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Investigation of Anatomical and Histological Structures of Superficial and Lacrimal Glands in Tuj Sheep

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ABSTRACT This study was carried out to investigate the anatomical, histological and histochemical properties of superficial and lacrimal glands in tuj sheep. A total of 10 superficial and lacrimal glands were used in the study. Anatomical, histological and histochemical techniques were applied to the glands. As a result, the superficial gland was found to be 18-19 mm long, 8-9 mm wide and 3-4 mm thick. Lacrimal gland was determined as 20-23 mm in length, 17-26 mm in width and 3-4 mm in thickness. Hyaline cartilage was observed at the base of the superficial gland. Superficial and lacrimal glands were found to consist of acinuses, interlobular and intralobular ducts and many blood vessels. While Periodic Acid Schiff (PAS) positive reaction was observed in superficial glands, no reaction was observed in goblet cells in lacrimal glands. The secretion in the interlobular and intralobular ducts of superficial and lacrimal glands showed PAS positive reaction. In both glands, elastic fibers around the ducts and vessels showed positive reaction to orcein staining. As a result, when the values given in the literature were taken into consideration, it was seen that the length value of the superficial gland was low and the width and thickness values were high. While the length of the lacrimal gland was in the middle of the values given in the literature, it was found to have high values in terms of width and thickness. It is thought that this study will contribute to veterinary medicine regarding eye diseases and eye-related surgeries.

Keywords: *Eye, Glandula membranae nictitans, Tear gland.*

ÖZ

Tuj Koyunlarında Superficial ve Lakrimal Bezlerin Anatomik ve Histolojik Yapılarının İncelenmesi

Bu çalışma, tuj koyunlarında superficial ve lakrimal bezlerin anatomik, histolojik ve histokimyasal özelliklerini araştırmak amacıyla yapıldı. Çalışmada toplam 10 adet yüzeysel ve lakrimal bez kullanıldı. Bezlere anatomik, histolojik ve histokimyasal teknikler uygulandı. Sonuç olarak, superficial bezin 18-19 mm uzunluğunda, 8-9 mm genişliğinde ve 3-4 mm kalınlığında olduğu tespit edildi. Lakrimal bez 20-23 mm uzunluk, 17-26 mm genişlik ve 3-4 mm kalınlık olarak belirlendi. Superficial bezin tabanında hiyalin kıkırdak gözlendi. Superficial ve lakrimal bezlerin asinüsler, interlobüler ve intralobüler kanallar ve çok sayıda kan damarından oluştuğu tespit edildi. Superficial bezlerde Periyodik Asit Schiff (PAS) pozitif reaksiyon gözlenirken, lakrimal bezlerde goblet hücrelerinde reaksiyon gözlenmedi. Superficial ve lakrimal bezlerin interlobüler ve intralobüler kanallarındaki salgı PAS pozitif reaksiyon gösterdi. Her iki bezde de kanallar ve damarlar etrafındaki elastik iplikler orcein boyamasına pozitif reaksiyon gösterdi. Sonuç olarak literatürlerde verilen değerler dikkate alındığında superficial bezin uzunluk değerinin düşük, genişlik ve kalınlık değerlerinin ise yüksek olduğu görüldü. Lakrimal bezin uzunluğu literatürde verilen değerlerin ortasında yer alırken, genişlik ve kalınlık bakımından yüksek değerlere sahip olduğu tespit edildi. Bu çalışmanın veteriner hekimlik mesleğine katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler: *Glandula membranae nictitans, Göz, Gözyaşı bezi.*

INTRODUCTION

The eye, together with extraocular muscles, ligaments, adipose tissue, blood vessels, nerves and glands, is located in the bony orbit. The eyeball consists of three layers surrounding the chambers containing the refractive media. These are the fibrous layer (tunica fibrosa bulbi) on the outside, the vascular layer (tunica vasculosa bulbi) in the

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middle and the neuroepithelial layer (tunica interna bulbi) on the inside. The fibrous layer consists of the sclera and cornea. (Eurell and Frappier 2006). The superficial gland (gl. profunda palpebra tertiae, gl. membranae nictitans) is located deep within the orbit in some mammals such as pigs, cattle and poultry, except fish and aquatic amphibians. The superficial gland is located at the base of the T-shaped cartilage of the third eyelid. It was reported that this gland has an important role in porphyrin biosynthesis in rodents, has receptors for gonadal hormones such as estrogen and testosterone in hamsters and also has an important role in sex differentiation. Superficial gland is responsible for cleaning and washing the cornea and lubricating the third eyelid in poultry. The most important feature of this gland is the presence of plasma cells originating from bursa fabricius (Özer 2010; NAV 2017). The superficial gland is serous in cattle, sheep and dogs, serous in cats and mucous in pigs. The pig also has a second, deeply located glandula palpebra tertia profunda (König and Liebich 2022).

