data were submitted to Pearson´s linear correlations test ($p < 0.05$ was considered significant). No correlations were found between motility and testicular hemodynamic. The percentage of total sperm defects was positively correlated to left and right parenchymal score and to left RI and PI. On the other hand, the pampiniform plexus score was positively correlated to the number of colored pixels and negatively correlated to the RI and PI, for both sides. This study showed that the increase of sperm defect can be related to increase of testicular blood flow; however, more studies are need.

Keywords: Ovine. Spermatozoa. Blood flow. Doppler. Testicle.

Resumo

A artéria testicular é responsável pelo fluxo de sangue que chega aos testículos e tem grande importância na termorregulação. Mudanças vasculares nos testículos podem levar à queda da produção espermática, refletindo na motilidade e morfologia. O objetivo deste trabalho foi avaliar as correlações entre a vascularização testicular e as características espermáticas. Foram utilizados oito carneiros adultos Santa Inês com diferentes status reprodutivos. A vascularização testicular e as características seminais foram avaliadas por um período de 90 dias. A ultrassonografia Doppler colorida foi utilizada para avaliar a hemodinâmica testicular. Foram calculados os índices de resistência (RI) e os índices de pulsatilidade (PI) com o modo espectral do Doppler. O modo colorido do Doppler foi utilizado para analisar o fluxo sanguíneo do plexo pampiniforme e do parênquima testicular. As características seminais avaliadas foram o volume, concentração, motilidade e morfologia. Os dados foram submetidos ao teste de correlação linear de Person ($P < 0.05$ foi considerado significativo). Não foram encontradas relações entre motilidade e a hemodinâmica testicular. A porcentagem de defeitos totais correlacionou-se positivamente com o escore de vascularização dos parênquimas direito e esquerdo, e com o RI e PI esquerdos. Também o escore de vascularização dos plexos se correlacionou positivamente com a média de pixels e negativamente com o RI e PI, de ambos os lados. Este trabalho mostrou que o aumento de defeitos espermáticos pode estar correlacionado com o aumento do fluxo sanguíneo nos testículos; contudo, mais estudos são necessários.

Palavras-chave: Ovino. Espermatozoide. Fluxo sanguíneo. Doppler. Testículo.

Introduction

Ram sperm quality depends on several variables. Since ovine is photoperiod dependent, significant differences in sperm quality parameters are found depending on the season, showing lower quality in breeding season (autumn) compared with early

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nonbreeding (spring) or late nonbreeding season (summer) (MARTÍ et al., 2012)

In lambs submitted to high ambient temperatures there were a significant decrease in testicular weight, degeneration in germ cells, vacuolization or disappearance of seminiferous tubules epithelium lining, formation of intratubular multinucleate giant cells, spermatogenic arrest at the spermatocyte stage and decreasing thickness of germinal epithelium layer, thickened basement membranes with interstitial fibrosis and increased peritubular connective tissues and an increase in serum cortisol concentrations (RASOOLI et al., 2010).

In addition, other causes can also lead to degenerative process of the testis. Examples include the various epididymal and testicular abnormalities of hereditary and/or congenital origin, such as cryptorchidism and hypoplasia, or acquired ones, such as inflammatory responses and neoplasic processes (LAGERLOF, 1938; VAN CAMP, 1997).

The color-Doppler ultrasonography is an effective and noninvasive method to evaluate vascular changes in the scrotum (LAM et al., 2005), and provides information about vascular architecture, direction and velocity of blood flow. By a two-dimensional image (mode B), a colored map of the vascularity in tissues and organs is visualized (POZOR; MCDONNELL, 2002; POZOR; MCDONNELL, 2004). On the other hand, the analysis by spectral-Doppler mode provides information of velocity and flow resistance of the blood, and has been used to characterize blood flow in the testicular artery of stallions (POZOR; MCDONNELL, 2002)

The RI is a reliable indicator for routine clinical use to identify infertile/dyspermic men (BIAGIOTTI et al., 2002). Subjective appearance of the color Doppler images and RI and PI values had been used as a diagnostic parameter in dogs (GÜNZEL-APEL; MOÈHRKE; POULSEN NAUTRUP, 2001; GUMBSCH; GABLER; HOLZMANN, 2002). In camelids, color pulsed-wave Doppler ultrasonography has been used to measure testicular blood flow and compare with fertility (KUTZLER et al., 2011). In stallions, the Doppler ultrasonography was efficient in the characterization of testicular blood flow (POZOR; MCDONNELL, 2002).

The aim of the present investigation was to evaluate the correlation between the vasculature of the testicles and sperm characteristics in rams. The different reproductive status of the rams represented contrasting sperm characteristics and was compared with the testicles vascularity. The hypothesis that the high percentage of defective sperm is related to the increase on the vascularity of the testis was tested.

Materials and Methods

Animals

Eight adult Santa Ines rams aged between one to six years old were used during May through July in the southern tropical zone. Rams were randomly selected for the experiment. All rams remained healthy and in good body condition throughout the experiment. The experiment was in agreed with Ethical Principles in Animals Research adopted by "Ethic Committee in the use of animals" of the School of Veterinary Medicine and Animal Science of University of São Paulo.

