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Study of the Histological Structure of Hardin's Glands in Khalkhali Goats

Azin Beiranvand[a,*], **Ali Kalantari-Hesari**[a], **Mohammad Babaei**[b]

[a] Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
[b] Department of Basic Sciences, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

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Abstract

The Khalkhali goat is a black dairy breed found in the Azerbaijan region. The Harderian gland is only found in some species. These glands secrete an oily substance rich in porphyrin that facilitates the movement of the eyelids over the eye and creates an immune response. So far, no study has been conducted on the histological structure of the Hardin glands in the eyes of Khalkhali goats. In this study, 4-goats slaughtered at the Zanjan slaughterhouse were used. After, the heads of the goats were separated from the trunk and the eyelids along with the Hardin glands were separated from the skull bone. The samples were placed in 10% formalin. The sample was fixated and entered the tissue passage and after preparing paraffin blocks, 5-μm thick sections were prepared and stained using the hematoxylin-eosin method. Histological examination showed that this gland in the Khalkhali goat is covered from all sides by a capsule of tissue. Blades of the capsule enter the parenchyma of the gland and divide it into lobules. The lobules of this gland include serous, mucous, and seromucous secretory units, and the secretions of these units are collected by intralobular ducts. It can be concluded that the Harderian gland in the Khalkhali goat breed was similar to the Harderian glands of the alpaca in terms of the type of secretions of the acini units and in terms of the interlobular and extralobular ducts, it was very similar to the existing reports on cattle, other goat breeds, and sheep.

1. Introduction

The goat, scientifically known as (Capra aegagrus hircus), is a domesticated animal of the order of the ungulates, the family of the Bovids, the subfamily of the Capidae, and the genus of the Goats. The first signs of goat domestication were found in Iran, dating back to about 10,000 BC. The anatomy of goats is not much different from that of sheep, except for some things like tail and hair, which also vary between breeds. The pupil of the eye in goats is a horizontal rectangle with convex corners (Rana, 2023).

The Khalkhali goat is a black dairy breed. The distribution area of this breed is in the Moghan plain and around Khalkhal city. The hair coat of this breed is long and thick and the ears are wide and short. The amount of milk produced by this breed of goat in one period is 130 liters (Nasirain *et al.*, 2009).

Among the anatomical and morphological features of the eye in small ruminants, one can mention the orbital bone, which is approximately 37 to 38 mm wide and approximately 41.2 mm long. The angle between the two eyes is approximately 90 degrees. The volume of the orbital cavity is approximately 1.1 compared to the eyeball. The orbital depth is 45 to 50 mm and the angle between the two orbital cavities is 115 to 120

*Corresponding author: A. Beiranvand (M.Sc. Student)
E-mail address: azin770823@gmail.com
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degrees. The orbital rim is completely surrounded by bone and is formed by the junction of the cheekbones, frontal bone, and lacrimal bone. The orbital rim is fissured and rough in small ruminants. The ducts of the eye glands are connected to each other by bones. The duct is a protruding bone located in the anterior and posterior parts of the eye canal and is approximately 3 mm wide and 2 mm long.

The eyelids of goats are movable and much thinner than those of cattle. The upper eyelid is thicker than the lower eyelid. These folds of skin protect the eye from external damage. They also help keep the cornea moist and regulate the amount of light entering the eye. The upper, lateral, and third eyelids are the lateral organs of the eye whose normal structure is essential for proper eye function. The eyelids protect the eye from damage and control the amount of light entering the eye by opening and closing the palpebral fissure. The inner eyelid or third eyelid is referred to in veterinary terminology as Palpebra tertia, but it also has other names such as eyelid membrane (Schlegel *et al.*, 2001).

The superficial glands of the third eyelid surround the base of the stalk of the T-shaped cartilaginous plate. These glands are structurally similar to the lacrimal glands of Boh and also participate in the secretion of a thin layer of tear film. These glands are of the serous type in horses and cats, of the seromucous type in cows and dogs, and of the mucous type in pigs. Most acinar cells secrete lipids. The deep third eyelid gland is only present in cattle and pigs (Heatley, 2005).

The lacrimal system consists of the lacrimal gland, the lacrimal sac, and the duct system that runs from the eye to the nasal area. The lacrimal gland is a broad, oval, pink-colored gland that surrounds the orbital tissue and is covered by bone, and may be partially hidden by the fat surrounding the eyeball. The lacrimal gland measures 2.8 cm in the mediolateral direction, about 2 cm in the anterior-posterior direction, and 5 mm when the layers are compressed between the bone and the eyeball. Both lacrimal ducts start at a corresponding point and converge towards the medial margin, passing through the orbit and finally opening into the lacrimal sac. The superficial layer of the canal may change from squamous to cuboidal, and a few goblet cells may be seen. The lacrimal sac is located in the depression of the lacrimal bone and transports tears from the lacrimal canal to the nasolacrimal bone canal. The lacrimal duct passes through the lacrimal canal and then the lacrimal groove ends in the maxillary bone and the posterior part of the nostril. When the duct leaves the lacrimal canal and enters the lacrimal groove, its medial surface is covered by nasal mucosa (Coulombre *et al.*, 1962; Kassab and El-Zoghby. 2010; Olopade *et al.*, 2005; Gelatt *et al.*, 2008).