The lacrimal gland (glandula lacrimalis) is located between the eyeball and the dorsotemporal wall of the orbit. Its secretion enters the conjunctival sac from the dorsotemporal margin of the upper eyelid via small ductuli excretori (König and Liebich 2022). The lacrimal gland is a complicated tubuloacinar or tubuloalveolar gland. It is seromucous in dogs and ungulates and serous in cats. Acinar cells are lined by myoepithelial cells and have lipid inclusions in their cytoplasm. The intercalated and secretory ducts are lined with single- and multilayered cuboidal epithelium, respectively. Lacrimal ductules are lined with multilayered cuboidal epithelium. The lacrimal gland produces approximately 60% of the aqueous portion of the tear film and the superficial gland produces 35% (Eurell and Frappier 2006).

Based on the literature review, it was observed that there were limited number of studies on the lacrimal glands in tuj sheep. Therefore, our study aimed to investigate the anatomical and histological examination of the superficial and lacrimal glands in tuj sheep.

MATERIAL AND METHODS

Materials

In this study, lacrimal and superficial glands were obtained from Tuj sheep of 2 years of age and gender indiscriminate were used as material. A total of 10 eyes, 5 right and 5 left, were studied. This study was conducted with the permission of Kafkas University Animal Experiments Local Ethics Committee on 04.07.2024 with the number 2024-147.

Methods

Anatomical Investigations

After dissecting the skin, superficial muscles and adipose tissue, the glands around the eyeball were removed from the orbit with blunt dissections. After the glands were identified, the width, thickness and length of each gland were measured using a digital caliper. SPSS 20.0 package program was used for statistical analyses. Mean and standard deviation values of the parameters were analysed.

Histological Investigations

Lacrimal and superfacial gland samples were fixed in 10% formol and then blocked in paraffin using routine tissue tracing procedure. The 5µm sections taken from the paraffin blocks were stained with haematoxylin-eosin and triple staining to examine the general structure of the glands. Periodic Acid Schiff (PAS) (HX87411546/ Merck KGaA, 64271 Darmstadt, Germany) staining was performed to demostrate neutral mucins and orcein staining was performed to show elastic fibres. Sections were evaluated under light microscope and photographed (ZEISS Primostar 3).

RESULTS

Anatomical Results

The third eyelid was localised in the medial angle of the eye (Figure 1). The cartilage of the third eyelid and the glands in this region were all revealed together by dissection. It was determined that all of these structures were seen together in the shape of a fish from the caudomedial angle (Figure 2). It was seen that the tip of the musculus retractor bulbi terminated just near the superficial gland. The results of statistical analyses of the lacrimal and superficial gland are presented in Table 1 and Table 2.

Histological Results

Hyaline cartilage was observed at the base of the superficial gland. Superficial and lacrimal glands were found to consist of acinuses, interlobular and intralobular ducts and many blood vessels. It was observed that the acinuses were serous and mucous and serous acinuses were more dense. The surface of the interlobular ducts was found to be lined with multilayered cubic epithelial cells with goblet cells secreting between them. The surface of the intralobular ducts consisted of single layer cubic epithelial cells but no goblet cells were observed (Figure 3-6). Superficial and lacrimal glands of the right and left eyes had similar histology.

While PAS positive reaction was observed in superficial glands, no reaction was observed in goblet cells in lacrimal glands. The secretion in the interlobular and intralobular ducts of superficial and lacrimal glands showed PAS positive reaction. In both glands, elastic threads around the ducts and vessels reacted positively to orcein staining and stained brown (Figure 7- 8).

Table 1: Some measurement results of lacrimal gland.