Ultrasonography

Ultrasound scanning (Mindray, model M5Vet, Digital Ultrasonic Diagnostic Imaging System) of the testicles were performed in duplex B-mode (grey scale), color-Doppler flow mode and spectral mode functions, using a 6 MHz convex transducer (Mindray, model 6LE5Vs Vet). Analyzes were done fortnightly for 90 days (six analyses per animal).The extent and direction of blood flow in the vessels of the testicles are indicated by color signals (red or blue) (GINTHER, 2007), and the color-Doppler was used to display signals of blood flow in the vessels of pampiniform plexus and parenchyma of the testicles. All color-Doppler scans were performed at a constant gain setting, filter setting, and velocity range setting. The transducer was placed

caudally in the middle of the pampiniform plexus and testicular parenchyma. The vascular perfusion was estimated subjectively by scoring the extent of colored signals for blood flow in the pampiniform plexus and testicular parenchyma during real-time cross-sectioning in a continuous span of 1 min. The score ranged from 1 to 5 for the pampiniform plexus, indicating nil, minimal, intermediate and maximal vascular perfusion (Table 1 and Figure 1) and a score ranging from 0 to 4 for the testicular parenchyma, indicating no apparent vascularity, low, intermediate, high, very high vascularity (Table 2 and Figure 2), similarly as described for uterus in mares (SILVA et al., 2005). Real-time B-mode/color-Doppler images of the continuous scans were captured for validation and confirmation purposes.

Table 1 – Ranking of scores used for evaluating of the image obtained by color-mode ultrasonography from pampiniform plexus of the rams in scores from 1 to 5

Score	Percentage of staining	Visualization
1	0% - 20%	Extremely small colored area
2	20% - 40%	Small colored area
3	40% - 60%	Average colored area
4	60% - 80%	Large colored area
5	80% - 100%	Great majority of colored area

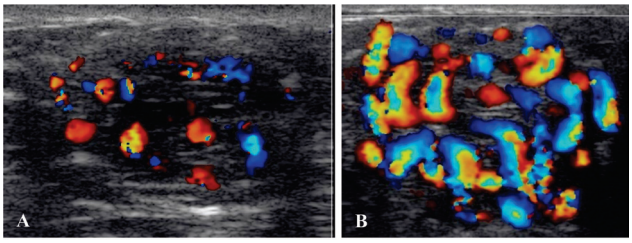

Figure 1 – Sample images of the blood flow from pampiniform plexus by color Doppler ultrasonography (M5vet, Mindray). A: score 1 (1 to 5). B: score 5 (1 to 5)

Table 2 - Classification of scores used for evaluation of the image obtained by color-mode ultrasonography of the testicular parenchyma of rams in scores from 0 to 4

Score	Percentage of staining	Visualization
0	0%	No apparent vascularity
1	1% - 25%	Low vascularity
2	25% - 50%	Intermediate vascularity
3	50% - 75%	High vascularity
4	75% - 100%	Very high vascularity

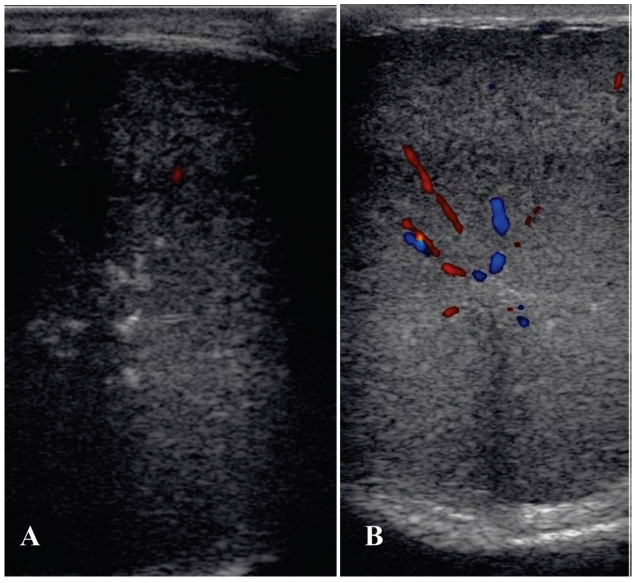

Figure 2 - Sample images of the blood flow of the testicular parenchyma by color Doppler ultrasonography (M5vet, Mindray). A: score 1 (0 to 4), B: score 4 (0 to 4)

The subjective scoring of the extent of vascular perfusion of the pampiniform plexus and testicular parenchyma was validated by objective assessment of color changes in the pixels of still images, as described for mares and heifers (SILVA et al., 2005; GINTHER et al., 2007; ARAUJO; GINTHER, 2009). To assess the validity of the subjective real-time estimation of vascular perfusion, objective off-line measurements of the total number of colored pixels, total pixel intensity and average intensity were done (Figure 3), as described by Silva and Ginther (2010). For each 1 min continuous color-Doppler scan, three still images were selected and used for the determination of the pixel-related end points, and the average was used in the analyses.