The Harderian gland (deep gland of the third eyelid) is present only in some species. It is absent in species such as carnivores, primates, and humans. However, it is well developed in many laboratory animals, amphibians, reptiles, and birds (Sisson *et al.*, 1975). These glands secrete an oily substance rich in porphyrin that facilitates the movement of the eyelids over the eye. They are a site for the immune response, a source of lipids for thermoregulation and pheromones, and a protective organ for the retina-pineal axis (Emily *et al.*, 2015). Considering the role of this gland in the function and health of the eyelids and eyes, and also considering the fact that no study has been conducted so far regarding the histological structure of the Hardin glands in the eyes of Khalkhali goats, the histological structure of this gland was examined in the present study.

2. Materials and Methods

In this study, 4 goats (2 male goats and 2 female goats) slaughtered at the Zanjan livestock slaughterhouse were used. After slaughter, the goats' heads were separated from the trunk, the heads were placed on a table, and then the eyeballs and eyelids, along with the Hardin glands, were separated from the skull bone. The samples were placed in 10% formalin. After the minimum time required for tissue fixation, the samples were dehydrated, clarified, and embedded in paraffin using a tissue passager, and finally paraffin blocks were prepared from the samples. Each sample was stained with hematoxylin-eosin after being sectioned at 5 μm thickness. The stained sections were examined under a light microscope at magnifications of $\times 4$, $\times 10$, $\times 40$, and $100\times$.A Dino late camera and Dino capture version 2 software were used to capture images of the slides (Morovvati and Kalantari-Hesari, 2019).

3. Histological Results

Regarding the histological examination of the Hardin glands in the Khalkhali goat, it should be noted that this gland is surrounded by a capsule of relatively hard connective tissue, along with connective fibers and adipose tissue. Blades of the capsule enter the gland parenchyma and divide it into lobules. The lobules of this gland contain serous, mucous, and seromucous secretory units (Fig. 1, parts A and B), and the secretions of these units are collected by intralobular ducts. These intralobular ducts are lined with simple cuboidal epithelium or stratified cuboidal epithelium with a few visible layers (Fig. 1, part C). The intralobular ducts end in the interlobular ducts. The interlobular ducts are larger, located between the lobules, and are lined by a stratified cubic epithelium with a higher number of layers (Fig. 1, Part D).

