

## TESTICULAR FIBROTIC LESIONS AND SEMEN QUALITY IN ADULT MONTANA TROPICAL COMPOUND BULLS\*

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**ABSTRACT.** Pinho R.O., Costa D.S., Siqueira J.B., Martins L.F., Miranda Neto T., Pereira J.V.T. do N., Guimarães S.E.F. & Guimarães J.D. **Testicular fibrotic lesions and semen quality in adult Montana Tropical Compound bulls.** [Lesões fibróticas testiculares e qualidade seminal em touros adultos da raça Composto Montana Tropical]. *Revista Brasileira de Medicina Veterinária*, 35(2):105-110, 2013. Departamento de Zootecnia, Universidade Federal de Viçosa, Avenida PH Rolfs, s/n, Viçosa, MG 36570-000, Brasil. E-mail: rogerio\_op@yahoo.com.br

This study investigated the use of ultrasonography as a complementary test in the diagnosis of testicular fibrosis, and the relationship of fibrotic lesions with semen quality in adult Montana Tropical Compound breed bulls. For this purpose 109 adult Montana Tropical Compound breed bulls, aged between 2.9 and 11.8 years, and raised extensively were used. The animals were evaluated for breeding soundness, and classified as sound and unsound for breeding by physical and morphological semen features. All animals underwent ultrasound examination of the testes, forming images which were transferred to a computer, capturing the average pixel intensity of each testicular image region. Additionally, a testicular fibrosis and/or calcification points score, ranging from 0 to 6 was assigned, to quantify the patterns, size, and frequency of fibrotic lesions. From the animals studied 87.2% (95/109) were found sound for breeding, and 12.8% (14/109) unsound for breeding. The correlation between pixel intensity of the left testis and testicular volume was low ( $r = -0.16$ ). The testicular fibrosis point score demonstrated a low correlation with scrotal circumference ( $r = 0.25$  and  $0.24$  for left and right testis, respectively) and testicular volume ( $r = 0.27$ ), and a medium correlation with animal's age ( $r = 0.35$  and  $0.34$  for left and right testis, respectively). Pixel analyses of testicular ultrasonograms were not predictive of semen quality of adult Montana Tropical Compound breed bulls. Moreover, the varying degrees of testicular fibrosis did not affect sperm quality.

**KEY WORDS.** Bulls, semen quality, testicular fibrosis, ultrasonography.

**RESUMO.** O presente estudo investigou a utilização da ultrassonografia como teste complementar no diagnóstico de fibrose testicular e verificar a relação das

lesões fibróticas com a qualidade seminal em touros adultos da raça Composto Montana Tropical. Foram utilizados 109 touros adultos da raça Composto Mon-

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tana Tropical, com idade variando entre 2,9 e 11,8 anos, criados extensivamente. Os animais foram avaliados por exame andrológico e classificados como aptos e inaptos à reprodução pelos aspectos físicos e morfológicos do sêmen. Todos os animais foram avaliados pelo exame ultrassonográfico dos testículos para se avaliar a média da intensidade de pixels de cada imagem das regiões testiculares. Adicionalmente foi atribuído um escore de pontos de fibrose testicular, de 0 a 6, a fim de quantificar os padrões, dimensões e frequência das lesões fibróticas. Os animais subdividiram-se em 87,2% (95/109) de animais aptos à reprodução e 12,8% (14/109) de animais inaptos à reprodução. A correlação entre intensidade de pixels do testículo direito e volume testicular foi baixa ( $r = -0,16$ ). Os escores de ponto de fibrose testicular demonstraram baixa correlação com o perímetro escrotal ( $r = 0,25$  e  $0,24$  para testículo esquerdo e direito, respectivamente) e volume testicular ( $r = 0,27$ ), além de média correlação com a idade dos animais ( $r = 0,35$  e  $0,34$  para testículo esquerdo e direito, respectivamente). A quantificação da intensidade de pixels por meio da avaliação ultrassonográfica dos testículos isoladamente não foi eficaz em determinar a qualidade seminal em touros adultos da raça Composto Montana Tropical. Além disso, os diversos graus de fibrose testicular não afetaram a qualidade seminal.

