

## COMPARATIVE HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF THE LACRIMAL GLANDS OF LOCAL SHEEP AND GOATS.

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Received: 17 April 2016; Accepted: 28 July 2016

### ABSTRACT

This research was carried out on the lacrimal glands (LGs) of male adult local sheep and goats. The aim of this study was to elucidate the normal histological and histochemical characteristics of the LGs. For this purpose, the histological specimens were stained with the H&E, van Gieson's, periodic acid Schiff (PAS), diastase-PAS, alcian blue (AB) at pH 2.5 and pH 1, alcian blue-periodic acid Schiff (AB-PAS) and toluidine blue (TB). The present study revealed that the LGs of both animals were mixed tubuloacinar gland composed of secretory units (mainly serous and mucous as well as mucous acini opened into serous tubules) and excretory duct system. The mucous secretory units were predominant in sheep LGs comparing with those of goats LGs. The mucous cells in both animals contained mucins (neutral, carboxylated and sulphated) with the prevalence of carboxylated group in cells of sheep. The duct system in sheep and goats consisted of intercalated, intralobular, interlobular and main excretory ducts. The lining epithelia of the interlobular and main excretory ducts in both animals had light cells, but the same ducts contained goblet cells in goat only. The mucin content of the goblet cells was similar to that of the glandular tissue of goats.

**Key words:** Lacrimal glands, sheep and goats, histology, histochemistry

### INTRODUCTION

The tear film consisted of a superficial lipid layer, a middle aqueous layer and inner mucin (glycoprotein) layer covering the cornea (Walcott, 1998 and Davidson and Kuonen, 2004). The main function of lacrimal gland (LG) was the production of the aqueous layer (Stein and Hurwitz, 1996 and Samuelson, 2007). The LG also involved in the production of a component of the mucin layer of that film (Stein and Hurwitz, 1996 and Arango *et al.*, 2001). The LG played an important role in the protection of the cornea and the conjunctiva from drying up and also nourished and lubricate the eye, contributed in metabolite exchange, protected the corneal surface from injuries caused by foreign bodies and had bactericidal and bacteriostatic properties (Stern *et al.*, 2004; Zagon *et al.*, 2012 and Al-Ramadan and Ali, 2012).

In literature, the morphology and histochemistry of LGs had been described in ruminants including; sheep (Gargiulo *et al.*, 1999 and Gargiulo *et al.*, 2000) goats (Sinha and Calhoun, 1966) cattle (Pinard

*et al.*, 2003b) buffalo (Maala *et al.*, 2007; Shadkhist and Bigham, 2010) European bison (Klećkowska-Nawrot *et al.*, 2015).

The LGs of sheep and goat were regarded as a compound tubuloacinar gland, distinctly lobulated and highly vascularized (Sinha and Calhoun, 1966; Gargiulo *et al.*, 1999). They were composed of serous, mucous and mixed acini (Daryuos and Ahmed, 2012).

The objectives of the present research were to describe the normal histological and histochemical features of the LGs of local sheep and goats.

### MATERIALS AND METHODS

#### Animals:

Twelve LGs of adult male (six Karadi sheep and six local black goats) were immediately collected after slaughtering at Duhok abattoir, Kurdistan region, Iraq.

#### Tissue preparation:

Small samples (0.5-1 cm thick) obtained from the LGs and fixed in buffered 10 % formalin (pH 7.0) and Bouin's fluid for 24 hours. The fixed specimens were dehydrated, cleared and then embedded in paraffin wax. Histological sections with 5-6  $\mu$ m thick were prepared and stained with the following stains.

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**Stains:**

Histological study:

The following stains were utilized to demonstrate the general tissue structure:

1. Harris hematoxylin and eosin (H&E) (Luna, 1967).
2. Van Gieson's (Luna, 1967).

Histochemical study:

It was conducted in order to identify the presence of carbohydrate types depending on the classification of Culling *et al.* (1985):

- I. Glycogen or neutral polysaccharides (NPS).
- II. Acid mucopolysaccharides (MPS) or Glycosaminoglycans (GSGs).
- III. Glycoproteins.

