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Morphological and morphometric characterization of the preputial ostium, internal preputial leaflet, and free part of the penises of Aberdeen Angus and Nellore bulls

[*Caracterização morfológica e morfométrica do óstio prepucial, do folheto prepucial interno e da parte livre do pênis de touros Aberdeen Angus e Nelore*]

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ABSTRACT

The morphological characteristics of the external genitalia of the bull may contribute to the etiopathogenesis of preputial diseases that trigger the *impotence coeundi* and losses in farms. The goal of this study was to establish morphometric parameters of the preputial ostium, the internal preputial leaflet (IPL), the free part and glans of the penis; describe the histological characteristics of the IPL; perform a count of the preputial sebaceous glands and compare these variables between 24-month-old Aberdeen Angus and Nellore bulls. At the slaughterhouse, 20 preputial pieces were collected from each breed. A device composed of base, rod, ring, and funnel was designed to hold each suspended and distended part. The IPL, the free part of the penis and the glans were measured. The ostium diameter was measured, and the radius, perimeter, and area of the preputial ostium were calculated. Samples were collected from the dorsal and ventral regions and from the cranial, medial, and caudal portions of the IPL. By electronic and optical microscopy, IPL characterization and sebaceous gland counts were performed. Analysis of variance was performed using the F test at 5%. It was concluded that Nellore bulls have a more extensive IPL and free part of the penis and more preputial sebaceous glands than Aberdeen Angus bulls. In both breeds, there are more glands in the ventral region of the ostium.

Keywords: bovine, smegma, sebaceous glands, foreskin

RESUMO

As características morfológicas da genitália externa do touro podem contribuir para a etiopatogenia de enfermidades prepuciais que desencadeiam a impotência coeundi *e prejuízos nos criatórios. O objetivo deste estudo foi estabelecer parâmetros morfométricos do óstio prepucial, do folheto prepucial interno (FPI), da parte livre e da glande do pênis; descrever as características histológicas do FPI; realizar contagem de glândulas sebáceas prepuciais; e comparar essas variáveis entre touros Aberdeen Angus e Nelore, com 24 meses de idade. No abatedouro, colheram-se 20 peças prepuciais de cada raça. Elaborou-se um dispositivo composto por base, haste, anel e funil para sustentar cada peça suspensa e distendida. Realizou-se medição do FPI, da parte livre do pênis e da glande. Aferiu-se diâmetro do óstio, e calculou-se o raio, o perímetro e a área do óstio prepucial. Colheram-se amostras nas regiões dorsal e ventral e das porções cranial, medial e caudal do FPI. Por microscopia eletrônica e óptica, fez-se a caracterização do FPI e a contagem de glândulas sebáceas. Realizou-se análise de variância pelo teste F a 5%. Concluiu-se que touros Nelore possuem o FPI e a parte livre do pênis mais extensos e mais glândulas sebáceas prepuciais que touros Aberdeen Angus. Nas duas raças, há mais glândulas na região ventral do óstio.*

Palavras-chave: bovino, esmegma, glândulas sebáceas, prepúcio

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INTRODUCTION

Nellore bulls (*Bos taurus indicus*) are efficient in covering cows, mainly by natural mating in extensive breeding. Aberdeen Angus bulls (*Bos taurus taurus*) are used in artificial insemination and industrial crossbreeding with Nellore breeds to benefit from heterosis (Baruselli *et al*., 2019).

The external genitalia of the bull is composed of the foreskin and penis and has as its vital function the effectuation of copulation. However, these organs become vulnerable and susceptible to lesions due to variation in morphological characteristics, such as the width of the orifice of the preputial ostium, pendulousness, and the length of the preputial sheath (Rabelo *et al*., 2017).

These particularities, especially in zebu bulls (*Bos taurus indicus*), may justify the incidence of preputial diseases and, consequently, economic losses due to expenses with medicines and veterinarians or, commonly, the discarding of non-pregnant cows and sick bulls for failure in coverage or coital impotence (Rabelo *et al*., 2015).

The foreskin or preputial sheath is made up of two laminae or leaflets. One of them is the external preputial leaflet (EPL), formed by a fold of thin, elastic, and mobile skin, adhered to the belly of the abdomen from the umbilical fold to the base of the scrotum. The other is the internal preputial leaflet (IPL) or preputial mucosa, characterized as a tubular organ that houses the free part of the penis (Wolfe, 2018).

