

# Daily sperm production and evaluation of morphological reproductive parameters of Murrah buffaloes in an extensive breeding system

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**Abbreviations:** SEM, scanning electron microscopy; TEM, transmission electron microscopy; FMVZ/USP, School of Veterinary Medicine at the University of São Paulo; SEC, seminiferous epithelium cycle; STTL, total length of the seminiferous tubules; VTTs, total volume of the seminiferous tubules; NTCS, total number of Sertoli cells; DSP, daily sperm production; FAPESP, Foundation for Research Support of São Paulo

The development of male sexual maturity varies among buffaloes. The Murrah buffalo is considered the most important and efficient milk and fat producer, but aspects of its reproductive biology are still unknown. The present study aimed to evaluate the daily sperm production (DSP) and spermatogenesis in developing Murrah buffalo bulls by evaluation of the seminiferous tubules, testicular morphometry and using light microscopy and scanning electron microscopy. The testes of Murrah buffalo bulls at 18 mo was immature and at 24 mo could still be considered an average-efficiency breed based on their DSP. At 24 mo, the DSP rate was 0.97 billion sperm per testis and 13 million sperm per gram of testis. However, the animals had superior morphometric parameters compared with those of other livestock animals, except for the seminiferous tubule volume and diameter, which were inferior. In conclusion, our data support former views that the testes of the Murrah breed does not reach sexual maturity before 2 y of age and that important developmental steps occur later than Murrah crossbreeds from Brazil.

## Introduction

The onset of puberty and sexual maturity shows variations in buffalo breeds from different continents.<sup>1</sup> The Murrah breed is considered the most important and efficient milk and fat producer,<sup>2</sup> but aspects of its reproduction are unknown. Some data suggest that male Murrah buffalo from the Indian continent reached full productive sexual maturity late in life, i.e., from 2 to 3 y onwards.<sup>3,4</sup> In contrast, crossbreeds derived from Murrah x Mediterranean buffaloes, maintained in an extensive breeding system in Brazil, may enter into puberty earlier than other breeds, i.e., between 10 to 15 mo, and may reach sexual maturity between 16 to 24 mo.<sup>5</sup> However, data related to reproductive characteristics, as well as a histological evaluation of its seminiferous tubules, are scarce for Murrah breeds. Moreover, data on testicular structure and function, focusing on testicular morphometry and an estimate of daily sperm production, have not been evaluated in Murrah buffaloes.

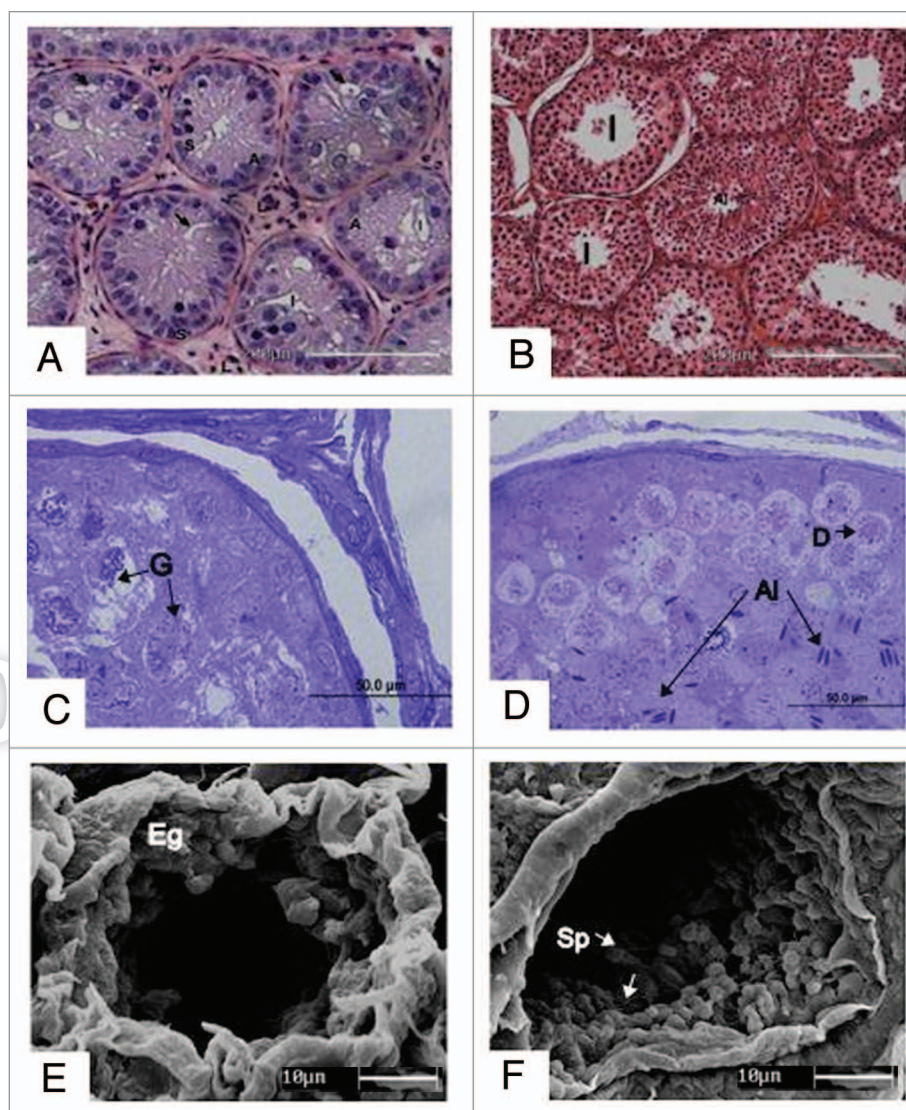
In order to better understand these aspects of male reproduction in Murrah buffaloes and its relationship to maintenance

