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Microscopic anatomy of harderian gland in goats

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Abstract
The aim of this study was to describe the gross anatomy, morphometry, histology and histochemistry of the harderian gland in goats. A total number of six goat heads from three adult male and three adult females with an average age ranged from (7-9) months were collected from the corporation slaughter house, Perambur, Chennai. Right and left eyes were dissected out from six heads and harderian glands were obtained. The harderian gland of both male and female goats was heart shaped with two lobes (dorsal and ventral). The mean size of the gland was recorded as length 11.1 ± 0.11 mm, width 12.1 ±0.11 mm and thickness 5.0 ± 0.08 mm on the right side and length 12.1± 0.11 mm, width 12.5 ± 0.11 mm and thickness 6.0 ± 0.08 mm on the left side. Histologically, the gland was a holocrine compound tubuloalveolar gland. A thin connective tissue capsule surrounded the gland and septa divided the gland into lobes and lobules. The acini were lined with a single layer of columnar cells with small intracytoplasmic eosinophilic vacuoles apically and spherical nucleus with prominent nucleolus basally and were frequently binucleated. Fusiform shaped myoepithelial cells with elongated nucleus were found between the basal surface of the epithelial cell and the basement membrane. Duct system started with intralobular and interlobular duct and was drained with main excretory duct which opened into the inner surface of the nictitating membrane. Intralobular duct was lined with columnar epithelium. They converged into an interlobular duct to join the main collecting duct, with lining epithelium from stratified columnar to stratified squamous near its end. Histochemical studies with PAS stain revealed weak reaction in the capsule and septa and strong reaction in the cytoplasm of the acinar cells.

Keywords: Goat, harderian gland, morphology, histology

1. Introduction
The harderian gland is a paraorbital tubulo-alveolar gland located within the orbit on the medial aspect of the eyeball in animals which possess nictitating membrane (third eyelid). It is a nictitating membrane which is comparable to the conjunctival fold of the human eye, but is less prominent in mammals. Harderian gland is primarily found in terrestrial vertebrates which support to the fact that it evolved with the lacrimal glands and lubricated the nicticating membrane and cornea [26]. A prominent harderian gland is present in mammals, amphibians and birds with a well-developed nictitating membrane but the gland is absent in primates and carnivores [26]. The harderian gland is compound tubular or compound tubulo-alveolar which indicates that the gland has branched duct system and tubular alveoli.
Functions of this gland are lubrication of the eye in mammals, thermoregulation, photoprotection in rodents and a source of either pheromones or growth factors in rodents [9]. Contrasting biochemical and physiological characteristics of the harderian gland namely sexual dimorphism, photosensitivity, synthesis of hormones, assisting the thermoregulatory behaviours and production of pheromones have been identified [21, 26]. In addition to that it also plays an immunological role [17].
There are many investigations of the harderian gland in camel [2], Mongolian gerbils [26], domestic fowl [31], domestic duck, fowls, turkeys and ducks [5]. The reports on the harderian gland in the goats are scanty. In the present work, gross studies, histological and histochemical observations have been performed to study the structure of the harderian gland of both male and female goats.

2. Materials and Methods
A total number of six goats heads from three adult male and three adult females with an average age ranged from (7-9) months were collected from the corporation slaughter house, Perambur, Chennai.