The IPL originates at the entrance of the preputial ostium, follows caudally as mucosa lining the preputial cavity, up to the preputial recess or blind cul-de-sac, and finally returns as a penile layer lining the surface of the penis and inserts into the preputial raphe. The preputial ostium is the opening through which the exteriorization of the penis occurs. It is known that this organ has preputial sebaceous glands and that these produce lipid secretion that make up the smegma, which is important for preputial and penile hygiene (Rabelo *et al*., 2012; Silva *et al*., 2019).

However, although there are studies on the sebaceous glands in various body regions in human and veterinary dermatology (Souza *et al*., 2009; Szöllősi *et al*., 2018; Costa *et al*., 2020), there is no comparative information on these glands in the preputial ostium of bulls.

Thus, the objective of this study was to establish morphometric parameters of the preputial ostium, the length of the internal preputial leaflet (IPL), the free part and the glans penis; to describe the histological characteristics of the IPL; to perform the count of the preputial sebaceous glands and compare these variables between bulls of the Aberdeen Angus and Nellore breeds.

MATERIAL AND METHODS

Forty uncastrated male cattle were used, randomly distributed in two groups of pure breeds and without crossbreeding: 20 bulls of the Aberdeen Angus breed and 20 bulls of the Nellore breed, 24 months old, average of 540 kg live weight. We selected bulls with preputial hygiene and without eversion or preputial prolapse. These animals were slaughtered at the Marfrig slaughterhouse, Mineiros-GO unit. Slaughter monitoring was performed by the federal inspection service and by the research team responsible for sample collection and conditioning. The study was approved by CEUA of UFG, protocol nº 023/18.

After the bulls were slaughtered, the skin of the foreskin was flapped, with reference to the ventral abdominal wall and the umbilical scar, preserving the integrity of the ostium and the preputial mucosa or IPL. From the penis, the free part of the penis was harvested, including the glans and the portion of the body of the penis surrounded by the penile layer of the IPL The penis was resected as a cranial reference to the base of the scrotum.

The anatomical parts of foreskin and penis were identified and conditioned in isothermal boxes, wrapped in plastic bags, and immersed in 0.9% sodium chloride solution. Then, all the material was transported to the Laboratory of Animal Anatomy of the Centro Universitário de Mineiros UNIFIMES for the preparation, measurements, and collection of samples.

In the laboratory, the specimens were laid out on a table, and the preputial hair was trichotomized to facilitate the identification of the transition of skin and mucosa of the preputial ostium. Then, the preputial mucosa was exposed or exteriorized,

and the specimen was placed in a device made to take measurements.

This device was made from a universal support composed of a 14x20 cm polypropylene base, a 70cm long stainless-steel rod, a 10cm diameter iron ring used to support the funnel, and an adjustable muffle that fixed the ring to the rod (Fig.1A). Inside the funnel, each anatomical piece remained suspended in the vertical position. Consequently, there was distension of the mucosa of the IPL and the free part of the penis, only by the force of the weight of the piece itself, simulating the exposure in live animals.

To measure the internal diameter of the preputial ostium we used a digital pachymeter (Instrutherm® São Paulo - SP - Brazil), with results in millimeters (mm). From the diameter, the radius, perimeter, and area of the preputial ostium were calculated. Then the length of the IPL was measured in centimeters, having as reference points the extension from the preputial ostium to the foreskin. Next, the free part of the penis was measured, from the foreskin to the glans, and finally the glans alone (Figs. 1B, 1C, and 1D).

