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Testicular biometry of free-ranging feral pigs (*Sus scrofa* sp)

Biometria testicular em porcos Monteiros ("Sus scrofa" sp*) de vida livre*

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SUMMARY

This study evaluated the testicular biometry of free-ranging feral pigs with a focus on the gonadosomatic index, seminiferous tubule diameter, seminiferous epithelium height, volumetric proportion, volumetric proportion of the of testicular parenchyma components, and length of seminiferous tubules per testis and per gram of testis. Twenty free-ranging feral pigs were captured, castrated and released back into nature. The testes were fixed by tissue perfusion and embedded in plastic resin for histological examinations. Testis weight was approximately 121.0g and gonadosomatic index was 0.21%. Seminiferous tubule diameter was around 242µm and epithelium height was 85µm. The relative length of the seminiferous tubules was 15.7m per gram of testis. The testicular parenchyma was composed of 70% seminiferous tubules and 30% intertubular tissue. In conclusion, the testicular biometry of free-ranging feral pigs is quite similar to that observed in other Suidae species, varying only in terms of the volumetric proportion of testicular parenchyma components and the gonadosomatic index.

Keywords: gonadosomatic index, morphology, seminiferous tubule, testicle, volumetric proportion,

RESUMO

Objetivou-se com esta pesquisa avaliar a biometria testicular de porcos monteiros adultos de vida livre, dando ênfase ao índice gonadossomático, ao diâmetro dos túbulos seminíferos, à altura do epitélio seminífero, à proporção volumétrica, ao volume dos componentes do parênquima testicular e ao comprimento total dos túbulos seminíferos por testículo e por grama de testículo. Utilizaram-se 20 porcos monteiros adultos de vida livre que foram capturados, castrados e soltos novamente na natureza. Os testículos foram fixados por perfusão tecidual e incluídos em resina plástica para as análises histológicas. O peso testicular médio foi de aproximadamente 121,0g, o índice gonadossomático foi de 0,21%. O diâmetro tubular e a altura do epitélio seminífero foram, respectivamente, 242 e 85µm. Cada grama de testículo possuía aproximadamente 15.7m de túbulos seminíferos. Os túbulos seminíferos ocuparam cerca de 70%, enquanto o tecido intertubular ocupou cerca de 30% do parênquima testicular. Conclui-se que a biometria testicular de porcos monteiros é relativamente semelhante ao observado em outras espécies de suídeos, variando apenas na proporção volumétrica dos componentes do parênquima testicular e no índice gonadossomático.

Palavras-chave: índice gonadossomático, morfologia, proporção volumétrica, testículo, túbulo seminífero

INTRODUCTION

The feral pig (*Sus scrofa* sp), also known as Monteiro pig, is an exotic species (2n=38) that descends from domestic pigs brought to Brazil in 1778 by the founders of Albuquerque city (actually Corumbá city). Since the 18th century a large numbers of feral pigs have escaped and spread through Brazilian forests, where they found suitable reproductive conditions that gave rise to wild lineages. After successive transformations over 200 years, feral pigs currently resemble wild boars and are widely distributed throughout Brazil, particularly in the Pantanal region (DESBIEZ et al., 2009).

Feral pigs cause an ecological impact in the Pantanal because they compete with native species such as the collared and the white-lipped peccaries (family *Tayassuidae*). On the other hand, they are socioeconomically important to the local population as one subsistence hunting (LOURIVAL & FONSECA, 1997).

In recent years, the biotechnologies devoted to the reproduction of domestic animals have significantly improved production rates through the selection of individuals with high genetic value. However, the elementary reproductive physiology of most wild animals has not been thoroughly studied. Thus, the basic knowledge of the reproductive physiology of male feral pigs may be guiding their reproductive management to enlighten the factors mentioned above, the present study evaluates the testicular biometry of free-ranging feral pigs. The investigation focused on the gonadosomatic index (GSI), seminiferous tubule diameter, seminiferous epithelium height, volumetric proportion, volumetric proportion of testicular parenchyma components, and length of seminiferous tubules per testis and per gram of testis.