The harderian gland was dissected out and gross observations were recorded. Morphometrical parameters like length, width, thickness of the gland were measured using a scale and thread. Then the glands was washed in normal saline, mopped with blotting paper and fixed in 10 per cent neutral buffered formalin, Bouin's solution and Zenker fixatives. After 24 hours of fixation, the tissues were processed by dehydrating in ascending grades of isopropyl alcohol (70%, 80%, 90%, 95%, 100% and 100%). For each grade of alcohol one hour was provided. After dehydration, the tissue was transferred to three changes of xylene for ninety minutes for clearing purpose. Then the tissues were impregnated in the liquid paraffin at 60ºC temperature for ninety minutes and were repeated again. Finally the tissues were embedded in paraffin wax (melting point 58-60˚C) to prepare paraffin tissue blocks. Afterwards 3-5 µm thickness tissue sections were prepared by using Leica rotary microtome. After sectioning of paraffin block, the slices were floated on warm water in a water bath at 45ºC for stretching. Then the sliced tissue was placed on grease free clean glass slide using adhesive like Mayer's egg albumin. Then the glass slides were dried at 37ºC temperature for 24 hours in an incubator. After drying the slides, the tissue sections were stained by using the following methods; Haematoxylin and eosin (H&E) stain for general tissue structure, Masson's trichrome stain for demonstration of collagenous fibers and smooth muscle cells, Gomori's method for Reticulum for demonstration of reticular fibers, Weigert's stain for demonstration of elastic fibers, Periodic acid- Schiff (PAS) stain for demonstration of glycoprotein (neutral mucosubstances), Alcian blue ph 2.5 for demonstration of acidic mucosbstances, Unna's method for the demonstration of mast cells and Van Geison's method for demonstration of Collagen fibres [3]. The images were photographed by the Leica computed image analyser and stored. The above morphometric data were analysed statistically and presented as Mean ± Standard deviation.

3. Results and Discussion

3.1 Gross Observation

The gross observation of the harderian gland of both male and female goats revealed that they are the second largest orbital gland next to the lacrimal gland and was located in the medial side of the orbital cavity (Fig. 1). The harderian gland was observed to be bilobed with small dorsal lobe and large ventral one and seen as the heart shaped in the medial canthus below the nictitating membrane (Fig. 2) but the gland was dissimilar to the present study as it was oval in the European bison and elongated-triangular with a cobble-stoned proximal part and a smooth distal portion in the American bison and cattle [22]. According to El-leithy [9], the gland was bilobed with small white lobe and large pink one in rabbits which was similar to the present study. The gland was located between medial rectus, inferior oblique muscle and third eyelid. This finding is similar to the findings for rat and other rodents [19]. The outer surface of the gland was lobulated and was pale pink in colour.

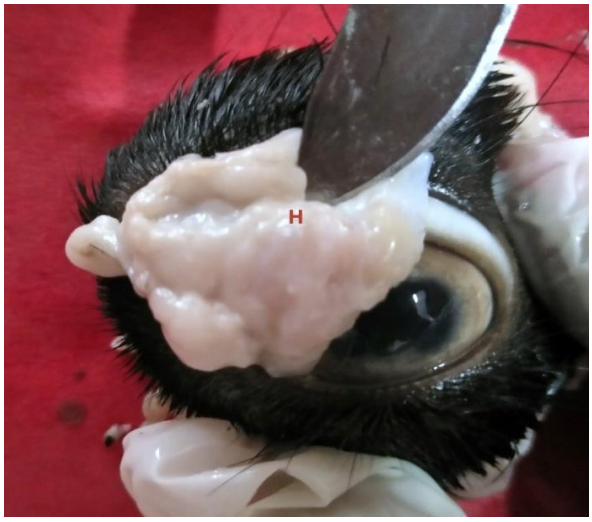

Fig 1: Photograph showing the left harderian gland (H) of adult goat

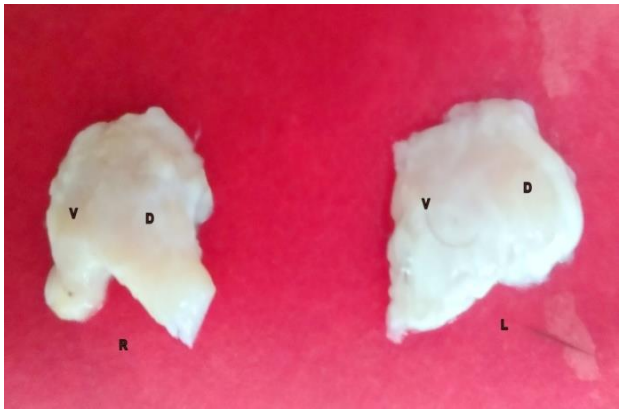

Fig 2: Harderian gland of right (R) and left (L) eye each showing dorsal (D) and ventral (V) lobes

3.2 Morphometry

The mean size of the gland in the present study was recorded as length 11.1 ± 0.11 mm, width 12.1 ±0.11 mm and thickness 5.0 ± 0.08 mm on the right side and length 12.1± 0.11 mm, width 12.5 ± 0.11 mm and thickness 6.0 ± 0.08 mm on the left side. This is contrary to the finding for the European Bison [17] and pigs [23] in which the measurements were found to be larger when compared to the goat. These may be due to the species difference and size of the eyeball in relation to the size of the head.