In addition to the color-Doppler evaluation of the pampiniform plexus and testicular parenchyma, spectral Doppler scans were made by placing the spectral cursor on the testicular artery in the pampiniform plexus. For spectral examinations, the

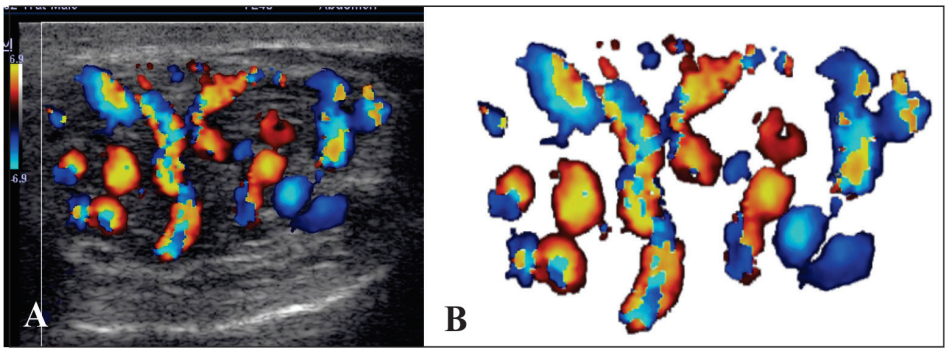

Figure 3 - Example of an image obtained by the color Doppler mode at the ultrasound (M5Vet, Mindray) from pampiniform plexus (A) and a "clean" image of the same through the use of the Adobe Photoshop program (B), in order to obtain the number of colored pixels mean

velocity-range setting and the size of spectral gate were adjusted during each examination to obtain a sequence of spectral Doppler graphs with symmetrical and distinct systolic and diastolic cardiac cycles (Figure 4) without aliasing. Aliasing is a Doppler artifact generated when the blood flow velocity is higher than the velocity of the ultrasound beam (GINTHER, 2007). Settings of gain and filters were uniform for all spectral examinations. One cardiac cycle was chosen and was used for the measurement of RI and PI using preset functions in the ultrasound scanner. The formula of the RI and PI is well established and has been reviewed (GINTHER, 2007). No attempt was made to measure blood-flow velocity and volume; the tortuosity of the vessels prevented the placement of a cursor for obtaining the insonation angle.

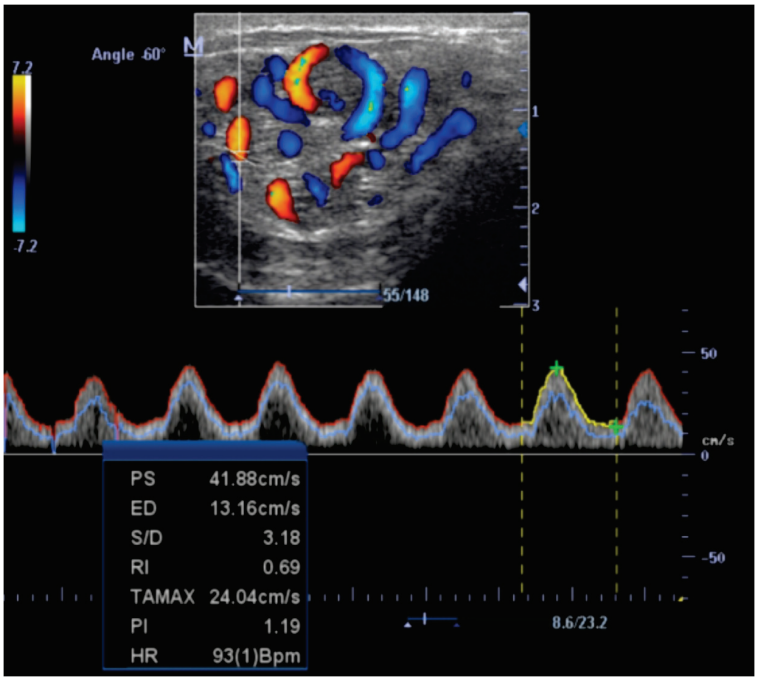

Figure 4 - Ultrasonography image of data collected from pampiniform plexus of ram to obtain the pulsatility index (PI) and resistance index (RI) in the spectral mode (M5Vet, Mindray)

Evaluation of the testicles and semen

The testicles were evaluated for scrotal circumference and the parenchyma was scanned by B-mode ultrasonography.

The semen was obtained by electroejaculation. After collection the semen sample was kept in a water bath at a temperature of 37°C and evaluated for the following characteristics: volume, sperm motility, concentration and morphology. The volume was determined by direct reading on the collection tube (scale of 0.1 mL). Vigor was ranged on a scale of 1 to 5 according to the movement of the spermatozoa under optical microscopy (100x amplification). Motility was expressed in percentage of mobile spermatozoa under optical microscopy (100x amplification). The sperm concentration count was performed in a Neubauer chamber. Sperm morphology was assessed using a phase contrast microscope (1,000 x 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 (FRANKEN; OEHNINGER, 2012).

Statistical analysis

The data of eight ram from six evaluations (48 samples) were analyzed according to its mean ± standard deviation mean (S.D.M.). All data were statistically analyzed and compared using the Pearson linear correlation through the software SAS (Statistical Analysis System), with previous verification of normality of the residues by the Shapiro-Wilk (PROC univariate). The significance level was 5%.