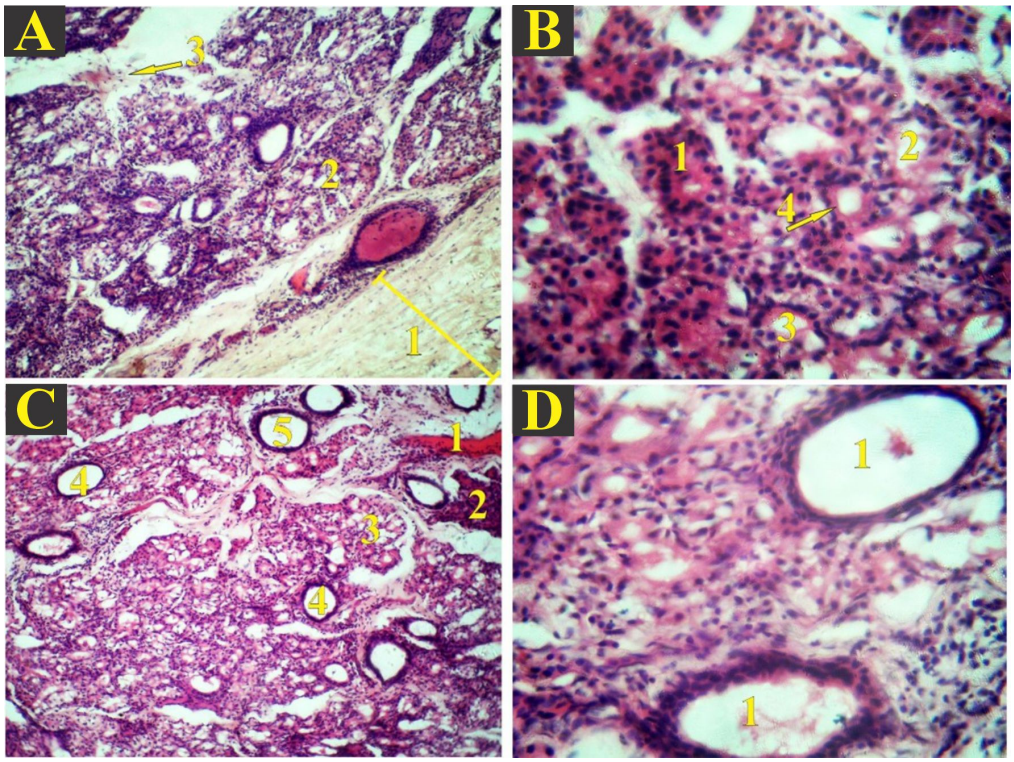

Fig. 1. Cross-section of the Harderian gland in the third eyelid of a Khalkhali goat with hematoxylin-eosin (H&E) staining and ×100 magnification. A) No. 1: gland capsule, No. 2: gland lobules, and No. 3: septa branching from the capsule. B) Secretory units of the third eyelid Harderian gland of a Mahabadi goat with H&E staining and ×400 magnification (No. 1: serous unit, No. 2: mucous unit, No. 3: seromucous unit, and No. 4: intralobular duct with simple cuboidal epithelium). C) No. 1: Septa branching from the capsule, No. 2: Serous secretory units, No. 3: Mucous secretory unit, No. 4: Intralobular collecting duct with stratified cuboidal epithelium and interlobular collecting duct with stratified cuboidal epithelium. D) No. 1: Interlobular collecting duct with stratified cuboidal epithelium.

Given that goats play an important role in the economy of some countries, knowledge of the anatomical condition and function of the various systems and structures of the animal's body can help diagnose diseases and abnormalities and also be useful in maintaining their health. The predominant glands in the eyes of mammals and domestic animals are the lacrimal glands, which may have different locations. The shape of these glands may also vary (Klećkowska-Nawrot *et al.*, 2015).

It has been stated that the secretory acinar units in the Harderian glands of alpaca are composed of long, irregular cone cells with a very narrow lumen in the middle, while the ducts or excretory ducts are lined by a layer of simple or stratified cuboidal epithelium (Klećkowska-Nawrot *et al.*, 2015). Similar reports have been reported in cattle, buffalo, goats and sheep in Iran (Gargiulo *et al.*, 1999; Gargiulo *et al.*, 2000; Kühnel 1968a, b; Shadkhast and Bigham. 2010; Sinha and Calhoun. 1966). Secretory units in the Hardin glands of Khalkhali goats were also composed of long and irregular cone cells. Also, the intralobular ductal epithelium was composed of simple cuboidal to stratified cuboidal type with a low number of layers, which was consistent with previous reports.

It has been shown that the Harderian glands in deer and goats are of the serous type (Kühnel., 1968a; Klećkowska-Nawrot *et al.*, 2013). In histological examination of the Harderian glands of alpaca, these glands were diagnosed as seromucous type (Klećkowska-Nawrot *et al.*, 2015). While in the present study, serous, mucous and seromucous secretory units were observed.

Histological examination of the Harderian gland in goats revealed that this gland is of the lobar-alveolar type with a holocrine secretion that is surrounded by a thin connective tissue capsule. Blades from this capsule penetrate into the gland tissue and divide it into lobules (Rajathi *et al.*, 2019). The results of the present study were consistent with previous reports regarding the connective capsule surrounding the gland.

It has also been reported that secretory units in goats consist of acini with a single layer of columnar cells lined with intracellular eosinophilic vacuoles (Rajathi *et al.*, 2019). The results of the present study were also consistent with previous reports regarding

the shape of secretory cells. It has also been stated that the duct system of the Hardin glands in goats starts from the intralobular ducts and ends in the main excretory duct on the surface of the eyelid. The intralobular ducts are covered by simple cuboidal or cylindrical epithelial tissue, which is mostly in a single layer. The union of these ducts forms interlobular ducts that may be lined by simple columnar to stratified squamous epithelium (Rajathi *et al.*, 2019). In the present study, secretions from acinar units were observed through intralobular ducts lined by simple cuboidal epithelium or stratified cuboidal epithelium with a low number of layers. The intralobular ducts end in larger interlobular ducts, which are located between the lobules and are lined with stratified cubic epithelium with a higher number of layers. According to the results of the present study and the existing reports in this field, the histological structure of the Hardin glands in the Khalkhali goat breed was examined in terms of the shape of the secretory cells similar to most animals. In terms of the type of secretions, the acini units were similar to the Hardin glands of the alpaca, and in terms of the interlobular and extralobular ducts, it was very similar to the existing reports on cattle, other goat breeds, and sheep.

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Conflict of Interest
The authors declare no conflict of interest in this research.

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