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Research Article
Morphological, Histochemical and Morphometric Studies of the Preorbital Gland of Adult Male and Female Egyptian Native Breeds of Sheep (*Ovis aries*)

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Abstract

Background: Reproductive communication in ungulates is thought to be accomplished by means of odors associated with many cuteneous glands; preorbital, interdigital and tarsal glands. The preorbital gland varies in its morphology according to animal species. **Objective:** The purpose of the current study was to describe the histomorphology, histochemical structure and morphometric analysis of the preorbital gland of male and female adult Egyptian native breeds of sheep in order to determine its secretory function. **Methodology:** Forty adult and apparently healthy sheep were used for gross and histological examination. Some morphometric parameters of the used glands were measured and the obtained data was analyzed by using the independent samples T test, SPSS package 2007, version 15. **Results:** The results of the current study showed that the gland is pear shaped structure lies in external lacrimal fossa of the lacrimal bone rostral to medial canthus of the eye. Histologically, it composed of outer sebaceous and inner apocrine gland portions under epidermis. The proportion of sebaceous glands is larger than apocrine ones. The former glands are of two types; ordinary and modified type, while the later is composed of apocrine acini lined by simple cuboidal cells. Both two glands reacted positively with Sudan III and Sudan Black but only apocrine acini reacted positively with periodic acid schiff. **Conclusion:** Based on the macroscopic observation of waxy secretion within the glandular pocket and the microscopic observation of both sebaceous and apocrine glands, it is possible to suggest that the preorbital gland of male and female sheep may produce phermonal secretion used in olfactory communication between animals.

Key words: Histomorphology, skin gland, sebaceous gland, sinus infraorbitalis, apocrine gland

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reproductive communication in ungulates is thought to be accomplished by means of odors that associated with many skin glands such as preorbital (infraorbital gland or sinus infraorbitalis), tarsal and interdigital gland[1-3]. The preorbital gland is one of the most important skin glands[2,4-6]. Scent released from such glands have been frequently associated with different forms of olfactory communication in several ungulates; mate recognition[7,8], territory marking[9-11], sexual attraction and selection[12,13] and help in threat animal behaviour pattern[14].

Meanwhile, the released scent from preorbital gland used for pheromonal communication between animals[11] and establishment of mother-offspring communication[15]. Furthermore, Bartos *et al.*[4] reported that the opening of the infraorbital gland in red deer calves acts as indication to the mother that her calf is hungry. On the other hand, when the calf closes gland, a signal to the mother that her calf has received a sufficient amount of milk was given, thus stop suckling.

Histologically, most scent glands consisted of sebaceous and apocrine glands that secrete different odoriferous substances[16]. The proportion of two glands varies according to species[6,17]. The sebaceous gland may be of ordinary[6], or a modified[11] or both ordinary and modified type[17]. The apocrine glands frequently located between the epidermal and dermal layer[11]. Contraction of encircled myoepithelial cells facilitates the movement of synthesized secretion into ducts[4].

However, scarce literatures and so little morphological data with their related functions are available on the preorbital gland of the Egyptian native breed of sheep (*Ovis aries*). Hence, this study is the first to describe the histomorphological and histochemical features as well as morphometric analysis of the preorbital glands of such animal to determine their secretory functions.

MATERIALS AND METHODS

Sample collection: Preorbital glands from forty adult and apparently healthy sheep of both sexes (aged 2-3 years, mean weight of 50 kg) were collected just after slaughtering sheep in Beni-Suef Abattoir, Egypt following the guidelines given by the Institutional Ethical Committee.

Gross examination: Gross examination of the gland was conducted on all specimens used in the current study. The gland was carefully dissected to show its position and relations. Some morphometric parameters (length, width and depth) of the glands were measured.

Microscopic examination: After determination of previously mentioned morphometric parameters, specimens were collected from different parts of the gland and transferred immediately to bouin's fixative for 24 h. The fixed samples transferred to 70% ethanol, dehydrated in ascending grades of ethanol, cleared with xylene and embedded in parablast.

The prepared blocks were cut serially at 5 µm thickness using rotary microtome. Sections were stained with Harris Haematoxylin and Eosin, Crossmon's trichrom stain and Periodic Acid Schiff (PAS) reaction. Two fresh samples, one from male and the other from female were cut using cryostat then stained by Sudan black and Sudan III stains. The above mentioned stains were used as outlined by Bancroft and Gamble[18].

Morphometric measurements: By using light microscopy, the density (number of SG or AG mm^{-2}) of sebaceous and apocrine glands could be measured. Also, the diameters of both gland acini of 20 randomly chosen sections for male and equal sections for females were measured by the aid of Image J analysis software program, Microsoft Company using LEICA DFC290 HD system digital camera connected to the light microscope using 10X objective lens. In addition, the thickness of epidermis and volume of sebaceous gland (according to modified method described by Haffner[19] were measured by the same system but using 20X objective lens.

Statistical analysis: The obtained morphometric data were analyzed by using the independent samples T test, SPSS package 2007, version 15 in order to compare these parameters in male and female sheep under study.

