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Original Research

Anatomical Studies On the Infraorbital Glands of Male Sheep of Tirunelveli Region of Tamil Nadu

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Abstract

Chemical communication plays a crucial role in mammalian behavior starting by understanding olfactory communication. The work is undertaken to study the morphology, morphometry, histology, and micrometry of the infraorbital gland in the male sheep of the Tirunelveli region of south Tamil Nadu to provide baseline information about the gland's function. The infraorbital gland was exocrine and found within an infraorbital pouch. Histologically, the gland was covered with an epidermis of skin – stratified squamous epithelium. Below the epidermis was the dermis showing the connective tissue fibres and cells, striated muscles, and hair follicles. The dermis region contains the outer sebaceous, holocrine portion of glands, and an inner apocrine portion of glands. The sebaceous holocrine part of the gland was found in the dermis region in high thickness and the apocrine part of the gland was found below. The apocrine glandular part was seen in less diameter and was a simple coiled tubular gland.

Keywords: Anatomy, Histology, Infraorbital Gland, Sheep.

Introduction

Chemical communication plays a crucial role in mammalian behaviour starts by understanding olfactory communication on body sources of odours by putative chemosignals. A pheromone is an odour that elicits a predictable and stereotypical behaviour or response, provides information and modifies responses. Some of those chemosignals are of skin origin namely tarsal gland of deer, inguinal gland of rabbits, preputial glands of pigs and infraorbital glands of sheep (Mykytowycz, 1974). All cervids and antelops are possessed with infraorbital glands with gland and pouch located near to the nasal corner of eye (Rajagopal and Archunan, 2011). the infraorbital glands were involved in mate recognition, territory marking sexual selection and mother-offspring bonds (Rajagopal and Archunan, 2011). The scent is released from this infraorbital gland when a deer rubs its face against bushes or trees (Osborn *et al* 2000). As Scully *et al*. (2000) stated that most of the scent glands are normally composed of sebaceous and apocrine glands which produce different odouriferous molecules. the morphology and histology of the infraorbital glands in male black buck (Rajagopal and Archunan, 2011), male barking deer (Adnyane *et al*, 2011) and Egyptian native breeds of sheep (Maowad, 2016) has been studied. But the literature regarding the detailed anatomical study on the infraorbital glands in Indian native breeds of sheep were scanty. So, present work is undertaken with the objective to study the morphology, morphometry, histology and micrometry of the infraorbital gland in the male sheep of Tirunelveli region of south Tamil Nadu.

Materials and Methods

The infraorbital glands were collected from the 12 heads of the healthy adult male sheep (average body weight 21 kgs) of Tirunelveli region from the nearby mutton shops around Tirunelveli region. The gland (Right and left side) was carefully dissected from the head and the morphometerical measurements like length, width and thickness of the gland were measured. Then the gland was cut into small pieces and fixed in 10& neutral buffered formalin and Bouin's solution for 48 hours. Then the tissues were dehydrated in ascending grades of alcohol, cleared in xylene, paraffin infiltrated and embedded with paraffin wax. The blocs were cut into 3-5 µm thickness sections and stained with haematoxylin and eosin stain and Masson's trichrome stain (Luna, 1968). The slide was examined under microscope and photographed using Lynx Trinocular microscope with imaging system software. The micrometrical observations namely thickness of epidermis, sebaceous portion and apocrine portion were measured. Two types of sebaceous glands were found namely common type and modified type.