Direction	Length (mm)	Width (mm)	Thickness (mm)
Right eye lacrimal gland	20.58 ± 3.25	17.02 ± 9.62	3.89 ± 0.72
Left eye lacrimal gland	23.81 ± 0.55	26.87 ± 0.37	3.27 ± 0.67

*mm: millimeters.

Table 2: Some measurement results of the superficial gland.

Direction	Length (mm)	Width (mm)	Thickness (mm)
Right eye superficial gland	18.89 ± 0.64	9.05 ± 0.77	3.97 ± 0.63
Left eye superficial gland	18.53 ± 0.23	8.72 ± 0.24	3.59 ± 0.12

*mm: millimeters.

Figure 1: The anatomical position of the lacrimal gland (L) and superficial gland (S) in Tuj sheep.

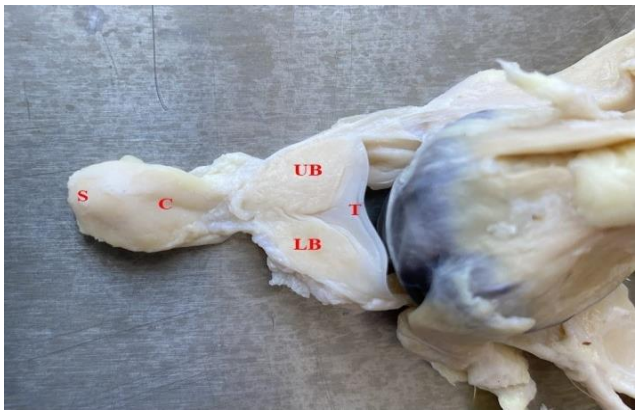

Figure 2: Caudomedial view of the superficial gland in Tuj sheep (C: crossbar, UB: upper branch, LB: lower branch, S: superficial gland, T: cartilage of the third eyelid).

DISCUSSION AND CONCLUSION

The secretions of the lacrimal glands protect the eye by removing foreign bodies and keeping the eye constantly moist. Failure of these glands to function causes disturbances in visual function. Determining the anatomical and histological structure of this region will be useful in eye diseases and operations. In the literature reviews, it was determined that there were studies on the anatomical and histological structure of the lacrimal glands in different animal species (Alsafy 2010; El-Naseery et al. 2016; Klećkowska-Nawrot et al. 2015b; Klećkowska-Nawrot et al. 2016). Based on these studies, superficial and lacrimal glands of tuj sheep were investigated anatomically, histologically and histochemically.

In Sulawesi bear cuscus (Klećkowska-Nawrot et al. 2019), the superficial gland was measured as 5.65 ± 0.2 mm in length, 7.29 ± 0.3 mm in width, and 3.59 ± 0.1 mm in thickness. In Alpaca, the length of this gland was 24.73 ± 1.1 mm, width 1.605 ± 0.08 mm, and thickness 0.77 ± 0.1 mm (Klećkowska-Nawrot et al. 2015a). In Tuj sheep, the length of the superficial gland was found to be 18-19 mm, width 8-9 mm, and thickness 3-4 mm. When we compare with the literature, it is seen that the length value is low and the width and thickness values are high.

In Sulawesi bear cuscus, lacrimal gland length was reported as 9.24 ± 0.7 mm, width 3.61 ± 0.1 mm, thickness 2.55 ± 0.1 mm (Klećkowska-Nawrot et al. 2019). The length of lacrimal gland in Alpaca females was 31.39 ± 0.7 mm, width 0.84 ± 0.05 mm, thickness 0.46 ± 0.03 mm, and in males the length was 32.37 ± 0.6 mm, width 0.905 ± 0.05 mm, thickness 0.42 ± 0.02 mm (Klećkowska-Nawrot et al. 2015a). The length of the lacrimal gland in Tuj sheep was determined as 20-23 mm, width 17-26 mm, and thickness 3-4 mm. While the length was in the middle of the values stated in the literature, it was found to have high values in terms of width and thickness.

