MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (6): 1157-1161, 2009
ISSN: 1680-5593

Morphology and Histology of the Interdigital Gland in an Iranian Native Breed of Sheep

[1]M. Abbasi, [2]A. Gharzi and [3]S. Mohammadzadeh and [4]H. Karimi
[1]Department of Anatomy and Histology, School of Veterinary Medicine,
[2]Department of Biology, Faculty of Sciences,
[3]Department of Technology of Animal Products, Faculty of Agriculture,
University of Lorestan, Khorram-Abad, Iran
[4]Department of Anatomy and Histology, Faculty of Veterinary Medicine,
University of Tabriz, Tabriz, Iran

Abstract: Many species of Artiodactyls possess well developed interdigital glands, which differ in terms of morphology and histology among different species. The aim of present study was to characterize the anatomical and histological characteristics of interdigital gland in Lori sheep. To conduct this research we selected 20 mature and healthy Lori sheep (in 2 different periods including breeding and non-breeding seasons) from a local slaughterhouse. The selected sheep were then sacrificed and their manus and pes were detached from the rest of limbs. After anatomical evaluation of interdigital glands in the laboratory, the samples were fixed in 10% formalin and afterwards processed for histological examinations. Seven micrometer sections were stained with H and E, PAS and Alcian blue methods. Anatomical and histological results that were observed in this study can be summarized as follows: the mean weight, length, width, thickness of the interdigiral gland and its duct length in this sheep were 1.16 g, 15.80, 7.60, 7.71 and 26.06 mm, respectively. The characters that were measured in 2 different seasons showed a significant increase in breeding season compared to non-breeding season. The lumen of gland and its duct is filled with wool fibers along with secretions of apocrine and sebaceous glands and desquamated cells detached from lining epithelial tissue.

Key words: Anatomy, histology, interdigital, gland, sheep, breed

INTRODUCTION

There are several native breeds of sheep in Iran. One of the main breeds is Lori sheep found in West and Southwest of the country especially in Lorestan province. There are different kinds of skin glands in mammals in respect to size, shape and location. In ungulates, the communication of reproductive information is thought to be accomplished by odors associated with cutaneous glands localized to specific areas, such as infraorbital, tarsal and interdigital areas (Konig and Liebich, 2007). The interdigital glands belong to a group of cutaneous apocrine and holocrine glands, varied in morphology, located in cleft between the hooves, in several species of Artiodactyla. As regards their function, these glands are considered scent glands producing odorous signals and pheromones that play important biological roles in the conspecific chemical communication, such as active territorial demarcation and in the expression of social behavior (Parillo and Diverio, 2008). In some species, like sheep these glands are found on all 4 limbs but other species shows the gland only on the hind feet (Janicki *et al*., 2003). The present study was carried out to identify the anatomical and histological characteristics of interdigital glands in Lori sheep. Moreover, in order to better understand more aspects of the glands, possible sex and season related differences in anatomy and morphology of the interdigital glands were also investigated.

MATERIALS AND METHODS

In order to conduct this research, the whole interdigital glands of both fore and hind limbs were collected from adult male (n = 10; aged >1 year old) and adult female (n = 10; aged >1 year old) Lori's sheep during the breeding season (5 males and 5 females) and non-breeding season (5 males and 5 females). These limbs samples were excised immediately after slaughter of the animals. After gross dissecting, the interdigital glands were taken out and immediately fixed in 10% formalin. In order to determine a possible sex and season-related

Corresponding Author: M. Abbasi, Department of Anatomy and Histology, School of Veterinary Medicine, University of Lorestan, Khorram-Abad, Iran

variation, the glands were weighed with an aid of a balance (to the nearest 0.01 g) and their dimensions were also measured using the digital Vernier calipers. In biometrical examination, the length of excretory duct and its diameter at the proximal and distal ends were also considered. The General Linear models procedure of SAS/STAT software program was used to analyze the data. Following the above mentioned biometrical examinations, we processed the specimens for histological surveys. In order to perform this task, we routinely dehydrated the fixed specimens in graded series of alcohols, cleared in xylene and subsequently embedded in paraffin. Serial sections 7 μm thick were cut and subjected to conventional H and E, PAS and alcian blue staining (Humason, 1979).

