Original article

# Morphology and biometric of the vesicular and bulbourethral glands in castrated and non-castrated *Santa Ines* breed sheep

**Neves, CC.[1], Artoni, SMB.[1], Pacheco, MR.[1], Feliciano, MAR.[2]*, Amoroso, L.[1] and Melo, DG.[3]**

[1]Department of Animal Morphology and Physiology, Agricultural and Veterinary Sciences College, São Paulo State University – USP, Via de Acesso Professor Paulo Donatto Castellane, s/n, CEP 14884-900, Jaboticabal, SP, Brazil
[2]Department of Animal Reproduction, Agricultural and Veterinary Sciences College, São Paulo State University – USP, Av. Pedro Marques, 282, CEP 14882-222, Jaboticabal, SP, Brazil
[3]Department of Surgical Clinic of Agricultural and Veterinary Sciences College, São Paulo State University – USP, Via de Acesso Professor Paulo Donatto Castellane, s/n, CEP 14884-900, Jaboticabal, SP, Brazil
*E-mail: marcusfeliciano@yahoo.com.br

## Abstract

**Introduction:** The purpose of this paper is to determine the topography, biometry and light microscopy image of the vesicular and bulbourethral glands in order to analyze morphologic features of the accessory sexual glands in castrated and non-castrated animals. **Materials and Methods:** The morphology of the accessory sexual glands was investigated in 14 adult Santa Ines breed sheep, weighing 32 kg, on average. Six of them were castrated, and eight, non-castrated. For macroscopic study, the description of these two glands was carried out, as well as dissection and biometry study. Moreover, weight, length, height and width measurements were evaluated. For histological analysis, the vesicular and bulbourethral glands were sampled. **Results:** The topography of the reproductive glands was similar to bovine species. However, lower macroscopic measurements ($p < 0,05$) in the glands of the castrated sheep were evidenced when compared with the non-castrated ones. Characteristics such as shape of the glands, composition of the layer mucosa, the lamina propria, muscular, the excretory ducts and the adventitia were determined. **Conclusion:** Sheep castration promoted changes in the biometric measures of the glands, which were lower in castrated animals. The morphological and biometric characteristics of the vesicular and bulbourethral glands in sheep were determined.

**Keywords:** biometric, accessory reproductive glands, histology, ruminant.

## 1 Introduction

Domestic sheep (*Ovis aries*), family Bovidae, were the first animals domesticated by men. For centuries, sheep were exploited for meat and wool production (HAFEZ, 2003).

The accessory glands of the male sheep reproductive system correspond to ampulla, vesicular glands, bulbourethral or Cowper's glands and prostate. Their secretions contribute for the most of ejaculate volume (ASHDOWN and HAFEZ, 1995; CAMPOS, 2003).

The contribution of each gland to sperm volume varies among species, due to differences in gland size, and even absence of one, or more of them, in some species (GONZÁLEZ, 2002).

Since no parameters were found in the morphology of the accessory reproductive glands of castrated and non-castrated sheep, this study characterized the morphology of the vesicular and bulbourethral glands through topographical, histological and biometric analysis, evaluating weight, length, height and width in the vesicular and bulbourethral glands of sheep.

## 2 Materials and Methods

### *2.1 Animals and experimental design*

We used 14 sheep (*Ovis Aries*), adult males, Santa Ines, with average weight of 32 kg. Six of them were castrated and eight non-castrated from Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Brazil.

The present study was performed in accordance with Brazilian College of Animal Experimentation.

### *2.2 Morphological features*

In the topographical study of the accessory reproductive glands, two non-castrated male sheep of approximately eight months old were used. After slaughter, the animals were positioned in right lateral decubitus with the left pelvic limb abducted.

Craniocaudal incision was carried out in the pelvic region, three centimeters below the lumbar vertebrae. After that, the skin, the subcutaneous and the fat tissues were folded. The body of the iliac bone was cut transversely with a common saw, removing the b one fragment and immediately showing the vesicular and bulbourethral glands caudally.