RESULTS

Gross examination: The preorbital gland of Egyptian native breeds of sheep was paired exocrine gland. They appeared as deep pocket, external lacrimal fossa, in the lacrimal bone covered with loosely distributed hair and located below the medial corner of the two eyes in both male and female sheep (Fig. 1a, b). Dried waxy secretions were observed adhering to the hairs within the pocket of the glands (Fig. 1c, d).

The gland was pear-shaped structure embedded in the external lacrimal fossa of the lacrimal bone (Fig. 2a, b). It measured 2.8-3.3 cm in length, 2.5-2.8 cm in width and

Fig. 1(a-d): Photographs exhibits (a) Male sheep (b) Female sheep showing the position of preorbital gland (arrows) in front of the eye. Dried secretions adhered to the hairs within the pocket of preorbital gland (arrow heads) of (c) Male sheep and (d) Female sheep

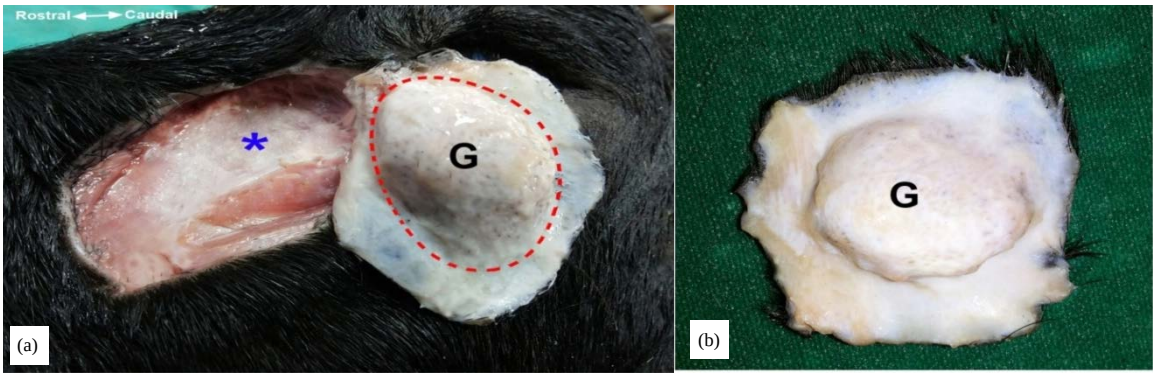

Fig. 2(a-b): Photograph shows pear-shaped preorbital gland (G) and preorbital gland pocket (astrick)

0.3-0.5 cm in depth in males; 2.4-2.7 cm in length, 2.0-2.2 cm in width and 0.2-0.4 cm in depth in females. The gland was bounded rostro-ventrlly by the malaris muscle, dorsally by the external surface of the nasal bone and caudally by the medial canthus of the eye (Fig. 3).

Histological observations: Histologically, both male and female sheep had preorbital gland (outer sebaceous and inner apocrine acini) located under the epidermis of skin (epidermis has mean diameter 72.95±1.78 μm in male and 59.36±1.81 μm in female) and surrounded internally by connective tissue and striated muscle fibers (Fig. 4a, b). The outer sebaceous gland portion was occupied the superficial region below the skin epidermis and the apocrine portion was deeper in position. The proportion of the sebaceous gland lobules in both sexes was larger than the apocrine gland units.

Two types of sebaceous gland; ordinary and modified types were seen in most sections prepared. The ordinary type was located mainly at the superficial region directly under the epidermis (Fig. 5a, b) and their volume was very small compared to that of the modified type. In females, the ordinary type was smaller and less lobulated than that of males (Fig. 5a, b).

The modified type was larger in size than the ordinary one. Their ducts showed a narrow lumen and their lobules ramified in a branching tree-like manner throughout the gland. In males, it was larger in size, highly branched and had numerous glandular lobules (Fig. 6a, b). Some glands were seen emptied directly on the skin surface and others were opened into the hair follicles (Fig. 7).

The lining epithelium of the excretory duct of the large gland was similar to that of the epidermis, stratified squamous

Fig. 3: Photograph illustrating head of sheep showing preorbital gland (arrow) bounded 1: Rostro-ventrally by malaris muscle, 2: Dorsally by external surface of the nasal bone and 3: Caudally by the medial canthus of the eye