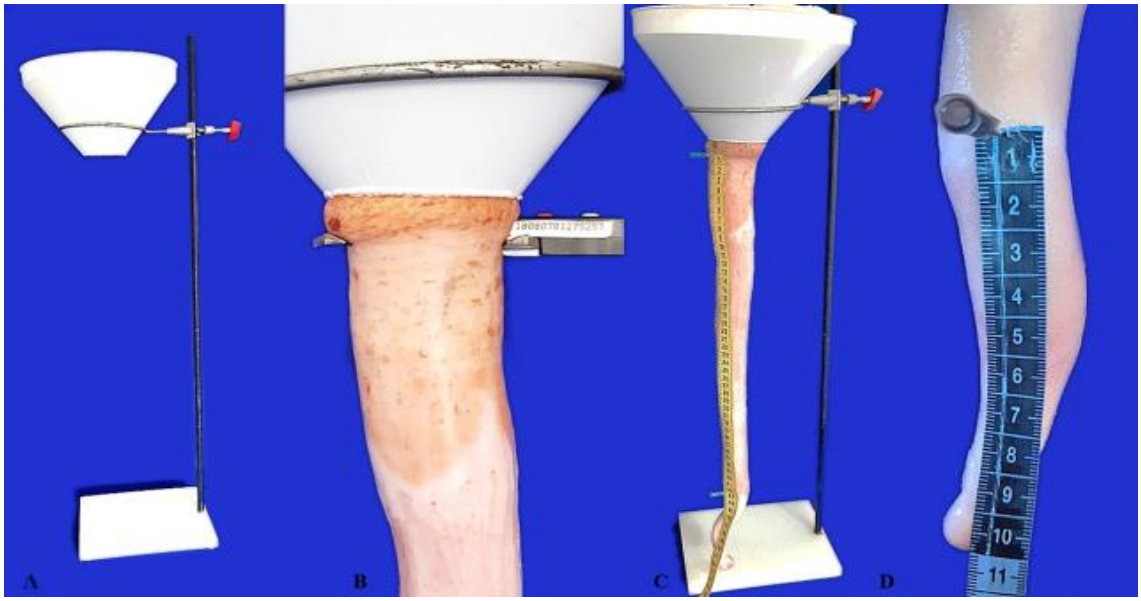

Figure 1. Method for obtaining measurements of the preputial ostium and IPL. In (A), device made from a universal support composed of a polypropylene base, a stainless-steel rod 70 cm long, an iron ring 10 cm in diameter used to support the funnel and a muffle adjustable from the ring to the rod. (B) Measurement with a digital pachymeter of the diameter of the preputial ostium (mm). (C) Measurement with a tape measure of the length of the IPL (cm), from the preputial ostium to the raphe of the foreskin or insertion of the foreskin into the free part of the penis. (D) Measurement of the free part of the penis from the foreskin including the glans.

Six 1cm^2 fragments were collected from each foreskin, one dorsal and one ventral, from the cranial, medial, and caudal portions of the IPL (Fig. 2A). These samples were fixed in 10% buffered formalin solution, then dehydrated in alcohol solution at increasing concentrations of 70-100%. Subsequently, diaphanization in Xylol, paraffin impregnation, and embrocate were performed. Serial sections, approximately 5µm thick, were made. Hematoxylin-eosin staining, and two other staining methods were used to highlight the connective tissue, Mallory's trichrome and Gomori's trichrome. Permanent slides were mounted with piermount balsam and a coverslip (Tolosa and Behmer, 2003).

Visualization of histological sections was performed on a Leica DM 750 optical photomicroscope with an ICC50 built-in digital camera (HD-521420221). The tissue constituents of the preputial ostium and IPL were observed for both breeds.

The number of sebaceous glands in the samples from the cranial portion of the IPL was counted. From each breed, 10 slides from the dorsal region and 10 slides from the ventral region at the transition of the skin from the preputial ostium and IPL were used. On each slide, 10 random fields were captured, called regions of interest (ROI - Regions of interest of 399.22 x 399.22 µm2), according to methodology adapted from Xu *et al*. (2017) (Figure 2B). Counting was always started in the upper left corner and shifted to the right of the field, with 400-fold magnification.

