



Body and testicular biometry of Crioulo stallions

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ABSTRACT: Brazilian Association of Crioulo Horse Breeders (ABCCC) establishes minimal and maximal body biometrics evaluation; although, nothing is mentioned about testicular measurements. Body and testicular growth are associated and related to age. We described data regarding body and testicular biometry of Crioulo stallions, with a comparative study between young and adult categories. Evaluation was performed in 56 stallions, Crioulo breed, split in: youngsters (3 and 4 years-old, n=16) and adults (above 5 years-old, n=40). Body biometry included weight, height, cannon bone and chest circumference, body score condition and neck fat accumulation. Testicular biometry included height, length, width, volume, combined volume and daily sperm output (DSO). Statistical analyses included descriptive statistic, Pearson correlation, comparison between means by Kruskal Wallis, being $P < 0.05$ considered significant. There was no significant difference between the parameters of body and testicular biometrics between categories young and adult. In relation to height, the average was very close to the lower limit established, while chest and cannon bone circumference were above the minimum recommended by ABCCC. Cannon bone circumference presented a positive correlation with height. Most of stallions presented excessive body fat, with a body score condition above 8 (scale 1-9). Neck fat accumulation presented a positive correlation with body score condition. Testicular height, length, width and volume presented a positive correlation between the ipsi and contralateral testicle, total testicular volume and DSO. In conclusion, no difference in the body and testicular biometric evaluation was observed between young and adult Crioulo stallions. Testicular and body growth are associated and also related to age, so our finding suggested that after 3-4 years-old most of Crioulo stallions have already reached maximum growth.

Key words: growing, foal development, puberty, morphology, morphometry.

Biometria corporal e testicular de garanhões da raça Crioula

RESUMO: A Associação Brasileira de Criadores de Cavalos Crioulos (ABCCC) define parâmetros mínimos e máximos para algumas aferições biométricas no corpo do animal, embora nada seja mencionado sobre medidas testiculares. O crescimento corporal e testicular estão associados e relacionados a idade do animal. Objetivamos descrever parâmetros relacionados a biometria corporal e testicular de garanhões Crioulos, comparando o grupo de jovens e adultos. Foi realizada avaliação em 56 garanhões Crioulos, divididos em: jovens (três e quatro anos, n=16) e adultos (acima de cinco anos, n=40). A biometria corporal incluiu peso, altura, circunferência de canela, perímetro torácico, escore corporal e acúmulo de gordura no pescoço. A biometria testicular incluiu altura, comprimento, largura, volume de cada testículo, volume combinado dos testículos e estimativa de produção diária de espermatozoides (DSO). Com os dados, foi realizada estatística descritiva, correlação de Pearson, comparação entre médias com Kruskal Wallis, sendo considerado significativo quando $P < 0,05$. Não foi observada diferença significativa entre as avaliações biométricas e testiculares de garanhões das categorias jovens e adultos. Em relação à altura, a média foi muito próxima ao limite inferior estabelecido, enquanto a circunferência torácica e de canela estavam acima do limite mínimo recomendado pela ABCCC. A circunferência de canela apresentou correlação positiva com altura. A maioria dos garanhões apresentou alta deposição de gordura, com condição corporal acima de 8 (escala 1-9). O acúmulo de gordura no pescoço apresentou correlação positiva com escore corporal. A altura, largura, comprimento e volume testicular apresentaram correlação positiva com testículo ipsi e contralateral, volume testicular total e DSO. Em conclusão, não houve diferença entre dados de biometria corporal e testicular de garanhões Crioulos adultos e jovens. O crescimento corporal e testicular estão associados e relacionados a idade, sendo assim, nossos resultados sugerem que após 3-4 anos de idade, a maioria dos garanhões Crioulos já atingiu seu pico de desenvolvimento.

Palavras-chave: desenvolvimento de potros, crescimento, morfologia, morfometria, puberdade.

INTRODUCTION

The Crioulo horse of southern Latin America is a direct descendant of the horses brought to the New World by the Spanish and Portuguese

conquers, during the sixteenth century (RODERO et al., 1992). This breed is a descendant of native horse from those territories, being originated from *Equus caballus asiaticus*, which also originated Arabian breed, and *Equus caballus africanus*. In Brazil,

many of these animals escaped or were abandoned, reproducing freely in herds, returning to a wild state and being submitted to natural selection.

The main characteristics inherited from their ancestors are average height; short, triangular, straight or subconvex head; short, well-separated ears, broad at the base, raised neck, slightly sloping rump, abundance of horsehair and tail and active temperament (ABCCC, 2020). The Crioulo horse has morphological, functional and behavioral characteristics inherent to the breed. The selection methods currently used in reproduction farms are subjective, based on the owners' experience and personal preferences. Also, mares and stallions, which won competitions, are elected to become breeders.