Results and Discussion

Morphology and Morphometry

The infraorbital gland of native sheep of Tirunelveli region was exocrine gland and found paired within a deep pocket called infraorbital pouch in the external lacrimal pit or preorbital pits (Fig. 1). The lacrimal pit was located on the external surface of the lacrimal bone (Fig. 2). The gland was surrounded by fascia, skin with hairs. The lacrimal pit was located below to the nasal corner of the eye in the male sheep. Secretions were found as waxy dark brown coloured and located within the pouch. The shape of the gland was pear shaped to oval and measured 1.8 – 2.1 cm in length, 0.6 cm in width and 0.1 cm in thickness in the right side and 1.7- 2.0 cm in length, 0.5 cm in width and 0.09 cm in thickness in the left side. In white tailed deer, the gland measured 2.2 cm in length, in mule deer, it was 4 cm and in black tailed deer, it measured 3.2 cm in length (Rue, 2004). In Egyptian native breed of sheep, the gland measured 2.8-3.3 cm in length, 2.5-2.8 cm in width and 0.3-0.5 cm in depth ((Maowad, 2016). In barking deer, the gland measured 2.3 -2.7 cm in length and 2.0-2.3 cm in width (Adnyane *et al*., 2011). In male black buck, the gland measured 2.8 to 3.5 cm un length, 1.5 to 1.7 cm in width and 1.0 to 0.2 cm in depth in territorial males and 2.0 to 2.5 cm in length, 0.8 to 1.5 cm in width and 0.8 to 1.0 cm in depth in non-territorial males (Rajagopal and Archunan, 2011). The differences in the measurements might be due to species and breed variation. The measurements were almost same by comparing with other sheep breeds and this concludes that the gland secretion process was almost uniform in the sheep breeds. The gland was related cranially to the malaris muscle, caudally to the medial canthus of eye and dorsally to the nasal bone.

Histology

Histologically both the left and right infraorbital gland showed no variation. The outer surface of the gland was covered with epidermis of skin (Fig. 3) showing the five layers namely stratum basale, stratum germinativum,

stratum granulosum, stratum lucidum and stratum corneum. Below the epidermis, there was dermis showing the connective tissue fibres and cells, straited muscles and hair follicles (Fig. 3). Within the dermis region contained outer sebaceous, holocrine portion of glands (Fig. 3) and inner apocrine portion of gland. Sebaceous holocrine part of the gland was found in the dermis region and the apocrine part of the gland was found below in this region. Similar findings were also observed in barking deer (Adnyane *et al*., 2011) and in Egyptian breed of sheep (Maowad, 2016). Whereas in serow, the apocrine part was more in diameter and found above the sebaceous part (Atoji *et al*., 1995). This might be due to species variation and pheromone secretion. The proportion of sebaceous holocrine part of the gland was more when compared to the proportion of the apocrine part of the gland (Fig. 6). Similar observations were also observed by Maowad (2016) in Egyptian native breed of sheep, Rajagopal and Archunan (2008) in Indian blackbuck, Atoji *et al* (1995) in serow. In contrast to this, the thickness of apocrine portion was found more than the sebaceous portion in barking deer (Adnyane *et al*., 2011) which might be due to species variation. The sebaceous and apocrine portions of the gland secreted different volatile substances and were responsible to allow pheromonal communication between animals as per the statement of Rajagopal and Archunan (2008) in Indian black buck. The pheromone secretion was dependent upon the combination of secretions by both the sebaceous and apocrine portions of the glands as per the statement of Adnyane *et al* (2011) in barking deer. The high proportion of sebaceous gland might be responsible for the thick waxy secretion as per the statement of Maowad *et al* (2016) in Egyptian breed of sheep.