For this purpose, the following dyes were applied:

1. Periodic acid Schiff (PAS) for identification of neutral polysaccharides (NPS) and different types of glycoproteins (Humason, 1972).
2. Diastase-PAS for establishment of glycoproteins (Humason, 1972).
3. Alcian blue at pH 2.5 (AB, pH 2.5) for identification different types of MPS or GSGs, sulfated and carboxylated glycoproteins (Lev and Spicer, 1964 and Spicer *et al.*, 1967).
4. Alcian blue at pH 1 (AB, pH 1) for demonstration of sulfated MPS and sulfated glycoprotein (Lev and Spicer, 1964).
5. Alcian blue-PAS (AB-PAS) for detection of neutral and carboxylated glycoproteins (Mowry, 1956 and Lev and Spicer, 1964).
6. Toluidine blue (TB) for explore various types of MPS or GSGs (Humason, 1972).

The tissue sections were photographed by a photomicroscope (Olympus, Japan) and camera (Leica, Germany).

**RESULTS**

The histological and histochemical description of the LGs included both sheep and goat with some variations in between.

Histological study:

The present study showed that the LG was surrounded by relatively thick capsule. The components of the capsule comprehend collagen fibers, smooth muscle fibers, nerves, blood and lymphatic vessels. Layer of the adipose tissue was partially covering the capsule. The septa originated from the capsule dividing the gland into lobules with variable sizes. Individual or clusters of adipocytes were present in the septa and parenchyma of sheep only (Fig.1). The interstitial tissue was composed of fibroblasts, lymphocytes, plasma cells and capillaries.

In the current study, the LG was regarded as a mixed tubuloacinar gland consisting of secretory units and duct system.

**1. Secretory units:**

The LG comprised two types of secretory units; serous and mucous. The mucous secretory units were predominant in the gland of sheep, while the gland of goat had dominant serous ones. Both serous and mucous secretory units consisted of tubular and acinar portions. The acinar portions were more prevalent than that of tubular portions.

**Serous secretory units:**

The acinar portion was lined by single layer of pyramidal cells, whereas the tubular portion was lined with one layer of cuboidal cells. The cells of both portions had acidophilic cytoplasm. The cells of some acini had basophilic granules in their basal part. The nuclei of both portions were spherical in shape and basally located.

**Mucous secretory units:**

The lining epithelial cells of acinar and tubular portions were varied from tall to low pyramidal, respectively. Their cytoplasm was vacuolated and did not stain with eosin. The limits of the cells were distinct. The nuclei of both portions were oval or spherical in shape. Some mucous acini opened into the serous tubular portions. These patterns were located at the periphery of lobules in both animals (Fig. 2).

The myoepithelial cells were present between the secretory cells (serous and mucous) and the basement membranes. These cells had distinctive scant cytoplasm and flattened heterochromatic nuclei.

**2. Duct system:**

The duct system consisted of intercalated ducts, intralobular ducts, interlobular ducts and main excretory ducts.

Both intercalated and intralobular ducts were characterized by wide lumina. The lining cells of intercalated and intralobular ducts ranged from low cuboidal to cuboidal, respectively. The cytoplasm of these ducts was scant and faintly stained with eosin. The nuclei had spherical shaped which occupied almost all the space of the cells.

The interlobular ducts were lined with double layer of cuboidal cells and surrounded by connective tissue of septa. The interlobular ducts of goats had goblets cells, whereas those of sheep were devoid from these cells.

The main excretory duct was left the gland, where it encircled by a sheath of collagenous fibers containing arterioles, venules, capillaries, lymphatics, nerves and

aggregation of plasma cells. The main excretory ducts of sheep (Fig. 3) and goat (Fig. 4) were lined by stratified columnar epithelium containing numerous goblet cells in goat only.