The topographic anatomy of the accessory reproductive glands were described, then they were photographed with a digital camera, Olympus® 7,0. Later, they were dissected for microscopic study. The anatomical terminology used follows Schaller (1999). Fragments of the vesicular and bulbourethral glands were collected and fixed for three hours in Bouin's solution. After that, the samples were reserved to histological routine. They were embedded in

paraffin and sections of 5m were stained using Hematoxilin-Eosin technique. The obtained slides were evaluated by the photomicroscope Olympus® BX 50.

### *2.3 Biometry of the vesicular and bulbourethral glands*

Twelve male sheep were divided into two experimental groups (G1, six castrated animals, and G2, six non-castrated sheep). The vesicular and bulbourethral glands were dissected, weighed on digital scales and evaluated using digital calipers.

Measurements of weight, length, height and width of the vesicular and bulbourethral sheep glands of those two experimental groups were carried out.

### *2.4 Statistical analysis*

Experimental design was completely randomized. The data were previously tested for normality of residuals and variance homogeneity (F Test). We used PROC MEANS, SAS® and GraphPad Prism 4.

Real or transformed averages were evaluated by analysis of variance. For significance analysis, deployment of statistical averages was achieved by paired means (Student's t test). A significance level of 5% for all tests was used.

## 3 Results

In the present study, a pair of lobular accessory sex glands was covered by a thick capsule constituted of fibrous tissue and partly located in the urogenital fold. Each gland was lateral to the ampulla of its corresponding ductus deferens (Figure 1). These anatomy macroscopic findings in the sheep vesicular glands corroborate with Getty (1986) and Núñez (1993).

The glandular secretion was stored in an intralobular space, and the vesicular duct was observed in seminal colliculus. The excretory duct of the gland and the terminal portion of the ductus deferens formed a short ejaculatory duct that discharged into the seminal colliculus situated on the dorsal wall of the pelvic urethra.

Next to the urethral area, the vesicular glands were ventrally syntopic with the ampulla of the ductus deferens. In the cranial extremity of the pelvic urethra, the ostia of the excretory ducts of the right and left vesicular glands were located.

When the ductus deferens reached caudal region of urinary bladder, they were in apposition by a distance of eight centimeters, parallel, overlapping with vesicular glands. The ductus deferens were like ampullas, with 5.0 cm of length and 0.6 cm of width.