Results

Externally, no abnormality was found on the testes or genital structure of the rams. Ultrasound examination found some spots of calcifications in the testicular parenchyma of four animals. The mean of scrotal circumference values was 32.93 ± 2.20 cm, with values ranging from 29 to 36.5 cm (Figure 5A).

The Figure 5 shows the variation among rams in the different days of analysis. The average volume was 0.7 ± 0.4 mL, with values ranging from 0.2 to 2 mL. The vigor mean was 2 ± 1,42, with values ranging from 0 to 4. The motility mean was 64.0 ± 24.8%, ranging from 0 to 90%, and the mean concentration was 2.6 x 106 ± 2.1 x 106sperm/mL, with values ranging from 0.2 x 106 to 9.5 x 106. The evaluation of sperm morphology showed 22.8 ± 12.0% of minor defects, varying from 3 to 56%, 12.2 ± 11.6% of major defects, varying from 2 to 56%, and 35.6 ± 16.1% of total defects, varying from 7 to 82%. Two rams in the second analysis showed necrospermy.

The mean, minimum and maximum values regarding the hemodynamic characteristics of rams are shown in Table 3. Hemodynamic variation among animals and days of evaluation, showing a high variability (Figures 6 to 8), was also found.

Table 3 - Values of mean, standard deviation mean (S.D.M.), and minimum and maximum values of hemodynamic characteristics of testicles from six evaluation of the eight ram evaluated

	Mean	S.D.M.	Minimum Values	Maximum Values
Left Resistance Index	0.60	0.10	0.34	0.88
Right Resistance Index	0.62	0.11	0.40	0.85
Left Pulsatility Index	1.01	0.29	0.43	1.98
Right Pulsatility Index	1.06	0.35	0.52	2.02
Left Plexus Score	3.61	0.82	1.66	5.00
Right Plexus Score	3.65	0.78	2.00	5.00
Left Parenchyma Score	1.47	0.83	0.33	4.00
Right Parenchyma Score	1.53	0.87	0.33	3.66
Left Plexus Pixel Mean	5.513,239	2.895,553	1.535,290	20.851,980
Right Plexus Pixel Mean	5.204,266	1.966,936	1.615,328	9.329,625
Left Parenchyma Pixel Mean	59.769	46.691	9.623	263.662
Right Parenchyma Pixel Mean	83.863	84.035	6.410	430.517