Regarding body biometry, measures adopted by Brazilian Association of Crioulo Horse Breeders (Associação Brasileira de Criadores de Cavalos Crioulos – ABCCC) for males are: height between 140-150 cm; minimum chest circumference of 168 cm; minimum cannon bone circumference of 18 cm. For females the measures are height between 138-150 cm; minimum chest circumference of 170 cm; minimum cannon bone circumference of 17 cm.

Regarding testicular biometry, the amount of sperm that a stallion produces varies according to the amount of functional testicular tissue, which is correlated with testicular size. Measurement of testicular size is an important factor, considering the selection and management of breeders aiming maximum reproductive efficiency (GEBAUER et al., 1974; PICKETT, 1993; SUDA et al., 1992).

According to ABCCC, from 2001 until the end of 2010 there was an increase of 143.7 percent in applications for registration of temporary records, jumping from 10.271 in 2001 to 25.020 in 2010. Based on this growth, the Association estimates that this number could reach 57.502 in the year 2020, an increase of approximately 129.82 percent over 2010.

Growing expansion and appreciation of Crioulo breed encourages scientific research in the area, since this activity has a significant economic impact in Brazil. However, currently there are still few studies describing the biometric characteristics of Crioulo stallions. Considering this situation, this research aimed to describe data related to body and testicular biometrics of Crioulo stallions, comparing young and adults.

MATERIALS AND METHODS

Body and testicular biometrics were evaluated in 56 Crioulo stallions, located in the state

of Rio Grande do Sul (RS), Brazil. Age of the animals was obtained in the Genealogical Registration section of ABCCC, ranging between 3-18 years. The animals were classified as young, between 3-4 years ($n = 16$) and adults, above 5 years ($n = 40$).

All stallions had the ABCCC mark on the right hind limb. This mark means that the stallion is within the reference body measures (height, chest and cannon bone circumference) and morphological features adopted by ABCCC; consequently, able to be used as breeders.

Body biometrics was assessed with a measuring tape, weight tape, hypometer and ultrasound, including: Weight (circumference measured with a weight tape positioned just after the withers end); Height (distance between the ground and the height at the withers, measured with a hypometer); Cannon bone circumference (circumference of the left thoracic limb in the metacarpus middle third); Chest circumference (circumference measurement using a tape positioned just after the end of the withers); Body condition score (ECC – scale of 1-9 by visualization and palpation, according to Henneke Chart scale); Neck fat (measurement of subcutaneous fat at the neck crest by ultrasound, with a linear transducer and a frequency of 5MHz).

Testicular biometry was measured with a pachymeter, in the left and right testicles, being evaluated: Width (distance between the lateral and the medial side); Height (distance between the dorsal and the ventral surfaces); Length (distance between cranial and caudal pole); Testicular volume (width x height x length x 0.5333); Combined testicular volume (right testicular volume + left testicular volume); Daily sperm output (DSO) [(combined testicular volume * 0.024) - 0.76], as described by LOVE et al., (1991), which estimates sperm production $\times 10^9$.

Descriptive statistical analysis (minimum, maximum, average and standard error of the mean) of variables were performed, as well as normality test, comparison of means by Kruskal Wallis and Pearson's correlation. Tests were executed with Statistix 9® (Statistix, Statistix 9 for Windows, Analytical Software, Tallahassee, FL, USA, 2008), being $P < 0.05$ considered significant.

RESULTS AND DISCUSSION

In body biometry data, there was no difference between young and adult stallions (Table 1). In the present study, only animals from 3 years old (after the end of the growth phase) were included and; consequently, animals that had already reached their

Table 1 - Average \pm standard error mean (SEM) of Crioulo stallions body biometry.

Body biometry	Young (n=16)	Adult (n=40)
Height (cm)	141.1 \pm 2.3	141.4 \pm 0.4
Weight (kg)	449.4 \pm 11.5	421.7 \pm 7
Chest circumference (cm)	179.4 \pm 4.3	175.7 \pm 4.2
Cannon bone circumference (cm)	19 \pm 0.2	19.2 \pm 0.1
ECC (scale 1-9)	7.4 \pm 1.2	7.4 \pm 0.1
Neck fat (cm)	9.4 \pm 0.1	8.7 \pm 0.4

*No statistical difference was observed between young and adult.

Young: 3-4 years-old.

Adult: 5 or more years-old.

ECC: body score condition.

maximum growth and height. In Crioulo breed horses, PIMENTEL et al. (2017) reported that immediately after birth the foal has 62.14% of the adult height; at 12 months about 89.28%; at 24 months 95.71%; and at 36 months 98.57%.