The light cells were occurred in the epithelia of both interlobular and main excretory ducts of goats and

sheep (Fig. 3 and Fig. 4). They possessed clear unstained cytoplasm with oval, pyknotic nuclei.

Histochemical study:

The histochemical study of the LGs of sheep and goats was summarized in tables 1 and 2.

**Table 1:** Histochemical reactions of the lacrimal glands of sheep and goats.

Animals	Types of secretory units	Stains *				
		PAS	Diastase- PAS	AB pH 2.5	AB pH 1	TB
Sheep	Serous	-	-	-	-	-
	Mucous	+++	+++	+++	++	-
Goats	Serous	-	-	-	-	-
	Mucous	++	++	++	+	-

(\*) : Include all the stains except, the AB-PAS.

(+++): Intense reaction.

(++) : Moderate reaction.

(+) : Faint reaction.

(-) : Negative reaction

**Table 2:** Types of carbohydrates within the lacrimal glands of sheep and goats.

Animals	Types of secretory units	Types of carbohydrates				
		Glycogen (NPS)	All types of Glycosaminoglycans (MPS)	Glycoproteins		
				Mucin (Neutral)	Carboxylated (Sialomucin)	Sulphated (Sulphomucin)
Sheep	Serous	-	-	-	-	-
	Mucous	-	-	+	+++	+
Goats	Serous	-	-	-	-	-
	Mucous	-	-	+	++	+

(+++): Intense occurrence

(++) : Moderate occurrence

(+) : Faint occurrence

(-) : Negative occurrence

Serous secretory units:

The serous cells had negative reaction with PAS, diastase- PAS, AB pH 2.5, AB pH 1, AB-PAS. Also, there was no metachromachia, when they stained with TB. These reactions showed that the serous cells did not contain any kinds of carbohydrates in their cytoplasm.

Mucous secretory units:

The mucous cells reacted positively with PAS and diastase-PAS (purple) in sheep (Fig. 5) and goats (Fig. 6). These data revealed that the mucous cells did not contain glycogen granules and included glycoproteins. The mucous cells of sheep LGs were stained more intensely with PAS than those of goat, so the presence of glycoproteins were more abundant in sheep comparing with those of goat.

The mucous cells of sheep LGs reacted intensely with AB pH 2.5 (deep blue) and moderately with AB pH 1 (blue), whereas the same cells in LGs of goats reacted moderately and faintly (Fig. 7) with the pervious stains. The tissue sections of the sheep and goat LGs were stained with AB pH 2.5 (deep blue) and then

followed by PAS (purple), most mucous cells reacted intensely with AB and faintly with PAS (Fig. 8).

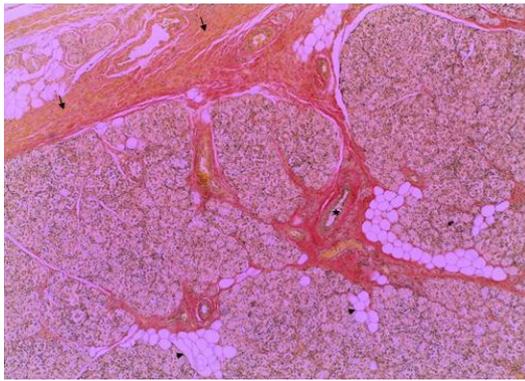
All above-mentioned reactions revealed that the mucous cells of sheep and goats comprised neutral, carboxylated and sulphated glycoproteins, but the carboxylated group was predominant in sheep cells. The mucous cells had no metachromachia, when they stained with TB in both sheep (Fig. 9) and goats. This was indication for the absence of glycosaminoglycans (MPS).