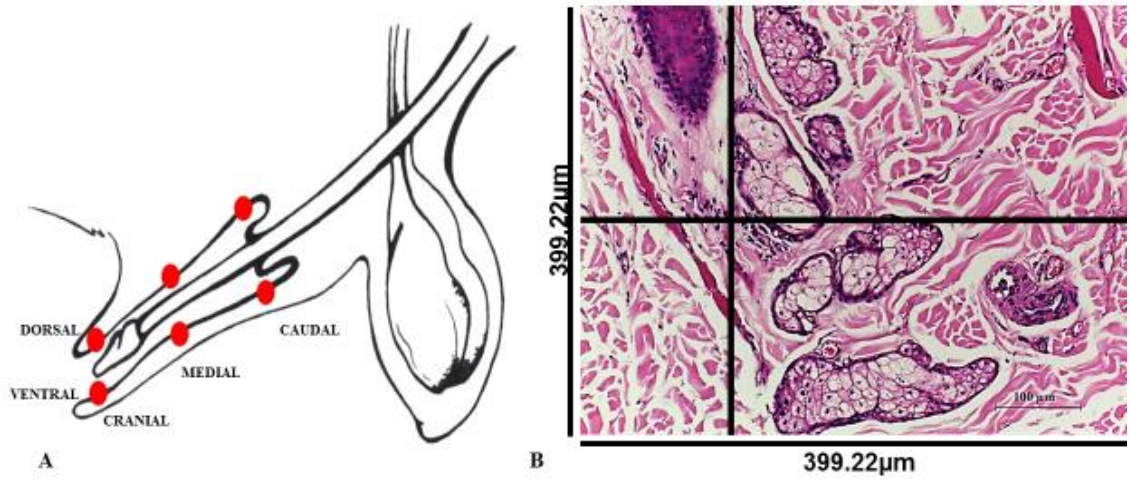

Figure 2. In (A) illustrative drawing of the dorsal and ventral regions and cranial, medial and caudal portions of the IPL for sampling for light microscopy. In (B) Method counting sebaceous glands. Note the histological image of the Region of Interest (ROI = 399.22 X 399.22 µm). Magnification 400x. Each slide with 10 fields.

For transmission electron microscopy (TEM) analysis, the preputial ostium was incised with a scalpel blade to collect one cubic millimeter fragments. The samples were then placed in a fixative solution of 2.0% glutaraldehyde and 2.0% paraformaldehyde in 0.1M sodium cacodylate buffer, pH 7.2 for 12 hours in refrigeration at 10ºC. After this period the samples were kept in sodium cacodylate buffer solution (0.1M, pH 7.2) until the moment of processing.

In the laboratory, the material was post-fixed in 2% osmium tetroxide, 1.6% potassium ferricyanide (1:1) in 0.2M sodium cacodylate buffer, pH 7.2 for 1 hour. Next, dehydration in increasing acetone solution (30-100%) was performed for 10 minutes at each concentration. After dehydration, the sample was embedded in Spurr resin at 60°C to obtain 30 nanometer ultrathin sections in a Leica ultramicrotome (UCT, AG, Vienna, Austria) and contrasted in uranyl acetate and lead citrate. A Jeol 1011 Transmission Electron Microscope (TEM) from the Microscopy and Microanalysis Laboratory of the University of Brasília (UnB) was used. The images were obtained with a GATAN camera (BioScan Camera, model 792 Gatan, Warrendale, IL, USA).

For the statistical analysis of the variables of ostium, IPL, and free part of the penis measurements the entirely randomized design (ERD) was used, with 20 repetitions. The data were analyzed for normality by Lilliefors test ($p<0.05$) and homogeneity of variance by Bartlett test ($p<0.05$). Once normality and equality of variance were confirmed, the results were submitted to analysis of variance by the F test at a 5% significance level. For the variable quantity of preputial sebaceous gland, the ERD was used in a 2 x 2 factorial arrangement, with 10 repetitions. In this design, there were two treatments, and the F test was significant, conclusive, and sufficient, without the need for other complementary tests.

RESULTS

The results in Table 1 refer to the mean values, standard error of the mean, and coefficient of variation of the measurements of diameter, radius, perimeter, area of the preputial ostium, length of the internal preputial leaflet (IPL), free part of the penis, and glans of Aberdeen Angus and Nellore bulls.

Aberdeen Angus bulls presented statistically smaller mean length values of the IPL and free part of the penis ($p<0.05$) in relation to Nellore bulls. However, there was no statistical difference in the diameter, radius, perimeter, ostium area and glans of the penis between the breeds evaluated.