The average height was close to the lower limit (141.3 cm young and 141.4 cm adults) recommended by ABCCC (minimum 140 and maximum 150 cm). However 10.7% (6/56) of stallions were 138 cm tall at the time of the evaluation. These data corroborate with those reported in the literature, by KURTZ FILHO and LÖF (2007), where the authors reported that males (n = 866, age 2-13 years) had an average height close to the minimum limit and attributed this finding to the probable use of ascendants with short stature. In some countries, such as Chile, this characteristic is attributed to greater racial purity and adaptation to national equestrian events (FCCC, 2020).

Regarding the chest circumference, mean values (179.9 cm young and 175.7 cm adults) were above the minimum recommended by the breed association (168 cm). The thoracic perimeter showed a positive correlation with ECC and weight. As reported by KURTZ FILHO & LÖF (2007), average thoracic perimeter of horses in an agricultural show was 179 cm (n = 866, age 2-13 years), with a minimum value of 168 and a maximum of 196 cm.

Regarding cannon bone circumference, all stallions showed values equal or greater (19 cm young and 19.2 cm adults) than recommended by the breed association (18 cm). The cannon bone circumference showed a positive correlation with height. Similar results have been reported in the literature, by

KURTZ FILHO & LÖF (2007), with a mean cannon bone perimeter of 20 cm and an amplitude of 18-22 cm (n = 866, age 2-13 years).

In the group of young horses, all of them were kept most of the time in stables, in intensive management, in preparation for morphological exhibitions or sports competitions. In the group of adults, most of the stallions were in a semi-extensive management, being kept in stables during the night and released in paddocks during the day. In youngster, average weight (449 kg) and neck accumulated fat (9.4 cm) were higher than in adults (421 kg and 8.7 cm, respectively) ($P > 0.05$). This finding can be attributed to the intense preparation for morphological exhibitions, especially in young animals. Neck fat showed a positive correlation with ECC. Overweight associated with physical exercise increases the risk of musculoskeletal injuries, in addition to these, those are stressful for the joints (MCILWRAITH, 2004). Fat accumulation can cause chronic inflammatory process that affects the entire organism, including joints, muscles, hooves, liver, pancreas, uterus and testicles. Consequently, fat accumulation predisposes to numerous clinical-metabolic problems (FRANK, 2009), being undesirable considering the animal's general health.

Body score assessment in horses is a simple and easy method that is used to estimate and monitor body fat accumulation. In mares, this parameter is related to reproductive efficiency, including estrous cycle duration, conception rate, interval between births, duration of pregnancy and embryonic losses (HENNEKE et al., 1983). About 34% (19/56) of the evaluated stallions had a body score condition below or equal to 7 and 66% (37/56) had a body score

between 8 and 9. Therefore, most animals had excess of fat at the time of assessment. In humans, obesity is associated with subfertility and infertility, due to changes in sperm quality parameters (DU PLESSIS et al., 2010). In ruminants, a high body score impairs sperm quality. However, no difference was observed between sperm parameters after thawing of semen from Crioulo stallions, with low or high body score, suggesting that only the assessment of body fat is not able to explain sperm quality after thawing (NOVELLO et al., 2020).

Data regarding testicular biometry are shown in table 2. Among the parameters evaluated, there was no significant difference between the linear measurements of right and left testicles, similar to that described in the literature by GREGORY (2013) and MENDES (2012) in Crioulo horses; by BORGES et al. (2010), PARLEVLIE et al. (1994) and WADDINGTON et al., (2017) in other breeds. Testicular height, length, width and volume showed a positive correlation between ipsi and contralateral testis, testicular volume and DSO.

Testicular growth occurs gradually and proportional to body growth from the 12th month of life until puberty (NADEN et al., 1990). In Crioulos foals, the first epididymal sperm was reported at 16 months of age, when the testicular volume reached 16 cm³ and testicular weight of 23 grams (GREGORY, 2013). The number of Sertoli cells, weight and testicular size increase with age, stabilizing when the stallion reaches sexual maturity, about 4-5 years in most breeds (THOMPSON, 1979). In the present study, no

difference was reported between testicular biometric data between young and adult horses. Corroborating with the literature, these findings suggest that after 3-4 years of age, testicular measurements have little variation, considering that they have already reached their maximum growth size.

About testicular biometry, there are no data with minimum and maximum values recommended by the breed association (ABCCC). Corroborating with Mendes (2012), biometric evaluation of Crioulo horses over 3 years of age (n = 19) showed similar values to the ones reported in our study, being TE width (48.2 ± 1.9), TE length (82.8 ± 2.6), TE height (44.4 ± 2.0), TD width (49.2 ± 1.9), TD length (85.2 ± 2.0) and TD height (43.8 ± 1.9), in millimeters (MENDES, 2012).