Goblet cells:

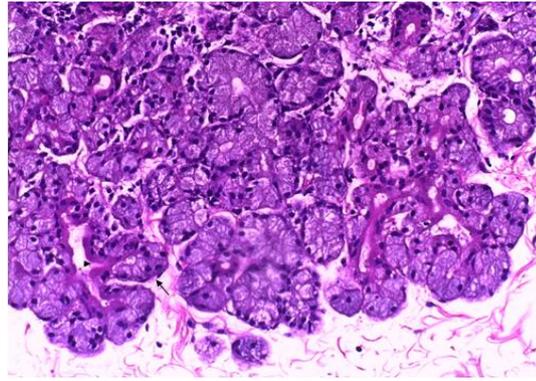
The goblets cells within the epithelia of intralobular and main excretory ducts of goats were stained moderately with PAS and diastase-PAS (Fig. 10), strongly with AB pH 2.5 (Fig. 11) and faintly with AB pH 1 (Fig. 12). They had negatively reactions with TB. These reactions showed that the goblet cells contained different types of glycoproteins with the prevalence of carboxylated group.

Light cells:

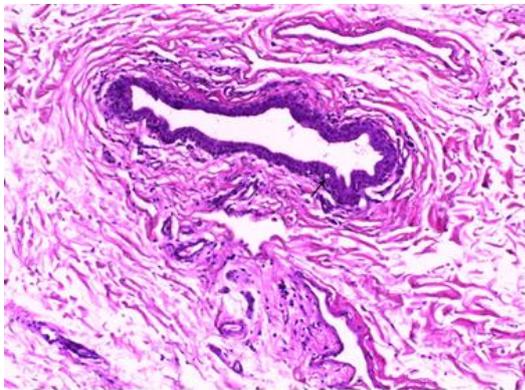
The light cells had no any reactions with stains applied for histochemical study (Fig. 10 and Fig. 11).



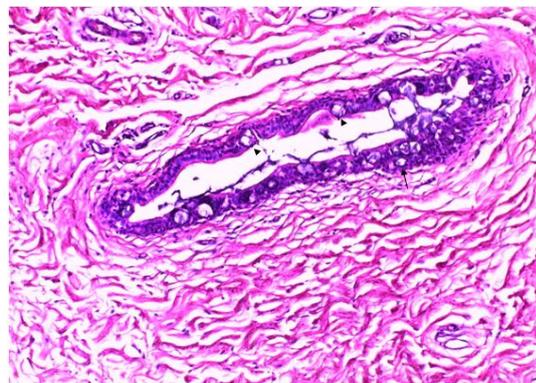
**Figure 1:** A photograph of the capsule and septa of sheep lacrimal gland showing smooth muscle fibers (arrows). Interlobular ducts (star). Adipocytes (arrow heads).  
Stain: Van Gieson's X 4



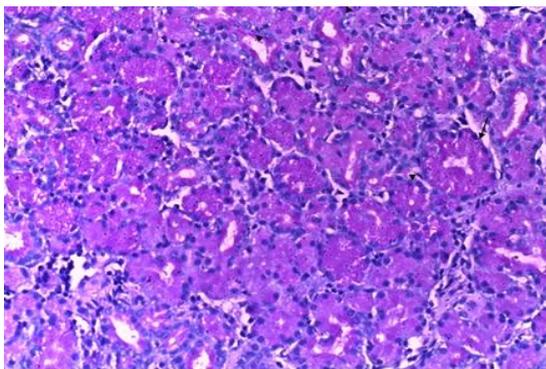
**Figure 2:** A photograph of sheep lacrimal gland showing mucous acinus (arrow). Serous tubule (arrow head).  
Stain: H&E. X 20



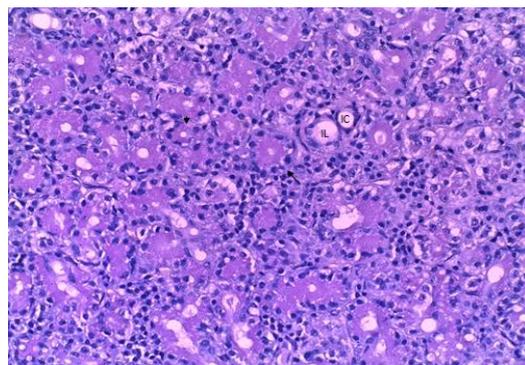
**Figure 3:** A photograph of the main excretory duct of sheep showing light cells (arrow).  
Stain: H&E X 10