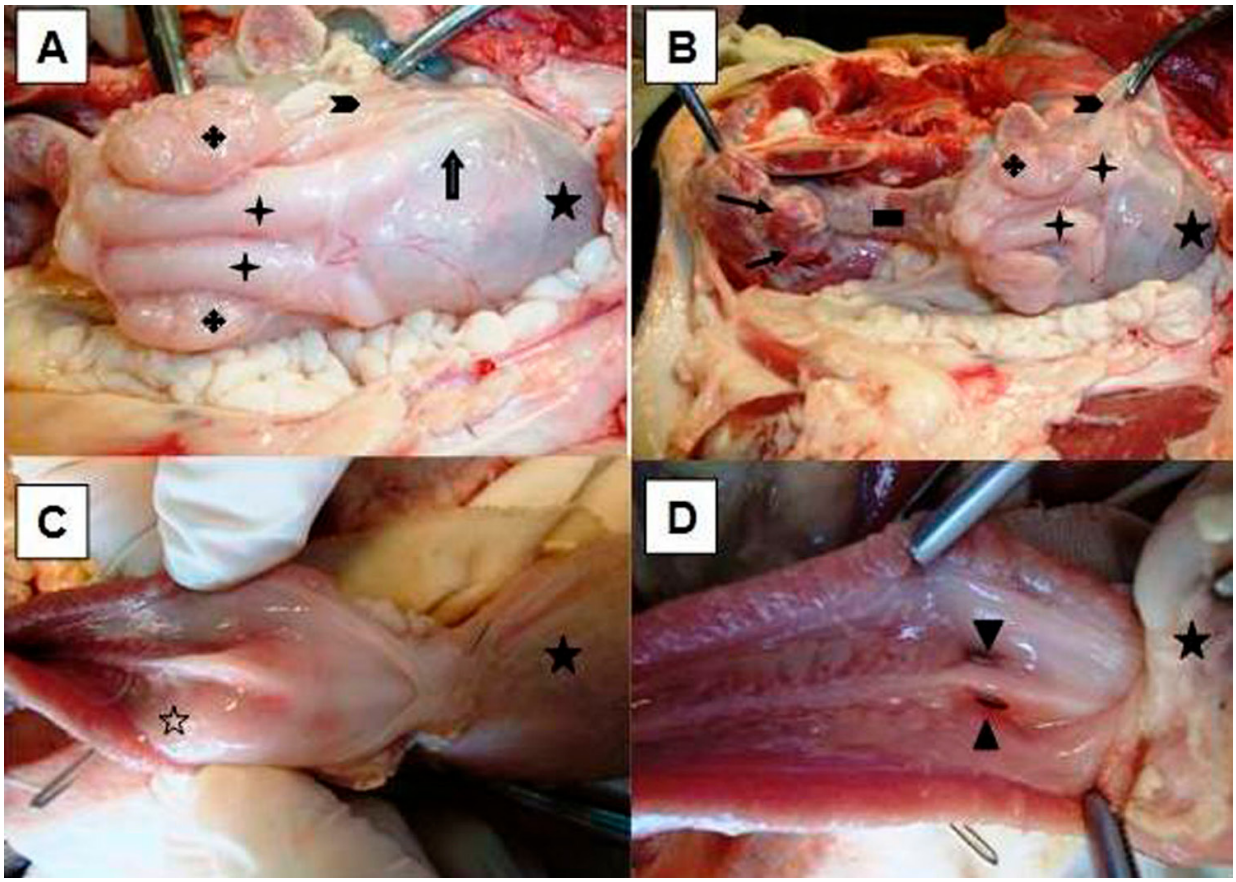


**Figure 1.** (A) and (B) Topographic view of the accessory reproductive glands of non-castrated sheep, showing: (1) urogenital fold, (2) bladder, (3) ductus deferens, (4) ampullae of ductus deferens, (5) vesicular glands, (6) and pelvic urethra (7) bulbospongiosus muscle covering the bulbourethral glands. (C) and (D) cranial portion of the pelvic urethra opened dorsally, (a) lumen of the pelvic urethra (b) ostium of the vesicular gland ducts.

It was verified that this anatomical arrangement in the gland ductus studied is similar to that observed in other domestic animals, described in ruminants by Getty (1986).

The vesicular gland was constituted by a tube that was intensely wound around it, which is sectioned into different positions (Figures 2 and 3).

Analyzing the tube, it was observed that the mucosa was highly folded with simple columnar epithelium with tall cells. The lamina propria was rich in elastic fibers and surrounded by a smooth muscle layer, which consisted of two laminas: an inner, constituted by circular fibers and an outer, with longitudinal fibers. The tunica adventitia showed loose connective tissue, typical and well-marked in some areas.

The glandular secretion was accumulated inside the gland and the main excretory ducts were lined by a bi-stratified columnar epithelium. The overall appearance of this gland presented was a structure with pinnate shape with central duct, which branched secretory terminals parts. Lobular subdivisions were present.

Distinct from sheep, and supported by Hib (2003), the men seminal vesicle is a simple tubular gland, long, which flows between the boundary of the ampulla of the ductus deferens and the ejaculatory duct. However, it was observed that sheep had similar structure to the ampulla of the ductus deferens of men and its course is sinuous.