Epidermis

The Epidermis of the skin formed the outer portion of the infraorbital gland. The epithelium was keratinized stratified squamous epithelium (Fig. 3) as stated by Maya *et al* (2019) in deer, goat ad sheep. It consisted of five layers namely stratum basale, stratum germinativum, stratum granulosum, stratum lucidum and stratum corneum from inside to outside (Fig. 4). Stratum basale consisted of single layer of columnar cells. Above this layer was stratum germinativum layer which consisted of many layers of polygonal cells with centrally located oval to round vesicular nucleus with mitotic figures. Within this layer, melanocytes with brown coloured melanin pigment within the cytoplasm was also observed in the deep layers of stratum germinativum. Above this layer found stratum granulosum with rhomboid cells in 2-3 layers. The cytoplasm of these cells contained keratohyaline granules which stained dark purple by H & E staining. The nucleus was elongated oval in appearance. The above layer was stratum lucidum with 2 layers of squamous cells with occurrence of flat nucleus in some cells and absent in others. The outermost layer was stratum corneum with cornified cells without showing any cells morphology or nucleus and difficult to differentiate it from stratum lucidum layer. This layer contained the diffuse distribution of dark brown to black coloured stained area which might be the pigment of stratum corneum layer (Fig. 4). In contrast to this, Maya *et al* (2019) observed rete pegs in the epithelium of deer, goat and sheep skin which was not observed in the present study.

Fig. 1: Photograph showing infraorbital gland (IOG) within infraorbital pouch (IOP) of sheep in relation to medial canthus (MC) and eye (E).

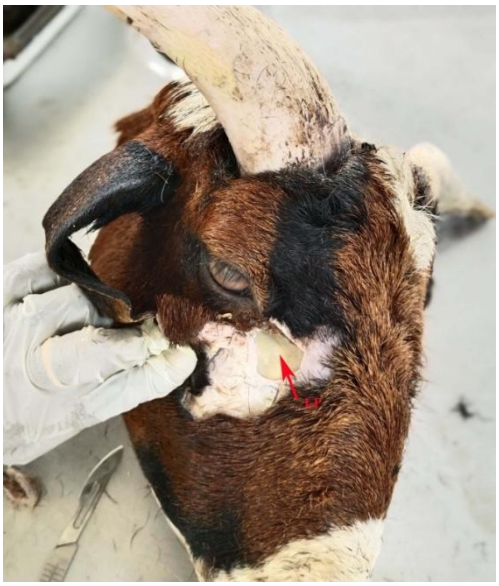

Fig. 2: Photograph showing the location of infraorbital gland in the lacrimal pit (LP).

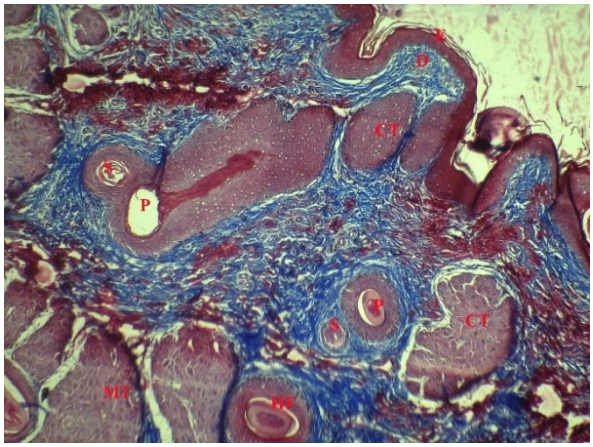

Fig. 3: Photomicrograph of left infraorbital gland of native breed of sheep of Tirunelveli region showing epidermis (E), Dermis (D), primary hair follicle (P) secondary follicle (S), common type (CT) and modified type (MT) of sebaceous gland Masson's Trichrome stain x 100

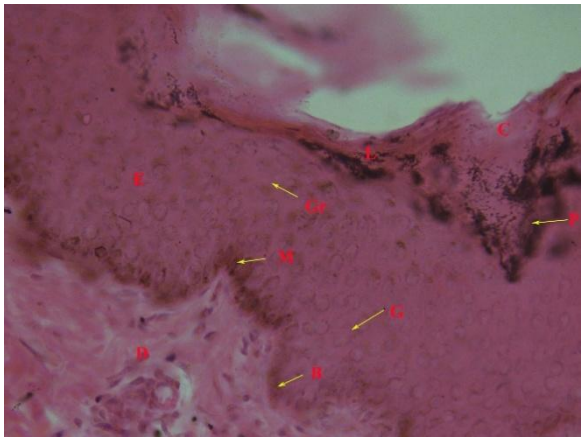

Fig. 4: Photomicrograph of right infraorbital gland showing epidermis (E) and Dermis (D), Stratum basale (B) Stratum germinativum (G), Stratum Granulosum (Gr), Stratum Lucidum (L) and Stratum corneum (C), Melanocytes (M) and Pigment granules (P) H & E x 400