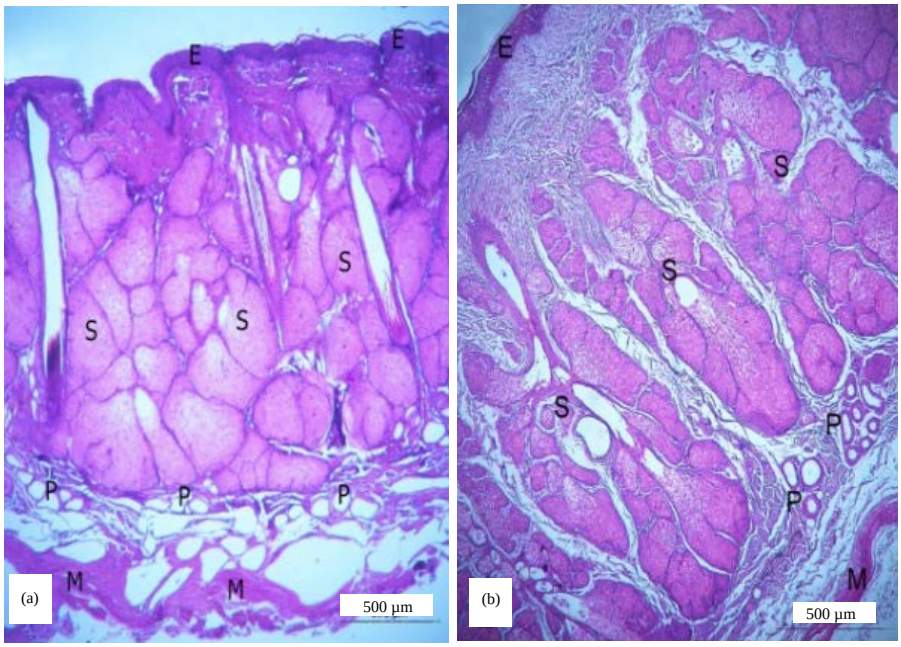

Fig. 4(a-b): A photomicrograph of preorbital gland of (a) Male sheep and (b) Female sheep showing epidermis (E), outer sebaceous gland portion (S), inner apocrine gland portion (P) and striated muscle fibers (M). H and E stain, X40

epithelium keratinized, while that of the small glands was covered by stratified squamous epithelium. Secretory acini of the modified sebaceous gland were tear-shaped, larger than that of the ordinary type and their ducts opened either in into hair follicles or directly into skin surface. Cells close to the central portion of the sebaceous gland were much bigger in size with faintly stained cytoplasm and invisible nuclei (Fig. 8).

The apocrine portion was composed of groups of apocrine acini that showed histological differences in male and female. In male's gland they were more abundant, had wide lumens and larger diameter (Fig. 9a). In contrast, they were fewer in number, had narrower regular lumens and smaller diameter in female's gland (Fig. 9b). Each acini was lined with single layer of simple cuboidal secretory cells containing eosinophilic cytoplasm and rounded centrally

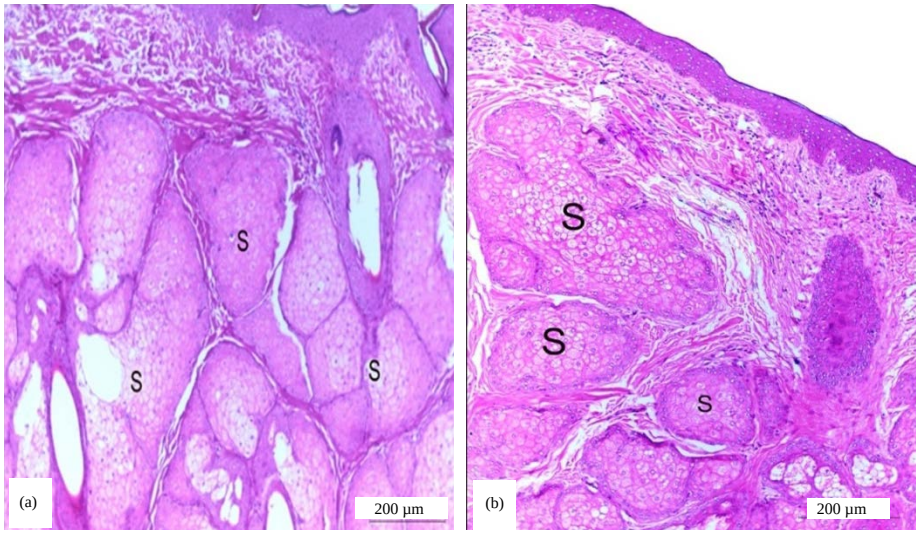

Fig. 5(a-b): A photomicrograph of preorbital gland of (a) Male sheep and (b) Female sheep showing ordinary sebaceous glands (S) occupy superficial position under the skin epidermis. The ordinary type of male is larger in size than that of female and more lobulated. H and E stain, X100

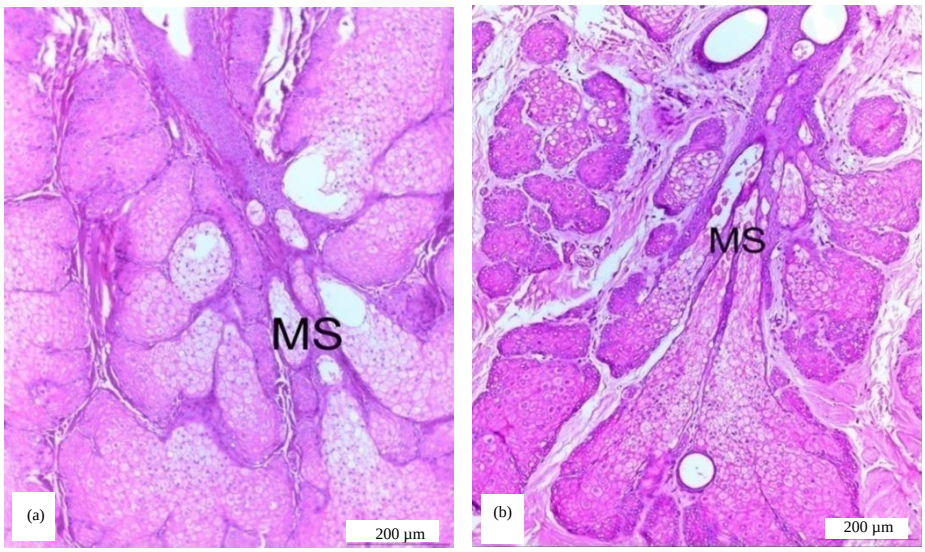

Fig. 6(a-b): A photomicrograph of preorbital gland of (a) Male sheep and (b) Female sheep showing modified sebaceous gland (S) ramify in a branching tree-like manner. The number of sebaceous glandular lobules in male gland more abundant and had a larger size than that of female. H and E stain, X100