In agreement with observations made in sheep in this study, the vesicular gland mucosa of men reported by Hib (2003) has a pseudostratified epithelium with high non-ciliated columnar cells, basal and oval cells. The lamina propria is surrounded by two layers of smooth muscle cells, an inner circular and an outer longitudinal one. Also, the muscle layers are surrounded by loose connective tissue, which is rich in elastic fibers that constitute the adventitia.

### *3.1 Morphological characterization of the bulbourethral glands*

The bulbourethral glands of sheep were pairs, small, oval, and flattened in dorsoventral position. These structures were covered by bulbospongiosus muscle throughout its length and its dorsal apex by a thick layer of dense fibrous tissue (Figure 4).

These glands were located in the dorsal and caudal portions of the pelvic urethra, cranial to the ischial arch. They showed the ostia of the excretory ducts (right and left), which opened at the caudal extremity in the pelvic urethra.

Anatomical characteristics of the bulbourethral glands in the present study corroborated with Dyce, Sack and Wensing (2010), Getty (1986) and Vasquez and Del Sol (2001).

The bulbourethral glands are classified as compound tubulo-acinar, with prismatic cells, acidophilic cytoplasm and rounded nucleus basalis (Figures 5 and 6).

The duct system of this gland is lined by pseudostratified epithelium. The capsule is composed by dense connective tissue containing striated skeletal muscle. The capsular tissue presents septals components, constituted by the loose connective tissue of the lamina propria and occasionally by the diffuse lymphatic tissue.

The illustrations of Bacha Junior and Bacha (2003), as well as Hib (2003) and Junqueira and Carneiro (2008) mention that histologically, the bulbourethral glands in humans are tubuloalveolar formations in pairs, with mucous-type cells and striated skeletal muscle in septa that separate their lobes, as well as in capsule, confirming findings of this research.

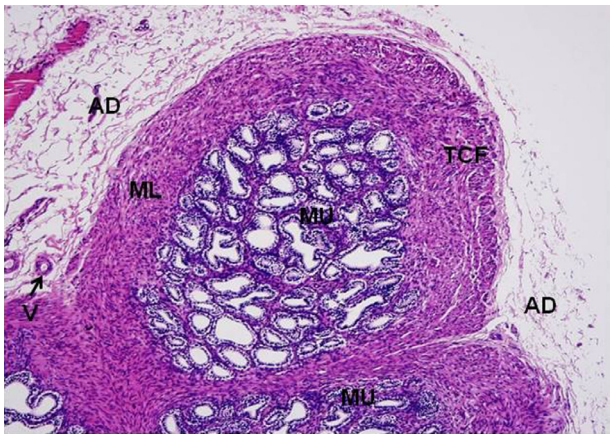


**Figure 2.** Photomicrograph of the vesicular gland of non-castrated Santa Ines sheep, showing mucous layer with secretory portions (MU), the lamina propria of loose connective tissue (TCF), smooth muscle layer (ML), adventitial layer (AD) and blood vessels (V), HE 10×.