MATERIAL AND METHODS

Twenty adult free-ranging feral pigs were captured fron a farm in the region of Pantanal do Rio Negro, state of Mato Grosso do Sul, duringthe months of June and July (IBAMA license # 1916054), year using several rope loops. After being sedated with an intramuscular injection of 1.0mL / 20kg Azaperone (Suicalm®) and 10.0mg Diazepan (Diazepan®), the animals were subjected to bilateral orchiectomy using the routine procedure. The pigs were released and observed until they were completely recovered and had returned to their natural environment.

Immediately after removal, the testes were separated from the epididymides and their length and thickness measured with a caliper. Net testis weight was determined by subtracting tunica albuginea and mediastinum testis weight from testis weight. The gonadosomatic index (GSI) was calculated according to Costa et al. (20011).

The left testis of each animal was perfused for tissue fixation with Karnovisky solution, and the testicular fragments embedded in plastic resin (COSTA et al., 2004, 2007). The 4.0µm-thick histological sections were stained with 1% toluidine blue/sodium borate. The slides were mounted with Entellan® (Merck). The right testis was stored frozen and used to calculate the relative proportion of tunica albuginea and mediastinum.

The volumetric proportion of testicular parenchyma components was measured indirectly using digital images of the histological sections captured at 400x magnification and ImageJ 1.34 software (RASBAND, 2005). Calculations were based on 432 reference points located at the intersections of the graticule traced

over the areas of interest, i.e. the seminiferous tubules, Leydig cells and stroma (cells and fibers of connective tissue, nerves, blood vessels and lymphatic vessels). Twenty fields, randomly selected during horizontal scanning, were examined on each slide. Therefore, volumetric proportion (%) was calculated from 8640 points per testis.

The mean diameter of the seminiferous tubules of each testis was obtained from 20 cross-sectioned tubules, irrespectively of developmental stage. These sections were used to measure seminiferous epithelium height, which extends from the basement membrane to the luminal surface. The epithelium height of each tubule was the mean between two height measures taken at different portions of the tubule. Measurements were also performed on digital images captured at 400x magnification using ImageJ 1.34 software (RASBAND, 2005).

The total length of the seminiferous tubules was calculated according to Costa & Silva (2006). A linear retraction factor of 5% was considered, as proposed by Costa et al., (2007a).

The mean and standard deviation of data obtained in this study were determined using BioEstat 3.0 software (AYRES et al., 2003).

RESULTS

Feral pig weight ranged from 45.50kg to 76.60kg, with a mean body weight of approximately 57kg (Table 1). Mean testis weight was about 121.0g, while mean GSI was around 0.21%. Gross and net testis weight, and tunica albuginea and mediastinum weight and percentage in adult feral pigs are shown in Table 1.

Table 1. Means of Body weight, testis biometry, gonadosomatic index, tunica albuginea and mediastinum weight, and percentage (testis/body weight) proportion of 20 adult free-ranging feral pigs

Variables	Mean ± sd
Body weight (kg)	57.24 ± 8.24
Testis	
Gross Weight (g)	121.13 ± 39.25
Net Weight (g)*	110.92 ± 36.71
Length (cm)	8.39 ± 0.89
Width (cm)	5.26 ± 0.44
Thickness (cm)	5.12 ± 0.81
Gonadossomatic index	0.21 ± 0.05
Albuginea	
Weight (g)	9.33 ± 2.42
Proportion (%)	7.86 ± 0.92
Mediastinum	
Weight (g)	0.87 ± 0.31
Proportion (%)	0.72 ± 0.17
Albuginea+mediastinum	
Weight (g)	10.20 ± 2.67
Proportion (%)	8.58 ± 0.93

*Net weight is albuginea and mediastinum weight subtracted from gross testis weight.

The proportion of tunica albuginea corresponded to 7.86% of gross testis weight, whereas mediastinum represented only 0.72%. These structures accounted for 8.56% of testicular weight. Net testis weight was approximately 111g, as shown in Table 1.

The mean seminiferous tubule diameter of feral pigs was about 241 µm, with a seminiferous epithelium height of around 84 µm (Table 2). The total length of seminiferous tubules was approximately 1 898 m, representing 15 m of tubule per gram of testicle.

