MICROSTRUCTURE AND MORPHOQUANTITATIVE STUDY OF PINEAL GLAND IN SANTA INES SHEEP

ESTUDO MORFOQUANTITATIVO E DA MICROESTRUTURA DA GLÂNDULA PINEAL EM OVINOS SANTA INÊS

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SUMMARY

This study examined morphoquantitative aspects of the pineal gland in Santa Ines sheep in anoestrus physiological and reproductive activity. Seven female adult sheep collected from slaughterhouses in the Brasilia-DF region, with no clinical signs related to diseases of the nervous system were used. The brains were removed and their dimensions were measured. The brains were sectioned to expose and measure the pineal gland. The fragments of the diencephalon containing the pineal gland were immersed in 20% formaldehyde solution. Subsequently, the fragments were submitted to conventional histological techniques. The Hematoxylin-eosin, Toluidine blue and Gomori trichrome staining were used for a morphoquantitative analysis. The brain had an average length of 67.25 ± 1.75 mm and average width of 58.97 ± 4.0 mm. The pineal gland had an average length of 6.98 ± 0.79 mm and average width of 6.40 ± 1.35 mm. The quantitative analysis showed an average 86.27 ± 30.44 of pinealocytes per field. According to the Pearson correlation test, the number of pinealocytes showed a weak negative linear correlation (r = -0.11) with the length of the pineal gland and a weak positive linear correlation (r = 0.39) with the width. Therefore, the number of pinealocytes has a stronger correlation with the width of the gland compared to the length. Mastocytes were present in only one animal (14.28%). Calcareous concretions were observed in two animals (28.57%). The connective tissue formed the capsule surrounding the gland with no projections into the parenchyma.

KEY-WORDS: Calcareous concretions. Mastocytes. *Ovis aires*. Pineal gland. Pinealocytes. Santa Inês.

RESUMO

O presente estudo avaliou os aspectos morfoquantitativos e qualitativos da glândula pineal em fêmeas de ovinos da raça Santa Inês em atividade reprodutiva e anestro fisiológico. Foram utilizados sete ovinos, fêmeas e adultas, coletados em frigoríficos da região de Brasília-DF. Estes não apresentavam sinais clínicos relacionados a afecções do sistema nervoso. Os encéfalos foram retirados e suas dimensões mensuradas. Posteriormente foram seccionados para expor a glândula pineal e mensurar suas dimensões. Os fragmentos do diencéfalo, contendo a glândula pineal, foram submersos em solução aquosa a 20% de formaldeído e posteriormente submetidos a técnicas histológicas convencionais. Para a avaliação morfoquantitativa desta glândula, foram empregadas as colorações Hematoxilia-Eosina, Azul de Toluidina e Tricrômio de Golmori. O encéfalo teve um comprimento médio de 67,25 ± 1,75 mm e a largura média de 58,97 ± 4,0 mm. Já a glândula pineal apresentou o comprimento médio de 6,98 ± 0,79 mm e a largura média de 6,40 ± 1,35 mm. Diante da análise microscópica, foi obtida uma média de 86,27 ± 30,44 pinealócitos por campo. Ao ser aplicado o teste de correlação de Pearson, o número de pinealócitos apresentou fraca correlação linear negativa (r = -0,11) em relação ao comprimento da glândula pineal e uma fraca correlação linear positiva (r = 0,39) em relação à largura da mesma. Portanto o número de pinealócitos possui uma maior relação com a largura da glândula do que com o comprimento. Mastócitos estiveram presentes em apenas um animal (14,28%) e as concreções calcareas foram observadas em dois animais (28,57%). A glândula se mostrou envolta por uma cápsula de tecido conjuntivo com ausência de projeções para o parênquima.

PALAVRAS-CHAVE: Concreções calcáreas. Glândula pineal. Mastócitos. *Ovis aires*. Pinealócitos. Santa Inês.

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INTRODUCTION

For a long time, it was believed that the pineal gland in higher vertebrates represented only an organ that had atrophied during species evolution and became an epithalamic appendix without much function (HOLANDA-BARROS, 2002). However, the finding that the pineal gland produces melatonin has opened a vast field for research related to their physiology. The hormone melatonin is involved with the circadian control and other organic functions such as reproductive cycle, thermoregulation and thermal tolerance (MACHADO, 1993; TILDEN & HUTCHINSON, 1993).