**PALAVRAS-CHAVE.** Fibrose testicular, sêmen, touros, ultrassonografia.

## INTRODUCTION

The main difficulty for the ultrasonography use in the breeding soundness evaluation has been the absence of association studies between lesions disclosed by the ultrasound images and the current stage of the pathology (Eilts & Pechman 1988). Barth et al. (2008) did not detect an association between fibrosis and semen quality, suggesting that the fibrotic lesions of the testicular parenchyma are common, and necessary to the determination of the prevalence of these injuries through testicular ultrasound in bulls of different age classes in order to relate the ultrasound image information with seminal quality.

Therefore, the establishment of normal ultrasound parameters for the testicular dimensions and the characterization of normal testicular images are necessary to allow more detailed studies related to degenerative and pathologic conditions of bulls' testis (Cardilli et al. 2010).

Thus, this study investigated the use of ultrasonography as a complementary test in the diagnosis of testicular fibrosis, and the relationship of fibrotic

lesions with semen quality in adult Montana Tropical Compound breed bulls.

## MATERIAL AND METHODS

There were used 109 adult Montana Tropical Compound breed bulls, aged between 2.9 and 11.8 years old, selected in a herd raised in the state of Mato Grosso do Sul, Brazil, at the latitudes between 20° and 21° South and the longitudes between 55° and 56° West, with an average temperature of 21°C during the period of the experiment, and annual precipitation between 1,000 and 1,400 mm<sup>3</sup>. These animals were extensively raised, with predominantly *Brachiaria decumbens* pasture, mineral salt and water ad libitum.

This is a breed constituted by 4 biological types (NABC), according to its similarities of type, function, physiology, growth and breeding features. The N group is constituted by *Bos taurus indicus* (Nelore) animals, the A group by animals from *Bos taurus taurus* breed adapted to the tropics (Bonsmara and Senepol), the B group is composed by *B. t. taurus* animals of British origin (Aberdeen Angus and Red Angus), and the C group includes animals like *B. t. taurus* from Continental Europe (Limousin and Simental) (Ferraz et al. 1999a, Ferraz et al. 1999b). Based on this knowledge, the predominantly blood degree of animals under this study, related to the biological types, was from bovines of *B. t. taurus* breed origin adapted to the tropics (A Group), mainly Bonsmara or Senepol breeds.

The animals were individually restrained in appropriate frames for breeding soundness evaluation and their scrotal circumference (SC) was obtained through a tape measure. A caliper was used to get the testis width and length. The ratio between width (W) and length (L) was calculated to determine the testicular shape, as defined by Bailey et al. (1996), and the formula of the cylinder volume recommended by Fields et al. (1979) was used to calculate the total testicular volume (TV).

Semen was collected through the electroejaculation method for physical evaluation, according the Brazilian College of Animal Breeding (CBRA 1998). In addition, a semen sample was stored in a graduated cylinder with 1 mL of buffered formalin-saline solution (Hancock 1957) for the morphological analysis of the spermatozoa, determining the normal spermatozoa percentage and anomalies of acrosome, head, middle piece, and tail, as established by the Brazilian College of Animal Breeding (CBRA 1998), and classified as major (MAJDEF), minor (MINDEF), and total sperm defects (TDEF), following the criteria described by Blom (1973). The classification recommended by CBRA (1998) was used to interpret the breeding soundness evaluations, with the breeding potential being predicted through recorded values for the physical and morphological semen characteristics, where: minimum of 70% of progressive sperm motility, major sperm defects lower than 20% and total sperm defects lower than 30%. Additionally, according to physical and morphological semen characteristics and sex organ diseases, the animals were allocated into sound and unsound for breeding.