RESULTS

Anatomically, the interdigital glands in Lori sheep were located between the digits of the fore and hind-limbs and their opening ducts lie in the space between 2 hooves just above interdigital cleft. These glands lie between the distal part of first phalanx and proximal part of the 2nd phalanx but in different samples there might be some variations in their location (Fig. 1). The size of the opening duct varies in different specimens. Within this opening, we can observe a group of hair extended externally. In general, the glands comprise two distinct parts including a broad and pouch-like followed by a narrow duct, which open the skin surface. The pouch-like part is referred to secretory portion and the narrow canal is called excretory duct. The excretory duct started at the pouch bottom and curves dorsocranially and terminated at dorsal surface of interdigital cleft. Therefore, the secretory and excretory parts collectively assume a tobacco pipe shape. Both secretory and excretory portions reveal a similar structure in terms of histology. In some cases, the gland ducts move directly and open to dorsal surface behind the phalanx above the hoof. However, in case of large glands and because of little space, the duct is bent several times. We observe that the lumen of gland is filled with a dense secretory material and quite a number of wool fibers embedded in the luminal content. The wool fibers are produced by the follicles that exist in the wall of the duct and excretory portion (Fig. 2). At the opening of the gland, we can generally find a dense and oily material. This accumulation is the result of sloughed cells and glandular secretions. The material often has a foul, rancid odor. There is a significant difference ($p<0.05$) in size (length, width and thickness) and weight of interdigital gland between breeding season and non-breeding season as this character was larger in breeding season. However,

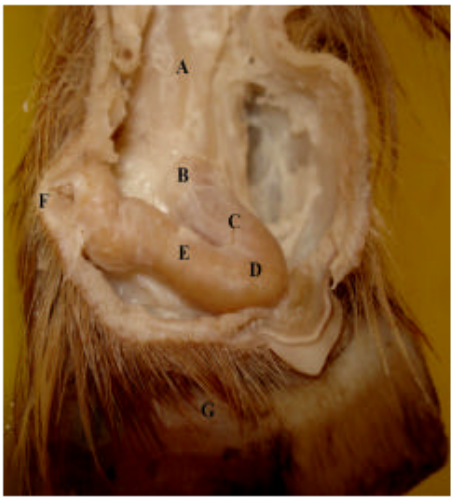

Fig. 1: Gross anatomy of an interdigital gland after removing a hoof. The 1st phalanx (A), the proximal extremity (B), the mid part (C) and distal extremity (D) of the gland, which connects to excretory duct (E) and finally ends to opening duct (F) at the skin surface above the hoof (G)

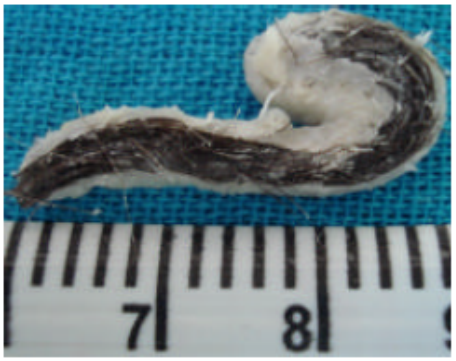

Fig. 2: A detached interdigital gland which its wall is partially removed in order to show the excretory duct and its lumen content. Wool fibers fill the excretory duct

the length of excretory duct did not show any significant difference in breeding season when compared with its length in non-breeding season. We also found a sex-related variation ($p<0.05$) in respect to the all biometric features (except for the diameter of proximal part of the duct diameter) of interdigial gland, so that males exhibited higher values than females. Moreover, when we compared the biometric characters of interdigital gland in fore limbs with hind limbs, it was revealed that the glands on fore limbs are significantly ($p<0.05$) larger than the hind limb's glands. In histological examination, we observed that the