located nuclei. Cytoplasmic rounded bleb like projections were often noticed protruded from the apical surfaces of the epithelial cells into the lumen of the acini (Fig. 10a, b). Rounded apocrine droplets were found to be stored in lumina of some acini and myoepithelial cells were observed between the epithelial cells and the basement membranes (Fig. 10c, d). In some stained sections, both prominent apical blebs and apocrine droplets were seen within their lumens (Fig. 11).

Layers of collagenic and muscle fibers of differing density were demonstrated between the sebaceous lobules and apocrine units (Fig. 12a, b).

By using the PAS reaction, the epithelial cells lining the apocrine acini, the apical border and their basement membranes were reacted positively. In addition, the apically located bleb-like projections were also strongly reacted. On the other hand, the connective tissues surrounded the apocrine acini show moderate reaction (Fig. 13).

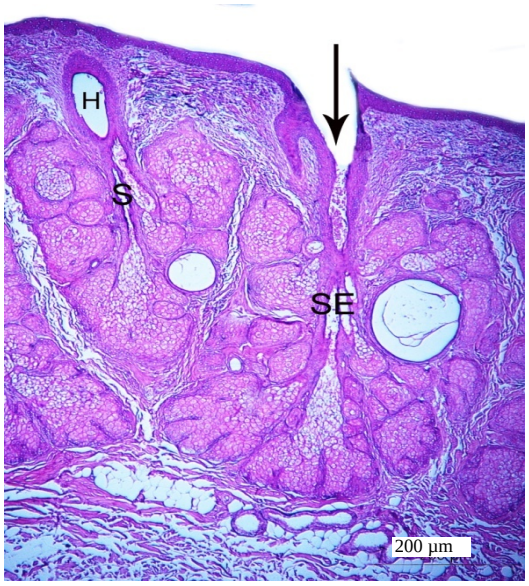

Fig. 7: A photomicrograph of preorbital gland of male sheep showing large modified sebaceous gland (SE) opens directly into skin surface (arrow) and small one (S) opens into the hair follicle (H). The large sebaceous gland is highly branched. Hand E stain, X100

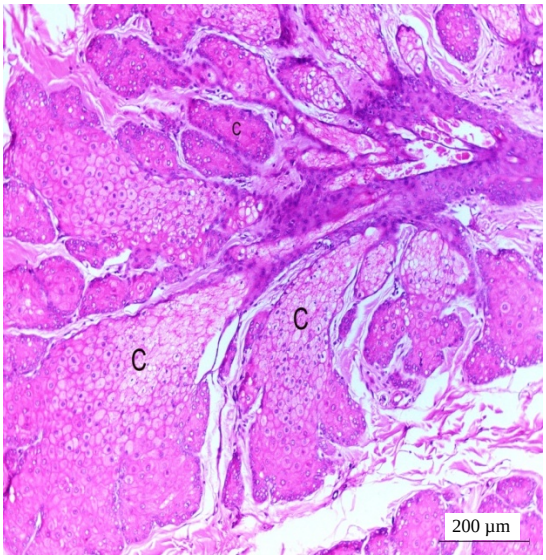

Fig. 8: A photomicrograph of modified sebaceous gland showing lightly stained cells (C) close to the center of lobules. These cells devoid of nuclei. H and E stain, X100

High concentration of sudanophilic substance was noticed in all layers of the sebaceous glands and the apocrine acini when stained with Sudan Black (Fig. 14).