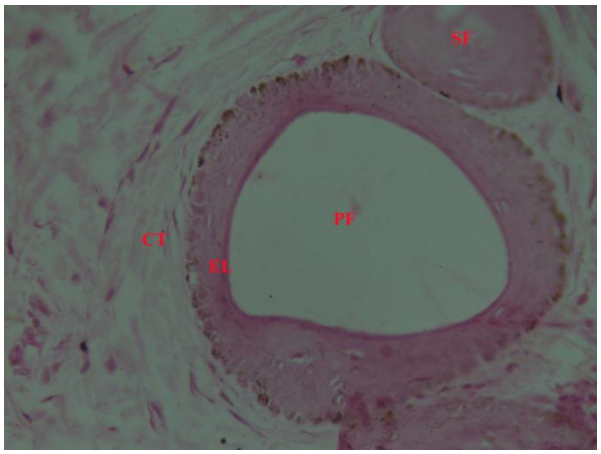

Fig. 5: Photomicrograph of right infraorbital gland showing Primary follicle (PF) and Secondary Follicle (SF), Epithelial layer (EL) and Connective tissue layer (CL) H & E x 400

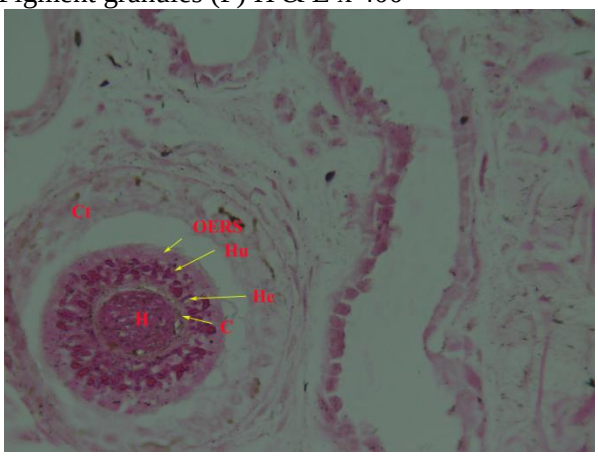

Fig. 6: Photomicrograph of left infraorbital gland showing Primary follicle (PF) with Hair (H), Cuticle (C), Henley's layer (He), Huxley's layer (Hu), Outer epithelial root sheath (OERS) and connective tissue layer (CL) H & E x 400

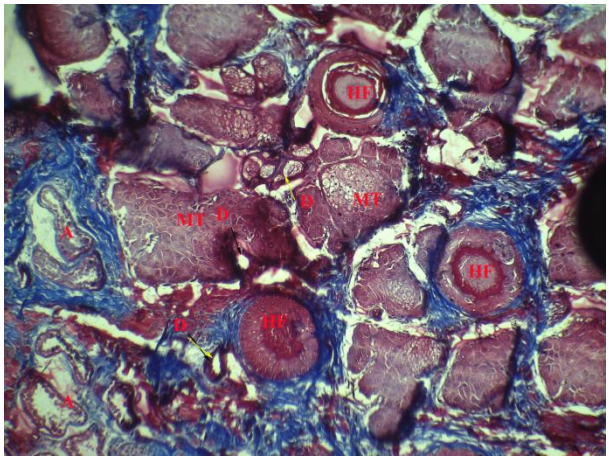

Fig. 7: Photomicrograph of left infraorbital gland showing hair follicle (HF) with Modified type of sebaceous gland (MT), Duct (D) and Apocrine gland (A) Masson's Trichrome stain x 100

