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Characterization of vesicular glands, study of vesiculitis and its association with other accessory sex glands in bulls

[*Caracterização das glândulas vesiculares, estudo da vesiculite e sua associação com as demais glândulas sexuais acessórias em touros*]

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ABSTRACT

The aim of this study was to elucidate histological characteristics of the vesicular glands as well as to investigate vesiculitis and its association with ampolititis and prostatitis. Sex gland samples from 40 bulls were collected. The vesicular glands were descriptively evaluated, the number of acini was counted in an area of 25mm², compared to the number of acini between healthy and pathological glands, the data were submitted to the Student's T test at 5%. Inflammatory tissue was investigated in the ampullae of the vas deferens and in the prostates, the chi-square test at 5% was performed to assess the association between the inflamed glands. Regarding inflammation in the gland (vesiculitis), 30 animals (75%) were positive. The vesicular glands presented an average of 355 ±76.4 acini/25mm². When comparing the amounts of acini between the glands with and without vesiculitis, no significant difference was observed, p=0.930. When performing the chi-square test to assess the association between vesiculitis and ampolititis or vesiculitis and prostatitis, the results were p=0.356 and p=0.300 respectively. Despite the high incidence of inflammatory reaction in the glands, no association was observed between the accessory sex glands studied here.

Keywords: vesicular adenitis, prostate, ampullae of vas deferens, bovine

RESUMO

O objetivo deste estudo foi elucidar características histológicas das glândulas vesiculares bem como pesquisar sobre a vesiculite e sua associação com a ampolite e a prostatite. Amostras de glândulas sexuais de 40 touros foram coletadas. As glândulas vesiculares foram avaliadas descritivamente. Realizou-se a contagem da quantidade de ácinos em uma área de 25mm², e comparou-se a quantidade de ácinos entre glândulas sadias e patológicas. Os dados foram submetidos ao teste T de student a 5%. Foi pesquisado tecido inflamatório nas ampolas do ducto deferente e nas próstatas e realizou-se o teste qui-quadrado a 5% para se avaliar a associação entre as glândulas inflamadas. Com relação à inflamação na glândula (vesiculite), 30 animais (75%) foram positivos. As glândulas vesiculares apresentaram uma média de 355±76,4 ácinos/25mm². Quando comparadas as quantidades de ácinos entre as glândulas com e sem vesiculite, não foi observada diferença significativa (P=0,930). Ao se realizar o teste de qui-quadrado para se avaliar a associação entre a vesiculite e a ampolite ou a vesiculite e a prostatite, os resultados foram P=0,356 e P=0,300, respectivamente. Apesar da alta incidência de reação inflamatória nas glândulas, não foi observada uma associação entre as glândulas sexuais acessórias aqui estudadas.

Palavras chave: adenite vesicular, próstata, ampola do ducto deferente, bovino

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INTRODUCTION

The vesicular glands are a pair of accessory sex glands, they are relatively large and can be easily palpated rectally. They are firm in consistency and have lobulated surfaces. The vesicular glands are the ones that contribute the most to semen (Alexander *et al*., 1971), producing substances that are responsible for the nutrition and survival of the sperm cell (Campos, 2003, Brackett, 2006; Garner and Hafez, 2004), as well as decreasing its antigenicity and neutralizing the vaginal pH for fertilization to occur (Veselský, 1981; Gonzalez, 2002). The gland is an alveolar tubule type and has a relatively large lumen for storing secretions between ejaculates with pseudostratified columnar epithelium and sparse basal cells (Ghonimi *et al*., 2014a; Adhikary *et al.*, 2015; Gofur, 2015).

Vesiculitis is the most diagnosed pathology among those that affect the accessory sex glands in production animals and can be caused by various agents such as viruses, protozoa, or bacteria. The prevalence of vesiculitis varies greatly in herds depending on the age of the animals, production system, diagnostic methods and even the breed of the animals (Bagshaw and Ladds, 1974; Cavaliere and Van Camp, 1997; Martínez and Barth, 2007; Rovay, *et al*. 2008; Gonzalez, *et al*. 2019). The routes of infections can be ascending, descending or hematogenous, as well as congenital anomalies can predispose to the appearance of this pathology (Dargatz, *et al*., 1987; Cavaliere and Van Camp, 1997; Foster, 2009).