conditions, we investigated spermatogenesis and daily sperm production (DSP) in two age groups (18 and 24 mo) that are close to the transition from puberty to sexual maturity in other buffaloes, especially from Brazil. In general, measurement of the DSP rate in different species can highlight genetic potential and therefore improve the management of these animals.<sup>6</sup> An important criterion of superior sire selection in buffalo bulls is the number of sperm that a bull produces.<sup>1</sup> The DSP per gram of testis is an effective parameter to estimate the spermatogenic efficiency of an animal.<sup>6</sup>

## Results

**Organization of testicular cells.** The testes from animals at 18 mo showed a particular uniformity in histological sections, including the formation of small gaps in the sex cords, in the form of small vacuoles. The gonocytes could be visualized; however, the spermatogonia were observed to be in the process of differentiation; their nuclei were round, and the major axis was located close to the cord basement membrane. Few primary spermatocytes were

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**Figure 1.** Photomicrographs of buffalo testis at 18 mo (A, C and E) and 24 mo (B, D and F) in transverse sections. (A and C) Seminiferous tubules showing spacing (arrows), gonocytes (G), spermatogonia (A) and supporting cells (S). (B and D) In the seminiferous tubules in the process of lighting (I), elongated spermatids (AL) and diplotene primary spermatocytes are seen (D). (E) Seminiferous tubules of animals at 18 mo presenting the germinal epithelium (Eg). (F) Seminiferous tubules of animals at 24 mo presenting sperm (Sp) and flagellated sperm (arrow). HE staining, 200x (A and B); toluidine blue, 1,000x (C and D). Bar: 10  $\mu\text{m}$  (E and F).

found in this age group. The supporting cells were differentiated in a triangular shape located close the basement membrane. The interstitial space still presented clustered Leydig cells and blood vessels (Fig. 1A). These differences emphasize the fact that at 18 mo, the animals presented sex cords at the beginning of the lighting process and other cords that were still completely devoid of vacuoles (Fig. 1C and D).