Regarding the microscopic aspects of the gland, Hullinger (1993) stated that the pineal parenchyma is composed of pineal endocrinocytes surrounded by neurofilaments. He also characterized central gliocytes as support cells. The pia mater limited the glandular tissue, and the moderate amount of conjunctive tissue was disposed on the capsule to form the septa and trabeculae. Blood vessels and post-ganglionic sympathetic fibers were observed throughout the stroma. Concerning the analysis of the postnatal development of the pineal glands of females at different times, Lewczuk et al. (2004) and Nowicki et al. (2006) while studying sheep and goats, respectively, reported on the structural changes of the pineal gland and observed that the changes of the pinealocytes were related to different developmental stages and also substructures were involved with the secretory activity of this gland.

According to Chemineau & Malpaux (1998) the secretion by the pineal gland is sensitive to light. This secretion in vertebrates was stimulated by the lack of light and seemed to interfere with reproductive function adaptation to the lighting conditions, especially in animals that breed seasonally, allowing birth during more favorable season (GOMES, 2003; RESENDE 2006).

The objective of this study was to evaluate morphologically Santa Ines sheep pineal gland by determining gland and brain dimensions. The microstructure was also evaluated qualitatively by determining the presence of pinealocytes, mastocytes and calcareous concretions, as well as the arrangement of connective tissue. Quantitatively, it was determined the number of pinealocytes, mastocytes and calcareous concretions.

MATERIAL AND METHODS

Seven adult female Santa Ines sheep, during breeding season, were used in the study. Their brains were collected after slaughtering from slaughterhouses in the region of Brasília, DF. The absence of clinical changes in the central nervous system was a requirement when choosing the animals. The study was reviewed and approved by the Ethics Committee of Animal Use of the Instituto de Ciências Biológicas, Universidade de Brasília, protocol nº43.

The brains, while still fresh, were accessed through a midline incision while the skin was folded along with the muscles of the temporal region. Then, the skull was opened by cutting the frontal and parietal bones in a rectangular-like shape using a saw tape. After longitudinal sectioning of the brain meninges, the brain was exposed. Subsequently, the brain was removed while trying to preserve its structures, from the olfactory bulb to the medulla oblongata. Brain width and length were measured using an electronic caliper model Starrett® 799. The length of each cerebral hemisphere was measured from the rostral end of the frontal lobe to the transition region between the medulla oblongata and the spinal cord. The width was estimated by the distance between the lateral rinal groove of each antimeres. The brains were then isolated and sectioned along the brain longitudinal fissure to reach and cut the corpus callosum and then completely separate the brain hemispheres, thereby exposing the pineal gland. The macroscopic measurements, width and length, of this gland were obtained using the electronic caliper model Starret® 799. Subsequently, the fragments containing part of the diencephalon and pineal gland were immersed in 20% aqueous solution of formaldehyde (LABSYNTH - Produtos para Laboratórios, Ltda).

The material containing the pineal gland was cut into a 1-cm³ cube and immersed in McDowell solution (phosphate-buffered 4% glutaraldehyde with 1% paraformaldehyde 0.1M and pH=7.4), during 24 hours. After this, the material was submitted to conventional histological techniques, it was dehydrated in ethanol, cleared in xylene, and embedded in paraffin blocks. The blocks were then cut into 5 μm sections using a manual microtome (Spencer-Lens Co.). Stains used were hematoxylin-eosin, toluidine Blue and Gomori trichrome.

The histological analysis was performed using the optical microscope BX51 Olympus®, coupled to the reading equipment, with mobile and fixed imaging, using Windows and software Image-Pro Plus® in order to evaluate qualitatively the microstructure of the cells such as pinealocytes, mastocytes, calcareous concretions and connective tissue. Quantitatively, the number of pinealocyte and the presence of calcareous concretions, mastocytes and connective tissue in this gland were determined. From each slide, ten fields were analyzed at 40x magnification. Data were analyzed using descriptive statistics and by the Pearson correlation test between microscopic and macroscopic dimensions.

RESULTS

Macroscopically, the length and average width of the brain hemispheres were 67.25 ± 1.75 mm and 58.97 ± 4.0 mm, respectively. On the other hand, the pineal gland average length and width were 6.98 ± 0.79 mm and 6.40 ± 1.35 mm, respectively.