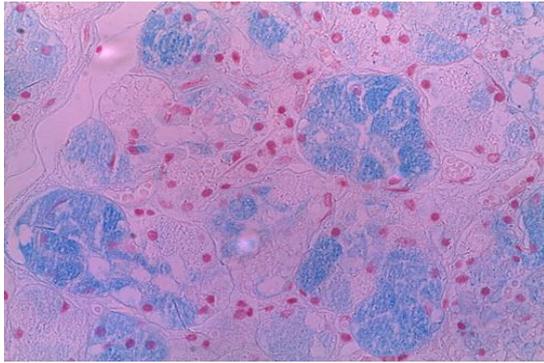
**Figure 4:** A photograph of the main excretory duct of goat showing goblet cells (arrow heads). Light cell (arrow).  
Stain: H&E. X 10



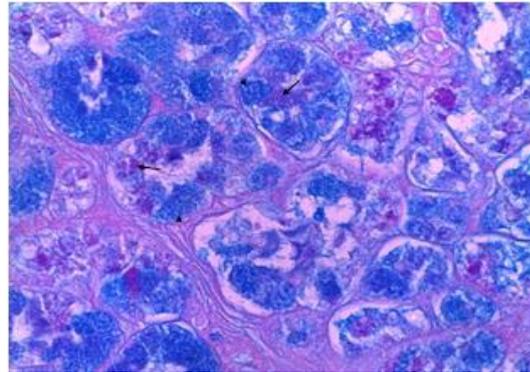
**Figure 5:** A photograph of sheep lacrimal gland showing cells of mucous secretory units stained intensely purple; acinar portion (arrow) & tubular portion (arrow head).  
Stain: PAS X 20



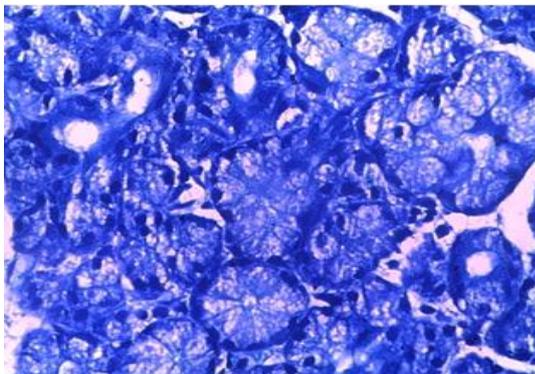
**Figure 6:** A photograph of goat lacrimal gland showing cells of mucous secretory units stained moderately purple; acinar portion (arrow) & tubular portion (arrow head). Intercalated (IC) and intralobular ducts (IL).  
Stain: PAS X 20



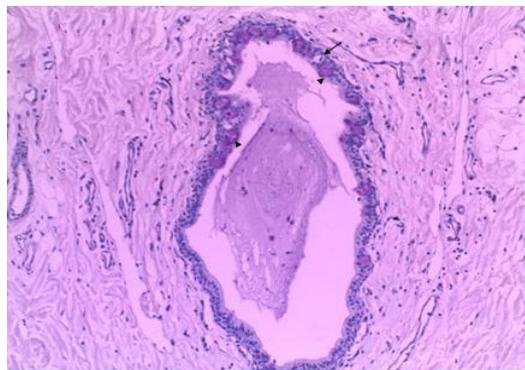
**Figure 7:** A photograph of goat lacrimal gland showing cells of mucous secretory units stained faintly blue.  
Stain: AB pH 1 X 40