The nictitans (eyelids) are structures that provide barrier protection to the eyeball, remove ocular debris, help distribute tears, add mucin to the preocular film and house the third eyelid gland (superficial gland) (Sandmeyer et al. 2022). The superficial gland produces an average of 30-60% of tears. It has tubuloacinar and seromucoid gland structure (O'Neill et al. 2022). In the dog, it is located in the ventromedial part of the eye. It has a structure suitable for the shape of the cornea. It is supported by a T-shaped hyaline cartilage and this cartilage structure merges with the conjunctival mucosa (Dugan et al. 1992; Schlegel et al. 2001). In cat, horse and pig, the superficial gland is supported by elastic cartilage (Bacha and Bacha 2012). It is stated that the superficial gland in the pig produces mucous secretion, is compound multilobar and tubular branched (Paszta et al. 2022), serous in the horse and cat, serous in the dog and ruminating, and mucous in the pig (Bacha and Bacha 2012). In Tuj sheep, the hyaline cartilage at the base of the superficial gland, serous and mucous acinuses, interlobular and intralobular ducts forming the gland were determined. It was observed that serous acinuses were mostly present. The acinuses forming the gland showed PAS positive reaction and the elastic fibres in the structure of the gland were stained brown in orcein staining.

The lacrimal gland is a multilobed and tubulo-acinar gland. Depending on the species, it may be serous, mucous or seromucous (Prince 1977; Gargiulo et al. 1999). It is serous in cats, rabbits and pigs, mucous in goats, seromucous in rats and dogs. The secretion of the lacrimal gland contributes to the formation of the aqueous layer of the tear film covering the eye. It also helps to nourish and protect the ocular surface. Its structure includes water, electrolytes and proteins (Hodges and Dartt 2003). The secretion content and the amount of secretion of the lacrimal gland varies between species. In the monkey, the lacrimal glands show a compound tubuloalveolar gland structure divided into lobes and lobules by connective tissue. It produces a watery secretion rich in lysozyme. The secretory cells are composed of numerous serous acinus with round, basally located nuclei (Gartner and Hiatt 2014). It has been reported that the lacrimal gland in the dromedary camel (Camelus dromedarius) has a compound tubulo-acinar gland structure. It was also reported that there is a thick connective tissue capsule surrounding the lacrimal gland and this capsule is rich in elastic and collagen fibres. The acini of the gland have irregular lumens and the cells vary from conical to pyramidal cells. It was observed that the secretory cells in the acini had basally located nuclei, and the cytoplasm was eosinophilic and granular. It was stated that serous, mucous and seromucous secretory cells were present in the glandular tissue, mucous cells showed PAS-positive reaction, acinus contained neutral mucopolysaccharide, and seromucous cells showed weak PAS-positive reaction (Roshdy et al. 2024). In sheep lacrimal glands, it has been reported that there are three cell types in the acinus. It is stated that mucous, serous and seromucous cells may be present in varying proportions in the structure of the gland and the gland may consist entirely of serous or mucous acinus (Gargiulo et al. 1999). It was stated that the lacrimal gland

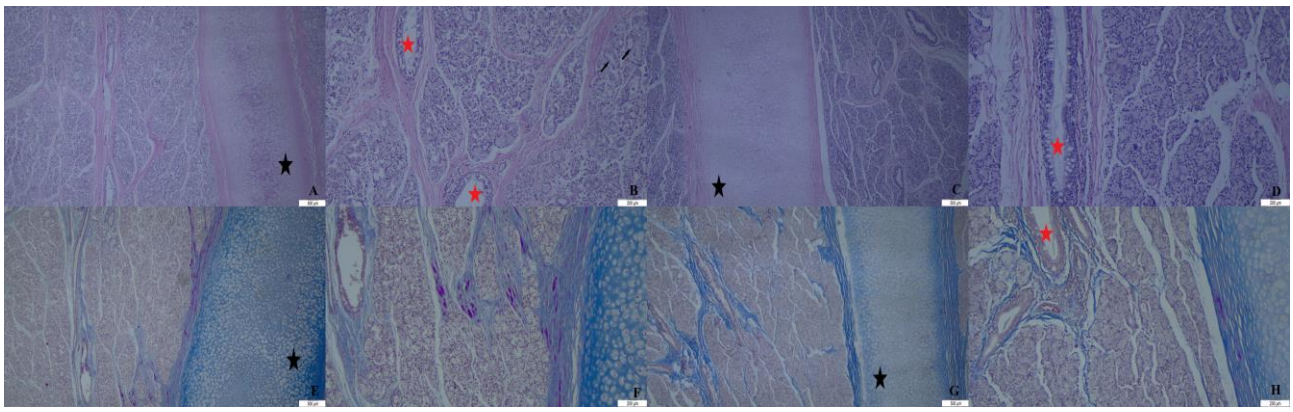

Figure 3: Tuj sheep. Histological structure of the superficial gland. A, B, E, F: Right eye superficial gland. C, D, G, H: Left eye superficial gland. Hyaline cartilage (black star), interlobular canals (red star), intralobular canals (arrow). A, B, C, D: H-E staining. E, F, G, H: Triple staining. A, C, E, G: Bar 500µm. B, D, F, H: Bar 200µm.