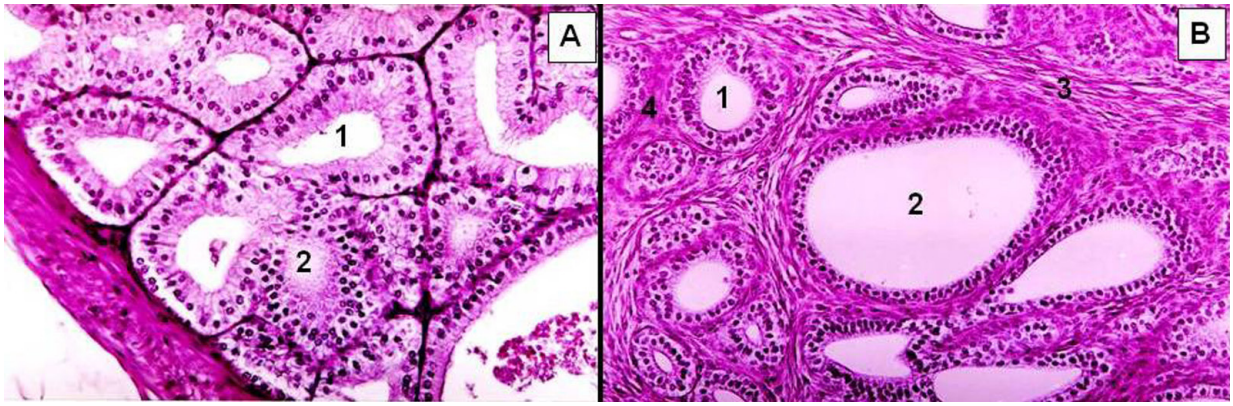


**Figure 3.** (A) Photomicrograph of the vesicular gland of non-castrated and (B) castrated Santa Ines sheep, showing the secretory regions constituted by simple columnar epithelium (1), duct lined by biestratified epithelium (2), fibromuscular septum (3) and loose connective tissue (4), HE 10×.