Table 1. Distribution of the mean values, standard error of the mean and coefficient of variation of the measurements of diameter, radius, perimeter and area of the preputial ostium and length of the internal preputial leaflet (IPL), free part of the penis and glans of Aberdeen Angus and Nellore bulls

Breed	DOP (mm)	ROP (mm)	POP (mm)	AOP (mm^2)	IPL (cm)	PL (cm)	Glans (cm)
Aberdeen Angus	30.60±1.27a	15.30±0.64a	96.13±4.00a	759.65±5.9a	36.41±0.57b	11.02±0.18b	2.98±0.06a
Nelore	30.90±1.29a	15.45±0.65a	97.08±4.06a	774.79±6.3a	38.30±0.67a	11.55±0.18a	3.16±0.08a
CV (%)	9.50	9.47	9.50	18.66	7.48	3.52	5.50

Averages followed by the same letters are statistically equal by the F test, 5% probability.
Legend: AOP (area of the preputial ostium); DOP (diameter of the preputial ostium); IPL (internal preputial leaflet); glans penis; PL (free part of the penis); POP (perimeter of the preputial ostium); ROP (radius of the preputial ostium).

In the middle and caudal portions of the IPL, optical microscopy images revealed that the preputial mucosa of both breeds is composed of epidermis and dermis. The epidermis consists of keratinized stratified sidewalk epithelium (Fig. 3A) and keratinocyte layers called basal, spinous, granular, lucid, and horny (Fig. 3B). The basal is formed by a cuboidal cell layer, the spinous by polyhedral cells in a spiny arrangement, and the granular by accumulation of granules. In the lucid layer, there are paved cells without organelles. In the horny layer, desquamation of the epithelium.

The dermis consists of irregular dense connective tissue. The papillary dermis presented a glove finger shape, in connection with the epidermis and, more deeply, the reticular dermis (Fig. 3A). The papillary layer of the mucosa showed a higher concentration of leukocytes in relation to the dermis of the preputial skin in both breeds. These defense cells were similar to what occurs in the lymphoid tissue associated with the mucosa of other organs (Fig. 3C and 3D). In the middle and caudal portions of the mucosa of the IPL we did not observe the presence of hairs, hair follicles and sebaceous glands in any of the breeds evaluated in this study.

In the cranial portion of the IPL, light microscopy images revealed that the skin of the preputial ostium of both breeds is composed of epidermis and dermis (Fig. 4A). The epidermis consists of keratinized stratified sidewalk epithelium. It also has basal, spinous, granular, lucid, and horny layers (Fig. 4B). The dermis consists of irregular dense connective tissue. In the reticular layer of the dermis, denser fibers and a smaller cell population were evident, especially with fewer leukocytes. Also, predominantly in the reticular layer, hair follicles, hairs, and sebaceous glands were observed (Fig. 4C and 4D).

Transmission electron microscopy (TEM) revealed the image of the microstructure of the preputial sebaceous gland. Observed in each sebocyte, the lipid-filled cytoplasm that makes up the smegma and nucleus compressed on the side of the cell (Fig. 5).