The qualitative analysis of the pineal gland microstructure was performed in relation to

pinealocytes and mastocytes, as well as the calcareous concretions and the arrangement of the connective tissue. Pinealocytes and calcareous concretions were stained by hematoxylin- eosin, mastocytes by toluidine blue and connective tissue by Gomori trichrome.

The microstructure of the pineal gland showed that calcareous concretions were arranged in the center of the gland (Figure 1.a). On the other hand, the pinealocytes were homogeneously distributed in the parenchyma. The connective tissue formed a coating capsule (Figure 1.d). This tissue neither projected towards the parenchyma nor formed septa, and therefore, this gland did not show lobular structure.

Quantitatively, concretions were present in only two animals, which represented 28.57% of all studied animals. The pinealocytes showed an average of 86.27 ± 30.44 cells present per field (Figure 1.b). And mastocytes (Figure 1c) were found in only one animal, that is, 14.28% of the animals.

The results were analyzed by the Pearson linear correlation test to determine correlation between microscopic and morphometric analysis. The number of pinealocytes was weakly positively correlated with the length of the pineal gland (r = 0.110) and strongly positively correlated (r = 0.834) with the width. Regarding the brains, the number of pinealocytes was weakly negatively correlated with both brain length (r = -0.374) and width (r = -0.291). Regarding the size of the pineal gland and brain, length showed a weak negative correlation (r = -0.257) and width a weak positive correlation (r = 0.305).

DISCUSSION

The mean length and width of the brains of Santa Ines sheep were 67.25 ± 1.75 mm and 58.97 ± 4.0 mm, respectively. In monkeys of the species *Cebus apella,* mean brain length and width were respectively, 61.08 ± 5.50 mm and 49.08 ± 2.93 mm, as reported by Barros (2006). Finally, Gomes (2003) while studying dogs, reported brain length 70.05 mm and width 36.65 mm, without standard deviations. The dimensions of the brains are important to establish a correlation with the dimensions of the pineal gland.