3.3 Histology and histochemistry

Histologically, the gland was surrounded by a thin irregular connective tissue capsule and septa from the capsule divided the gland into lobes and lobules (Fig. 3). These findings simulate the results for hamsters [20], pig [7] and bison [17]. In mice, Yamashita *et al.* [32] found an external endothelial layer on the outer surface of the capsule but was not found in this study and it was absent in the rat [30] and bison [17]. Capsule was composed of collagen, elastic and reticular fibres (Fig. 4, 5 and 6) with fibroblasts, melanocytes, adipose tissue, nerve

bundles (Fig. 7), lymph nodes (Fig. 8) and mast cells. This is in agreement with results for rabbits [10] in which mentioned that elastic fibers were detected in the capsule of rabbits. In the present study, the reticular fibers were thick in the capsule and septa and form a thin network between and around the acini. Similar findings also have been reported in domestic Geese [16]. The interstitial tissue was sparse and had fibroblast, collagen, blood vessels, lymphocytes and nerves. Numerous adipocytes penetrated into the glandular tissue together with the connective tissue and surrounded the lobules (Fig. 4). Similar reports were observed in Alpaca [18].

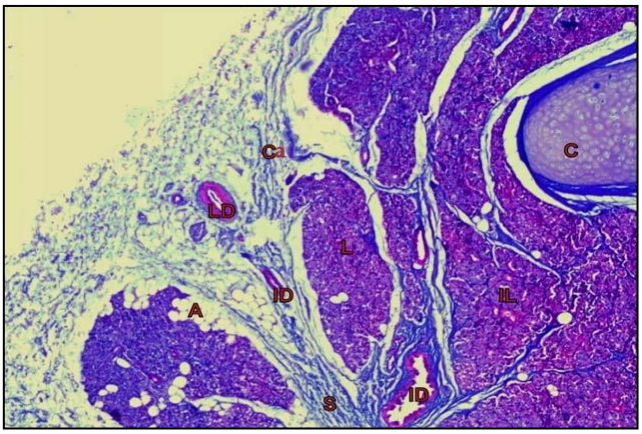

Fig 3: Photomicrograph showing the collagen fibres in the capsule (Ca) and Septa (S) dividing the parenchyma into lobes (L) and lobules (LL) C – Cartilage ID – Intralobar duct LD – Interlobar duct Massons Trichrome x 100

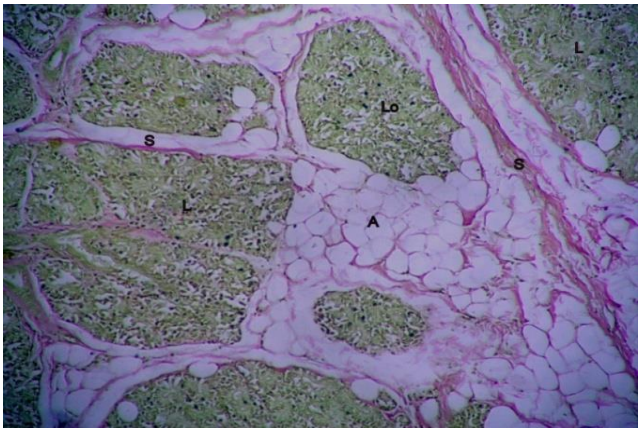

Fig 4: Photomicrograph showing collagen fibres in the septa (S) and adipose tissue (A) surrounding the lobes (L) Van Gieson x 100