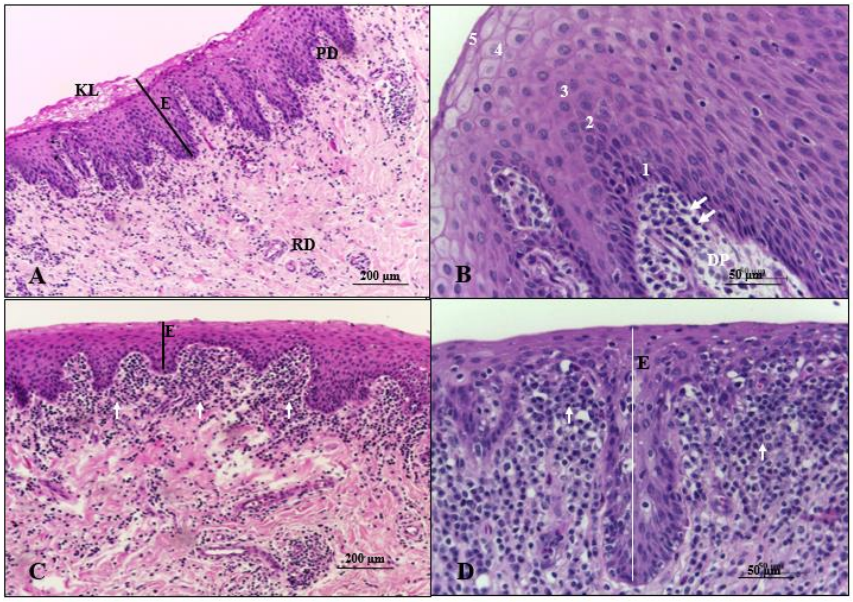

Figure 3. Histological images of the mid and caudal portions of the bovine IPL stained with H.E. Note the absence of hair and sebaceous glands in both portions of the IPL mucosa. In (A), middle portion of the IPL, keratinized stratified sidewalk epithelium "E", keratinized layer "KL", papillary dermis "PD" and reticular dermis "RD". In (B), middle portion of the IPL, observe the epithelium layers: 1 - basal; 2 - spiny; 3 - granular; 4 - lucid; 5 - keratinized; papillary dermis (PD) with the presence of leukocytes (arrows). In (C and D), caudal portion of the IPL, sidewalk stratified epithelium "E" and papillary dermis with concentration of leukocytes (arrows). In (A and C) Nellore bull. In (B and D) Aberdeen Angus bull.

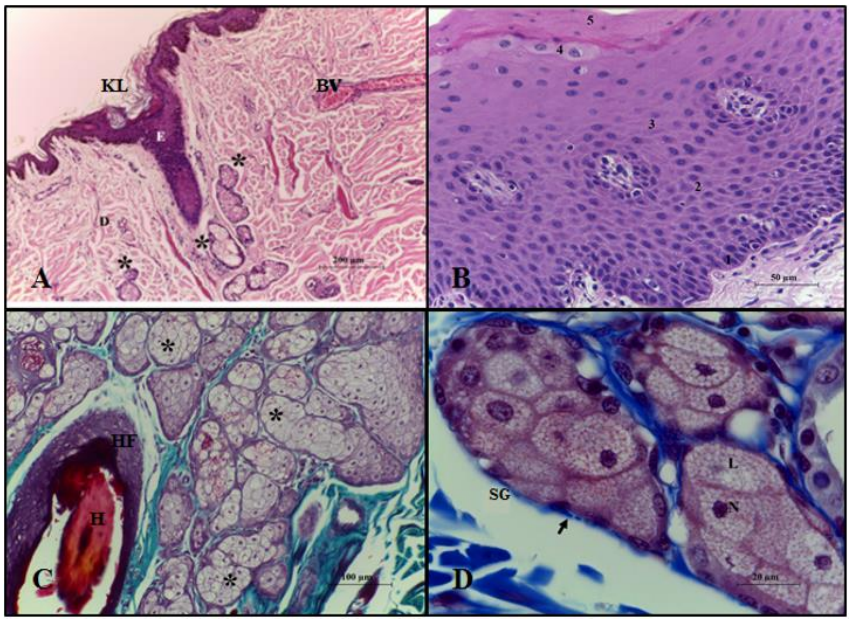

Figure 4. Histological images of the cranial portion of the IPL at the skin/mucosa transition of the bovine preputial ostium. Note the presence of sebaceous glands in this portion of the IPL. In (A), keratinized sidewalk stratified epithelium "E". The keratinized layer "KL" is observed; in the dermis "D" sebaceous glands "*" and blood vessel "BV" can be observed. In (B), epithelium and its layers: 1 - basal; 2 - spinous; 3 - granular; 4 - lucid; and 5 - keratinized. In (C), hair follicle "HF", traces of hair "H" and sebaceous gland "*". In (D), sebaceous gland "SG", basal lamina (arrow) and lipid vesicles "L" of the sebaceous cell. In (A and C) Nelore bull. In (B and D) Aberdeen Angus bull. In (A and B) H.E. staining. In (C and D) Mallory's Trichrome staining.