A Mindray brand equipment, DP - 2200 VET model, used for the ultrasound evaluation, was coupled to a linear transducer of 7.5 MHz to get images from testicular parenchyma of a pre-determined region at each image from the right and left

testis. Special attention was paid, so that regions were evaluated covering the testicular parenchyma only, and using acoustic gel on the scrotum, forming images in longitudinal plans, on the caudal face of the left and right testes. Furthermore, to ensure image quality, the same equipment was employed by the same operator for all examinations, in order to standardize the ultrasound evaluations of all animals, to permit the comparison between them.

All obtained images were transferred to a computer, and with the help of the software "Image J" (National Institutes of Health, USA) the image analysis was performed, capturing the average pixel intensity (PI) of each image of the testicular regions in a pixel scale varying from 0 (anechoic, dark image) to 255 (hyperechoic, white image). To evaluate the homogeneity of the testicular echotexture and the pixel representative area, each region of selected images was divided in squares with area of 200 mm<sup>2</sup>.

The fibrosis point evaluation and/or testicular calcification was also performed, through the lateral positioning of the ultrasound probe in the middle region of each testis, performing a complete scanning of the testicular parenchyma of approximately 90°, and assigning a score from 0 to 6, in order to quantify the patterns, dimensions, and frequencies of the fibrotic lesions, where the fibrosis points observed in the testicular parenchyma were classified as follows: 0 = zero; 1 = 1 to 10; 2 = 11 to 30; 3 = 31 to 50; 4 = 51 to 100; 5 = more than 100; and 6 = fibrosis areas of several shapes followed by fibrosis points.

In order to correlate the predominance of scores of testicular fibrosis points (STFP) with their ages, the animals were subdivided into three age classes: 1 = 2.9 to 3.9 years old; 2 = 4.0 to 6.9 years old; and 3 = 7.0 to 11.8 years old.

For the statistical analyses it was used the software SAEG version 9.1 (SAEG-UFV 2007). Descriptive analyses for averages and standard deviation were performed for all variables under study. The Lilliefors test was used to verify the data normality and the variation homogeneity among the treatment groups was evaluated using the Cochran-Bartlett test. ANOVA was used to analyze the effect of constituted groups related to testicular biometry, physical and morphological semen features, and intensity of the ultrasound resolution. When the effect was detected through F test, the averages were compared by the Tukey test (5%). The non-parametric analysis was used with the Kruskal-Wallis or Wilcoxon test (5%) for all characteristics that did not meet the ANOVA assumptions (data normality and/or variation homogeneities). Categorical data were organized in contingency tables and analyzed through Chi-square test ( $\chi^2_{g1} = 3.84$ ;  $p = 0.05$ ). Both the Pearson Single Correlations (quantitative characteristics) and the Spearman Correlations (categorical characteristics) were performed for testicular and seminal characteristics and ultrasound examination.

## RESULTS

The animals under study were classified as 87.2% (95/109) sound for breeding, and 12.8% (14/109) unsound for breeding. According to this breeding soundness class, the average values for SC and TV did not differ between them (Table 1;  $p > 0.05$ ). The

Table 1. Reproductive characteristics of adult Montana Tropical Compound breed bulls, raised extensively, classified as sound and unsound for breeding.

Characteristics	Sound	Unsound
SC (cm)	41.5 ± 3.1a	41.3 ± 3.8a
TV (cm <sup>3</sup> )	1,606.6 ± 314.9a	1,466.4 ± 310.3a
MOT (%)	72.6 ± 9.1a	65.0 ± 13.4b
VIG (0-5)	3.3 ± 0.5a	2.9 ± 0.5b
MAJDEF (%)*	12.0 ± 5.9b	41.2 ± 19.2a
MINDEF (%)*	4.0 ± 3.4b	7.2 ± 5.4a
TDEF (%)*	16.0 ± 7.4b	48.5 ± 22.4a

a,b = Average values followed by small letters in the same line differ among themselves through ANOVA ( $p < 0.05$ ); \*Non-parametric analysis through Wilcoxon test ( $p > 0.05$ ); Average ± sd = Average and standard deviation; MOT = Rectilinear progressive sperm motility; VIG = Sperm strength; MAJDEF = % of major defects; MINDEF = % of minor defects; TDEF = % of total defects.

