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Testis morphometry and kinetics of spermatogenesis in the feral pig (*Sus scrofa*)

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ABSTRACT

The feral pig (*Sus scrofa* sp) also known as Monteiro pig, originated from a domestic pig breed that was introduced into Pantanal region in Brazil in the eighteenth century. Although the feral pig has commercial potential, there are few reports in the literature concerning the reproductive biology of this species. Therefore, the aim of this study was to further describe the feral pig testis parenchyma as well as characterize the stages of the seminiferous epithelium cycle by tubular morphology method, and to evaluate the number of differentiated spermatogonia generations in this species. Eight sexually mature feral pigs were analyzed. Fragments of testes were embedded in plastic resin and used to prepare slides for morphometrical studies. It was concluded that the feral pig has six generations of differentiated spermatogonials (A1, A2, A3, A4, In, B) and that the cellular composition in the eight stages of the seminiferous epithelium cycle of these animals were very similar to those reported in species of suidae and tayssuidae already studied.

1. Introduction

The feral pig (*Sus scrofa* sp), also known as Monteiro pig, originated from a domestic pig breed that was introduced into Pantanal region in Brazil in the second half of the eighteenth century (Cavalcanti, 1985) coexisting with native peccaries (Tayassuidae) and this co-existence could be the cause of the decrease of the peccaries population (Alho and Lacher, 1991). Feral pigs are major contributors to biomass of mammals in the Pantanal, reflecting the ecological importance of this species to the region (Lacher et al., 1986). Previous studies showed that feral pig meat has less fat and cholesterol compared with the meat of most domestic animals and it is socioeconomically important to the local population as subsistence hunting (Sollero, 2006). Although the feral pig has commercial potential, there are few reports in the literature concerning the reproductive biology of this species (Costa et al., 2011; Macedo et al., 2011).

Spermatogenesis is a cyclical and highly organized process that occurs in the seminiferous tubules, where a diploid cell differentiates into a haploid cell, the spermatozoid. This process is made up of different cell associations called stages, which are established before puberty and classified based on changes in the shape of spermatid nucleus—occurrence of meiotic divisions and the arrangement of spermatids within the germinal epithelium. Spermatogenesis last from 30 to 75 days in mammals and this period is under the control of the germ cell genotype

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(Russell et al., 1990; Johnson, 1991; França et al., 1998, 2005; França and Russell, 1998).

The spermatogenesis involves three classes of germ cells: spermatogonia, spermatocytes and spermatids. This process can be divided into three functional and morphologically distinct phases named spermatogonial (proliferative or mitotic), spermatocytic (meiotic) and spermiogenic (differentiation) phases, each one characterized by morphological and biochemical changes in the components of the cytoplasm and cellular nucleus of the germ cells (Courot et al., 1970; Russell et al., 1990; Sharpe, 1994).

The spermatogenic cells (germ cells) are well arranged in the seminiferous tubules, consisting in cellular associations that characterize stages of the seminiferous epithelium cycle, which are segmental (only one stage per tubular cross-section) in most domestic mammals already investigated and helicoidal in some primates, including humans (Leblond and Clermont, 1952; Russell et al., 1990; França et al., 2005). Then, germ cells within each layer of the seminiferous epithelium change in synchrony with the other layers over time. The cells do not migrate laterally along the length of the seminiferous tubule. A coordinate order of the stages is observed, whereby sequential stages occur with repetition along the length of the tubules, in a wave of the seminiferous epithelium (Castro et al., 1997; Hess and França, 2008). In addition, the identification of the different stages of the seminiferous epithelium is essential to perform quantitative studies of the spermatogenesis, which is important to understand the normal spermatogenesis, as well as to determine the specific stages of the process that could be affected by treatment or drug administration (Berndtson, 1977).

Therefore, the aim of this study was to further characterize the sexually mature feral pig testis parenchyma as well as characterize the stages of the seminiferous epithelium cycle by tubular morphology method, and to evaluate the number of differentiated spermatogonia generations in free-ranging feral pigs (*S. scrofa* sp).

2. Material and methods

2.1. Animals

Eight fully sexually mature male free-ranging feral pigs were used in the present study. The animals were captured in Pantanal do Rio Negro, Mato Grosso do Sul, Brazil (IBAMA license for collection no. 1916054). After capture, the animals were sedated with intramuscular azaperone (Stresnil® Janssen Animal Health) 1.0 mL/20 kg associated with 10 mg of Diazepan® and submitted to bilateral orchiectomy. Then, the animals were monitored until complete recovery and returned to their natural environment. All surgical procedures were performed by a veterinarian and followed approved guidelines for ethical treatment of animals.

2.2. Tissue preparation

The testes were fixed by gravity-fed perfusion through the testicular artery with 0.9% saline containing 5000 IU of Liquemine® for 15 min at room temperature and, subsequently, with 4% buffered glutaraldehyde for 20 min (Costa et al., 2007). After fixation, testes were trimmed from the epididymis, weighed, and cut longitudinally with a razor blade. Tissue samples with dimensions of approximately 3.0 mm in diameter, 5.0 mm in width and 8.0 mm in length were obtained and the fragments were immediately refixated by immersion, in a new glutaraldehyde solution at 4% in phosphate buffer 0.1 M (pH 7.4), for at least 2 h. Testis fragments were routinely processed and embedded in glycol methacrylate (Leica Historesin Embedding Kit®). Subsequently, 4-μm-thick sections were obtained and stained with toluidine blue—1% sodium borate solution.

2.3. Testis morphometry

To perform light microscopic investigations, images were obtained using a digital camera (Leica DFC400) attached to a light microscope (Leica DM 2500) at 400× and 1000× magnification, and these images were analyzed with the aid of morphometry software ImageJ 1.34 (Rasband, 2005). To estimate the tubular diameter of the seminiferous tubules, at least 20 tubular profiles that were round or nearly round were chosen randomly and measured for each animal. The volume densities of the testis tissue components were determine using a 560-intersection grid in each image. A total of 6720 points were scored for each animal.

Points were classified as one of the following: seminiferous tubule (comprising tunica propria, epithelium and lumen), Leydig cell, connective tissue, blood and lymphatic vessels. The volume of each testis component was determined as the product of its volume density and testis volume. Artifacts were rarely seen and were not included in the data.

2.4. Cell counts

The seminiferous epithelium cycle was staged according to the tubular morphology method (Courot et al., 1970; Ortavant et al., 1977; Swierstra, 1968). The number of germ and Sertoli cells, for each animal, was estimated by the analysis of cell populations in 20 cross-sections of seminiferous tubules of circular profile that included different stages of the cycle. The following cellular types were counted: type A spermatogonia, in all eight stages; intermediate-type spermatogonia, in stage 6; type B spermatogonia, in stage 7; pre-leptotene/leptotene primary spermatocytes, in stages 8, 1 and 2; zygotene primary spermatocytes, in stages 3, 4 and 5; pachytene primary spermatocytes, in stage 3; round spermatids, in stages 5, 6, 7, 8 and 1; and Sertoli cells, in all eight stages.

The count obtained for each cellular type was corrected for the mean nuclear diameter and thickness of the section, using the Abercrombie (1946) formula modified by Amann (1962). Because Sertoli cells have irregular nuclei, the correction was made from the mean nucleolar diameter. Then, only nuclei with evident nucleolus were counted.