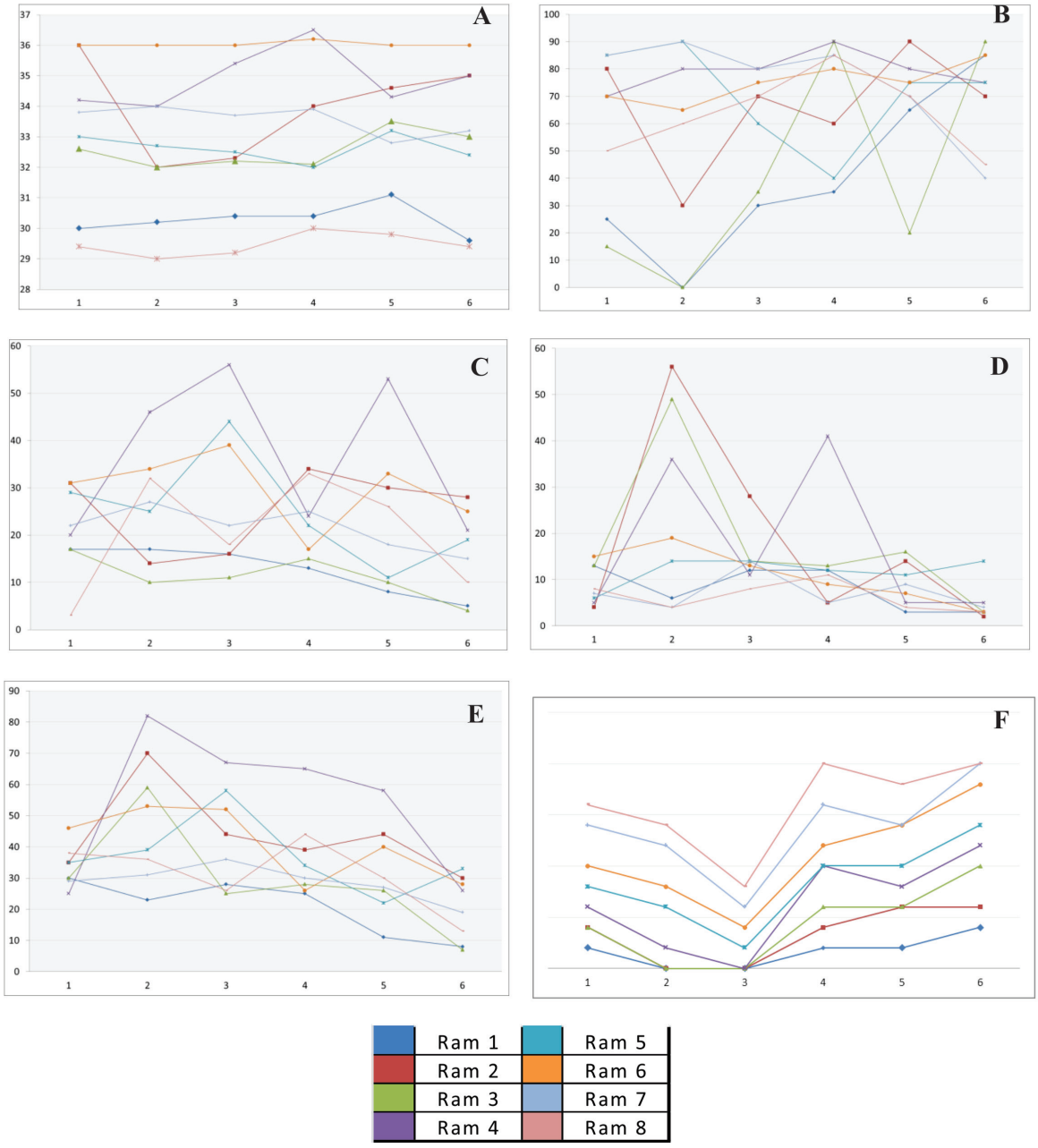

Figure 5 - Means of each ram during the six analyses: A. Scrotal circumference (cm); B. Sperm motility (%); C. Sperm minor defects (%); D. Sperm major defects (%); E. Sperm total defects (%); F. Vigor (1-5)

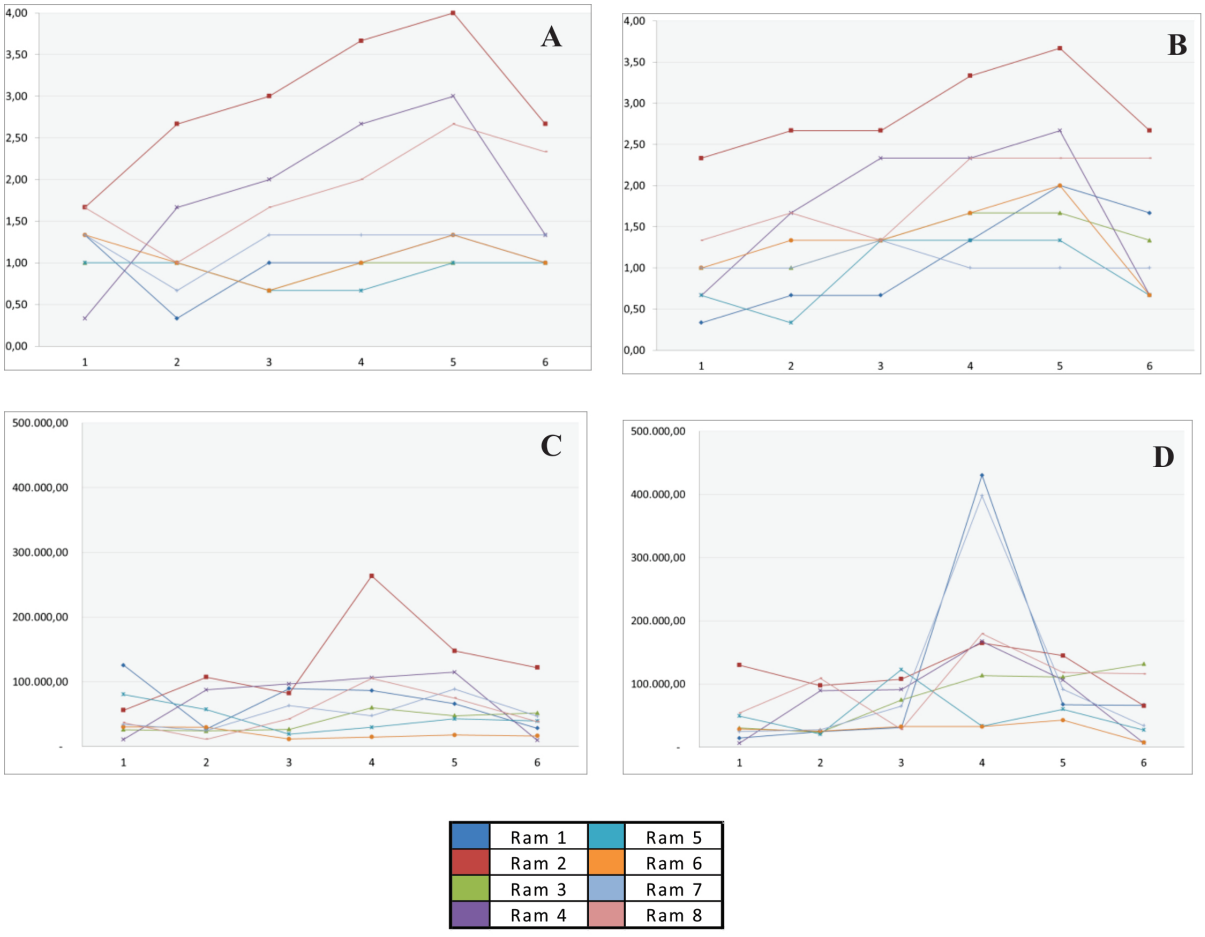

Figure 6 - Graphs showing the means of ultrasonographic evaluation by color Doppler mode from each ram during six analyses obtained with two-week interval: A. Left Parenchyma score (0 to 4); B. Right Parenchyma score (0 to 4); C. Mean of colored pixels number from left parenchyma; D. Mean of colored pixels number from right parenchyma