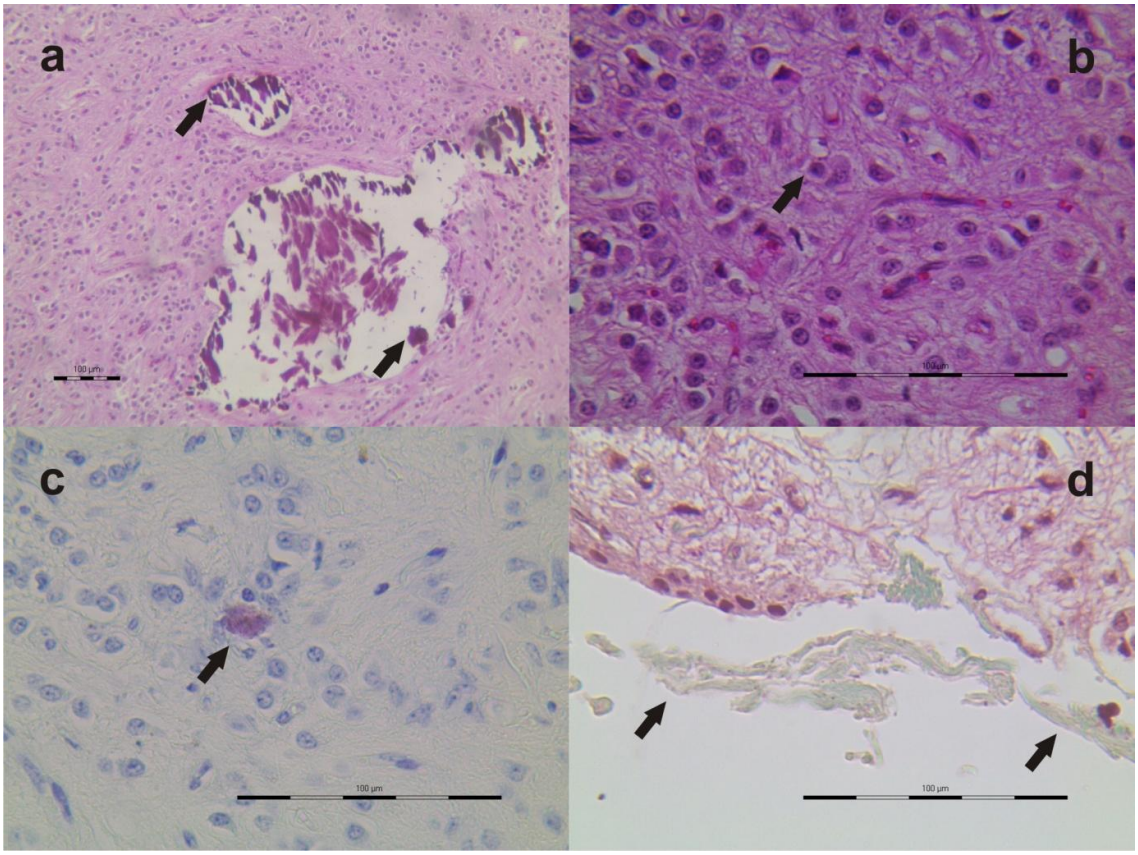

Figure 1: Photomicrograph of the parenchyma of the pineal gland of Santa Ines sheep showing: a) calcareous concretions (arrows) stained by hematoxylin-eosin enlarged to 100 µm; b) pinealocytes (arrow) stained by hematoxylin-eosin enlarged to 400µm; c) mastocytes (arrow) stained by toluidine blue enlarged to 400 µm; d) capsule formed by connective tissue (arrows) stained by Gomori trichrome enlarged by 400 µm . Bar100u.

The mean length and width of the pineal gland of Santa Ines sheep were 6.98 ± 0.79 mm and 6.40 ± 1.35 mm, respectively. Barros (2006) studying *Cebus apella* monkeys, reported 3.38 ± 0.48 mm mean length and 2.83 ± 0.44 mm mean width for the pineal gland. Gomes (2003) for dogs reported mean length of 2.05 mm and width 1.78 mm. While for skunks, *Didelphis sp*., these dimensions were microscopic according to Mançanares et al. (2007), Marques et al.(2010) reported that the pineal gland of *Procyon cancrivorus* had mean length of 7.5 mm and width of 4.5 mm. Favaron et al. (2008), while studying the pineal of *Nasua nasua*, observed mean length of 2.3 ± 0.45 mm and width 1.3 ± 0.21 mm. Montie et al. (2009) analyzed the neuroanatomy of the brain of a lion (*Zalophus californianus*), reported the pineal gland as surprisingly large, but did not give its dimensions. The pineal gland of an adult cat, according to Boya et al. (1995), has length between 1.5 and 2 mm and width between 2 and 2.5 mm. Carvalho et al. (2009) reported that the pineal gland of buffaloes (*Bubalus bubalis*) measures 7.9 ± 0.8 mm width and 8.8 ± 1.0 mm length. All these data show that different species have different gland size, even if the animals have similar dimensions. Nevertheless, we must emphasize that the variations found for the pineal gland size could also be justified by different phases of the reproductive cycles.

The mean number of pinealocytes in Santa Ines sheep was 86.28 ± 40.87 per field. Nowicki et al. (2006) while studying the pineal gland in goats, *Capra hircus*, from four months to three years old, concluded that the number of pinealocytes decreases with age. In contrast, the volume of the pinealocytes increases from birth until the fourth month and remains stable from the fourth month throughout the first year.

In this study, the cells were distributed evenly throughout the parenchyma of the pineal gland of each specimen, thus characterizing a particular arrangement that determines the architecture of the gland. Results that agree with Barros (2006) and Favaron et al. (2008) who characterized the pinealocytes as cell bodies distributed throughout the glandular parenchyma predominating over other cells. Gomes (2003) while analyzing pinealocyte distribution in relation to the structure of the pineal gland in dogs, reported that they were distributed throughout the gland with greater concentration in the center compared to the periphery, forming acini or cords. On the other hand, Barros (2006) reported that for *Cebus apella,* such cells were arranged as twisted cords aligned with the septum or randomly arranged forming cell groups that were mostly clustered in certain points and unclustered in others. Favaron et al. (2008) investigated the pineal gland of *Nasua nasua*, and reported a variable linear distribution between the linear and round cords scattered in the parenchyma. The distribution pattern in the shape of cords and randomly distributed cells in the parenchyma was also observed by Marques et al. (2010) in *Procyon cancrivorus*, by Silvino (1992) in golden agouti (*Dasyprocta aguti*) and by Carvalho et al. (2009) in buffaloes (*Bubalus bubais*). The sheep pinealocytes in this study were arranged in different ways, suggesting that possible differences could be related to the formation of lobes as reported by Silvino (1992), Gomes (2003), Barros (2006), Favaron et al.(2008), Carvalho et al. (2009) and Marques et al. (2010).