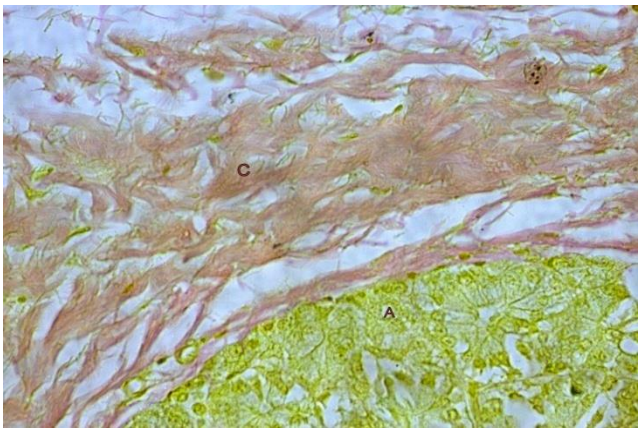

Fig 5: Photomicrograph showing elastic fibres in the capsule (c) Weigart's Stain x 400

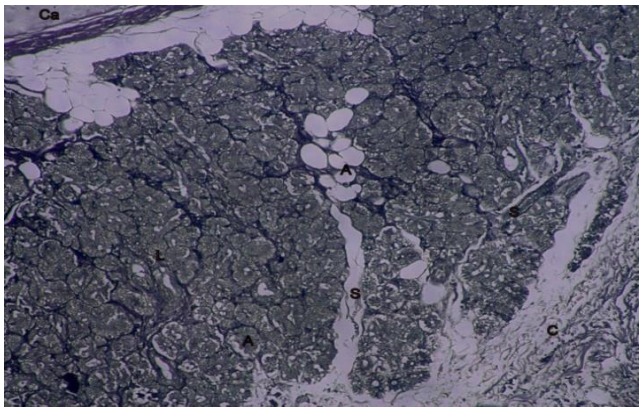

Fig 6: Photomicrograph showing reticular fibres in the capsule (C) and septa (S) and surrounding the acini (A) Gomori's reticulin method x 100

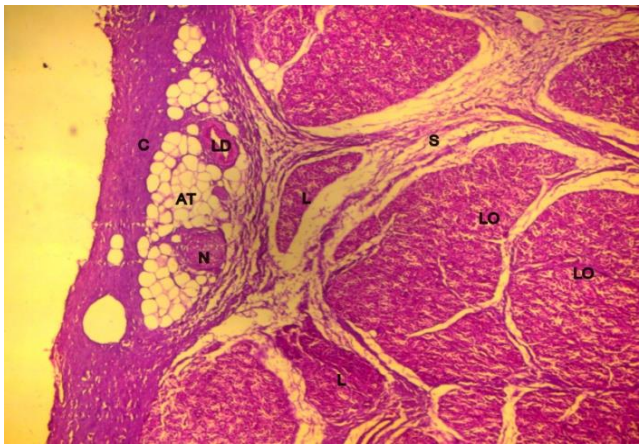

Fig 7: Photomicrograph showing the presence of Nerve fibres (N) surrounded by Adipose tissue (AT) below the capsule (C) L – Lobe LL – Lobule S – Septa LD – Intralobar duct H X E x 100

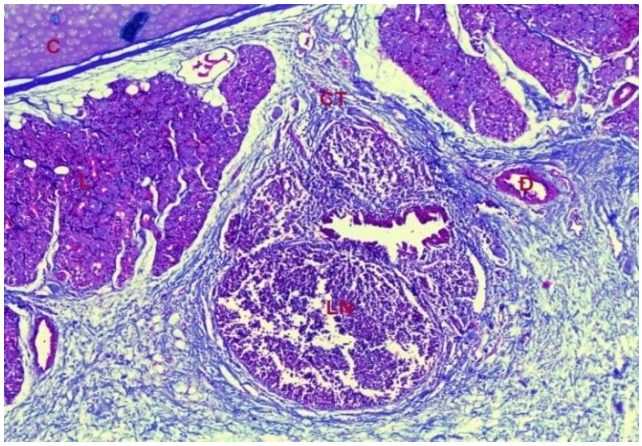

Fig 8: Photomicrograph showing the presence of Lymph node (LN) adjacent to the harderian gland and cartilage (C) CT – Capsule L – Lobes D – Intralobar duct Massons Trichrome x 100