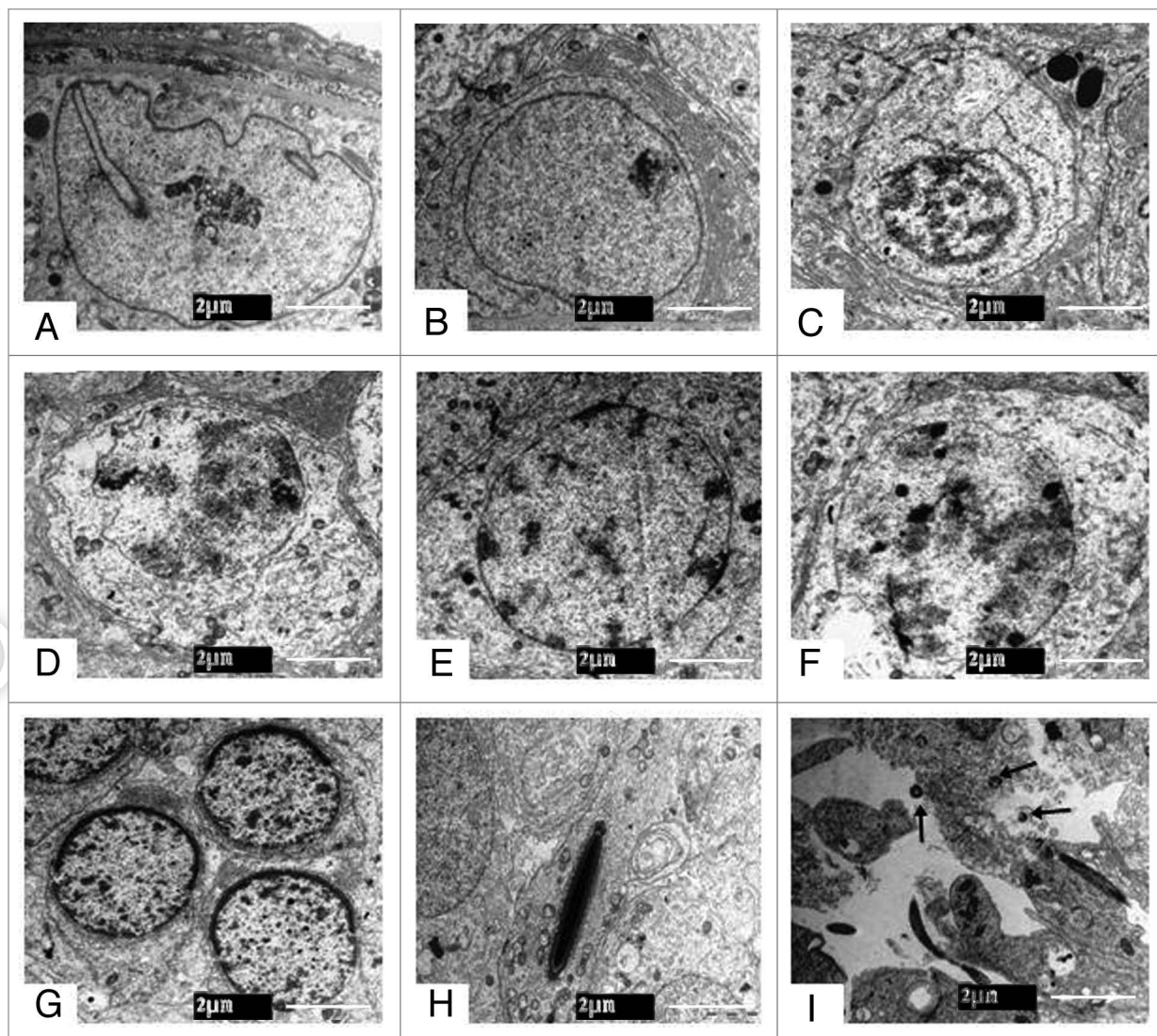
Testes at 24 mo still presented all cell types in the seminiferous epithelium: Sertoli cells, spermatogonia, pre-leptotene and leptotene primary spermatocytes, pachytene primary spermatocytes, zygotene primary spermatocytes, diplotene primary spermatocytes, round spermatids and sperm liberated into the tubular lumen in certain tubules (Fig. 2). The spermatogonia were more visible and were located close to the seminiferous tubule basal lamina, presenting an oval shape. Sertoli cells presented irregular,

almost triangular, light nuclei with characteristics of mature differentiated cells. The animals presented seminiferous tubules with sperm in the lumen (Fig. 1B and F).

The scanning electron microscopy and transmission electron microscopy analyses of the testes clearly demonstrated the differences in morphology of the seminiferous tubules in both groups.

*Testicular morphometry and daily sperm production (DSP).* The data on tubule diameter, seminiferous epithelium height and seminiferous tubule volume and length are shown in Table 1.

The numbers of cells per seminiferous tubule transverse section at stage 1 are presented in Table 2. The following cell types were found: Sertoli cells, spermatogonia, pre-leptotene primary spermatocytes, pachytene primary spermatocytes and round spermatids.



**Figure 2.** Electron micrograph of the buffalo testis for the identification of spermatogenic-lineage cells present in seminiferous tubules. (A) Sertoli cell; (B) spermatogonia; (C) pre-leptotene/leptotene primary spermatocyte; (D) zygotene primary spermatocyte; (E) pachytene primary spermatocyte; (F) diplotene primary spermatocyte; (G) round spermatids with deposition of chromatin on the superior base toward the sperm formation (arrow); (H) elongated spermatids; (I) sperm (arrow). Bar: 2  $\mu$ m.