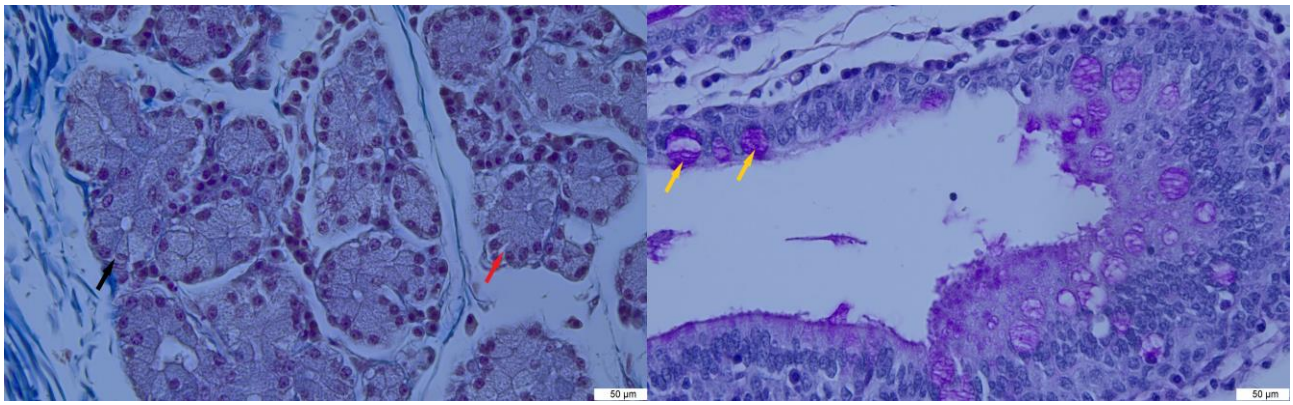

Figure 4: Tuj sheep. Histological structure of the superficial gland. Triple (a) and PAS staining (b). Mucous gland (black arrow), serous gland (red arrow) goblet cells (yellow arrows). Bar 50 µm.

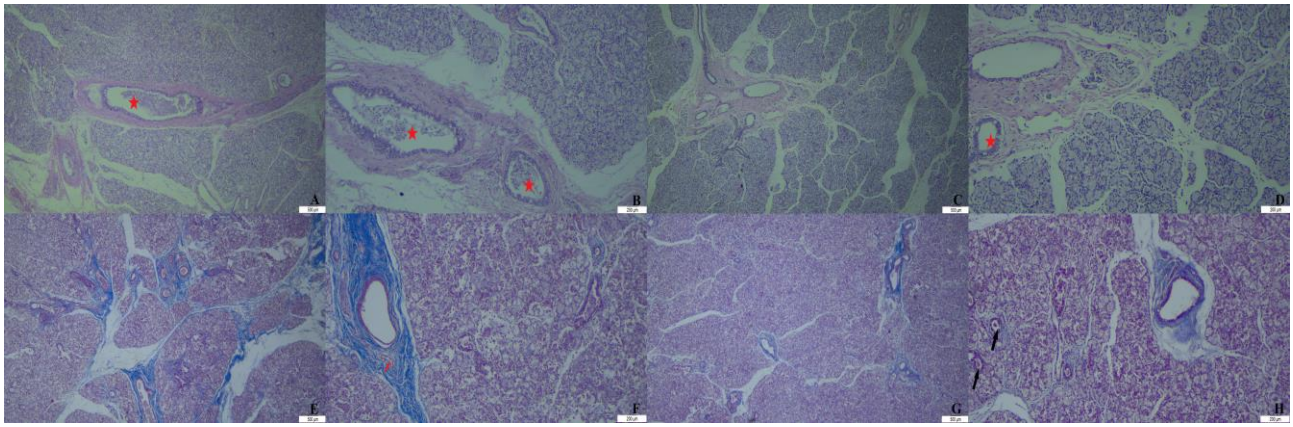

Figure 5: Tuj sheep. Histological structure of the lacrimal gland. A, B, E, F: Right eye lacrimal gland. C, D, G, H: Left eye lacrimal gland. Interlobular ducts (star), intralobular ducts (black arrow), blood vessel (red arrow). A, B, C, D: H-E staining. E, F, G, H: Triple staining. A, C, E, G: Bar 500µm. B, D, F, H: Bar 200µm.