average values of the physical and morphological semen features of animals evaluated according to the breeding soundness are presented in Table 1, where sound animals had higher averages of rectilinear progressive sperm motility (72.6% vs. 65.0%) than animals unsound for breeding ( $p < 0.05$ ). Regarding the morphological features, sound animals had lower averages in relation to the unsound animals ( $p < 0.05$ ), with highlight to the MAJDEF that were of 12.0% and 41.2%, for sound and unsound animals, respectively.

The average values of testicular echotexture for left and right testes and the average of both testes are recorded in Table 2. Left testis PI was different for sound (PI = 98.8) and unsound animals (PI = 90.8;  $p < 0.05$ ). However, there were no differences between sound and unsound animals for breeding ( $p > 0.05$ ) when the PI average of the testicular images under study was compared. There was a difference in the PI between left and right testis for sound animals ( $p < 0.05$ ), with higher averages to the left testis (PI = 98.8) than to the right testis (PI = 91.8).

Regarding the evaluation of the testicular fibrosis points, the general frequencies of animals within each score for animals considered as sound and

Table 2. Pixel intensity of the testes of adult Montana Tropical Compound breed bulls, raised extensively, classified as sound and unsound for breeding.

Characteristics	Sound	Unsound
LT	98.8 ± 12.5aA	90.8 ± 12.4bA
RT	91.8 ± 13.4aB	93.9 ± 14.7aA
LRT	95.3 ± 10.6a	92.4 ± 12.4a

a,b = Average values followed by small letters in the same line differ among themselves through ANOVA ( $p < 0.05$ ); A,B = Average values followed by capital letters in the same column differ among themselves through Tukey test ( $p < 0.05$ ); Average±sd = Average and standard deviation; LT = Left Testis; RT = Right Testis; LRT = Average between left and right testes.

unsound for breeding was verified both the score of fibrosis points of the left testis (STFPL), and the score of fibrosis points of the right testis (STFPR). Score 6 (fibrosis areas of several shapes followed by fibrosis points) was not observed in any breeding soundness classes of the current study. Also, there was no difference in the STFP between left and right testis within each age class, through Chi-square test ( $\chi^2_{gl_1} = 3.84$ ;  $p = 0.05$ ), with the exception of scores 1 and 2, where unsound animals presented higher frequency of these scores for the left (35.7 vs. 12.6% for unsound and sound animals, respectively, for score 1) and right (42.9 vs. 16.9% for unsound and sound animals, respectively, for score 2) testis.

Furthermore, the animal frequencies according to age classes were calculated, both for the score of fibrosis points of STFPL and STFPR. However, it was not possible to perform the statistical analysis for animals considered as sound and unsound for breeding, within each age class, due to the small number of animals under study.

Score 1 had higher frequency of animals between 4.0 and 6.9 years old (age class 2) when compared to age class 3, but it was not different from age class 1 ( $p < 0.05$ ). In score 4, there was a lower frequency of animals of age class 2 ( $p < 0.05$ ). There was no difference in the frequency of animals between age classes for the other scores of testicular fibrosis points ( $p > 0.05$ ).

The PI of the left testis had a low negative correlation with TV ( $r = -0.16$ ). There was also a low correlation of PI between both testes (LRT) with STFPL ( $r = 0.17$ ). The STFP had a low correlation with SC ( $r = 0.25$  and  $0.24$  for STFPL and STFPR, respectively), TV ( $r = 0.27$ ), and an average correlation with the age class ( $r = 0.35$  and  $0.34$  for STFPL and STFPR, respectively). The other characteristics had no correlation with the ultrasound examination features under study ( $p > 0.05$ ).