The level of sperm production represents the production of sperm per gram of testis per day. Based on the premise that during the spermatogenic process, there are no significant losses, the computed number of round spermatids at stage 1 of the buffalo SEC was considered to be the population size of the sperm. The buffaloes' daily sperm production in this study was  $0.97 \times 10^9$  sperm per testis, i.e., 13.26 million sperm produced per gram of testis per day.

## Discussion

**Organization of testicular cells.** In this study, it was observed that Murrah buffaloes showed immature at 18 mo and spermatogenic activity at 24 mo. According to histological analyses, variations in the buffalo species pubertal stage can be observed.<sup>5,7,8</sup> Ohashi,<sup>7</sup> analyzing crossbred buffaloes (Mediterranean x

**Table 1.** Morphometric parameters of Murrah buffalo testes: Tubular diameter, seminiferous epithelium height and seminiferous tubule volume and length.

Parameters	Group 1	Group 2
Tubular diameter ( $\mu$ m)	77.12 $\pm$ 6.83 <sup>a</sup>	156.96 $\pm$ 4.51 <sup>b</sup>
Seminiferous epithelium height ( $\mu$ m)	-	34.72 $\pm$ 2.77
Tubular volume (%)	-	79.35 $\pm$ 2.84
<sup>1</sup> STTL per testis (m)	-	3,003.93 $\pm$ 675.5
<sup>1</sup> STTL per gram testis (m)	-	42.94 $\pm$ 12.50

<sup>1</sup>STTL, seminiferous tubule total length. Means with different superscript letters in the same line are significantly different ( $p < 0.05$ ) as estimated by analysis of variance and Student's test.

**Table 2.** Adjusted numbers<sup>1</sup> of cells per transverse section of Murrah buffalo seminiferous tubules at 24 mo

Group	SPG	Primary spermatocyte		SPD Ar	Sertoli Cells
		PL/L	P		
G2	3.19 ± 2.34	7.39 ± 6.08	11.87 ± 18.12	33.94 ± 6.72	5.83 ± 3.93

<sup>1</sup>Adjusted according to Abercrombie (1946); SPG, spermatogonia; PL/L, pre-leptotene/leptotene primary spermatocytes; P, pachytene spermatocytes; SPD Ar, round spermatids at stage 1.

Jaffarabadi), observed that puberty began in animals aged 10 to 14 mo. Bongso<sup>8</sup> observed in the Carabao bull that puberty occurred at approximately 16 mo. Another study observed that in crossbred buffaloes (Murrah x Jaffarabadi), puberty occurred at 13 mo.<sup>5</sup>

The animals at 24 mo, when compared with crossbreeds in Brazilian conditions,<sup>5</sup> were considered delayed. Our histological analysis of the spermatogenic process indicated that these animals were not at sexual maturity. Melo,<sup>5</sup> analyzing crossbred buffaloes at 24 mo that weighed 468.6 ± 69.8 kg, found that these animals had not reached sexual maturity.

In addition to species specificity, the buffaloes' nutrition and breeding system are the main factors that affect buffalo and cattle reproductive performance, especially in *Bos taurus*; because the buffalo and *Bos indicus* are kept in extensive breeding systems, major nutritional deficiencies can result delay in reproductive development.<sup>9</sup>

Regarding the breeding system employed, the animals of this study were subject to a milk-production breeding system; therefore, the lactating calves might have received insufficient milk, which is a possibility that reinforces the delay of their reproductive development. Despite the genetic and nutritional improvements<sup>10</sup> provided by female buffaloes, buffalo calves that are breastfeeding are denied a portion of their mothers' milk,<sup>11</sup> which compromises their growth. However, to increase the amount of milk designated for commercialization, producers of buffaloes in intensive breeding programs in other countries resort to feeding buffalo calves cow's milk and milk substitutes, which does not significantly compromise the development or weight gain of neonates.<sup>12</sup>

**Testicular morphometry.** The data in Table 1 demonstrate that the tubule diameters differed between the groups and were both lower than those observed in crossbred buffaloes at the same ages.<sup>12</sup>

In many mammals, the seminiferous tubules occupy 70 to 90% of the testicular parenchyma.<sup>13</sup> The animals of this study presented a lower tubular volume compared with other farm animals, such as goats of the Pardo-Alpina breed (87.7%)<sup>13</sup> and pigs (86.9%).<sup>14</sup>

The seminiferous tubule length per gram of testicular parenchyma varies between 10 and 30 μm.<sup>15</sup> The animals of the present study exhibited a total length per gram of testis of 42.94 μm, which is higher than those observed in goats (26.16 μm).<sup>16</sup> The high seminiferous tubule total length could have been linked to the small tubule diameter because the length is determined from the ratio of the total volume of the tubule to the tubule diameter.