Considering other breeds, values of testicular biometry of Crioulo stallions are lower than those reported for Campolina (BORGES et al., 2010; MANSO FILHO et al., 2000) and Mangalarga Marchador (CRUZ et al., 2009; WADDINGTON et al., 2017). The Crioulo breed is considered small to medium sized, while the other reported breeds are considered medium to large sized. Therefore, it is believed that testicular size of Crioulo stallions could be related to breed size, with no reduction in fertility and/or sperm production capacity. All stallions included in the present study were used in the last breeding season with proven *in vivo* fertility.

The variation existing in the mean of testicular measurements between breeds has already been attributed to different sizes, especially when

Table 2 - Average ± standard error mean (SEM) of Crioulo stallions testicular biometry.

Testicular biometry	Young (n=16)	Adult (n=40)
Height LT (cm)	5.3 ± 0.1	5.4 ± 0.1
Width LT (cm)	4.7 ± 0.2	4.4 ± 0.1
Lenght LT (cm)	8 ± 0.3	7.9 ± 0.1
Volume TE (cm ³)	110 ± 10.8	103.3 ± 6.6
Height RT(cm)	5.7 ± 0.2	5.5 ± 0.1
Width RT (cm)	4.6 ± 0.1	4.6 ± 0.1
Lenght RT (cm)	7.6 ± 0.3	7.7 ± 0.2
Volume TD (cm ³)	107 ± 8.1	100 ± 14.3
Testicular combined volume (cm ³)	218 ± 16	210 ± 13
DSO (x10 ⁹)	4.4 ± 0.3	4.4 ± 0.2

*No statistical difference was observed between young and adult.

Young: 3-4 years-old.

Adult: 5 or more years-old.

LT: left testicle; RT: right testicle; DSO: daily sperm output.

these measurements are measured in equine ponies (PACCAMONTI et al., 1999). In addition, the minimum total testicular width for stallions should be 8 cm, according to PICKETT (1993); although, it is not specified about the breed or size of the animal.

According to CBRA (2013), in adult horses scrotal width cannot be less than 7 cm. In our study, all stallions had a total testicular width above this value (9.3 cm young and 9 cm adults). In addition, a testicular biometric pattern may exist among horses ranging between individuals of the same breed (FONSECA et al., 1997), with testicular size influenced by genetic inheritance, body score, feeding, age, among other factors.

Volume is a combination of linear testicle measurements (height, length and width) being this parameter used to suggest stallions with high or low reproductive potential. In dogs (OLAR, 1983), bulls (FRENEAU, 2006) and horses (LOVE et al., 1991) the largest testicular size coincided with the largest number of sperm produced and ejaculated. The testicular size and weight of the parenchyma are correlated with daily sperm production, being a parameter for predicting a stallion's reproductive potential (CHENIER, 2007).

Testicles size can be used to calculate the estimated number of sperm available in the ejaculation, which indirectly allows estimating daily sperm production (PICKETT & VOSS 1998). As testicular volume increases, the number of sperm in the ejaculate also increases (CLAY & CLAY, 1992), considering healthy animals. In stallions, DSO is between 4 to 6 x 10⁹ spermatozoa/ejaculate/day; however, this parameter is greatly influenced by the animal's age and season (HURTGEN, 1992), in addition to individual characteristics. In the present study, both young and adult stallions showed an average daily sperm production of 4.4 x 10⁹, being within the limits described in the literature (HURTGEN, 1992). Calculation of DSO uses only linear measurements of the testis, obtained by direct measurement or with ultrasound. Testicular biometrics can be easily included during breeding soundness examination of the stallion. These measurements associated with sperm quality evaluation help to predict the stallion's reproductive potential. However, the depletion of extragonadal sperm reserves, followed by breeding soundness exam, allows a better understanding of sperm quality and production of each individual. Consequently, the results of this evaluation help to adjust the individual's reproductive management, maximizing the use of the stallion, during breeding season.

CONCLUSION

Body and testicular biometry presented no difference between young (3 and 4 years old) and adults (over 5 years old) Crioulo stallions. Since testicular and body growth are associated and related to age, our finding suggests that after 3-4 years of age most of Crioulo stallions have already reached their maximum growth size.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This experiment used animals in the experimental model and had the approval of the Institutional Research Ethics Committee of Universidade Federal de Pelotas (UFPEL), Pelotas, Rio Grande do Sul, Brazil, by the number CEEA-9893-2014.

DECLARATION OF CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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AUTHOR'S CONTRIBUTIONS

All authors contributed to the development of this research.

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