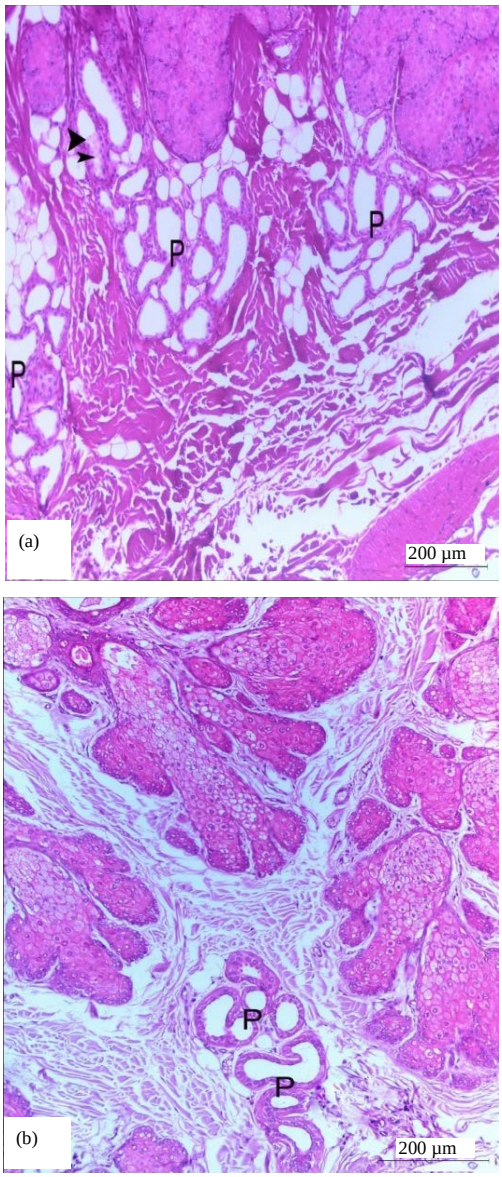

Fig. 9(a-b): A photomicrograph of preorbital gland of (a) Male sheep and (b) Female sheep showing apocrine gland acini (P) occupy the inner portion of the gland and contain excessive acidophilic droplets (arrow heads) in their lumen. The acini are more abundant and wider in diameter in male gland. H and E stain, X100

Using Sudan III showed intensive sudanophilic substances filled both sebaceous lobules and apocrine units (Fig. 15).

Morphometric and statistical analysis: The density and diameter of both sebaceous and apocrine glands as well as volume of sebaceous gland were found to vary in

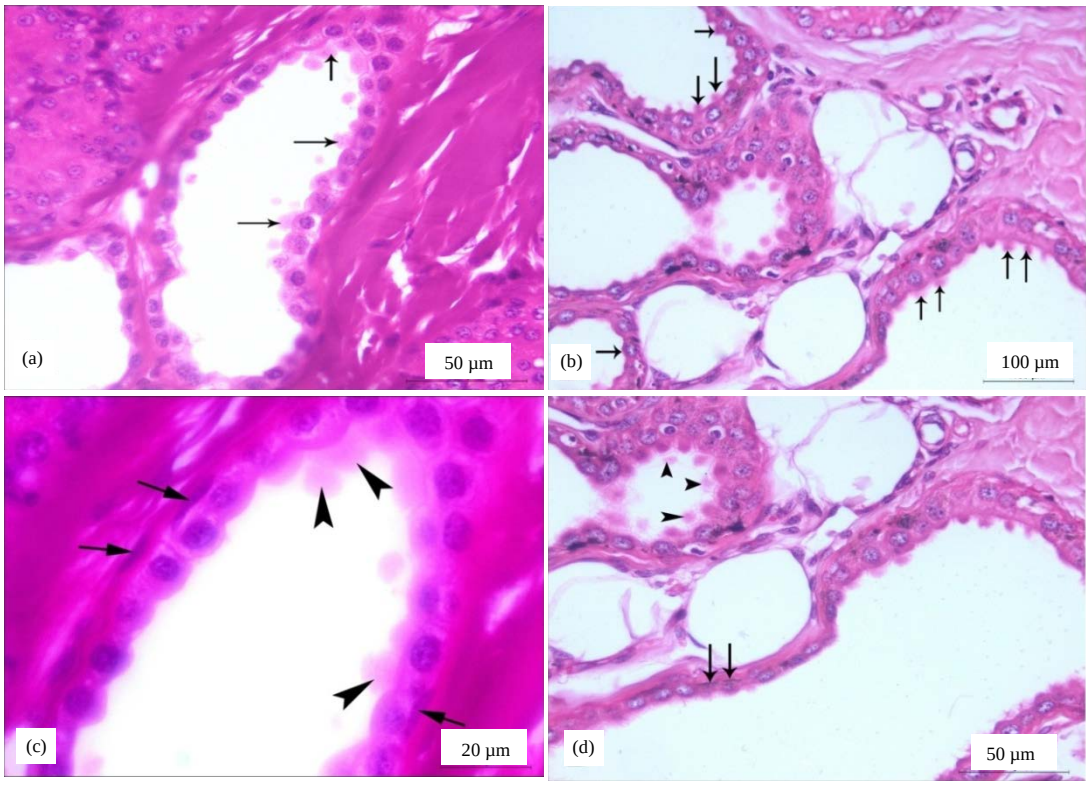

Fig. 10(a-d): A photomicrograph of apocrine acini of preorbital gland of (a) Male sheep and (b) Female sheep showing the apocrine acini lined by cuboidal cells with acidophilic cytoplasm and central rounded nuclei. Apical bleb like projections (arrows). Higher magnification of (a) and (b), respectively, showing apocrine droplets within the lumen of the acinus (arrow heads) and myoepithelial cells surround the acini (arrows). H and E stain, X400, X200, X1000 and X400