According to Castro,<sup>17</sup> studies on spermatogenesis should include not only the classification of the SEC stages but also the list of cell types present such that the evolution of each cell type throughout the cycle can be quantified.

This study observed fewer spermatogonia in the buffalo compared with those reported in pigs.<sup>18</sup> The number of spermatogonia observed at 24 mo was similar to Murrah buffaloes at 48 mo, which showed that spermatogenesis was established in both studies.<sup>4</sup> The proportion of pre-leptotene/leptotene buffaloes found in this study was similar to those observed in the literature for pigs<sup>18</sup> and higher than those in buffaloes.<sup>4</sup> The pachytene primary spermatocytes showed similar values, as previously reported. This study observed fewer Sertoli cells per seminiferous tubule transverse section compared with the aforementioned studies and also compared with buffaloes,<sup>12</sup> Murrah buffaloes.<sup>4</sup> The number of buffalo round spermatids per seminiferous tubule transverse section found in this study at 24 mo was lower than those found in goats,<sup>16</sup> and higher than those observed in Angus bulls<sup>19</sup> and Murrah buffaloes.<sup>4</sup>

**DSP.** The daily sperm production per gram of testis is an effective and easy comparison between species because it eliminates the disparity caused by testicular weight and the duration of spermatogenesis. Spermatogenic efficiency in mammals depends mainly on the testis seminiferous tubule volumetric density (%), the number of generations of spermatogonia and the spermatogenic efficiency of the process.<sup>20</sup>

The daily sperm production per gram of testis can be classified into three levels: (1) Species with high spermatogenic efficiency, which produce 20 to 30 million sperm; these species include domestic animals,<sup>20</sup> such as pigs, horses, sheep and rabbits, mice,<sup>21</sup> rhesus monkeys.<sup>22</sup> (2) Species with average spermatogenic efficiency, producing ten to 20 million sperm per day, among which are cattle,<sup>20</sup> domestic cats<sup>23</sup> and capybaras.<sup>24</sup> (3) Species that produce less than ten million sperm, for example, humans<sup>25</sup> and opossums.<sup>26</sup> As observed previously, the buffaloes in this study, which produced 13 million sperm per gram of testis per day, are among the species with average spermatogenic efficiency.

The animals of this study were classified as impuberal at 18 mo and pubertal at 24 mo. Therefore, under the breeding conditions of the present study, the testis morphological development of the buffalo began late and progressed slowly, resembling those reported in Murrah buffaloes.<sup>4</sup> This deficiency could be attributed to the type of feeding and breeding systems utilized. However, the animals presented superior morphometric parameters, except for the seminiferous tubule volume and diameter, compared with previously studied farm animals. Murrah buffaloes presented

fewer germ cells and Sertoli cells compared with livestock and exhibited an average-efficiency DSP.

## Materials and Methods

**Animals.** Seven male Murrah buffaloes from the Department of Animal Production, College of Veterinary Medicine and Animal Science, University of Sao Paulo State, Campus of Botucatu, SP were divided into two groups:  $G_1$  consisted of four animals at 18 mo and  $G_2$  consisted of three animals at 24 mo. The animals were raised in an extensive breeding system. They were fed with *Brachiaria* sp and salt ad libitum and supplemented with corn silage during dry season. The animals were regularly vaccinated and were treated to control endoparasites and ectoparasites.

After the animals were physically examined and anesthetized, orchietomy was performed to remove the testicles. The collected material was fixed for light microscopy and scanning electron microscopy. To characterize the stages of the seminiferous epithelium cycle, the tubular morphology method was used,<sup>27</sup> which consisted of observing the modifications in the shape and position of the spermatid nuclei and the occurrence of meiotic division in the seminiferous epithelium.<sup>28</sup> This study was approved by the ethics committee UNESP/Dracena-Protocol No. 17/2011.