The harderian gland of goat was an exocrine holocrine sero-mucoid compound tubulo-alveolar gland. This supports the results of harderian gland in rabbit [10], domestic geese [16], domestic chicken [15], alpaca [18] and pigs [23]. Sexual dimorphism was not observed in the harderian gland of goat morphologically and histologically, which is in agreement with findings for desert rodent [8]. However, a clear sexual dimorphism has been observed in the harderian gland of golden hamster in the number of the cell types, ultrastructural features and abundance of the interstitial cells [27, 6]

The acini of the harderian gland of goat were variable in shape and size but some appeared large with relatively wide lumen (Fig. 9). The acini were lined with a simple epithelial layer of columnar cells, this finding is in agreement with the study for bison [17] and pigs [23]. But in rabbits the presence of two epithelial cell types was found *viz*. one type with small intracytoplasmic lipid vacuoles and the other with large ones [9]. Harderian gland with two epithelial cell types was also found in male golden hamster [21] and in Gerbil [25]. Harderian gland with one epithelial cell type was found in female golden hamster [21].

The acini were lined with columnar epithelial cell layer with acidophilic cytoplasm at the apical part and small to medium sized intracytoplasmic lipid vacuoles with round nucleus with prominent nucleolus at the basal part (Fig. 9). Some columnar cells were often binucleated. These results are in line with the findings in type I cell of plains mouse [13], bison [17] and pigs [23]. Some small intracytoplasmic lipid vacuoles were also observed at the apical part of the cell. Goblet cells were not seen in the acinar cells.

Gesase and Satoh [12] mentioned that when the secretory process accompanied by the loss of the cytoplsamic fragments into the lumen which was considered as an apocrine secretion. The results in the present work showed that there were numerous cytoplasmic bleb like protrusions at the luminal surface of the epithelial cells. Some of these cytoplasmic blebs along with the nucleus were separated into the lumen of the secretory acini and hence was considered as a holocrine secretion (Fig. 9). This observation agrees with the results for bison [17] and pigs [23] and not supportive with the findings for the plain mouse [13], rodents [8], rabbit [10] who reported the secretion as apocrine.

Histochemical studies with PAS stain revealed weak reaction in the capsule and septa and strong reaction in the cytoplasm of the acinar cells (Fig. 10). The reaction was confined to the apical part, basement membrane and the luminal secretory materials and the cytoplasmic bleb like protrusion which proved the presence of neutral or weakly acidic glycoproteins (Fig. 11) whereas in domestic fowl [31], rabbits [10], domestic geese [16] and pigs [23], weak reaction was found.

Alcian blue staining in this study showed a negative reaction in agreement with the findings for rabbits [10] whereas it showed a strong reaction at ph 2.5 for domestic fowl [24], domestic geese [16] and in ospery [14].

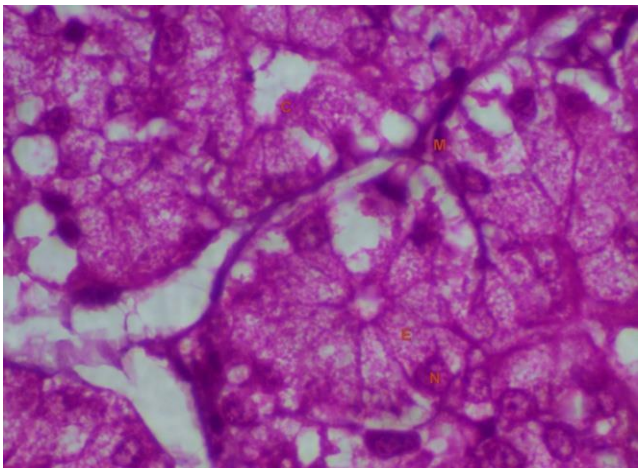

Fig 9: Photomicrograph showing acini in various size and shape with intracytoplasmic budding (c) and showing spherical nucleus (N) with apical eosonophilic granular cytoplasm with vacuoles (E) H & E x 1000