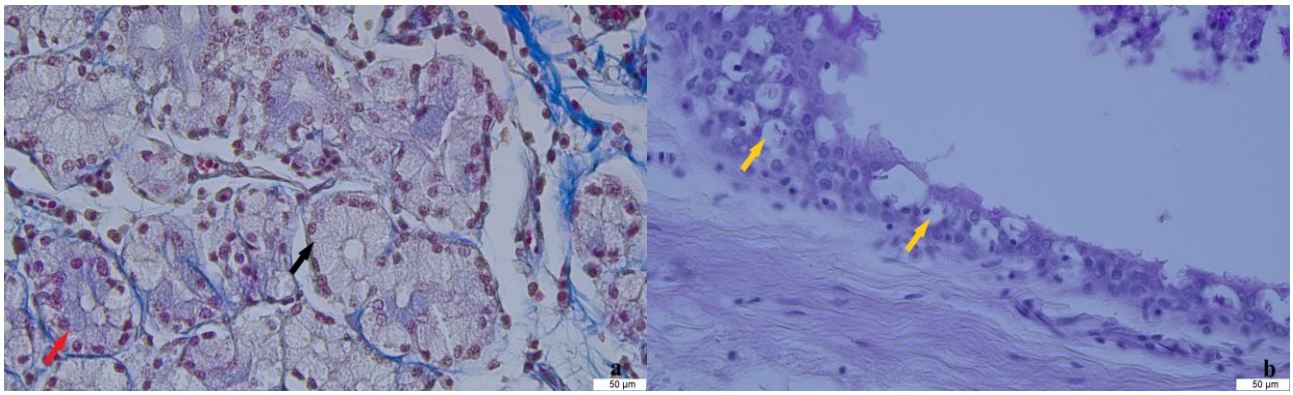

Figure 6: Tuj sheep. Histological structure of lacrimal gland. Triple (a) and PAS (b) staining. Mucous gland (black arrow), serous gland (red arrow) goblet cells (yellow arrows). Bar 50 µm.

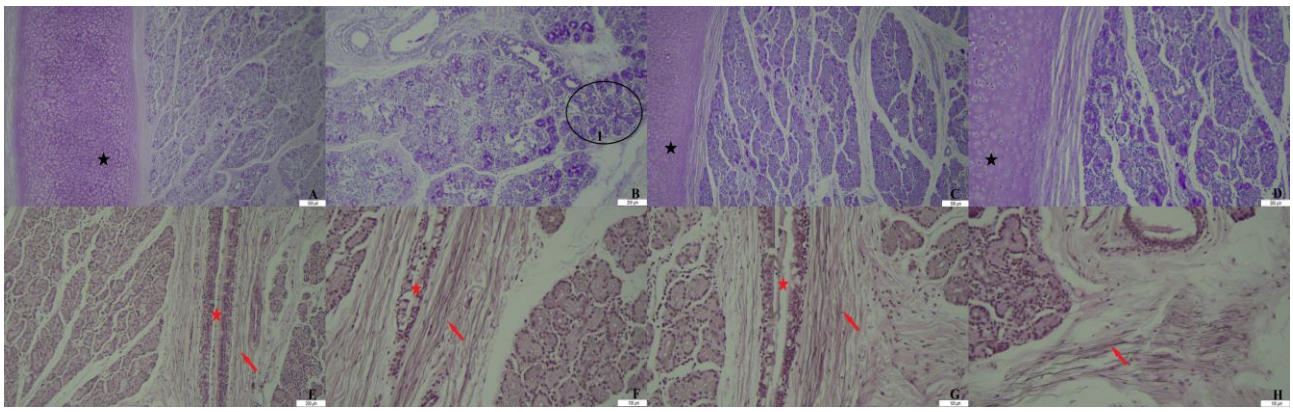

Figure 7: Tuj sheep. Histological structure of the superficial gland. A, B, E, F: Right eye superficial gland. C, D, G, H: Left eye superficial gland. Hyaline cartilage (black star), interlobular canals (red star), elastic fibers (arrow), acinus (1). A, B, C, D: PAS staining. E, F, G, H: Orcein staining. A,C: Bar 500µm. B, D, E: Bar 200µm, F, G, H: Bar 100µm.