The volumetric proportion of testicular parenchyma components is shown in Table 2. The intertubular tissue (Leydig cells and stroma) occupied 29.86 % of the parenchyma and the tubular tissue (seminiferous tubules) 70.14%.

Table 2. Morphometry of the seminiferous tubules and volumetric proportion of testicular parenchyma components of free-ranging feral pigs

Variables (n=20)	Mean ± sd
Tubule diameter (µm)	241. 60 ± 23. 38
Epithelium height (µm)	84. 83 ± 8.46
Tubule length per testis (m)	1,898.07 ± 737.62
Tubule length per gram of testis (m)	15.74 ± 3.58
Volumetric proportion (%)	
Seminiferous tubule	70.14 ± 4.34
Leydig cells	25.71 ± 4.56
Stroma*	4.16 ± 1.66
Interbular tissue	29.86 ± 4.34

*Includes cells and fibers of connective tissue, nerves, blood vessels and lymphatic vessels.

DISCUSSION

Adult feral pigs subjected to conventional management for slaughter purposes generally weigh around 45kg (LOURIVAL & FONSECA, 1997). This can be compared to the results of the present study as well as with those for unspecialized swine breeds such as the African pig, of approximately 34kg (OKWUN et al., 1996), and the Vietnamese pig of 42kg (EVANS & KO, 1990). By contrast, specialized swine breeds are heavier than the feral pigs studied here (FRANÇA & CARDOSO, 1998). This difference is somehow expected because specialized pig breeds have undergone years of genetic improvement and are being raised under special management and nutrition conditions. This same reasoning can be used to explain why specialized breeds have heavier testis than those of feral pigs (FRANÇA, et al., 2005).

As a consequence of body and testis weight, the GSI of feral pigs is only half the value reported for swine breeds Piau (0.39%), Yorkshire (0.36%), crossbred Landrace X Large White (0.37%) and the African pig (0.39%) (FRANÇA & CARDOSO, 1998; FRANÇA, et al., 2005).

In relation to the testis weight, the proportion of tunica albuginea in feral pigs was similar to that reported for the Piau breed and wild boars (FRANÇA, et al., 2005; ALMEIDA et al., 2006; COSTA & SILVA, 2006), but only half the value obtained for white-lipped peccaries 14.02% (COSTA et al.,

2007a) and collared peccaries 13.14% (COSTA et al., 2004). This divergence likely occurs because peccaries have light testes, resulting in higher relative tunica albuginea weight (COSTA et al., 2004).

To validate the comparison of testis weight as well as the proportion of tunica albuginea in different studies, the animals investigated must be about the same age. This is necessary because, although the phase of highest testis development lasts until sexual maturity, it continues to grow afterwards at a lower rate (MURTA et al., 2010). Therefore, any discrepancies in results must be carefully analyzed to prevent methodological differences from invalidating comparisons.

The differences observed in weight and percentage of mediastinum, even among same-aged animals, may be caused by the well-known difficulty to isolate and completely remove this structure during dissection, possibly resulting in underestimated values (COSTA et al., 2004).

Most amniotes have seminiferous tubule diameters ranging from 160 to 350µm (COSTA & PAULA, 2003). The tubular diameter of feral pigs lies within this range, similar to a number of Suidae such as domestic breeds (FRANÇA & RUSSEL, 1998), wild boars (ALMEIDA et al., 2006, COSTA & SILVA, 2006), collared peccaries (COSTA et al., 2004) and white-lipped peccaries (COSTA et al., 2007a).

When comparing results of seminiferous tubule diameters within a same species or among different species, it is important to determine if the authors used different methodologies, and if so, whether there was a linear correction of tissue retraction. Several authors have reported tissue shrinkage during histological processing, especially when using paraffin inclusion (COSTA et al., 2004, COSTA et al., 2007a, MURTA et al., 2010). Shrinkage of 15 % was reported in paraffin inclusions and 3-5% in inclusions with plastic resin (MURTA et al., 2010). Once the necessary corrections have been made, data can be readily compared, thus avoiding under or overestimation errors.