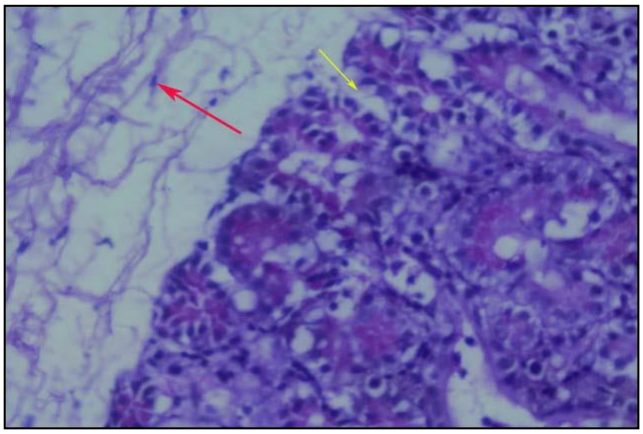

Fig 10: Photomicrograph showing mild PAS positive reaction in the capsule (Red arrow) and Septa (Yellow arrow) PAS x 400

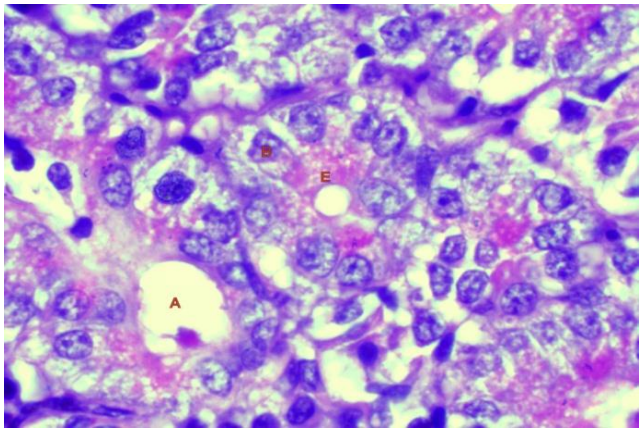

Fig 11: Photomicrograph showing strong PAS positive reaction in the apical (E) part of the acini (A) B – Nucleus PAS x 1000

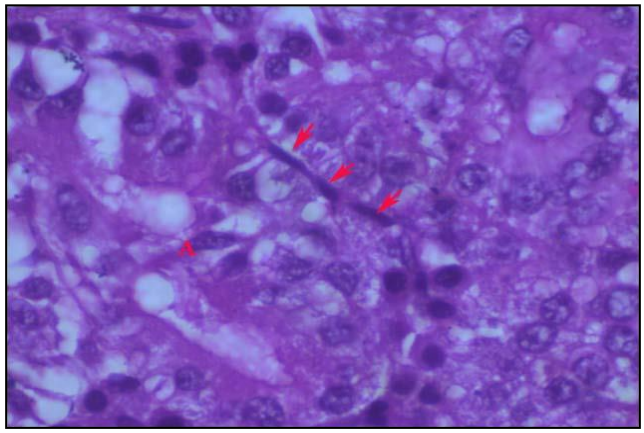

Fig 12: Photomicrograph showing Myoepithelial cells (Arrow) between basal part of acinar (A) epithelial cells and basement membrane H & E x 1000

In the present study, the alveoli were surrounded by basal myoepithelial cells. This finding simulated the results for the gerbils [26], mouse [29], one humped camel [2], rabbit [10], bison [17] and pigs [23]. The nuclei of myoepithelial cell were elongated oval, lied parallel and interposed between the basal surface and the basement membrane (Fig. 12). The main function of myoepithelial cells in any exocrine gland is to expel their secretory products into the lumen of the acini by their contraction [32].

In the present investigation, bundles of myelinated nerve fibers were detected above the capsule (Fig. 7). Plasma cells were detected beneath the capsule, in the interlobular septa

and in the interstitial tissue. This agreed with the findings for the rabbit, rat and guinea pig [4], domestic geese [16], osprey [19] and sheep [1]. Scott *et al.* [28] mentioned that the harderian gland was a site of unusual plasma cell proliferation. The plasma cells secreted different classes of immunoglobulins. He also stated that these immunoglobulins afforded the upper respiratory tract with protective antibodies through the tears.