The diagnosis is most often associated with macroscopic changes in shape and size by rectal palpation with the presence of pus in the ejaculate, but these changes do not always occur, the ultrasound examination can help in less serious changes observing the changes in the gland membrane or gland irregularity, small abscesses, areas of varied echogenicity (Lovell, 1957; Van Camp, 1997; Barth, 2007; Gnemmi and Lefebvre, 2009; Oliveira *et al*., 2018; Scheeren *et al*., 2018), however the absence of changes in this exam does not mean the absence of the pathology (Oliveira *et al*., 2018).Treatment is still a challenge due to difficulties in penetration of antibiotics into the gland, however injection of antibiotics directly into the gland or subcutaneously, endoscopic infusion and lavage are performed to try to combat the pathology (Blanchard *et al*. 1988; Wagenlehner and Naber, 2005; Pinto *et al.*, 2014; Zhang *et al.*, 2019; Scheeren *et al*., 2020).

Thus, it is necessary to better understand and standardize the cellular characteristics of the vesicular glands of cattle as well as better understand the pathological mechanisms that involve this organ, since they are important glands in the reproductive process and can cause genetic and financial losses. The understanding of tissue behavior in the face of aggression, as well as the possibility that the inflammatory process is not focused only on the vesicular glands but is simultaneously distributed in the other accessory sex glands, makes investigations regarding the association of these lesions important both knowledge and decision-making in face of the problem.

MATERIAL AND METHODS

All procedures were performed after approval by the Ethics Committee for the Use of Animals of the Federal Rural University of Pernambuco (CEUA – UFRPE), license n° 5495230620.

40 sets of accessory sex glands from bulls aged between 2-5 years were collected, the pieces were supplied by the municipal slaughterhouse of Garanhuns-PE, located 1 km from the laboratory where the samples were processed, 80 samples of the vesicular glands, 80 Samples of ampullae of the vas deferens and 39 samples of prostate were submerged in a 10% buffered formalin fixative solution for at least 24 hours, after which they were submitted to the process of impregnation in paraffin and then cuts of 5 µm thick were made to make slides and further observation under an optical microscope.

The vesicular glands were evaluated macroscopically and descriptively. The number of acini per area (25mm²) of each vesicle was also computed by means of an optical microscope at 40x magnification, the results were compared between the glands with and without inflammatory process and submitted to the Student's T test with a significance of 5%. To assess the existence of an association between vesiculitis, ampolititis and prostatitis, data were submitted to the chi-square test with a significance level of 5%.

RESULTS

The vesicular glands were paired, located dorsolateral to the neck of the bladder, lateral to the ampullae of the vas deferens (Fig 1). Macroscopically, the glands were lobulated, similar to grape bunches, with a firm consistency of whitish coloring on the surface (Fig. 1) with an average length of 12.8 ±1.74cm, the longest and shortest length obtained were 16cm and 8, 5cm respectively. When cut, the glands showed a yellowish color, some with the presence of viscous liquid with a whitish or translucent color (Fig. 1).