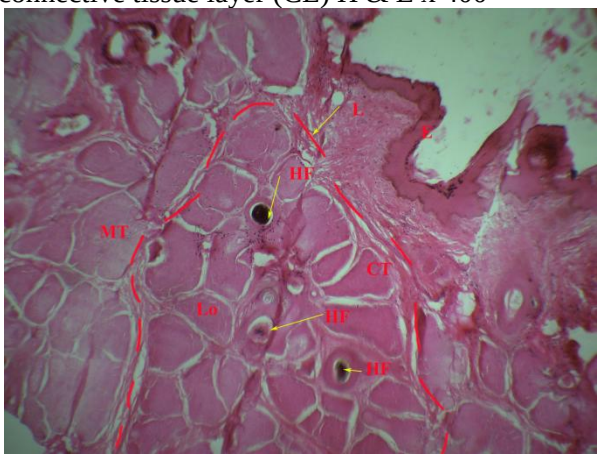

Fig. 8: Photomicrograph of right infraorbital gland showing hair follicle (HF) with Modified type of sebaceous gland (MT), and common type (CT), Lobe (L) and Lobule (Lo) H & E x 100

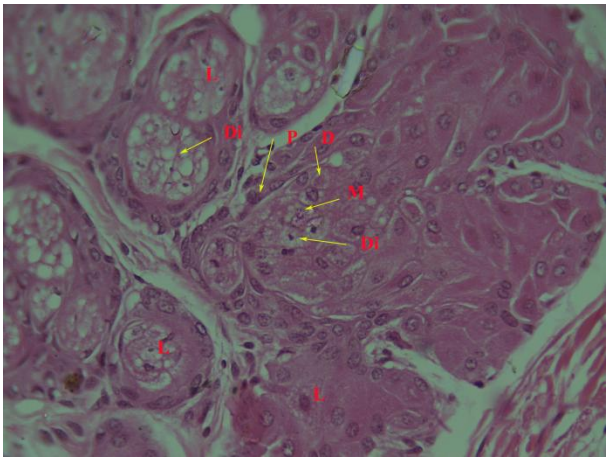

Fig. 9: Photomicrograph of right infraorbital gland showing Modified type of sebaceous gland lobe (L) with peripheral (P), differentiating (D), mature (M) and dissociative type (Di).H & E x 400

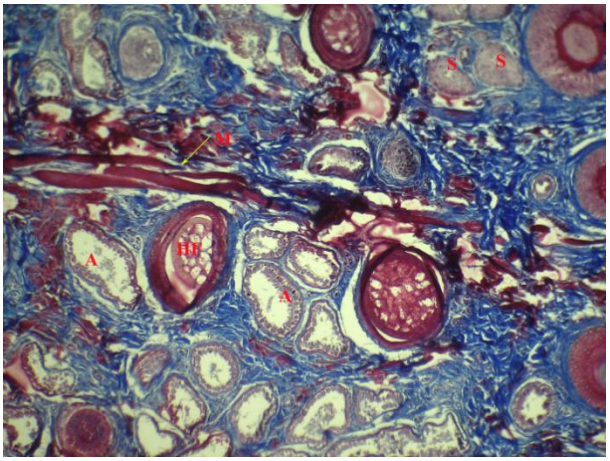

Fig. 10: Photomicrograph of left infraorbital gland showing plain muscle (M) separating Sebaceous gland (S) from Apocrine gland (A). Hair follicle (HF) Masson's Trichrome stain x 100