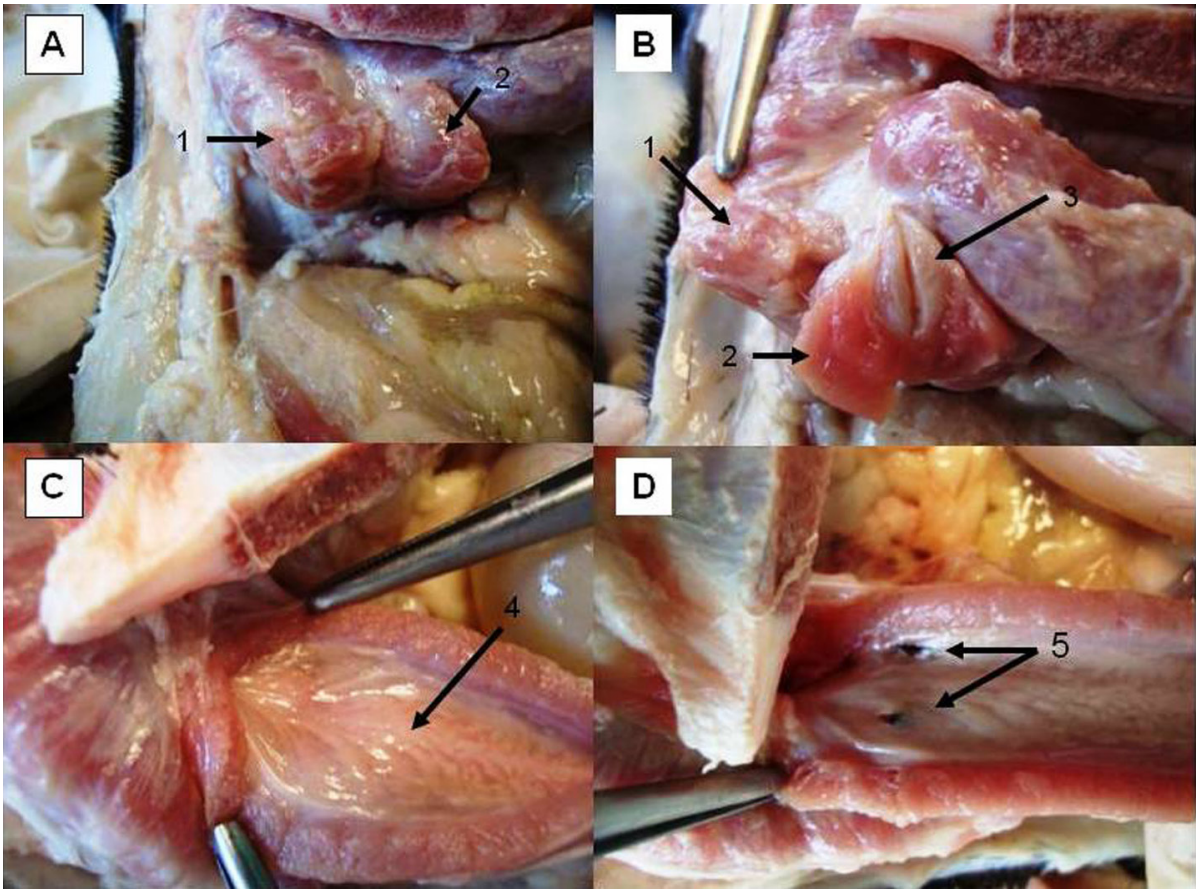


**Figure 4.** (A) and (B) Topographic view of the bulbourethral glands in non-castrated sheep, showing: (1) isqueocarvenoso muscle, (2) bulbospongiosus muscle covering bulbourethral gland, (3) Longitudinal incision in the left bulbourethral gland (C) and (D) pelvic urethra opened dorsally, (4) lumen of pelvic urethra pelvic (5) ostium of ductus in the bulbourethral glands.

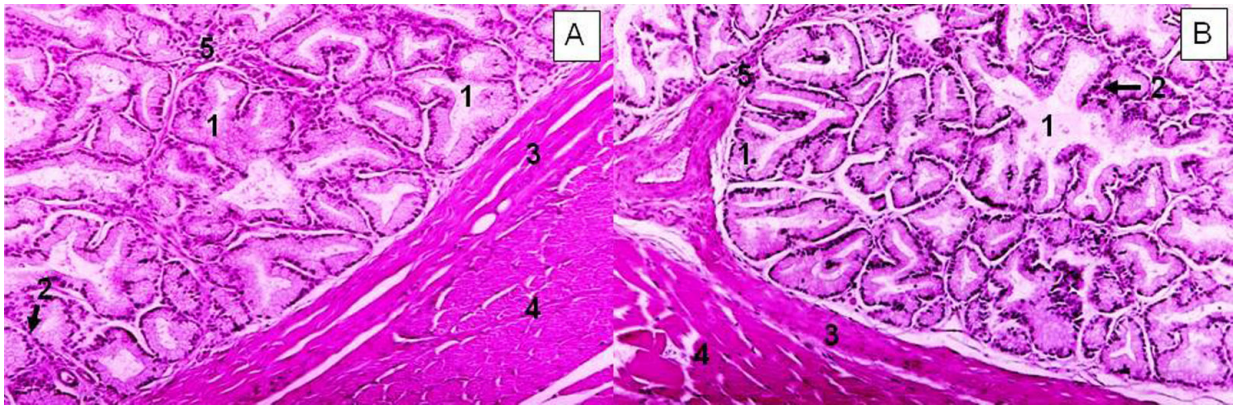


**Figure 5.** (A) Photomicrograph of the bulbourethral gland non-castrated Santa Inês breed sheep and (B) castrated, showing secretory regions (acini) (1), lamina propria (2), striated skeletal muscle in longitudinal section (3), striated skeletal muscle in cross section (4) and fibromuscular septum (5), HE 10×.

The vesicular glands weighed on average 0.052 mg, with 3.5 centimeter (cm) long, 2 cm wide, and 1,0 cm centimeter high. The bulbourethral glands weighed, on average, 0.016 mg, and presented 1.5 cm of length, 1.3 cm of height in middle portion and 1.0 cm in diameter.

Data of relative weights, lengths, heights and widths in the vesicular and bulbourethral glands of the castrated and non-castrated Santa Inês sheep are presented in Table 1.

Analyzing live weight and relative weight of the vesicular and bulbourethral glands, a significant difference was observed between experimental groups comparing castrated and non-castrated breed sheep. These results corroborate with the findings of Martins (2006) and Campos (2003).