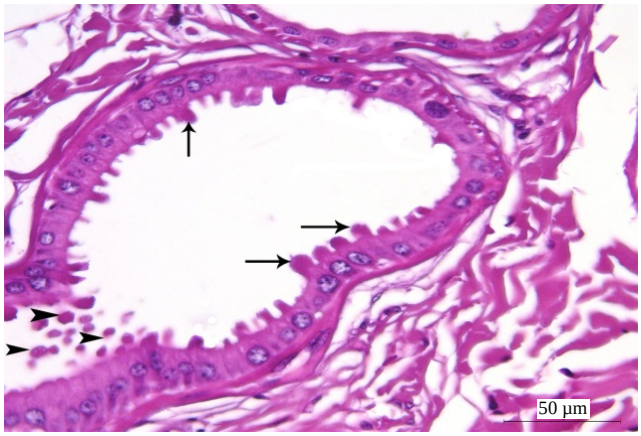

Fig. 11: A photomicrograph of an apocrine acinus of male's preorbital gland showing prominent apocrine belebs (arrows) on the apical borders of most epithelial cells and apocrine droplets (arrow heads) within the lumen of the acinus. H and E stain, X400

preorbital gland of male and female sheep under investigation. The independent samples T test, SPSS package 2007, version 15 clearly showed that the density of sebaceous and apocrine glands was significantly (SG: F = 0.57, df = 37;

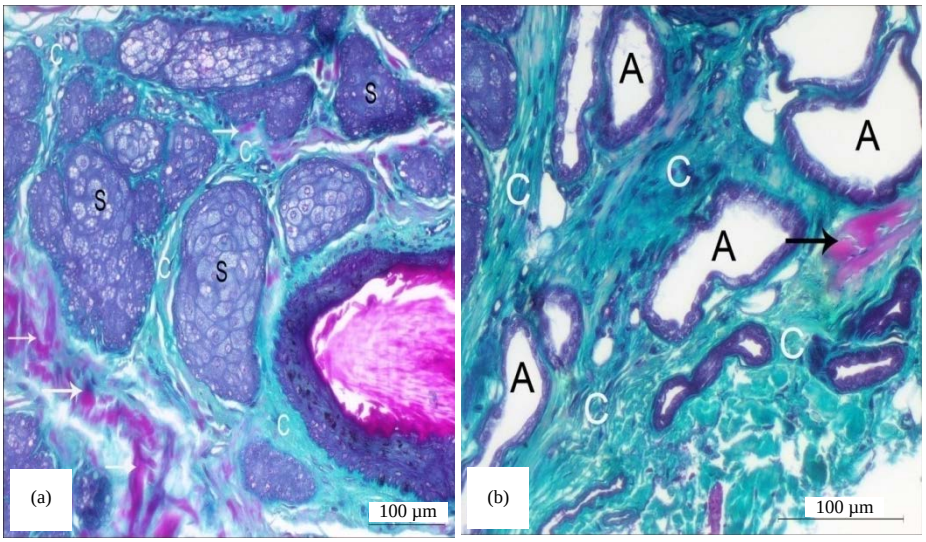

Fig. 12(a-b): A photomicrograph of (a) Sebaceous gland portion and (b) Apocrine gland portion of preorbital gland showing layers of collagenic fibers and muscle fibers (arrows) run between sebaceous glands (S) and apocrine acini (A). Crossmon's trichrom stain, X200

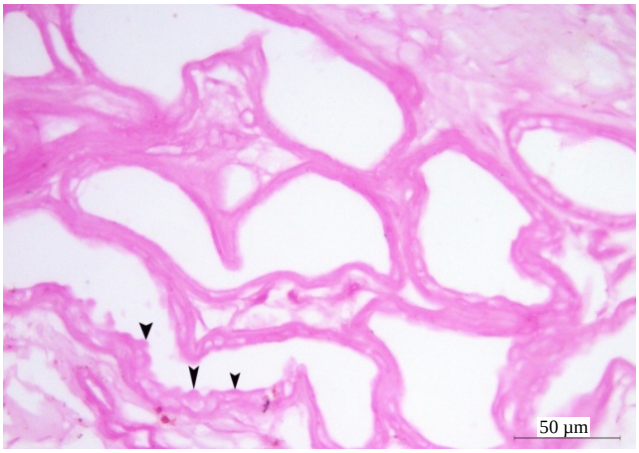

Fig. 13: A photomicrograph of apocrine portion shows positive PAS reaction in the acinar cells, apical and basal borders. Presence of apical fuchsinophilic blebs (arrow heads). PAS stain, X400

AG: F = 5.93, df = 29, $p<0.05$) higher in male sheep (SG: 65.35±2.71; AG: 13.47±1.08 U mm^{-2}) than female (SG: 56.58±2.56; AG: 7.6 ±0.62 U mm^{-2}). Furthermore, the diameter of sebaceous and apocrine glands varied significantly (The independent samples T test, SPSS, SG: F = 8.53, df = 242; AG: F = 3.4, df = 165, $p<0.005$) higher in males (SG: 231.4±8.93; AG: 126.96±7.22 µm) than that of females (SG: 153.14±7.41; AG: 107.38±3.77 µm).

Concerning the volume (log transformed) of sebaceous gland, the used statistical test show it was varied significantly (The independent samples T test, SPSS: F = 17.59, df = 83, $p<0.01$) higher in males 122.77±2787685×10^5 than that of females 120.657±1483882×10^5.