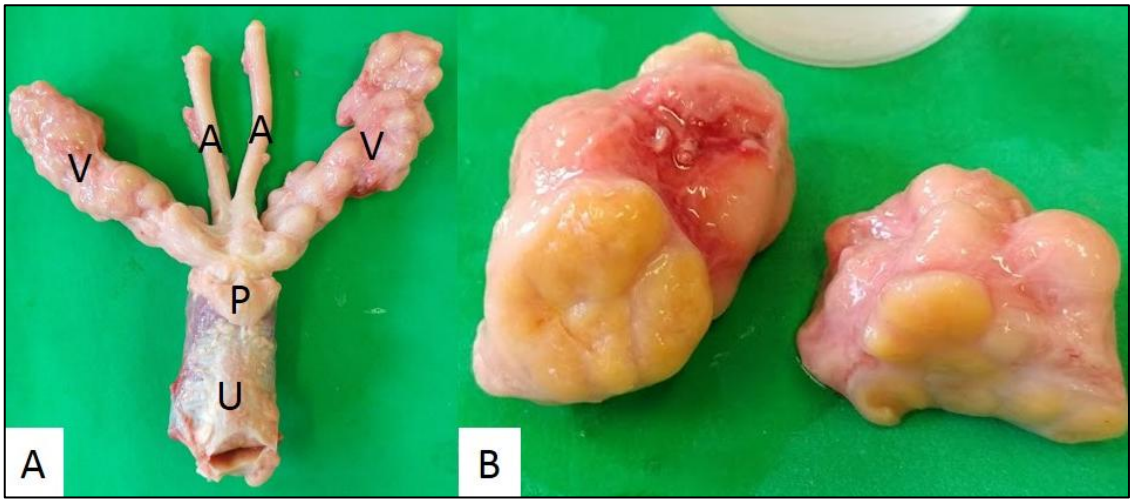

Figure 1. Bull accessory sex glands. Set of accessory sex glands, showing the vesicular gland: V; vas deferens ampulla: A; prostate: P; pelvic urethra region: U (A). Image B shows the vesicular gland in section (cross-sectional).

Microscopically, it was observed that the glands were of the tubulo-alveolar type, where the layers that compose it could easily be distinguished. The alveoli varied in size and shape, and eosinophilic content was commonly observed within them. The layers were differentiated into mucosa, submucosa, muscular and serosa; the muscular one varied in thickness depending on its location (Fig. 2 A).

The mucous layer is pseudostratified lined by columnar cells with a rounded or elongated nucleus located in the basal region of the cytoplasm, eosinophilic cytoplasm with the presence of granules mainly in the apical region, projections in the form of bubbles of eosinophilic content were observed, indicating the secretion of the gland (Fig. 2B). In some glands there were also vacuoles in columnar cells mainly in the basal region below the nucleus. In smaller numbers, narrower, rod-like, more strongly stained columnar cells with a smaller and elongated nucleus, scattered randomly, were observed (Fig. 2 B).

The basal cells with a more rounded and smaller shape, most of the time had vacuolated cytoplasm, the nuclei varied between round and flat shapes and may be displaced to one side in the cell (Fig. 2 B).

In the lumen of the acini it is common to find eosinophilic substances. The lamina propria is composed of connective tissue, smooth muscle and vessels that form inter-acinar septa, free defense cells (lymphocytes) are also found. There are thicker muscle layers that separate clusters of glands forming the interlobular septa. More externally, the muscular layer is composed of two layers of smooth muscle, an internal circular and an external longitudinal layer (Fig. 2 A).

Only basal cells were not PAS positive. Granules were observed at the apex of cells, bubble-shaped projections and rod cells, all PAS positive.