It was possible to observe differences in relative weight of the bulbourethral glands of castrated and non-castrated breed sheep. This difference was related by Nunes

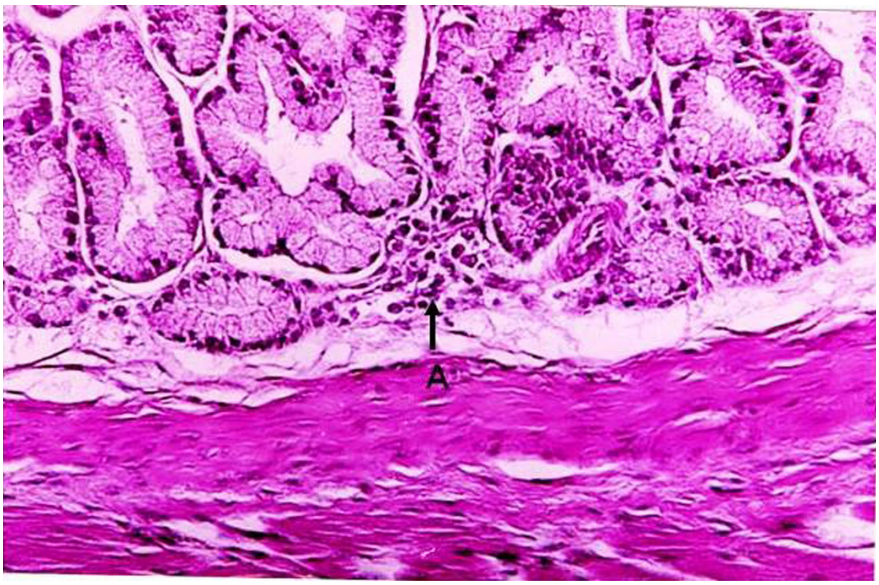


**Figure 6.** Photomicrograph of bulbourethral gland of the non-castrated Santa Inês breed sheep, showing diffuse lymphatic tissue (A), HE 20×.

**Table 1.** Mean values of lengths, heights and widths of the vesicular and the bulbourethral glands in castrated and non-castrated Santa Ines breed sheep.

| Biometric measures | Castrated animals | | Non castrated animals | | P (5%) |
|---|---|---|---|---|---|
| | Vesicular | Bulbo urethral | Vesicular | Bulbo urethral | |
| Live weight (Kg) | 32,17±1,72 | | 31,67±1,96 | | 0,64$^{ns}$ |
| Relative weight (mg) | 0,03±0,01 | 0,07±0,04 | 0,04±0,02 | 0,01±0,007 | ≤ 0,05* |
| Length (mm) | 0,50±0,06 | 0,96±0,13 | 21,61±3,10 | 10,71±1,35 | ≤ 0,0001* |
| Height (mm) | 0,34±0,05 | 0,41±0,13 | 11,60±4,88 | 7,45±1,51 | ≤ 0,0002* |
| Cranial width (mm) | 0,31±0,09 | 0,47±0,13 | 13,39±2,44 | 7,24±1,35 | ≤ 0,0001* |
| Caudal width (mm) | 0,36±0,09 | 0,49±0,17 | 12,08±2,66 | 7,84±2,28 | ≤ 0,0001* |

$^{ns}$ no significative ($p > 0,05$) and * significative ($p \leq 0,05$) by Student T test.

(1982), explaining that the vesicular glands are androgen-dependent, while the bulbourethral are prolactin-dependent, characteristic that makes biometric results different from each other after castration.

In values of length, width and height of the sheep reproductive glands, there was significant difference between castrated and non-castrated animals. These results confirm the assertions of Nunes (1982) and Risbridger and Taylor (2006), who had observed the functional dependence of the reproductive accessory glands on testosterone, providing different biometric data of the respective glands analyzed after castration.

Sheep castration promoted changes in the biometric measures of the vesicular and the bulbourethral glands, and they were lower in the castrated animals.

## 4 Conclusion

In the present study, it was possible to determine morphological and biometric characteristics of the accessory sexual glands. Their structures were topographically similar to the cattle sexual glands, but differing in shape, size and location. Histologically, findings of the sheep glands were also similar to those of the cattle.

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