## DISCUSSION

The average SC for sound and unsound animals was 41.5 and 41.3 cm, respectively (Table 1), and there was no difference between them ( $p > 0.05$ ). When Miranda Neto (2001) evaluated younger Montana Tropical Compound breed bulls, than in this study (24 months of age), he observed lower averages of 37.5 cm for SC. Likewise, when Fernandes Junior & Franceschini (2007) studied 3,636 bulls of the same genetic group, they had 36.0 cm

of SC, at the same age of Miranda Neto (2001). Pinho et al. (2012) registered similar averages for SC, with 37.2 and 35.2 cm for sound and unsound animals, respectively, when they worked with Montana bulls from 18 to 22 months of age. A higher SC was observed in this study, but in elder animals

Regarding TV, this study had averages of 1,606.6 and 1,466.4 cm<sup>3</sup> for sound and unsound bulls, respectively ( $p > 0.05$ ; Table 1). Fernandes Junior & Franceschini (2007) recorded an average of 780.95 cm<sup>3</sup>, but for younger Montana Tropical Compound breed bulls of 24 months of age.

The differences observed ( $p < 0.05$ ) in the physical and morphological semen features (Table 1) were already expected, since sperm analysis was the criteria used for the breeding soundness classification. For animals sound for breeding, the averages of rectilinear progressive sperm motility (72.6%) and sperm strength (3.3 – Table 1) are in accordance with the patterns recommended by the CBRA (1998) and higher than to the unsound animals. Nonetheless, Pinho et al. (2012) did not observe any differences between physical semen features for sound and unsound animals for breeding, although these results were obtained for younger animals.

In agreement with the present study (Table 1), Fernandes Junior & Franceschini (2007) also observed differences for MAJDEF, MINDEF and TDEF, respectively of 14.5; 5.2 and 19.7% for sound bulls, and 43.4; 7.6 and 51% for unsound bulls.

Regarding testicular echotexture (Table 2), there is no further literature referring to Montana Tropical Compound breed animals. A difference in PI between the testes was observed for sound animals only (Table 2;  $p < 0.05$ ), but there was no difference in PI averages between the classes of sound and unsound bulls for breeding ( $p > 0.05$ ). Nevertheless, Aravindakshan et al. (2000) and Cardilli et al. (2009b) did not record any difference in PI for the left and right testes.

The PI of the animals used in this study presented moderate echogenicity (95.3 and 92.4 for sound and unsound animals, respectively). For adult taurus animals, Abdel-Razek & Ali (2005) also observed moderately echogenic testicular parenchyma, while Cardilli et al. (2009a) and Cardilli et al. (2010) recorded testicular parenchyma for young Nelore bulls, as homogeneous and with low echogenicity. Thus, the animals in this study are more similar to taurus than zebu, regarding the testicular echotexture.

Carmo et al. (2012) observed, in Guzerat breed bulls, higher PI than the ones in this study (Table 2), with  $127.5 \pm 46.2$  in animals aged from 27.1 to 30 months. Likewise, Brito et al. (2002) studied animals with ages varying from 18 to 184 months, recording an average of PI of 196.1 for crossbred bulls, 192.7 for *B. t. indicus*, and 190.7 for *B. t. taurus*. Hahn et al. (1999) verified differences in the echogenicity of elder bulls when compared to younger bulls.

According to McEntee (1990), the testicular fibrosis could be a consequence of hits, backward kicks or headstalls, or even trauma caused by hits of testes on hamstring caused during the act of locomotion, and that could occur in any animal age. This occurs mainly with elderly bulls raised extensively under multiple breeding systems, as was the case of the animals under this study. This conclusion can be made due to the differences observed in the testicular fibrosis grades related to the age classes of the evaluated animals.

Concerning the identification of fibrotic lesions of the testicular parenchyma, there was no correlation of the testicular commitment grade by the STFP observed with the semen quality ( $p > 0.05$ ) between sound and unsound bulls. Likewise, Eilts & Pechman (1988) studied 78 bulls from 13 to 31 months of age, observing testicular fibrosis focus in 15 of them, but with any fibrosis focus correlations with sperm pathologies and, consequently, with reproductive capability. Hahn et al. (1999) evaluated 68 bulls aged from 1.6 to 12 years, observing the presence of fibrotic tissue in 26 animals (38%), but without affecting semen quality.