In goat harderian gland, there was a distinct duct system. It started with intralobular and then interlobular duct and was drained with main excretory duct which opened into the inner surface of the nictitating membrane. Similar findings were observed in the findings for one humped camel [11] and pigs [23]. However, these findings disagreed with that for wistar rat [8] and Eltony [10] in rabbit who mentioned that the duct became distinct only when it left the gland tissue.

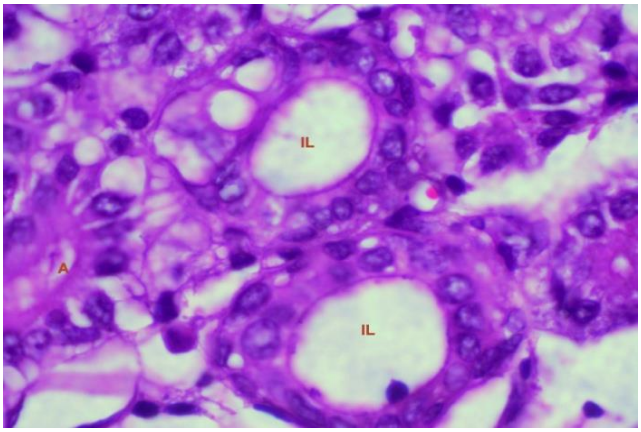

Fig 13: Photomicrograph showing Intralobular duct (IL) lined by simple cuboidal epithelium and Acini (A) H & E x 1000

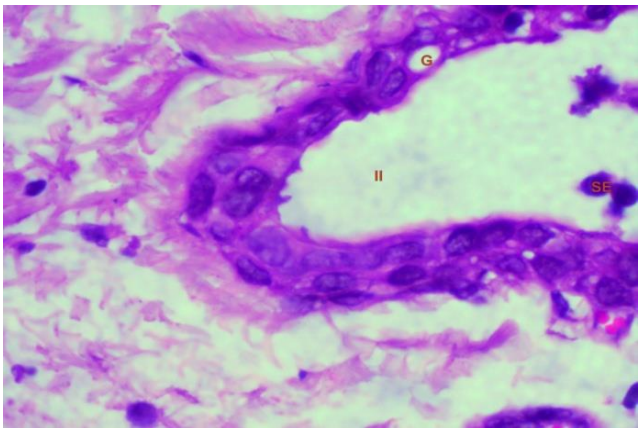

Fig 14: Photomicrograph showing Intralobar duct (II) lined by Stratified cuboidal epithelium with Goblet cells (G) H & E x 1000

The intralobular duct was lined with simple cuboidal epithelium (Fig. 13) which was similar to that found in one humped camel [11] and in pigs [23]. This duct along its course their epithelium becomes stratified cuboidal but in one humped camel, it was lined with simple columnar epithelium. The interlobular duct continued with simple columnar epithelium in one humped camel but in our investigation appears to be lined with stratified columnar epithelium. Payne [21] mentioned that, epithelial cells become squamous at the distal part of the duct which was similar to our findings. In the present investigation, the epithelial lining of the duct system showed PAS positive granules and the excretory duct showed goblet cells in between with the strong PAS reaction (Fig. 14). Burns and Maxwell [5] mentioned that the goblet cells in epithelial cells of ducts were weakly PAS-positive in turkey, duck, and aged hens. The mucous secreted from these cells might play a role in the lubrication and protection of the eye [8].

4. Conclusion

Harderian gland was the second largest orbital gland and was located medially in the orbit. It was bilobed with small dorsal lobe and large ventral one and heart shaped in goats of both sexes. The mean length, width and thickness of the gland corresponded to the eye size. Harderian glands were exocrine holocrine seromucoid compound tubuloalveolar gland. It was composed of lobes and lobules with acini of different sizes and shapes. Each acini was lined with a layer of columnar cells and a layer of flat basal cells resting on a basement membrane – myoepithelial cells. The duct system was initially intralobular, and then become interlobular, and finally empty into intralobar ducts. PAS staining revealed weak reaction in the capsule and septa and strong reaction in the cytoplasm of the acinar cells. The present study was carried out to understand the gross anatomy, morphometry, histology and histochemistry of harderian gland in goats.

5. References

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