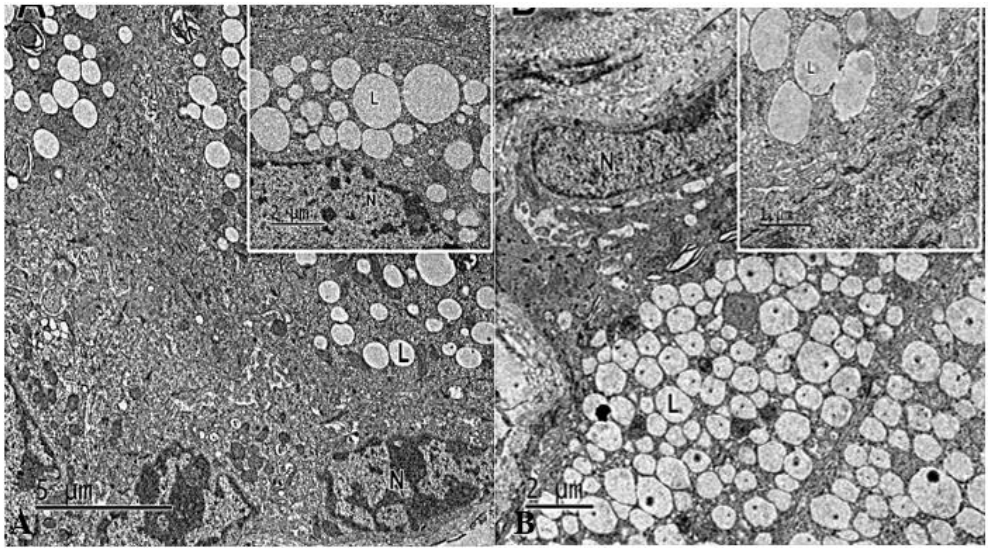

Figure 5. Electron micrograph of sebaceous glands in the cranial portion of the IPL of bulls. In (A) Preputial sebaceous gland of Aberdeen Angus bull. Note lower concentration of lipid granules. In (B) sebaceous gland of Nellore bull. The nucleus (N) presents loose chromatin and located at the side of the cell. In the cytoplasm are observed many vesicles or secretion granules with lipid content (L) in different sizes. In inset detail of the secretion vesicles. Magnifications: (A) 800 X, inset 2,000 X; (B) 1,000 X, inset 2,500 X.

The result of sebaceous gland counts by light microscopy are compiled in Table 2 and separated by dorsal and ventral skin region of the preputial ostium, cranial portion of the IPL, transition of the preputial skin, and mucosa of bulls of the Aberdeen Angus and Nellore breeds.

Table 2. Number of sebaceous glands per region of interest (ROI), in the dorsal and ventral regions of the preputial ostium, in the cranial portion of the IPL, in the transition of the preputial skin and mucosa of bulls of the breeds Aberdeen Angus and Nellore

Breed	Preputial ostium region		Averages
	Dorsal	Ventral	
Aberdeen Angus	1.81	2.02	1.92 B
Nellore	2.03	2.29	2.16 A
Averages	1.92 b	2.16 a	2.04
Coefficient of Variation (%) 15.77			
Standard error of the mean 0.1015			

Averages followed by the same capital letters in the column and lower-case letters in the row are statistically similar by the F test at 5% probability.

There was no significant interaction between the variables breeds and ostium region, but there was an independent effect of each variable. Aberdeen Angus bulls had fewer preputial glands compared to Nelore bulls. The ventral region of the preputial ostium had more sebaceous glands than the dorsal region of the ostium. There was coincidence with exceptionally equal results among the independent variables, but it was totally random. The effect of breed was independent of region and effect of region was independent of breed.

DISCUSSION

The equipment used to gather the information on the pieces from slaughtered animals proved to be effective and low-cost, allowing to simulate in a simple and easy way, the penile exposure of bulls, using only gravity, as occurs in live animals, after muscle relaxation. Therefore, the measurements were associated without interference from the stress of the animal's restraint and changes in the positioning of the foreskin and penis because of

the manual touch. Other authors reinforce that manipulation of these organs can pull or elevate the preputial sheath, retract the IPF mucosa and contract the preputial ostium (Ashdown, 2006; Rabelo *et al*., 2017).

In this study there was no difference between the breeds Aberdeen Angus and Nellore in the diameter, radius, perimeter, and area of the preputial ostium. Some authors suggest that bulls with a larger ostium diameter or wide preputial orifice have a greater chance of developing preputial prolapse syndrome, common in some cattle breeds, including Aberdeen Angus and Zebu breeds (Rabelo *et al*., 2012; Bodh *et al*., 2017).

To ensure the accuracy of the measurements in this study, the ostium perimeter was calculated from the diameter of the ostium, a fact rarely observed by other authors. Mendonça *et al*. (2012) measured the perimeter or circular length of the surface of the preputial ostium in live animals. In this case, possible interferences in the accuracy of the measurements may occur due to the stimulation of the preputial muscles during the manipulation.

The Nellore bulls stood out by presenting greater length of the internal preputial leaflet (IPL) than the Aberdeen Angus bulls. The explanation for this result is based on the principle of proportionality relationship between the length of the external preputial leaflet (EPL) and the internal preputial leaflet (IPL). Zebu breeds have more pendulous and longer EPL compared to taurine breeds, consequently, they have longer IPL length (Wolfe 2018), which justifies this finding.