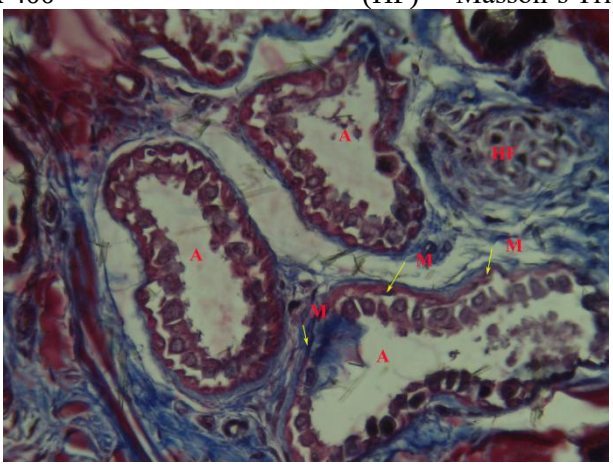

Fig. 11: Photomicrograph of left infraorbital gland showing Apocrine gland (A) with Myoepihtelial cells (M) and Hair follicle (HF) Masson's Trichrome stain x 400

Dermis

Below the epidermis was found the dermis region. It contained loose connective tissue with collagen fibres and other connective tissue fibres and cells (Fig. 4). It also contained hair follicles which showed inner and outer epithelial root sheath and connective tissue sheath (Fig. 3). In the present study compound hair follicles containing primary and secondary hair follicles were also found. Mostly one primary and one secondary follicle was occurred in one location (Fig. 5). The inner epithelial root sheath contained cuticle of root sheath which was found as homogenous layer next to cuticle of hair. Outer to this was Huxley's layer with 2-3 rows of elongated cells whose cytoplasm contained eosinophilic granules with spherical shaped nucleus. Next to it was the Henley's layer with a layer of flattened cells with flat nucleus. The outer epithelial root sheath simulated that of the stratum germinativum of the epidermis of the skin. Outer to this was the connective tissue layer which consisted of inner membrane like layer, narrow homogenous eosinophilic layer and collagen fibre layer (Fig. 6). the histological structure of primary and secondary hair follicle was almost same but lumen of the hair follicle was found empty and contained only outer epithelial sheath and connective tissue sheath and in inner epithelial sheath, cuticle layer was not found in the primary follicle and all layers were found in secondary hair follicle but the hair in the secondary follicle did not contained medulla.

Sebaceous Portion

Below the epidermis found the layer of holocrine sebaceous glandular portion. Two types of sebaceous glands were found namely common type and modified type (Fig. 3). Similar findings were also recorded by Maowad *et al* (2016) in Egyptian breed of sheep and by Atoji *et al* (1995) in serow. But Adnyane *et al* (2011) in barking deer found only single type of sebaceous gland which is common type and this may be due to species difference. Atoji *et al* (1989)

in Japanese serow found that females had modified type and males had common type. But in the present study of male Tirunelveli native breed of sheep we observed both common and modified type of gland. This might be due to species difference. The common type was found superficially below the epidermis (Fig. 3). The modified type was located deeper to the common type and found in larger diameter in comparison with common type. The modified type showed a centrally placed duct which opened into the hair follicle and surrounding the duct were found six to seven lobules of sebaceous gland (Fig. 7) and some ducts showed branching pattern too. The ducts emptying directly to the skin epidermis was not observed in the present study but was found in Egyptian native breed of sheep (Maowad, 2016). The duct of the gland was lined by stratified squamous epithelium and were surrounded by loose connective tissue with few fibres. The acinar cells of common type secreted sebum with secretory products (combination of mucous compounds and other serous secretory products) which was concluded from the staining properties and position of nucleus. The aninar cells of modified type secreted only lipid compounds and were concluded with the staining properties and position of nucleus. The common type of sebaceous gland was present in all over the skin but modified type of sebaceous gland was particular to the infraorbital gland which might be responsible for pheromone production.