**Histological processing.** The testes were separated from the epididymis and sectioned into fragments for placed in Bouin's solution for 24 h. After dehydration in increasing concentrations of ethanol followed by xylene, the fragments were embedded in paraffin (Histosec, Merck).<sup>29</sup> Sections of 5  $\mu$ m thickness were processed on a Leica Microtome (RM2145) and subsequently stained with hematoxylin-eosin (HE). Following the HE staining, digital photomicrographs were taken to identify and associate the cell types and to characterize the seminiferous epithelium cycle.

**Scanning electron microscopy (SEM).** The fragments of testicular parenchyma were fixed in 2.5% glutaraldehyde, cryofractured in liquid nitrogen and post-fixed in 1% osmium tetroxide (EMS®) for 1.5 h. The fragments were subsequently dehydrated in increasing concentrations of ethanol and were dried to the critical point (Balzers CPD 020). Next, the fragments were placed on stubs, covered with a gold plating ("sputtering" Emitech K 550) and analyzed by scanning electron microscopy (Leo 435 VP, University of Sao Paulo State, Department of Surgery, Anatomy Sector-FMVZ/USP).

**Transmission electron microscopy (TEM).** To perform TEM, the fragments were previously fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. The material was washed in 0.1 M sodium phosphate buffer, pH 7.4, three times for 10 min and postfixed in 1% osmium tetroxide for 1 h. Following additional washes in phosphate buffer, the fragments were dehydrated in 50%, 70%, 90% and 100% ethanol and washed in propylene oxide. For 12 to 16 h, the fragments were rotated in a 1:1 solution of propylene oxide and resin. Subsequently, this mixture was replaced with pure resin in molds for 4 to 5 h. Once embedded, the fragments remained in an incubator at 69°C for 72 h to consolidate the resin polymerization. Semi-thin sections

were obtained for staining in a hot solution of 1% borax in distilled water containing 0.25% toluidine blue for observation under light microscopy. Subsequently, the resin blocks were sectioned with an ultra-Microtome for TEM (Leica Ultracut UCT).

**Testicular morphometry.** To measure the seminiferous epithelium tubular diameter and height, 30 histological sections were analyzed per animal at stage 1 of the seminiferous epithelium cycle (SEC). Outlines that were as close to circular as possible were made and randomly selected. The seminiferous tubule volumetric proportion was determined using a counting grid with 336 points of intersection, where 10 fields in each animal were analyzed at 200x magnification, for a total of 10,080 points. The total length of the seminiferous tubules (STTL) was calculated based on the total volume of the seminiferous tubules (VTTS) using the formula proposed by Attal and Courot<sup>30</sup> and Dorst and Sajonski:<sup>31</sup>  $STTL = VTTS/\pi R^2$  ( $R$  = tubular diameter/2). The comparison between the average diameters of the seminiferous tubule at different ages was tested by Student's t-test.

**Quantification of cellular and daily sperm production.** In animals with complete spermatogenic activity, 10 circular outlines were analyzed of seminiferous tubules that were held in stage 1 of the SEC. The following cell types were present: Sertoli cells, spermatogonia, pre-leptotene primary spermatocytes, pachytene primary spermatocytes and round spermatids. To calculate the cell number, the formula proposed by Abercrombie<sup>32</sup> was used: no. of cells = corrected cell count  $\times$  [(section thickness)/(section thickness + nuclear diameter)].

In 20 cross-sections of seminiferous tubules, the number of nucleoli of Sertoli cells in stage 1 of the SEC was quantified. Based on the value of the STTL and the number of nucleoli of Sertoli cells ( $S$ ) per seminiferous tubule, cross-sections were used to calculate the total number of Sertoli cells (NTSC) per testis using the formula described by Hochereau-de-Reviere and Lincoln:<sup>33</sup>  $NTSC = (STTL \times n^\circ \text{ corrected from } S \text{ per transverse section})/\text{thickness section}$ . To determine the NTSC per gram of testis, the calculated NTSC was divided by the weight of the testis. The estimated daily sperm production (DSP) from the quantitative histology was calculated using the following formula:<sup>34</sup>

$DSP = (NTCS \times n^\circ \text{ round spermatids at stage 1 by CS} \times \text{frequency of stage 1})/\text{duration of the stage 1 in days}$ .