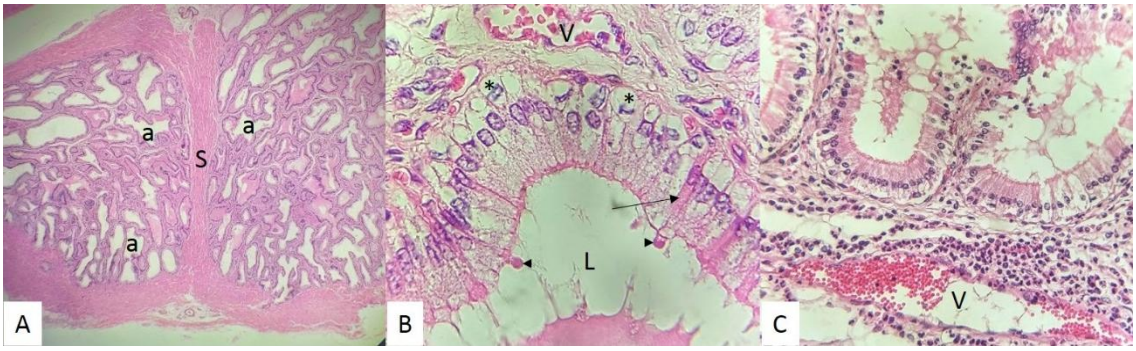

Figure 2. Histological characteristics of the sex glands of bulls stained with hematoxylin and eosin. Image A (40x) shows a vesicular gland fragment, showing acini: a; interlobular septum: S. Image B (1000x) shows part of an acinus with pseudostratified epithelium, formed mainly by columnar cells and basal cells, both may have vacuolated cytoplasm; acinus light: L; eosinophilic bubble-shaped projection: arrowhead; rod-shaped cell: arrow; basal cell: asterisk; blood vessel: V. Image C (400x) demonstrates a perivascular inflammatory reaction with partial desquamation of the acinar epithelium and blood vessel: V.

Regarding inflammation in the gland (vesiculitis), 30 animals (75%) were positive in the histological examination, 15 (37.5%) presented the disease bilaterally and the other 15 animals (37.5%) presented unilaterally, 8 in the right gland and 7 in the left.

Inflammatory lesions were most often multifocal (20) and focal (18) in isolated lobes, few showed a diffuse lesion (7), lymphoplasmacytic inflammatory infiltrates in the perivascular region was the most visualized lesion, a significant number of lesions was also seen (21) that showed neutrophils in the inflammatory reaction, which may be accompanied by epithelial desquamation in more severe cases (Fig. 2 C). To a lesser extent (13) hemorrhagic areas were visualized together with inflammation, in some cases inflammatory infiltrate could also be visualized in the lumen of the glands.

The vesicles presented an average of 355 ±76.4 acini in an area of 25mm², the highest and lowest number of acini found were 580 and 169 per 25mm² respectively. When comparing the amounts of acini between the glands with and without vesiculitis, no significant difference was observed p=0.930, indicating that there is no significant glandular loss even with vesiculitis installed.

Analyzing the data, it was possible to observe that 39 animals (97.5%) presented an inflammatory reaction in some sexual gland and only 1 animal (2.5%) did not present any lesion. The most common lesion was vesiculitis (30 animals) followed by prostatitis (26 animals) and ampolititis (17 animals). These lesions were distributed as follows: 11 animals (27.5%) had ampolititis, vesiculitis and prostatitis concomitantly, 7 animals (17.94%) had prostatitis and vesiculitis, 2 animals (5.13%) had ampolititis and prostatitis, 3 animals (7.69%) vesiculitis and ampolititis. Those that showed inflammation in only one of the glands were 15 animals (38.46%), 8 vesiculitis, 6 prostatitis and 1 ampolititis.

Table 1. Data on inflammatory lesions in the accessory sex glands of cattle. V = Vesiculitis, A = Ampolititis and P = prostatitis

Lesion	Qty animals	Lesion	Qty animals
vesiculitis	30 (75%)	V + A	3 (7.69%)
		V + P	7 (17.94%)
prostatitis	26 (66.6%)	A + P	2 (5.13%)
		V + A + P	11 (27.5%)
ampolititis	17 (42.5%)	V or A or P	15 (38.46%)
		None	1 (2.5%)

When performing the chi-square test to assess whether there is an association between vesiculitis and ampolititis or vesiculitis and prostatitis, the results were p=0.356 and p=0.300, respectively.

DISCUSSION

Macroscopically, the glands showed characteristics similar to those described by other authors, located cranially to the prostate, laterally to the ampullae and caudal-dorsal to the bladder, paired glands of firm, tense consistency, lobulated, of varying size.12.8 ±1.74cmshaped like a bunch of grapes (Krause, 1993; Sarangi *et al*., 2020).

Under microscopic evaluation, the presence of acini and duct system that conduct the secretion produced by the gland can be observed, giving the characteristic of a tubulo-alveolar type gland (Adhikary *et al*., 2015, Gofur, 2015; Scheeren *et al*., 2018).

The glandular epithelium is classified as pseudostratified, with the presence of columnar secretory cells as the main and basal cells sparsely distributed among the columnars, this is the description also used for other species (Ghonimi *et al*., 2014b; Adhikary *et al*., 2015; Emam *et al*., 2016) except for horses that have stratified epithelium (Scheeren *et al*., 2018).