DISCUSSION

The current study showed that the preorbital gland of sheep under investigation is one of skin glands composed of mixed sebaceous and apocrine glands, with proportion of sebaceous larger than apocrine gland. These findings are consistent with that reported by Rajagopal and Archunan[11], Atoji *et al.*[17], Agungpriyono *et al.*[20], Atoji and Suzuki[21],

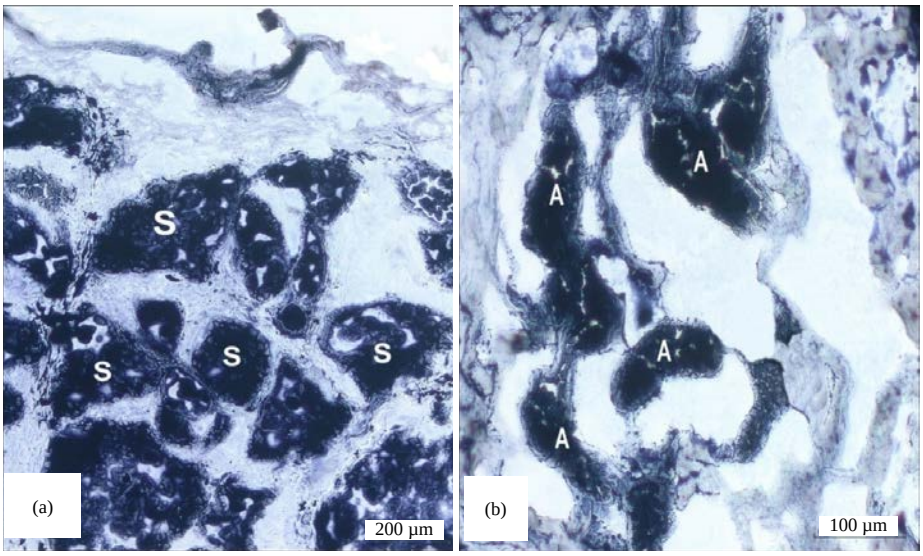

Fig. 14(a-b): A photomicrograph of (a) Sebaceous gland portion and (b) Apocrine gland portion showing intensive sudanophilic substances filled the sebaceous gland lobules (S) and apocrine gland acini (A). Sudan Black stain, X100 and X200

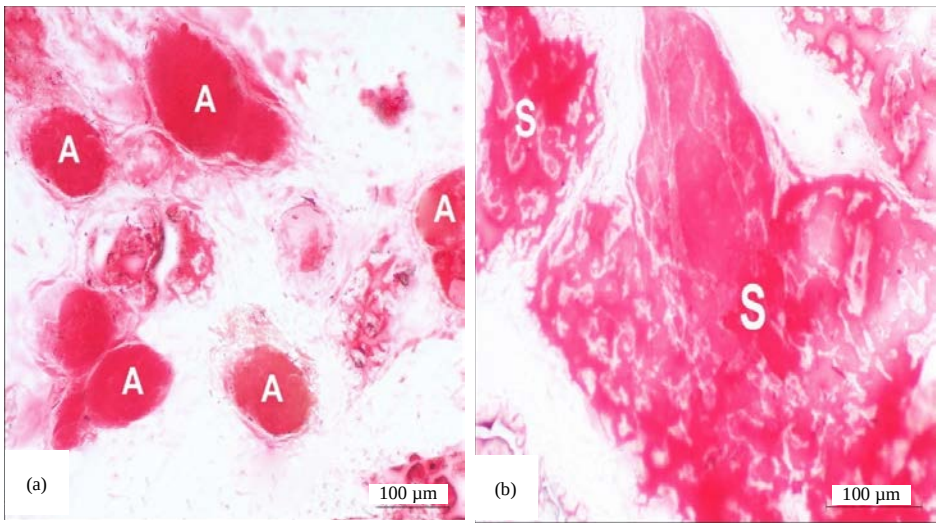

Fig. 15(a-b): A photomicrograph of (a) Sebaceous gland portion and (b) Apocrine gland portion showing sudanophilic substances fills all layers of sebaceous gland (S) and the apocrine acini (A). Sudan III stain, X200

Kozlowski and Calhoun[22] and Nickel *et al.*[23], but contradictive with that observed by Awaad *et al.*[1], Adnyane *et al.*[6], Atoji *et al.*[24], Avdic *et al.*[25] and Janicki *et al.*[26]. The sebaceous and apocrine portion of the preorbital gland secretes different odoriferous volatile substances. These substances seem to allow pheromonal communication between animals as reported elsewhere by Rajagopal and Archunan[11] and Scully *et al.*[16].

The presence of outer sebaceous and inner apocrine portion situated between the epidermis and the facial muscles as revealed by light microscopic examination in the present investigation are agreed with that obtained by Adnyane *et al.*[6], Agungpriyono *et al.*[20], Atoji *et al.*[24], Abbasi *et al.*[27] and Ajmat *et al.*[28] but contradictive with that reported by Atoji *et al.*[17] and Atoji *et al.*[29]. This may suggest that the waxy seromucoid secretions of this gland synthesized and secreted by a combination of sebaceous glands and apocrine tubules as reported by Adnyane *et al.*[6].