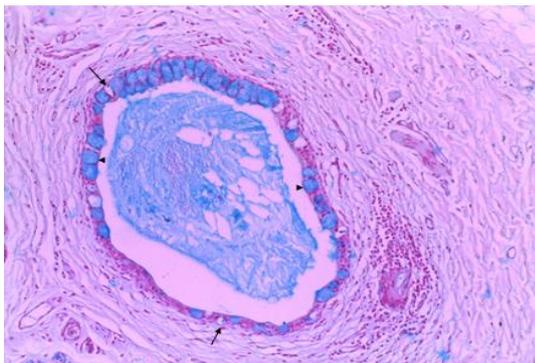
**Figure 8:** A photograph of sheep lacrimal gland showing cells of mucous secretory units stained intensely deep blue (arrow heads) and purple (arrows).  
Stain: AB-PAS X 40



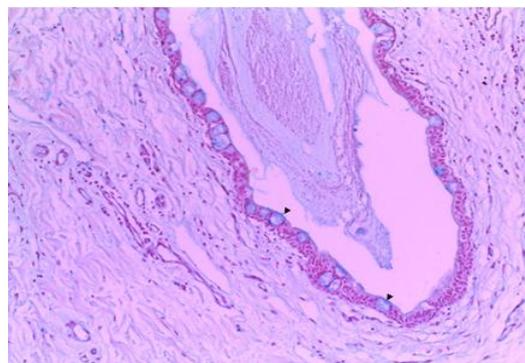
**Figure 9:** A photograph of sheep lacrimal gland showing absence of metachromacia.  
Stain: TB X 40



**Figure 10:** A photograph of the main excretory duct of goat showing goblet cells stained moderately purple (arrow heads). Light cell (arrow).  
Stain: Diastase - PAS X 10



**Figure 11:** A photograph of the main excretory duct of goat showing goblet cells stained intensely blue (arrow heads). Light cells (arrows).  
Stain: AB pH 2.5 X 10



**Figure 12:** A photograph of the main excretory duct of goat showing goblet cells stained faintly blue (arrow heads).  
Stain: AB pH 1 X 10

## DISCUSSION

In the current study, the histological and histochemical examinations of the LGs of sheep and goats observed similarities and differences, when they were compared with the other species.

In the present research, the capsule of the LG was partially covered by adipose tissue, while this tissue occurred beneath the capsule of alpaca (Klećkowska-Nawrot *et al.*, 2015). On the other hand, individual or clusters of adipocytes penetrated the parenchyma and septa were observed in the present study. This finding was in agreement with those of the LG of alpaca (Klećkowska-Nawrot *et al.*, 2015). The adipose tissue enclosed the eye ball and other orbital tissue structures which act as a cushion (Koorneef, 1979).

Our observation revealed that the smooth muscle fibers were restricted to the capsule of the LGs of both animals. Daryuos and Ahmed (2012) concluded that the smooth muscle fibers occurred in the capsule, as well as around the interlobular and main excretory ducts might facilitate the drain of secretion from the gland.

The present investigation pointed that the numerous plasma cells infiltrated in the gland interstitium. These cells played an important role in immune system (Walcott, 1998). In this respect, Moore and Whitley (1984); Rebhun (1984) and Dartt (2009) recorded that activated plasma cells secreted immunoglobulins protecting the sensitive mucosa and the ocular surface of the eye against disease, including many upper respiratory diseases in ruminants. The plasma cells in human LG produced predominantly IgA type (Franklin *et al.*, 1973). The source of this immunoglobulin in rat tears did not come from the serum, but come from the local synthesis within the gland interstitium (Sullivan and Allansmith, 1984). IgA immunoglobulin was transported across the secretory cell membranes and finally reached the glandular lumen (Walcott, 1998).

In accordance with the previous studies in the LGs of Philippine water buffalo (Maala *et al.*, 2007) Roe deer (Klećkowska-Nawrot *et al.*, 2013) and camel (Ibrahim, 2015), the myoepithelial cells in the present work were situated between the secretory cells and the basement membranes. Contraction of these cells was thought to "squeeze" the secretion away from the secretory units into the duct system (Walcott, 1998 and Samuelson, 2007).