Calcareous concretions were present in only 28.7% of the Santa Ines sheep. Barros (2006) after observing calcareous concretions in the parenchyma of the pineal gland, conducted a microanalysis by X-ray diffraction and showed that they are mainly constituted by phosphorus and aluminum, while calcium was also present. In this study, the concretions' composition was not determined; however, it has been suggested that among mammals the composition of the concretions is similar when present. Gomes (2003) reported that the concretions were absent in the gland structure of dogs. They were also absent from the structure of *Procyon cancrivorus* and *Cercopithecus aethiops* according to Marques et al. (2010) and Simmons (1977), respectively. On the other hand, in Landrace pigs, these structures were agglomerated into the shape of a morula as reported by Lima et al. (2003). Favaron et al. (2008) observed in the *Nasua nasua* coati a variety of sizes and shapes. Carvalho et al. (2009) while studying buffaloes (*Bubalus bubalis*), observed large amount of calcareous concretions in the parenchyma, but did not reported on their shape. These results seem to suggest that the concretions were present in different shapes and quantities among species. These variations indicate that they are susceptible to the dynamic processes of different species, as stated by Oliveira (1998), thus characterizing not a static structure, but a metabolically active one. Therefore, it was suggested by Cipolla Neto (1996) that the melatonin production by the pineal gland requires calcium, and that these calcareous concretions function as dynamic calcium storage. They may or may not be present and also vary in number according to the species.

Mast cells were observed in only 14% of the studied animals. This result agrees with Barros (2006) who characterized the mastocytes present in the gland parenchyma as large globular structures, without visible extensions and filled with metachromatic granules, randomly arranged or forming small clusters in the pineal gland of *Cebus apella.* Mançanares et al. (2007) while studying *Didelphis sp.* observed mast cells present in the peripheral region. The presence of these cells in the parenchyma of the pineal gland was also reported by Marques et al. (2010) for *Procyon cancrivorus* and by Carvalho et al. (2009) for buffaloes (*Bubalus bubalis*). The low frequency of these cells in sheep did not allow to characterize a standard arrangement throughout the pineal gland parenchyma. They may well be a determining factor in the differentiation of the pineal gland in Santa Ines sheep.

The connective tissue in the pineal gland in Santa Ines sheep formed a capsule around the gland, while septa and lobes were notably absent throughout the parenchyma. This same arrangement of the connective tissue was observed by Gomes (2003) in dogs and by Novotná et al. (1966) in *Macaca mulatta.* On the other hand, Barros (2006) while studying the pineal gland of

Cebus apella, observed that the connective tissue covering the gland protruded forming incomplete septa and divided the parenchyma into lobes that contained thin collagen fibers without definite orientation. This incomplete septa arrangement was also observed by Marques et al. (2010) in *Procyon cancrivorus*, by Lima et al. (2003) in Landrace pigs, by Favaron et al. (2008) in *Nasua nasua* and by Carvalho et al. (2009) in buffaloes (*Bubalus bubalis*). This division of the gland by incomplete septa of connective tissue agrees with the reports by Gartner & Hiatt (1994). Thus, the data by the authors aforementioned as well as the ones reported in this study show the diversity of arrangements of the connective tissue in the pineal gland among species.

The correlation of the morphoquantitative results of the pineal gland of Santa Ines sheep was determined by the Pearson correlation test. It was verified that the brain and the pineal gland showed weak negative correlation with length (r = -0.257) and weak positive correlation with width (r = 0.305). Therefore, there was no significant correlation between the structural dimensions. Gomes (2003) while studying dogs, also found no significant difference between these parameters. In the studied animals, it was possible to conclude that the amount of pinealocytes was directly related to the size of the pineal gland, and the number of pinealocytes displayed a higher correlation with width compared to length. The data obtained by such a correlation could not be compared to other authors since they did not use this tool for data analysis.

CONCLUSIONS

The results presented allow greater understanding of the pineal gland structure in sheep to allow comparison between species. It was observed that the mastocytes and the calcareous concretions were not seen in large quantities and were found in only one and two animals, respectively. The connective tissue formed a capsule that involved the gland and did not extended into the parenchyma. The average number of pinealocytes was 86.27 ± 30.41. The pineal gland of Santa Ines sheep had mean length 6.89 ± 0.79 mm and mean width 6.40 ± 1.35 mm. The amount of pinealocytes showed a strong positive correlation with gland width and weak with gland length.

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