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Original Research

Morphology and Morphometry of Seminiferous Tubules in Crioulo Horses

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ABSTRACT

This study aimed to assess the biometrics of the testes and the morphology of the seminiferous tubules of Crioulo horses. We studied 10 sexually mature stallions (3–6 years of age). After orchiectomy, testes were perfused with Karnovsky's solution and then embedded in glycol methacrylate. Testis sections (4 μm) were cut and stained with toluidine blue and a solution of 1% sodium borate. The histological images were digitized, and the morphometric analysis was performed using ImageJ software. The average weight of the stallions was 377.5 kg, and the average weight of both testicles was 162.9 g. The percentage of testicular parenchyma occupied by the seminiferous tubules and the intertubular tissue was 77.97% ± 6.34% and 22.03% ± 6.34%, respectively. The average tubular diameter was 205.00 ± 36.91 μm, whereas the average height of the seminiferous epithelium was 70.56 ± 2.82 μm. Average tubular length per testicle and average tubular length per gram of testicle were 4,085.10 ± 1,170.68 m and 26.09 ± 10.63 m/g, respectively. The characteristics of the eight stages of the seminiferous epithelium cycle were similar to those reported in other horse breeds. We conclude that the morphometry of the seminiferous tubules of Crioulo horse resembles what has been reported in other horse breeds. The volumetric proportion of the seminiferous tubules and the Leydig cells of the Crioulo horse is one of the highest ever reported for stallions.

1. Introduction

The Crioulo horse breed is found in several countries in South America, such as Brazil, Argentina, Uruguay Chile, and Paraguay. This breed underwent a process of natural selection over the course of four centuries, which resulted in a strong adaptation to the environmental conditions of South America, generating animals with great rusticity. This rusticity was accompanied by high fertility and longevity. Crioulo horses stand out for their ability to withstand adverse climate conditions and to survive in barren native lands because of their low nutritional demands [1]. However, despite the economic and cultural importance of Crioulo horses, there are only few studies that characterize some basic principles of their reproductive physiology, such as testes functional morphology.

Spermatogonia are found in well-organized and defined patterns in the seminiferous tubules, constituting cellular associations that characterize the stages of the seminiferous epithelium cycle (SEC). In the most mammals, the stages of the SEC present a segmented pattern, and

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normally there is only one stage per transversal section of tubule [2,3].

A stage in the SEC is considered as a set of defined generations of germ cells found, at a particular moment, in a transversely sectioned seminiferous tubule [4]. Because the spermatogenic cells develop at the same time and in close relationship with each other, over the course of time particular transversal section of seminiferous tubule begin to have a constant and progressive stage development, constituting the SEC. Identifying the various stages of the SEC is necessary for conducting quantitative studies on spermatogenesis; this is an important factor for understanding normal spermatogenesis, as well as for determining specific phases that might be affected by certain treatments or drugs [5].

The main methodology used to study the stages of the SEC in mammals is called the tubular morphology method, which is based on the variations in the shape of the nucleus of spermatogenic cells, the detection of meiotic divisions, and in the disposition of spermatids in the seminiferous epithelium. This method has enabled the description of eight stages in all species studied [5,6].

The testicular parenchyma of mammals is morphofunctionally constituted by two basic compartments: (1) the interstitial or intertubular compartment, also known as the intertubular space, which is responsible for producing steroids, and is constituted by blood and lymph vessels, nerves, connective tissue fibers, macrophages, mastocytes, and Leydig cells; and (2) the tubular compartment, which contains the seminiferous tubules that produce spermatozoids. These tubules are constituted by the tunica propria, the seminiferous epithelium, and the lumen. Myoid or peritubular cells, the basal membrane, and the collagenous fibers are found in the tunic. Sertoli cells and the male germ-line cells are in the seminiferous epithelium. The fluid secreted by the Sertoli cells and the spermatozoids are found in the tubular lumen [2,3].

The aim of this study was to evaluate the biometrics of the testes and the morphology of the seminiferous tubules in Crioulo horses, focusing on the volumetric proportion of testicular parenchyma, the characterization of the stages in the SEC, the diameter of the seminiferous tubules, the tubular length, and the height of the seminiferous epithelium.

2. Materials and Methods

2.1. Collection of Testes and Histological Processing

We collected the testes of 10 Crioulo horses between 4 and 6 years of age. Sampled animals were from the western border of the central region of Rio Grande do Sul State and the northern area of Espírito Santo State, during the winter season. All the animals used have been maintained grazing in native pastures of the corresponding region, and supplemented with minerals.

The animals were castrated under general anesthesia according to protocol no. 23081.013329/2009-20, approved by the Ethics and Animal Experimentation Committee of the Federal University of Santa Maria. Testicles were removed from the epididymis and weighed on a digital scale. The left testicular artery was cannulated for perfusion with 0.9% saline solution containing 5,000 IU/L of heparin for at least 15 minutes at room temperature. Immediately after this procedure, testes were perfused with Karnovsky's fixative solution (4% paraformaldehyde, 5% glutaraldehyde in a 0.1 M and pH 7.4 phosphate buffer) for 20 minutes, also at room temperature. The vials containing the perfusion solution were kept at 120 cm above the testis to ensure a pressure of approximately 80 mmHg [7].

Fragments of testicular parenchyma (approximately 8.0 × 5.0 × 3.0 mm) were cut from the middle third of the organ, and were then refixed in a new Karnovsky's solution for 24 hours. Following this, they were stored in a refrigerated phosphate buffer for 5 days until they were processed. The right testis of each animal was frozen, and used for further analysis of the volumetric percentage occupied by the albugineous tunic and mediastinum.

Fragments were dehydrated with increasing concentrations of ethanol, and embedded in a glycol methacrylate solution, following the methods of Costa et al [7]. Finally, using a glass blade microtome, 4 μm sections were cut and stained with toluidine blue and a 1% sodium borate solution, sections were then mounted on Entellan (Merck) glass slides, according to the manufacturer's instructions.

2.2. Morphometric Analyses of the Testes

The volumetric proportion occupied by different components of the testicular parenchyma was determined by means of the ImageJ 1.34s software [8], considering a graticule with 494 intersections as a point. The coincident points were computed with the seminiferous tubules (tunic, seminiferous epithelium, tubular lumen) and the intertubular tissue (Leydig cells, connective tissue, blood and lymph vessels) in 30 fields per animal. The fields were chosen randomly by means of a horizontal scan of the histological sections. The volumetric proportions were expressed as percentages, and estimated based on 14,820 points per testis. Analysis was performed at 400× magnification, under a light microscope.

The volume of the testicular parenchyma was calculated by subtracting the weight of the albuginea and the mediastinum, which were determined through the desiccation of the stored testes, from the weight of the corresponding testis. Testis density was considered to be very close to one [9]; therefore, the weight was considered to be equal to the volume.

The diameter of the seminiferous tubules was calculated by measuring 20 cross-sections of tubules, as circular-shaped as possible, per animal. For this, we considered the average between the greatest and the smallest diameter of each section. The average height of the seminiferous epithelium was measured from the basal membrane to the adluminal edge. Based on the same image used for calculating the tubular diameter and the height of the seminiferous epithelium, we determined the cross-sectional area of the seminiferous tubule. These three assessments were performed using ImageJ 1.34s software [8].

The total length of the seminiferous tubules was calculated according to the method described by Attal et al [10]; we divided the corrected value of the total volume of the