Similarly, when Barth et al. (2008) evaluated *B. t. taurus* bulls from 18 to 20 months of age ( $n = 105$ ), they also did not observe any relationship between the severity of the fibrotic injuries and the semen quality. However, Chapwanya et al. (2008) studied 32 bulls with an average of 5.6 years old, identifying seven bulls with testicular injuries detectable through ultrasound examination, with two of them having testicular fibrosis grades to the point that compromises semen quality.

There was also no difference between the left and right testes within each age class for the STFP. Eilts & Pechman (1988) and Barth et al. (2008) also did not observe any difference in STFP between left and right testis.

Opposite to this study, where no significant correlations of PI with the testicular biometry featu-

res were observed, Cartee et al. (1989) recorded correlation of PI with SC and TV in adult animals. Cardilli et al. (2009b), also recorded high and positive correlations of PI with TV ( $r = 0.77$ ) and SC ( $r = 0.83$ ). In the present study, no relationship of PI with sperm pathologies was found, since there was no difference in PI between sound and unsound animals. In Brito et al.'s study (2003), which evaluated animals during the recovering of the spermatid frame after scrotal insulation, PI presented positive correlation with MAJDEF in *B. t. taurus*.

## CONCLUSION

Pixel analysis of testicular ultrasonograms, were not predictive of semen quality of adult Montana Tropical Compound breed bulls. In addition, the several grades of testicular fibrosis did not affect the seminal quality.

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## REFERENCES

- Abdel-Razek A.Kh. & Ali A. Development Changes of Bull (*Bos taurus*) Genitalia as Evaluated by Caliper and Ultrasonography. *Reprod. Domestic Anim.*, 40:23-27, 2005.
- Aravindakshan J.P., Honaramooz A., Bartlewski P.M., Beard A.P., Pierson R.A. & Rawlings N.C. Pattern of gonadotropin secretion and ultrasonographic evaluation of developmental changes in the testis of early and late maturing bull calves. *Theriogenology*, 54:339-354, 2000.
- Bailey T.L., Monke D., Hudson R.S., Wolfe D.F., Carson R.L. & Riddell M.G. Testicular shape and its relationship to spermatid production in mature holstein bulls. *Theriogenology*, 46:681-887, 1996.
- Barth A.D., Alisio L., Avilés M., Arteaga A.A., Campbell J.R. & Hendrick S.H. Fibrotic lesions in the testis of bulls and relationship to semen quality. *Anim. Reprod. Sci.*, 106:274-288, 2008.
- Blom E. The ultrastructure of some characteristic spermatid defects and a proposal for a new classification of the bull spermiogram. *Nord. Vet. Med.*, 53:383-391, 1973.
- Brilo L.F.C., Silva A.E.D.F., Rodrigues L.H., Vieira F.V., Deragon L.A.G. & Kastelic J.P. Effect of age and genetic group on characteristics of the scrotum, testes and testicular vascular cones, and on spermatid production and semen quality in AI bulls in Brazil. *Theriogenology*, 58:1175-1186, 2002.
- Brilo L.F.C., Silva A.E.D.F., Barbosa R.T., Unanian M.M. & Kastelic J.P. Effects of scrotal insulation on spermatid production, semen quality and testicular echotexture in *Bos indicus* and *Bos indicus* x *Bos taurus* bulls. *Anim. Reprod. Sci.*, 79:1-15, 2003.
- Cardilli D.J., Toniollo G.H., Pastore A.A., Canola J.C. & Mer-