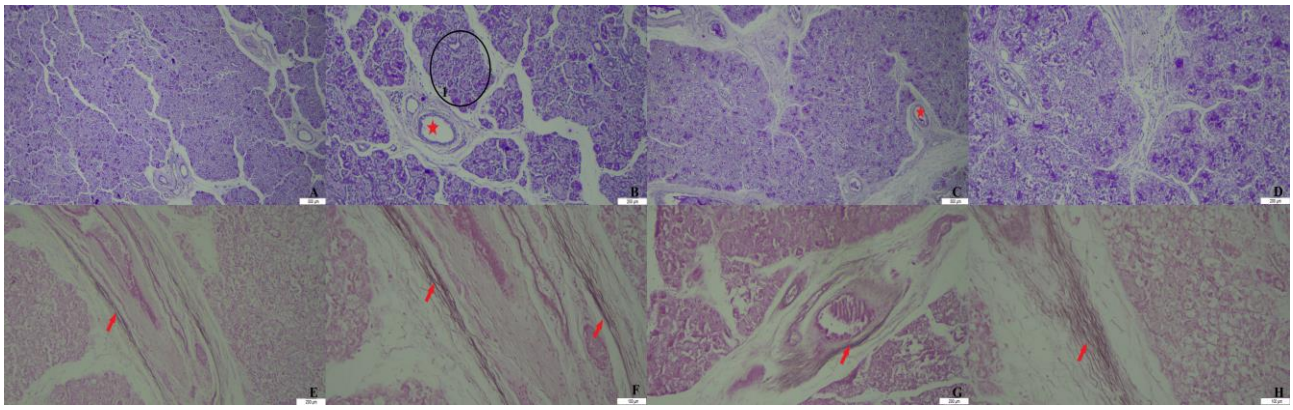

Figure 8: Tuj sheep. Histological structure of the lacrimal gland. A, B, E, F: Lacrimal gland of the right eye. C, D, G, H: Left eye lacrimal gland. Interlobular ducts (star), elastic fibers (arrow), acinus (1). A, B, C, D: PAS staining. E, F, G, H: Orcein staining. A, C: Bar 500µm. B, D, E, G: Bar 200µm, F, H: Bar 100µm.

of Lori sheep is lobulated and the parenchymal cells are a mixed type of gland containing serous and mucous cells. It was reported that the intralobular, interlobular and excretory ducts of the gland were lined with cuboidal, stratified cuboidal and pseudo stratified columnar epithelium respectively and goblet cells were found between epithelial cells in some ducts (Abbasi et al. 2014). The lobules forming the lacrimal gland in Tuj sheep and the acinus, interlobular and intralobular ducts and blood vessels in this region were determined. It was determined that the gland structure was similar to the histological features of the superficial gland. The epithelium lining the interlobular and intralobular ducts had the same histological characteristics as the superficial gland.

As a result, when the values given in the literatures were taken into consideration, it was seen that the length value of the superficial gland was low and the width and thickness values were high. While the length of the lacrimal gland was in the middle of the values given in the literature, it was found to have high values in terms of width and thickness. It was observed that the histological structure of the superficial gland was similar to the lacrimal gland, but PAS positive reaction was observed in goblet cells in the superficial gland, but not in the lacrimal glands. It is thought that this study will contribute to veterinary medicine regarding eye diseases and eye-related surgeries (Farjanikish et al. 2019).

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: ŞYA, GKD, EKS
Supervision / Consultancy: ŞYA, EKS
Data Collection and / or Processing: ŞYA, GKD, FY
Analysis and / or Interpretation: ŞYA, GKD, EKS, FY
Writing the Article: ŞYA, GKD
Critical Review: ŞYA, GKD, EKS, FY

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