To obtain the DSP g<sup>-1</sup> of testis, the calculated value from this formula was divided by the testis weight.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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## References

- Ahmad N, Umair S, Shahab M, Arslan M. Testicular development and establishment of spermatogenesis in Nili-Ravi buffalo bulls. *Theriogenology* 2010; 73:20-5; PMID:19815266; <http://dx.doi.org/10.1016/j.theriogenology.2009.06.034>.
- Cockrill WR. The husbandry and health of the domestic buffalo. *Trop Anim Health Prod* 1975; 7:40; <http://dx.doi.org/10.1007/BF02383241>.
- Pant HC, Sharma RK, Patel SH, Shukla HR, Mittal AK, Kasiraj R, et al. Testicular development and its relationship to semen production in Murrah buffalo bulls. *Theriogenology* 2003; 60:27-34; PMID:12620577; [http://dx.doi.org/10.1016/S0093-691X\(02\)01037-3](http://dx.doi.org/10.1016/S0093-691X(02)01037-3).
- Rana BK, Bilaspuri GS. A quantitative study of seminiferous tubular cells in the developing Murrah buffalo testis. *Vet J* 2004; 167:95-103; PMID:14623157; [http://dx.doi.org/10.1016/S1090-0233\(03\)00029-7](http://dx.doi.org/10.1016/S1090-0233(03)00029-7).
- Melo MIV, Vale Filho VR. Puberty in crossbred buffaloes. *Arq Bras Med Vet Zootec* 1992; 44:537-42.
- Johnson L, Varner DD, Roberts ME, Smith TL, Keillor GE, Scrutchfield WL. Efficiency of spermatogenesis: a comparative approach. *Anim Reprod Sci* 2000; 60-61:471-80; PMID:10844217; [http://dx.doi.org/10.1016/S0378-4320\(00\)00108-1](http://dx.doi.org/10.1016/S0378-4320(00)00108-1).
- Ohashi OM, Oba E, Nogueira JR, Souza SS, Silva AOA. Characteristics of the reproductive development of male buffaloes: testicular biometrics, puberty and sexual maturity. *R Bras Med Vet* 2001; 23:103-7.
- Bongso TA, Hassan MD, Nordin W. Relationship of scrotal circumference and testicular volume to age and body weight in the swamp buffalo (*Bubalus bubalis*). *Theriogenology* 1984; 22:127-34; PMID:16725943; [http://dx.doi.org/10.1016/0093-691X\(84\)90425-4](http://dx.doi.org/10.1016/0093-691X(84)90425-4).
- Vandemark NL, Fritz GR, Mauger RE. Effect of energy intake on reproductive performance of dairy bulls. II. Semen production and replenishment. *J Dairy Sci* 1964; 47:898-904; [http://dx.doi.org/10.3168/jds.S0022-0302\(64\)88799-3](http://dx.doi.org/10.3168/jds.S0022-0302(64)88799-3).
- Hishi E, Fruchi VM, Andrighetto C. Production and composition of the buffalo milk. In: IV Simpósio de Ciências e V Encontro de Zootecnia da Unesp de Dracena 2009; Dracena, Brazil.
- Bernardes O. Buffaloes breeding in Brasil: position and economic relevancy. *Rev Bras Reprod Anim* 2007; 31:293-8.
- Khan ZU, Khan S, Ahmad N, Raziq A. Investigation of mortality incidence and managerial practices in buffalo calves at commercial dairy farms in Peshawar city. *J Agric Biol Sci* 2007; 2:16-22.
- Leal MC, Becker-Silva SC, Chiarini-Garcia H, França LR. Sertoli cell efficiency and daily sperm production in goats (*Capra hircus*). *Anim Reprod* 2004; 1:122-8.
- Almeida FFL, Leal MC, França LR. Testis morphometry, duration of spermatogenesis and spermatogenic efficiency in the wild boar (*Sus scrofa scrofa*). *Biol Reprod* 2006; 75:792-9; PMID:16870941; <http://dx.doi.org/10.1095/biolreprod.106.053835>.
- Costa DS, Menezes CMC, Paula TAR. Spermatogenesis in white-lipped peccaries (*Tayassu pecari*). *Anim Reprod Sci* 2007; 98:322-34; PMID:16647229; <http://dx.doi.org/10.1016/j.anireprosci.2006.03.014>.
- Junior AA, Neto AC, Junior AS, Menezes DJA, Alves FR, Sousa AL, et al. Daily sperm production and testicular morphometry in goats according to external scrotal conformation. *Anim Reprod Sci* 2011; 127:73-7; PMID:21802871; <http://dx.doi.org/10.1016/j.anireprosci.2011.06.008>.