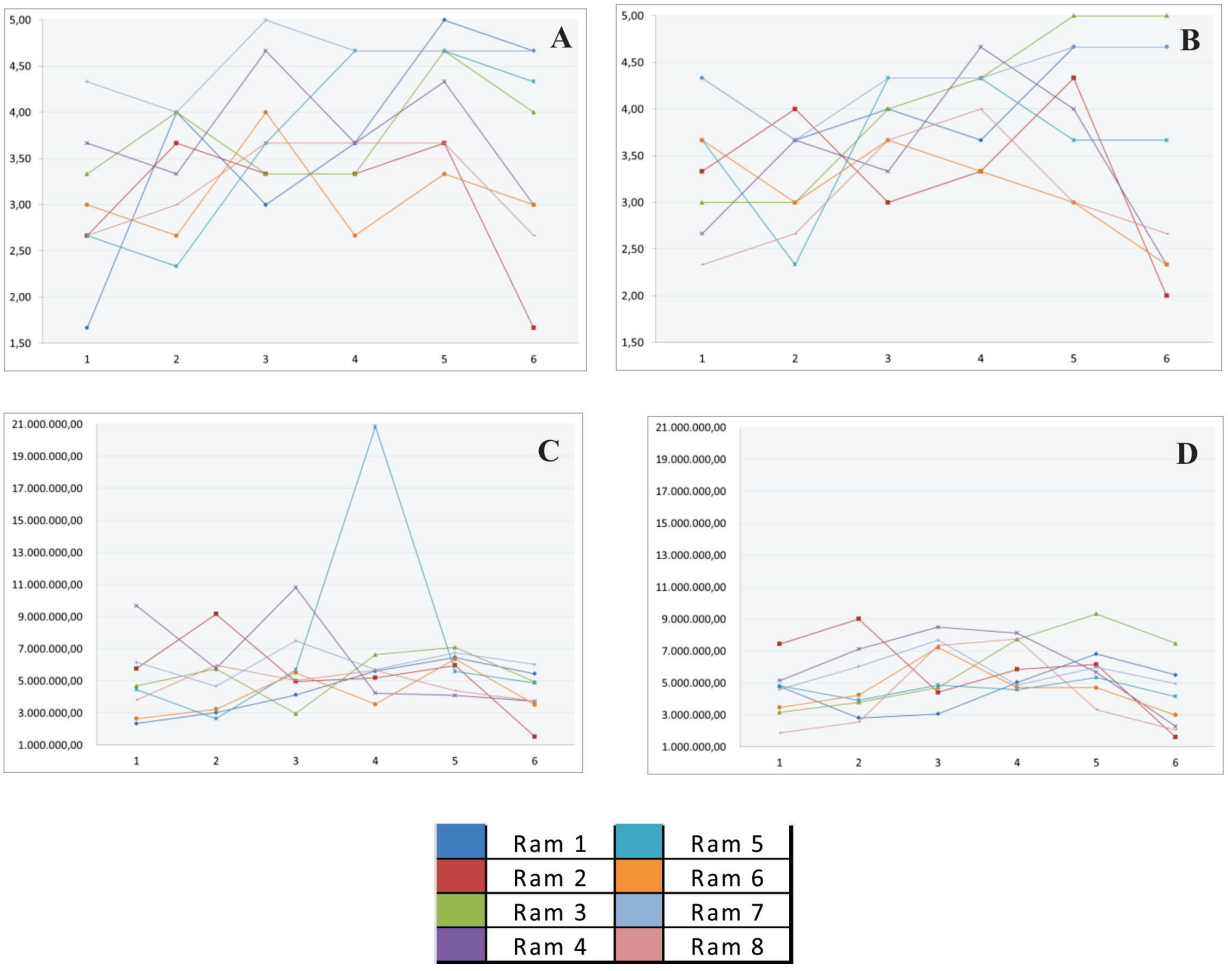

Figure 7 - Graphs showing the means of ultrasonographic evaluation by color Doppler mode from each ram during six analyses obtained with two-week interval: A. Left Pampiniform Plexus score (score 1 to 5); B. Right Pampiniform Plexus score (score 1 to 5); C. Mean of colored pixels number from Left Pampiniform Plexus; D. Mean of colored pixels number from Right Pampiniform Plexus