The morphological features of the secretory units in present investigation were similar to those of Abbasi, *et al.* (2015) in sheep, since comprised both serous and mucous. Daryuos and Ahmed (2012) mentioned that the LGs in sheep and goats contained mixed acini (possessed both serous and mucous cells) in addition to the serous and mucous acini. According to

Klećkowska-Nawrot *et al.* (2013) and Klećkowska-Nawrot *et al.* (2015), the LGs in Roe deer and European bison had been considered to be serous in nature.

Pinard *et al.* (2003b) demonstrated that the LGs of Americans bison and cattle consisted of acini containing PAS positive granules and serous tubules. Our results exhibited that some mucous acini opened into serous tubules. The cross and oblique sections of these secretory units were given a mistaken appearance which they comprised both serous and mucous cells.

Similar results were obtained by Gargiulo *et al.* (1999); Daryuos and Ahmed (2012) in small ruminants and Ibrahim (2015) in camel, the duct system in the present investigation was composed of intercalated ducts, intralobular ducts, interlobular ducts and main excretory ducts. Unlike that, the duct system of rabbit LG comprised the intercalated, intralobular, interlobular, intralobar, interlobar and main excretory ducts (Ding *et al.*, 2010).

The comparison of the duct system between the LGs in our study and the salivary glands in other studies (Elewa *et al.*, 2010 and Muthukrishnan *et al.*, 2013) showed that the intercalated and intralobular ducts were characterized by wide lumina and absence of the striated ducts. The lack of striated ducts in the LGs might be because the lacrimal secretion was isotonic, rather than hypotonic, as in the case of saliva (Ding *et al.*, 2010).

In the present work, the goblet cells were found in the lining epithelia of interlobular and main excretory ducts of goats only, while the former cells observed within the similar ducts of sheep and goats (Daryuos and Ahmed, 2012). This was due to breed differences between Iraqi sheep.

In our research, the mucous cells of LGs of both sheep and goat reacted positively with PAS. Furthermore, there was no any change in the staining, when we used diastase prior PAS. These data revealed that the mucous cells did not contain glycogen granules (NPS) and included glycoproteins. Mc Manus (1946) had stated that PAS stained glycogen as well as mucins, but tissue could be pre-digested with diastase to remove glycogen showing PAS an important mucin stain. Moreover, the PAS technique was especially sensitive to demonstrate neutral mucin and acid mucin which comprised significant quantities of sialic acid (Ali *et al.*, 2012).

Our study was identified that the mucous cells of sheep LGs reacted intensely with AB pH 2.5 and moderately with AB pH 1, whereas the same cells in LGs of goats reacted moderately and faintly with the previous stains. When we stained the LGs of both sheep and goats with AB-PAS, most mucous cells reacted intensely with AB and faintly with PAS.

The aforementioned reactions revealed that the mucous cells of sheep and goats contained neutral, carboxylated (sialomucin) and sulphated (sulphomucin) glycoproteins with the predominance of carboxylated group in the cells of sheep. Lev and Spicer (1964) used AB at pH 2.5 and pH 1 to distinguish between acid and sulphated mucopolysaccharids. Also, Spicer *et al.* (1967) explained that the carboxyl (COOH) groups stained well at AB pH 2.5, while sulphate (OSO<sub>3</sub>H) groups might stain poorly. Otherwise, Mowry (1956) used AB-PAS to differentiate between the acid and neutral mucins.

The mucous and seromucous glandular cells in sheep LG contained acidic glycoconjugates, mainly sulphated (Gargiulo *et al.*, 2000) and the mucous cells in alpaca LG comprised neutral and acid glycoproteins; sulphated and carboxylated (Kleckowska-Nawrot *et al.*, 2015). The mucin was constitute the inner layer of the tear film (Walcott, 1998 and Davidson and Kuonen, 2004) which secreted from the LG as well as the corneal and conjunctival epithelia (Arango *et al.*, 2001). This substance acts as a protective agent and produced a further reduction in surface tension. It also showed greater affinity for the water / oil interface than protein (Holly and Lemp, 1989; Gargiulo *et al.*, 2000 and Arango *et al.*, 2001). Additionally, the ocular mucin played a role in the treatment of dry eye syndromes and ocular surface microbial infections (Davidson and Kuonen, 2004).