The histomorphological findings reported in this study concerning the proportion of sebaceous and apocrine gland

with predominance of former gland on the expense of later one are in contrast with that observed by Adnyane *et al.*[6] in preorbital gland of male barking deer, *Muntiacus muntjak* and Gray *et al.*[14] in muskox preorbital glands in which the gland of the two animals was composed of a large aggregation of apocrine sweat glands portion with a relatively small sebaceous gland portion. On the other hand, this result passes hand by hand with that obtained by Atoji *et al.*[17] in male Formosan serow, *Capricornis crispus swinhoei* by Atoji and Suzuki[21] in Japanese serow, *Capricornis crispus*. The higher proportion of sebaceous gland is consistent with the waxy secretions that were noticed macroscopically in the glandular pocket of male and female sheep.

Light microscopic analysis of this study demonstrates that the observed sebaceous glands in male and female sheep are of ordinary and modified type. Similar findings were obtained by Atoji *et al.*[17] who reported the two types of sebaceous glands in the infraorbital gland of formosan serow. On the other hand, it is contraindicative with that reported by Adnyane *et al.*[6] in the infraorbital gland of the male barking deer by Van Lancker *et al.*[30] in skin glands of buck. Furthermore, sexual dimorphism was noticed in infraorbital gland of Japanese serow; the females have modified sebaceous gland, whereas the males have an ordinary type[31].

The observed dried waxy adhesive secretion adhering within the pocket of the as reported in this study were also noticed by Rajagopal and Archunan[11], Avdic *et al.*[25] and Aslan *et al.*[32]. The presence of this secretion in both male and female sheep under investigation may indicate that the gland is active in both sexes[14]. Moreover, this secretion may contribute to the production of volatile substances that may play an important role in chemical communication between animals in the herd[11].

Releasing secretion from preorbital gland may be influenced by several factors; contraction of a well-developed myoepithelial cells that encircles the apocrine portion of the gland[1,4,6,20,27,33] and presence facial muscles overlying the preorbital gland that compress the gland to express its secretions more effectively or mechanical release when the animal rubs the gland on firm objects such as its own foreleg (gland rubbing) or a prominent object in the surrounding environment (rubbing)[14]. The later researchers added that under normal conditions, the contraction of myoepithelial cells is probably accompanied by gland rubbing or rubbing on fixed object in the environment.

The higher development and density of both sebaceous and apocrine glands as well as the volume of sebaceous glands in males Egyptian native breeds of sheep than females which detected statistically may be based on the testosterone production. The obtained result augmented by Rajagopal and Archunan[11] and Prasad and Sinha[34] who mentioned that the higher size and density of both sebaceous and apocrine gland depend on male sex hormone (testosterone) production. In addition, this result confirmed by Miller *et al.*[7] who reported that during breeding season in dominant male white-tailed deer, the level of androgens and the quantities of ketones and ester volatile compounds secreted by interdigital gland were higher than that of the non-breeding season. Moreover, reduction of the sebaceous gland was noticed in castrated rats and implantation of testosterone propionate lead to increased in volume of the glands of these castrated males twice more than that of the non-castrated control rats as outlined by Sauter and Loud[35].

The current study clearly demonstrates that the peripheral cells that located peripherally next to the cell membrane of the modified sebaceous gland lobules are small and rounded in shape with clearly visible nuclei and cell membranes. In contrast, cells that are closer to the central portion of the alveoli are much bigger, lightly stained cytoplasm, a nuclear and had thin cell wall. These results are in agreement with that observed by Janicki *et al.*[26]. It is reported that all these pictures are points to their gradual fatty infiltration[26].

By staining the preorbital gland of the species under study with periodic acid schiff, it has been shown that only apocrin acini reacted positively with the stain indicate that these acini secrete neutral glycoconjugates. These findings augmented by Atoji and Suzuki[21] and Parillo and Diverio[36]. The later researchers reported that the apocrine acini of the infraorbital gland of Japanese serow secrete a large quantity of glycoconjugates.

The obtaining gross, histological and statistical findings in the present investigation refer to the fact that male's Egyptian native breeds of sheep have preorbital gland more developed than that of female. This finding confirmed by Rajagopal and Archunan[5] and Rajagopal *et al.*[37] who reported that the males perform efficiently scent marking behavior with their well-developed preorbital glands, while in females as they have less well developed preorbital gland this behavior is vestigial. In addition, Burger *et al.*[38] obtained more than 33 different chemical compounds from the preorbital gland's secretions of the common duiker. It is reported that these compounds are significantly present in higher concentrations in secretion of males than that of females, suggesting that they could use as sex recognition cues.

CONCLUSION

- Based on the morphological and histological pictures as well as the role of both apocrine and sebaceous acini in the preorbital gland supported by previous literature, it is possible to conclude that male and female Egyptian native breeds of sheep may produce pheromonal secretions through preorbital gland for olfactory communication
- The obtained results suggest that the preorbital gland of sheep under investigation contains lipids secreted from both sebaceous and apocrine glands and glycoconjugates secreted from apocrine acini only that play an essential role in territory and marking the area
- The higher development, density and volume of sebaceous and apocrine glands in male sheep may be based on the testosterone production.
- The present study provides a basis for further studies on the preorbital glands including immunohistochemical and lectin histochemical analysis which might be useful for determining their secretory functions

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