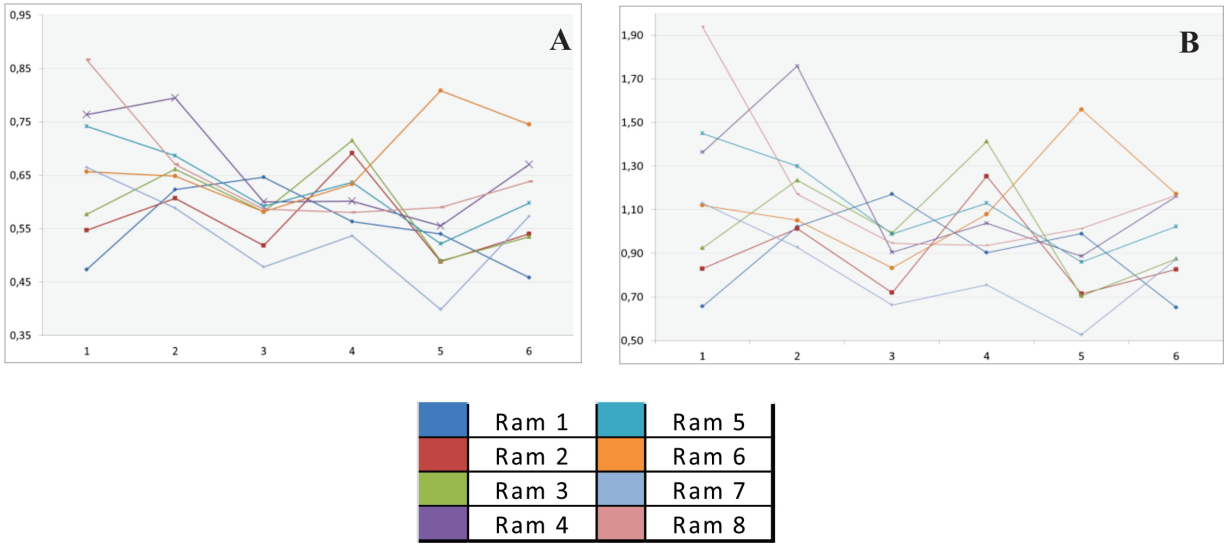

Figure 8 - Graphs showing the means of ultrasonographic evaluation of testicular artery by spectral mode from each ram during six analyses obtained with two-week interval: A. Resistance index (RI, 0-1); B. Pulsatility index (PI, 0-1)

Table 4 shows the significant ($p < 0.05$) linear correlation found in this study.

Table 4 - Pearson linear correlations between sperm characteristics and testicular vascularity of rams evaluated each two weeks for 90 days

Characteristics	r	P
Left Plexus score X Left Plexus Pixel Mean	0.51	0.0002
Right Plexus score X Right Plexus Pixel Mean	0.63	<0.0001
Total Defects X Left Parenchyma Score	0.32	0.02
Total Defects X Right Parenchyma Score	0.29	0.04
Total Defects X Left Resistance Index	0.31	0.03
Total Defects X Left Pulsatility Index	0.3	0.0351
Left Plexus Score X Left Resistance Index	-0.44	0.002
Left Plexus Score X Left Pulsatility Index	-0.41	0.003
Right Plexus Score X Right Resistance Index	-0.49	0.0003
Right Plexus Score X Right Pulsatility Index	-0.40	0.004
Left Parenchyma score X Right Parenchyma Score	0.83	<0.0001
Right Parenchyma Pixel Mean X Left Parenchyma Pixel Mean	0.34	0.01
Concentration X Minor Defects	-0.33	0.02
Scrotal Circumference X Motility	0.42	0.003

No correlation was found between motility and hemodynamic characteristics. Positive correlations were found between the percentage of total sperm defects and: left parenchymal score (r = 0.32), right parenchymal score (r = 0.29), left plexus RI (r = 0.31) and left plexus PI (r = 0.30).

The vascularity of the pampiniform plexus showed no correlation with the vascularity of the testicular parenchyma ($p > 0.05$). On the other hand, the left pampiniform plexus score showed positive correlation to the left pampiniform plexus pixel mean (r = 0.51) and negative correlation to RI (r = -0.44) and PI (r = -0.41) of the left pampiniform plexus. A similar situation was observed for the right side, and the right pampiniform plexus score was positively correlated to the right pampiniform plexus pixel mean (r = 0.63) and was negatively correlated with RI (r = -0.49) and PI (r = -0.40) on the right pampiniform plexus.

The correlation between RI and PI were identical for the right side (r = 0.96) and the left (r = 0.96). Also, left and right RI (r = 0.47) and left and right PI (r = 0.48) showed positive correlations.

Positive correlations were also found between left and right parenchyma score (r = 0.83), left and right plexus score (r = 0.61), left and right parenchyma pixel mean (r = 0.34) and left and right plexus pixel mean (r = 0.35).

Discussion

The values show discrepancies (Figure 5), with volume ranging from 0.2 to 2 mL, motility ranging from 0 to 90% and a concentration ranging from 0.2×10^6 to 9.5×10^6. These variations between analyses, if compared among individuals, are due to the different reproductive situations of each animal and the technology used in semen collection, the electroejaculation. Fourie et al. (2004) has reported motility ranging from 50 to 80% depending on the management. Volume is described in the literature, varying from 1.1 to 1.3 mL (FOURIE et al., 2004; KAFI; SAFDARIAN; HASHEMI, 2004).