The presence of vacuoles in columnar and basal cells is also described in several species such as buffaloes, bulls, goats, (Ghonimi *et al*., 2014b; Adhikary *et al*., 2015; Emam *et al*., 2016) but were not mentioned in swine nor in horses (Badia *et al*., 2006; Scheeren *et al*., 2018), which suggests that it is a characteristic of the vesicular glands of ruminants.

In related studies, there is confusion when classifying the cell types found in the glandular epithelium, this is because the different authors do not follow a standard in the classification, as well as by the method used in the staining or in the technique used itself, if optical or electron microscopy (Badia *et al*., 2006; Archana *et al*., 2009; Adhikary *et al*., 2015; Gofur, 2015; Mokhtar *et al*., 2016). In general, the cells classified differently are the same with different nomenclatures, which makes a standardized study of this organ difficult. In this study, it was observed that, both by HE staining and by PAS, there is a similar behavior in the staining by the cells, in which they were classified in a way considered more appropriate. Columnar cells are divided into two types: columnar cells, the main producers of glycoprotein substances, which may have vacuoles, and rod-shaped cells, are narrower and stain more strongly, finally, basal cells with a more rounded shape, cytoplasm in most sometimes vacuolated and may have a flattened and displaced nucleus.

The difference in both the shape and size of the cells is related to the cell development phase according to some authors, considering the basal cells as a reserve of cells, the rod-shaped cells as an intermediate phase, since in goats it was more quantified in prepubertal animals, and columnar cells as adult cells (Briz *et al*., 1993; Archana *et al*., 2009; Adhikary *et al*., 2015; Gofur, 2015). It was possible to clearly observe these three cellular phases, but the quantification of the different types was not performed.

Regarding the other layers, the submucosa composed of loose connective tissue, vessels and some muscle fibers the same composition was seen in other species (Adhikary *et al*., 2015; Mokhtar *et al*., 2016; Emam, 2016), this interglandular tissue may vary in size according to age and mainly due to the action of androgen hormones (Ghonimi *et al*., 2014a; Adhikary *et al*., 2015; Emam *et al*., 2016; Emam, 2016), it was not possible to observe this difference, since all the animals used in this study were adults. However, the presence of free defense cells, mainly lymphocytes, was also visualized, this finding corroborates Campero *et al*. (1989, 1990) and Dibarrat *et al*. (2006), despite this, they consider that this natural presence of immunoglobulin-carrying defense cells in the vesicular glands is very small when compared to other accessory sex glands and possibly because of this, there is a consequent predisposition of the vesicular gland to be affected by some infectious agents.

The muscular layer is visualized more externally, it is formed by two layers of fibers arranged in different ways, an internal circular and an external longitudinal one, they surround each lobe of the gland, forming interlobular septa, according to Porto (2007) and Ghonimi *et al*. (2014a) this form of muscle distribution helps in

the evacuation of substances produced by the gland at the time of ejaculation by the contraction of fibers. In the adventitial layer, the outermost layer, vessels, and connective tissue were observed surrounding the muscular layer, as already described by Ghonimi *et al*. (2014a).

The vesicular gland was the accessory sex gland that the presence of inflammatory reaction was most visualized, where 75% of the evaluated animals presented this alteration, confirming what has already been described by other authors (Cavaliere and Van Camp, 1997; Martínez and Barth, 2007; Oliveira *et al*., 2018), but the frequency of animals that present this pathology is very variable, especially when there is a difference in the method used for diagnosis, when by physical evaluation only or with the aid of ultrasound, the data show a significant discrepancy (Martínez and Barth, 2007; Oliveira *et al*., 2018). In our recent research carried out at LABRAPE, this difference in diagnoses was noticed (Unpublished data) and even when the same methods are used, there is a difference in the results (Oliveira *et al*., 2018; Gonzalez *et al*., 2019). This difference in the frequency of vesiculitis in different studies makes it difficult to understand the real situation that herds face in the face of this pathology.