The serous cells of sheep and goat had negative reactions with all stains using for histochemical study, so they did not contain any type of carbohydrates and their secretion was proteintous in nature. At ultrastructure level, the serous cells of sheep LG were regarded as protein -secreting cells, because contained all structural components of protein synthesis, e.g. extensive rough endoplasmic reticulum, well-developed Golgi apparatus and secretory granules (Gargiulo *et al.*, 1999).

During the course of this finding, the goat LGs contained numerous serous secretory units comparing with mucous ones. Furthermore, based on the histochemical analysis, the content of glycoprotein in mucous secretory units was less abundant. From these factors, the LG might produce small amount of mucin in their secretion. Therefore, the presence of goblet cells within the epithelia of their ducts would compensate this deficiency. Kim *et al.* (1991) stated that the mucin with a high molecular weight glycoprotein was secreted by epithelial mucosal cells, particularly the goblet cells.

Depending on the histological and histochemical studies in the present work, the cytoplasm of light cells in both animals did not stain with routine and histochemical dyes. Furthermore, the nuclei of these

cells had pyknotic appearance. From these criteria, the light cells might be undergoing apoptosis. Junqueira and Carneiro (2005) and Choudhury *et al.* (2012) recorded that the apoptotic cells displayed prominent morphological changes during apoptotic process, one of these; breakdown of chromatin in the nucleus often lead to nuclear condensation.

## CONCLUSION

In this study, the morphological structure of LGs could be reflected on their function. The LGs produced seromucous secretion, because they contain both serous and mucous secretory units. Furthermore, our results revealed the presence of mucin within the mucous cells. This substance was essential for maintaining the normal corneal as well as the optical integrity of the eye (Stein and Hurwitz, 1996).

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## دراسة نسيجية ونسجوكيميائية مقارنة للغدد الدمعية للأغنام والماعز المحلية

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اجري هذا البحث على الغدد الدمعية لذكور الاغنام والماعز البالغة المحلية. استهدفت الدراسة الحالية ايضاح الخصائص النسيجية والنسجوكيميائية للغدد الدمعية ولهذا الغرض صبغت الشرائح النسيجية بالصبغات الاتية: الهماتوكسيلين والايوسين' فان جيسون' كاشف الحمض البريودي' كاشف الحمض البريودي المقترن مع خميرة الدايبستاز، ازرق الالسيان عند الاس الهيدروجيني ٢.٥ و ١' ازرق الالسيان المقترن مع كاشف الحمض البريودي واخيرا ازرق التولودين. اظهرت نتائج الدراسة الحالية ان الغدد الدمعية هي غدد انبوبية عنبية مختلطة مؤلفة من الوحدات الافرازية (المصلية والمخاطية بشكل رئيسي فضلا عن بعض العنبات المخاطية المنفتحة على الانبوبات المصلية) والنظام القنوي الاخراجي. تغلبت الوحدات الافرازية المخاطية لغدد الاغنام مقارنة مع نظيراتها لغدد الماعز. احتوت الخلايا المخاطية لكلا الحيوانين على مادة المخاطين بانواعه الثلاث (المتعادل الكربوكسيلي والكبريتاتي) مع تغلب لمجموعة الكربوكسيل لخلايا الاغنام. يتالف النظام القنوي الاخراجي في كل من الاغنام والماعز على القنوات البيئية قنوات داخل الفصيصات قنوات بين الفصيصات والقنوات الاخراجية الرئيسية. امتلكت البطانة الظهارية لقنوات بين الفصيصات والقنوات الاخراجية الرئيسية في كلا الحيوانين على الخلايا الفاتحة بينما امتلكت البطانة الظهارية للقنوات نفسها في الماعز فقط على الخلايا الكاسية. احتوت الخلايا الكاسية على انواع المخاطين عينها المتواجدة في النسيج الغدي للماعز.