- Castro ACS, Bernedson WE, Cardoso FM. Kinetics and quantification of spermatogenesis: morphological bases and their applications in studies of mammalian reproduction. *Rev Bras Reprod Anim* 1997; 21:25-34.
- Costa DS, Macedo DB, Santos MD, Paula TAR, Faria FJC. Intrinsic rate of spermatogenesis in free-ranging feral pigs (*Sus scrofa* sp). *Anim Reprod Sci* 2011; 91:173-9; PMID:21441004; <http://dx.doi.org/10.1016/j.anireprosci.2005.03.013>.
- Aguiar GV, Araújo AA, Moura AAA. Testicular development, spermatogenesis and hormonal concentrations in Angus bulls. *R Bras Zootec* 2006; 35:1629-38; <http://dx.doi.org/10.1590/S1516-35982006000600008>.
- França LR, Russell LD. The testis of domestic animals. In: Martinez F, Regadera J, Editors. *Male Reproduction*. Madrid: Churchill Livingstone 1998; 197-219.
- Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *J Reprod Fertil* 1978; 54:103-7; PMID:712697; <http://dx.doi.org/10.1530/jrf.0.0540103>.
- Amann RP, Johnson L, Thompson DL Jr, Pickett BW. Daily spermatozoal production, epididymal spermatozoal reserves and transit time of spermatozoa through the epididymis of the rhesus monkey. *Biol Reprod* 1976; 15:586-92; PMID:826287; <http://dx.doi.org/10.1095/biolreprod.15.5.586>.
- França LR, Godinho CL. Testis morphometry, seminiferous epithelium cycle length and daily sperm production in domestic cats (*Felis catus*). *Biol Reprod* 2003; 68:1554-61; PMID:12606460; <http://dx.doi.org/10.1095/biolreprod.102.010652>.
- Paula TAR, Costa DS, Matta SLP. Quantitative histologic evaluation of adult capybara (*Hydrochoerus hydrochaeris*) testis. *Biosci J* 2002; 18:121-36.
- Amann RP. A critical review of methods for evolution of spermatogenesis from seminal characteristics. *J Androl* 1981; 2:37-58.
- Queiroz GF, Nogueira JC. Duration of the cycle of the seminiferous epithelium and quantitative histology of the testis of the South American white-belly opossum (*Didelphis albiventris*), Marsupialia. *Reprod Fertil Dev* 1992; 4:213-22; PMID:1438950; <http://dx.doi.org/10.1071/RD9920213>.
- Courot M, Hochereau-de-Reviere MT, Ortavant R. Spermatogenesis, p. In: Johnson AD, Gomes WR, Vandemark NL (Eds), *The Testis*. Academic Press, New York 1970; 1:339-432.
- Ortavant R, Courot M, Hochereau-de-Reviere MT. Spermatogenesis in domestic mammals. In Cole HH, Cupps PT (Ed.) *Reproduction de los animales domésticos*. New York: Academic 1977; 203-27.
- Luna LG. Manual of histology staining methods of the Armed Forces Institute of Pathology. New York: McGraw-Hill 1968; 3.
- Attal J, Courot M. Development testiculaire et établissement de la spermatogenese chez le taureau. *Ann Biol Anim Biochim Biophys* 1963; 3:219-41; <http://dx.doi.org/10.1051/rnd:19630302>.
- Dorst VJ, Sajonski H. Morphometrische untersuchungen am tubulussystem des schweinehodens wahrend der postnatalen entwicklug. *Monoths Vet Med* 1974; 29:650-2.
- Abercrombie M. Estimation of nuclear population from microtome sections. *Anat Rec* 1946; 94:239-47; PMID:21015608; <http://dx.doi.org/10.1002/ar.1090940210>.
- Houchereau-de-Reviere MT, Lincoln GA. Seasonal-variation in histology of testis of red deer, *Cervus elaphus*. *J Reprod Fertil* 1978; 52:209-13; PMID:633204; <http://dx.doi.org/10.1530/jrf.0.0540209>.
- Sharma AK, Gupta RC. Duration of seminiferous epithelial cycle in Buffalo Bulls (*Bubalus bubalis*). *Anim Reprod Sci* 1980; 3:217-24; [http://dx.doi.org/10.1016/0378-4320\(80\)90018-4](http://dx.doi.org/10.1016/0378-4320(80)90018-4).