Another important factor lies in the objectivity and perspicacity of the person performing the exam to recognize small variations in the normality of the organ (Hull and Vogel, 2008), since it is necessary for the examiner to have a good morphological knowledge of the sexual glands to have a more assertive diagnosis (Gnemmi and Lefebvre, 2009). Therefore, the histological examination can be the most accurate, it helps to understanding the dimension of the vesiculitis problem, as it reduces subjectivity on the part of the examiner, but in cattle, this examination is practically unfeasible for live animals, as there is no way to access the gland but through some surgical procedure, which is different in horses, which due to the anatomy of the urethra, an endoscope can be used to acquire a tissue sample via the transurethral route (Scheeren *et al*., 2018).

Also contributing to this oscillation in results is the fact that vesiculitis has multivariate etiological agents such as viruses, bacteria, fungi, protozoa, as well as several ways in which these agents can gain access to the gland, such as ascending through the urethra, descending by problems arising from the testis, epididymis, prostate and ampulla or from hematogenous route (Cavaliere and Van Camp, 1997; Foster, 2009; Nascimento *et al.*, 2016; Oliveira *et al*., 2020).

Despite the description of a reaction with the presence mainly of lymphocytes and plasma cells as the main inflammatory response in the accessory sex glands (Foster, 2009; Nascimento *et al*., 2016; Scheeren *et al*., 2018), it was observed in this study that, in some cases, the predominant cells in the reaction were neutrophils, especially in those with greater tissue damage, this can be explained by the type of agent or the time of injury, as they are attracted to the focus of the injury caused mainly by pyogenic bacteria as well as being the first cells recruited in inflammatory reactions (Ringler, 2000).

Among all the vesicles that presented an inflammatory reaction, 21 (46.6%) presented neutrophils, of which all that were classified as diffuse lesions also presented a predominance of neutrophils. The presence of neutrophils in the inflammatory reaction indicates that it is an acute inflammatory process, the cause of greater tissue injury may be related to the etiologic agent, however it may be a consequence of a greater presence of neutrophils in the tissue, as they could injure the tissue during the inflammatory process. (Ringler, 2000).

Visualization of hemorrhagic areas as well as tissue damage associated with the presence of inflammatory cells both in the interstitial tissue and in the lumen of the acini may explain the appearance of clinical signs such as hemospermia, pyospermia and semen agglutination (Sancler-Silva *et al*., 2020, Castiglione *et al*., 2014).

The histomorphometric data described in this work regarding the amount of acini per mm² can serve for future comparisons with other studies that find vesicles with macroscopic lesions, since no macroscopic alteration was observed in the studied glands, this indicates that, despite being observed microscopically inflammatory lesions, these were not sufficient for the morphological modification of the gland, which would explain

the result of the comparison of the amount of acini per mm² not having significance between healthy and diseased glands and the possible discrepancy in the diagnosis by rectal palpation or ultrasonography.

Since there is no association between inflammation of the accessory sex glands, it reinforces the idea that the way the etiologic agent reaches the vesicle is multivariate (Dargatz *et al*., 1987; Cavaliere and Van Camp, 1997; Foster, 2009; Nascimento *et al*., 2016; Oliveira *et al*., 2020). The fact that 39 animals (97.5%) had an inflammatory lesion in some sexual gland calls attention to the need for better care in the physical examinations performed on the animals, as this is a higher number than in previous studies (Bagshaw and Ladds, 1974); Cavaliere and Van Camp, 1997; Martínez and Barth, 2007; Rovay *et al*., 2008; Oliveira *et al*., 2018; Gonzalez *et al*., 2019).

CONCLUSION

Vesiculitis is a very common and the most diagnosed disease among the accessory sex glands, capable of causing important tissue changes. With the histopathological examination, a higher frequency of this pathology was observed because, in most cases, the lesions are not enough to macroscopically modify the gland, but it is noticed that the greater the presence of neutrophils, the greater the amount of tissue damage, although was not enough to decrease the amount of acini. There is no association between the inflammatory processes of the accessory sex glands, thus leaving the route of infection undefined. There is a high incidence of inflammatory processes in the sexual glands of bulls, with vesiculitis being the most frequent pathology, followed by prostatitis and ampollitis.

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