The secretory acini of modified type of sebaceous gland showed pear shaped lobule and were larger than common type which showed oval shaped lobule (Fig. 8). In common type, surrounding the duct, only two to four acinar lobules were observed. The lumen of the duct was also small in diameter. The acinar cells of the modified type and common type were of pyramidal or polygonal in shape with centrally located round to spherical shaped nuclei. The cells of modified type were found flat with dark blue stained nucleus at the periphery of the acini but in the centre, the cytoplasm of the cells found vesicular due to accumulation of secretory materials mainly pheromones in small vacuoles, other organelles like mitochondria, rough endoplasmic reticulum were not visible. When moved to the centre, the nucleus of the cells was not observed, cytoplasm showed big empty vacuoles showing the lodgment of pheromones in large vacuoles (Fig. 9). The cells of common type showed the same peripheral morphology of cells as in modified type and showed only the central part of the cells with round nucleus and darker eosinophilic stained cytoplasm with small vacuoles. So, the acinar cells of common type were darker stained and modified type were lighter stained with H & E (Fig 3). In both the type, the acini were surrounded by loose connective tissue with collagen fibres.

In modified type, the cells could be named as peripheral, differentiating, mature and dissociative type. The peripheral cells were flattened with eosinophilic cytoplasm with flattened nucleus. The differentiating cells were polygonal shaped with less eosinophilic small vacuolated cytoplasm with centrally located round nucleus. The mature cells were large polygonal shaped with large sized vacuolated less eosinophilic cytoplasm with centrally located vesicular round nucleus. The dissociative cells were located in the centre and mostly found anucleated and without clear cellular morphology stating these cells were in holocrine mode of secretion (Fig. 9). Similar findings were also observed in roebuck (Janicki *et al*., 2003).

Apocrine Portion

Below the sebaceous portion, skeletal muscle layers and collagen muscle layers were found as two to three layers in discontinuous pattern as a separation between the sebaceous and apocrine portion (Fig. 10). Below the muscular part, apocrine glandular part was seen in less diameter. It was found as group of apocrine acini with wide lumen. It was simple coiled tubular gland as stated by Adnyane *et al* (2011) in barking deer. Each acinus was lined with simple cuboidal epithelium containing eosinophilic cytoplasm and centrally located round nucleus. Cytoplasmic apical bleb-like projections were seen projected from the secretary epithelial cells towards the lumen. Apocrine secretions were also found as storage within the lumen of some acini. Myoepithelial cells were observed between the epithelial cells and basement membrane and whose contraction responsible for the release of secretion from the gland (Fig. 11). Similar results were also confirmed by Adnyane *et al* (2011) in barking deer. The release of secretion from the gland might be also due to contraction of facial muscles (malaris muscle) lying over and adjacent to the gland, or by mechanical release when the animal rubs any sharp ohjects. This was similar to the findings of Grey *et al*., (1989) in musk ox. The apocrine group of acini were separated by the bundles of collagen and muscle tissue.

Micrometry

The micrometrical observations namely thickness of epidermis, sebaceous portion and apocrine portion were measured as 22.3 µm, 215.2 µm and 98.3 µm respectively on the left side. The thickness of epidermis, sebaceous

portion and apocrine portion were measured as 24.6 µm, 223.8 µm and 99.1 µm respectively on the right side. In European breed of sheep, the measurements like thickness of sebaceous and apocrine portions were found as 231.4 µm and 126.96 µm respectively (Maowad *et al*., 2016). The varied measurements may be due to breed and location variation.

Conclusion

The infraorbital gland in Tirunelveli native breed of sheep showed the presence of epithelium, compound hair follicles with single primary and single secondary hair follicle, larger sebaceous portion with modified and common type of acinus, and lesser apocrine portion and was supported by literatures and this may be of proof that male sheep of Tirunelveli region releases pheromones as secretions for olfactory communication. the larger proportion of sebaceous gland over apocrine gland proves the waxy fatty secretion of the gland and was used by the sheep for territory marking behaviour.

Contribution by Authors

Each co-author contributes equally.

Conflict of Interests

There is no conflict of interest.

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