Previous studies reported that electroejaculation resulted in larger volumes and lower concentration (PINEDA et al., 1987; PINEDA; DOOLEY, 1991). The

physiological response to electrical stimuli resulted in large volume, probably from contribution of the accessory sex glands and the urinary losses caused in rams by the retrograde flow of spermatozoa (PINEDA et al., 1987). However, the total number of spermatozoa in the ejaculate seems to be similar (PINEDA et al., 1987).

The presence of spots of calcification was observed in four animals, which by soundness examination reinforced the statement that they result from a degenerative process. In men it is uncommon, and testicular calcification usually is dystrophic and caused by previous infections, trauma or tumor (KRONE; CARROLL, 1985). Ahmad and Nokaes (1995) found echogenic spots in caprine 30 days after testicular injury, noting through macro-and microscopic examinations that the images observed were actually testicular calcifications.

The mean of scrotal circumference values was 32.93 ± 2.20 cm, with values ranging from 29 to 36.5 cm. Souza et al. (2010) observed a mean of 31 ± 0.3 cm in Santa Inês rams aged more than 36 weeks. Clark, Schaeffer and Althouse (2003) found a high correlation between scrotal circumference of bovine and ovine with sperm production and the percentage of normal sperm.

The parameters of hemodynamic vary among animals in each analysis showing a discrepancy between animals (Figures 6 to 8).

In humans, significant correlations were found between semen analysis and ultrasonographic images in patients with varicocele (TARHAN et al., 2011). The testicular artery, besides having a large area for heat exchange, provides a decrease in arterial blood flow, increasing in this way, the contact time with the venous blood (BRITO et al., 2004), seen in the correlation between the values of RI and sperm abnormalities. It is suggested that the body acts as a compensatory mechanism in order to increase the time of heat exchange. High circulatory resistance in the veins results in a relatively low diastolic flow and a high value of RI. Hemodynamic changes involving the capillary bed and/or venous drainage have direct effects on arterial impedance (BALCI et al., 2008). It has also been shown in humans that patients with greater values of abnormal sperm cells had a higher value of RI when compared with normal patients (PINGGERA et al., 2008)

The negative correlation between scores of blood flow and RI and PI values of the respective plexus support the hypothesis that the increased flow observed in both plexus is related to the lowest values of RI and PI, i.e., there is a decrease of vascular resistance, leading to increased amount of blood passing through the vessel.

Some researchers have hypothesized that inadequate venous drainage causes an increase in venous stasis and a decrease in arterial blood flow, leading to hypoxia and a deficiency in testicular microcirculation (BALCI et al., 2008). Furthermore, it is believed that this hypoxia might be responsible for a defect in the energy metabolism at the mitochondrial level, causing dysfunction of the Leydig cells and germ cells (COMHAIRE et al., 1983; CHAKRABORTY; HIKIM; JHUNJHUNWALA, 1985; UNSAL et al., 2007).

Elevation of the testicular temperature results in increased metabolism and oxygen demand, but testicular blood flow is limited and this increased demand cannot be supplied, resulting in hypoxia, generation of reactive oxygen species, and deterioration of semen quality (SETCHELL, 1978; SETCHELL, 1998). With the increase of total morphological defects due to a degenerative process it is expected an increase in testicular vascularity, a mechanism that attempts to control the temperature and increase the blood supply to the testis.

In cattle, pigs and humans, it was observed that morphological abnormalities such as various head sizes, and vacuolar defects of the intermediate piece can arise in addition to genetic causes, an ineffective thermoregulation or a decrease in blood flow of the testis (KUTZLER et al., 2011). Unsal et al. (2007) reported that reduction of the spermatogenesis may

be secondary to a defect in energy metabolism at mitochondrial level when the arterial inflow decreases.

It is confirmed by observing the positive correlation between morphological defects and the vascular scores of left and right parenchymas, which would be increased in a regenerative process, also by the higher vascular resistance and positive correlation to total defects.

Testicular blood flow likewise seems to change with testicular and/or environmental changes in temperature. Setchell et al. (1995) demonstrated that the increase in scrotal temperature can result in changes in the movement of the vessels (spontaneous rhythmic change of blood flow). Waites, Setchell and Quinlan (1973) have documented increase of scrotal blood flow in the testicles and in the brains of anesthetized mice, when the scrotum of these animals were immersed in water at a temperature ranging from 28 to 45°C for 20 minutes. However, Glode, Robinson and Horowitz (1984) observed that after artificial cooling in dog testicles, there was a remarkable reduction of blood flow in the scrotal skin, but did not significantly affect testicular blood flow.

According to Setchell (1978), there is no increase in blood flow when the temperature increases in sheep testis (up to body temperature), but blood flow is increased when the testicular temperature is above body temperature. It was observed over the negative correlation between the scores of the plexuses and the PI and RI values on both sides, which shows the decrease of vascular resistance when the score of the plexus increases, thereby increasing blood flow, shown in the largest area filled with blood in the plexus seen in scores of them.

Conclusion

This study showed that the increase of sperm defects can be related to increase of testicular blood flow, but there is no relationship between pampiniform plexus and testicular parenchyma vascularization, and the characteristics evaluated by Doppler ultrasound are related; however, more studies are need to clarify these relationships.

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