- cadante M.E.Z. Alterações do padrão ultrassonográfico do parênquima testicular em bovinos jovens da raça Nelore. *Acta Sci. Vet.*, 37:10-13, 2009a.
- Cardilli D.J., Toniollo G.H., Pastore A.A., Canola J.C. & Mercadante M.E.Z. Ultrasonographic study of testicular development in young Nelore bulls raised in extensive management system. *Anim. Reprod.*, 6(supl.1):252, 2009b.
- Cardilli D.J., Toniollo G.H., Pastore A.A., Canola J.C., Mercadante M.E.Z. & Oliveira J.A. Padrão ultrassonográfico do parênquima, mediastino e túnicas testiculares em bovinos jovens da raça nelore. *Cienc. Anim. Bras.*, 11:899-905, 2010.
- Carmo A.S., Osorio J.P., Mendonça L.F., Lago L.A., Henry M. & Jaramillo L.C. Aspectos biométricos y ultrasonográficos del desarrollo testicular en bovinos de la raza Guzerat (*Bos taurus indicus*). *Rev. Cienc. Anim.*, 5:51-62, 2012.
- Cartee R.E., Gray B.W., Powe T.A., Hudson R.S. & Whitesides J. Preliminary implications of B-mode ultrasonography of the testicles of beef bulls with normal breeding soundness examination. *Theriogenology*, 31:1149-1157, 1989.
- Chapwanya A., Callanan J., Larkin H., Keenan H. & Vaughan L. Breeding soundness evaluation of bulls by semen analysis, testicular fine needle aspiration cytology and trans-scrotal ultrasonography. *Irish Vet. J.*, 61:315-318, 2008.
- CBRA. Manual Para Exame Andrológico e Avaliação de Sêmen Animal. 2ª ed. Colégio Brasileiro de Reprodução Animal, Belo Horizonte, 1998. 49p.
- Eilts B.E. & Pechman R.D. B-mode ultrasound observations of bulls testes during breeding soundness examinations. *Theriogenology*, 30:1169-1175, 1988.
- Fernandes Junior J.A. & Franceschini P.H. Maturidade sexual e biometria testicular de touros jovens Compostos Montana Tropical criados a pasto. *Ars Vet.*, 23:59-66, 2007.
- Ferraz J.B.S., Eler J.P. & Golden B.L. Análise genética do composto Montana Tropical. *Rev. Bras. Reprod. Anim.*, 23:111-113, 1999a.
- Ferraz J.B.S., Eler J.P. & Golden B.L. A formação do composto Montana Tropical. *Rev. Bras. Reprod. Anim.*, 23:115-117, 1999b.
- Fields M.J., Burns W.E. & Warnick A.C. Age, season and breed effects on testicular volume and semen features in young beef bulls. *J. Anim. Sci.*, 48:1289-1304, 1979.
- Hahn J., Stouffer J.R. & Foote R.H. Ultrasonographic and Other Testicular Characteristics of Holstein Bulls Revisited. *J. Reprod. Develop.*, 45:405-410, 1999.
- Hancock J.L. The morphology of boar spermatozoa. *J. Royal Microsc. Soc.*, 76:84-97, 1957.
- McEntee K. Scrotum and testis: Anatomy and congenital anomalies, p.224-251, In: McEntee K. (Ed.), *Reproductive pathology of domestic mammals*. New York, Academic press, 1990.
- Miranda Neto T. Puberdade e Maturidade sexual em touros compostos Montana Tropical. Dissertação (Medicina Veterinária), Universidade Federal de Viçosa, 2001. 72f. (Disponível em: <<http://www.yumpu.com/pt/document/view/13021318/tamires-miranda-neto-puberdade-e-maturidade-ufv>>).
- Pinho R.O., Martins L.F., Siqueira J.B., Domeneck F., Miranda Neto T. & Guimarães J.D. Avaliação da qualidade do sêmen fresco de touros jovens da raça Composto Tropical Montana e suas correlações com o teste hiposmótico. *Acta Vet. Bras.*, 6(3):192-198, 2012.
- SAEG. Sistema de análise estatística e genética - SAEG versão 9.1. Viçosa, Central de Processamento de Dados - UFV, 2007. 68f.