The difference between breeds with respect to IPL length provides pertinent information, especially for veterinary surgeons. When submitting a bull to corrective surgery for acropostitis-fimosis, there is the possibility of paraphimosis occurring as a surgical complication resulting from significant loss of injured preputial mucosa and scarcity of healthy mucosa. However, zebu bulls showed higher chances of successful surgical treatment, due to longer IPL length and greater mucosal availability compared to bulls of taurine breeds (Turner and Mclwraith 2002; Rabelo *et al*., 2017).

In this study, the length of the free part of the penis of Nellore bulls at 24 months of age was 11.5 cm, including the glans. Mendonça *et al*. (2012) evaluated Nellore bulls aged 30 to 38 months and obtained the length of the free part of the penis of 9.0 cm, but did not include the glans. However, according to veterinary anatomical nomenclature, the glans is a constituent of the free part of the penis (Schaller, 2017). Thus, it is considered that in the present work the measurements are more appropriate in relation to anatomical terminology, generating more accurate parameters.

Using optical microscopy, it was possible to verify that there was a difference in the amount of preputial sebaceous glands between the breeds of cattle. The smaller amount of preputial sebaceous glands in bulls of the Aberdeen Angus breed compared to bulls of the Nellore breed may be due to differences in the thickness of the skin of the ostium, although this study did not measure the skin between the breeds. Reinforcing this, it is notorious that the Zebu breeds, *Bos taurus indicus*, are more adapted and more tolerant to climatic conditions of tropical heat and, consequently, presenting less skin thickness, short hair, more sebaceous and sweat glands, than European breeds or *Bos taurus taurus* subjected to the same environmental conditions (Ribeiro *et al*., 2008; Batista, 2012).

Another justification for the superiority of Nelore bulls in the amount of preputial sebaceous glands in relation to Aberdeen Angus bulls can probably be explained by the different serum levels of testosterone between the breeds, although in this study the measurement of testosterone was not performed. However, it is known that these sebaceous glands are stimulated and dependent on androgen hormones (Szöllősi *et al*., 2018). Reinforcing this hypothesis, some authors concluded that *Bos taurus indicus* bulls have a better response to heat stress, managing to maintain blood concentrations of testosterone higher than in *Bos taurus taurus* bulls throughout the seasons in a tropical climate (Koivisto *et al*., 2009; Quezada-Casasola *et al*., 2016).

It was found that the ventral region of the ostium has a greater number of sebaceous glands than the dorsal region of the ostium. This result may be due to differences in the skin thickness of the ostium, although in this study the skin was not measured.

Generally, thinner skin has a greater number of sebaceous glands (Batista, 2012).

A practical application of the presence and quantity of preputial sebaceous glands is the possibility of their contribution to the response to surgical treatment of preputial diseases. It is believed that the more the skin of the ostium is preserved or the less surgical removal of this organ, the more glands will remain active and the greater the protection of the mucosa of the IPL. However, more studies are needed on the action of smegma on prevention and preputial hygiene.

CONCLUSION

Until this time, the emphasis in studies of the external genitalia of bulls has been on preputial diseases, which cause coeundi impotence. In this study, the objective was to establish morphometric parameters of the preputial ostium, the internal preputial leaflet, the free part of the penis and preputial sebaceous glands count by microscopy, clarifying points not mentioned until then in the specialized literature. It was concluded that Nellore bulls have more extensive FPI and free penis and more preputial sebaceous glands than Aberdeen Angus bulls.

These morphological characteristics of the bull's external genitalia may contribute to the etiopathogenesis and treatment of preputial diseases. Therefore, the greater length of the IPF of Nellore bulls is advantageous, mainly, in the surgical treatment of preputial conditions, with emphasis on the benefits of greater availability of the IPF mucosa in the postoperative period.

This research pioneered preputial sebaceous glands in cattle. However, further studies must be carried out to identify whether there is a possible relationship between the quantity and volume of sebaceous glands and smegma production. Beyond, it is necessary to investigate the relationship between the composition and amount of smegma and health and possible contributions to the pathophysiology of preputial and sexually transmitted diseases.

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