Under normal conditions, the mean tubular diameter does not change significantly in sexually mature, non-seasonal animals (FRANÇA & RUSSELL, 1998). However, different breeds and lineages of seasonal species have wide variations in tubular diameter outside the reproductive period (BARROS et al., 2007). In addition to differences arising from genetic selection, which benefits individuals with higher spermatic production per testis weight, other factors justify the significant differences in the tubular diameter of mammals. These include the evaluation of sexually immature animals; variations in the number of myoid cell layers that form the tunica propria of the seminiferous tubules; differences in Sertoli and spermatogenic cell populations; and differences in the fluid secretion patterns of Sertoli cells, which determines the size of the tubular lumen (COSTA & PAULA, 2003).

The seminiferous epithelium height of the feral pigs studied here (84.83µm) is in accordance with the values reported for domestic animals, which range from 60 to 100µm (FRANÇA & RUSSELL, 1998), but higher than those reported by Okwun et al. (1996) for swine. This is an intriguing result because the feral pigs in this study were not subjected to a genetic improvement program. Nevertheless , natural selection favored individuals with higher spermatic production potential, evidenced by the greater seminiferous epithelium height. Therefore, if feral pigs are similar to specialized swine breeds in terms of Sertoli cell support

capacity, spermatogenesis duration and percentage of spermatogenic cell loss in mitosis and meiosis, their intrinsic rate of spermatogenic and daily sperm production is likely higher.

Total tubular length depends on testis volume, volumetric proportion of seminiferous tubules and tubular diameter. Therefore, species with similar measurements in these parameters are expected to have comparable tubular lengths. However, it is difficult to compare different species, since testis weight varies considerably. To solve this problem, measurements of total tubular length per testis are converted into tubular length per testis weight, providing a relative measure, irrespective of animal or testis size.

The average length of seminiferous tubule per gram of testis was 15.7 m in the pigs studied. This corresponds to peak amplitude in the interval described for most domestic species, which ranges from 10 to 15m of tubules per gram of testis (FRANÇA & RUSSEL, 1998). Further regarding this parameter, wild boars are superior to feral pigs, with approximately 18 m of tubules per gram of testis (ALMEIDA et al., 2006). The benefits of a longer tubule per gram of testis as well as wider seminiferous tubules can be analyzed along with other morphometric parameters because the numerical superiority of these variables is not necessarily associated to higher daily spermatic production.

The seminiferous tubules occupied nearly 70% of testis parenchyma in the studied feral pigs. The remaining 30% of the parenchyma, was composed of 87% Leydig cells and 13% cells and fibers of connective tissue, nerves, blood vessels and lymphatic vessels.

Swine species generally have a volumetric proportion of seminiferous tubules between 60 and 90% (COSTA & PAULA, 2003; MURTA et al., 2009). This proportion was 70.14% in feral pigs, and although this value is within the expected range, it is under that reported for adults of the Piau breed (82.9% - FRANÇA et al., 2005). Comparisons of different experiments should consider the testis development phase because in the postpubertal stage, for instance, small age differences may result in huge variations in the volumetric proportion of seminiferous tubules (MURTA et al., 2010). These variations are reduced in sexually mature animals or if the methodologies used to prepare the histological sections are similar. Finally, the number of animal studied must be large enough to prevent inconclusive results arising from insufficient data.

In feral pigs, parenchymal occupation with intertubular compartments was higher than that observed in domestic swine (OKWUN et al., 1996). The proportion of Leydig cells in these pigs (approximately 26% of testicular parenchyma), is one of the highest values reported for Suidae or Tayassuidae. This result does not imply in a greater potential for testosterone production because steroid synthesis is more dependent on the individual production capacity of each Leydig cell than on their volume and number (COSTA et al., 2007b).

Finally, the volume occupied by the stroma is similar to that reported in other studies on Suidae and Tayassuidae. Stroma volume seems to be quite regular in animals of a same species as well as in different swine species (COSTA & PAULA, 2003).

In conclusion, the testicular biometry of feral pigs is quite similar to that observed in other Suidae species, except for the volumetric proportion of testicular parenchyma components and the gonadosomatic index.

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