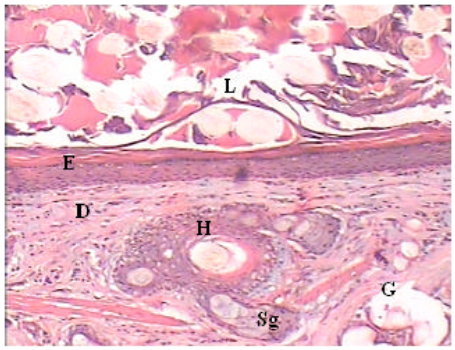

Fig. 3: A photomicrograph showing the stratified Epithelium (E) lines the interdigital gland. The lumen of the gland is filled with lumen content and wool fibers (H) along with Sebaceous glands (Sg) and apocrine sweet Glands (G) are seen in subcutis connective tissue (D). Alcian blue (×10)

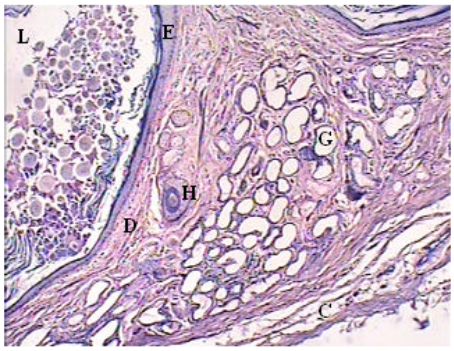

Fig. 4: Photomicrograph of the interdigital gland wall. Observe the stratified Epithelium (E) line the gland. The gland is encircled by a fibrous Capsule (C). Lumen (L), wool fiber (H), apocrine sweet Gland (G), subcutis connective tissue (D) (PAS, ×4)

covering wall of the glands is composed of 3 layers including epidermis, dermis and fibrous connective capsule. Stratified squamous epithelium with a prominent keratin layer faced the lumen (Fig. 3 and 4). This inner surface is similar to the skin covering the dorsal surface of manus and pes. The epidermis is almost smooth or has a few mucosal folds, which are projected into the lumen. The lumen of secretory portion is larger than the lumen observed within the excretory duct. The dermis contained common skin structures including sebaceous glands, hair follicles, arrector pili muscles and apocrine sweet glands (Fig. 3 and 4). The sebaceous glands lie closer to the surface than the deep sweet glands. A variation is observed in size and shape of the lumen of apocrine sweet glands whose secretory epithelium are lined with a simple layer of cuboidal and relatively flattened cells. The excretory ducts of these glands, however, are lined at least by 2 layers of cuboidal cells, which assume a darker appearance than its secretory portion (Fig. 5). Apical surface of secretory cells and their surrounding basal layer are PAS-positive. Hair follicles with different sizes are also clearly visible within the dermis. The fibrous capsule was composed mainly of dense connective tissue, containing parallel bundles of collagen. The capsule also, contained adipose cells, blood vessels and nerve fibers. The capsule is connected externally to skin surface. The lumen of interdigital glands is normally filled with secretions of sebaceous glands, apocrine sweet glands and of course the secretions of gland itself. Along with these secretions, we can notice to several wool fibers and desquamated cells of epithelial tissue that lines the lumens of secretory and excretory portions (Fig. 2 and 3). Staining with the PAS method revealed that these

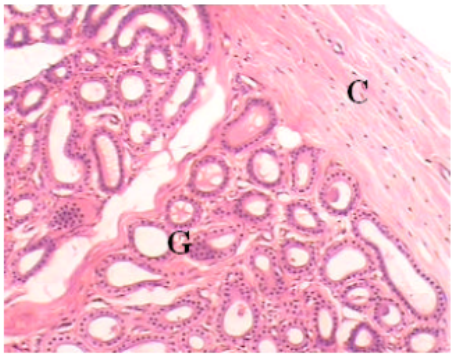

Fig. 5: Photomicrograph of apocrine sweet Glands (G), which are found in the wall of interdigital gland and encircle the gland like a belt. A fibrous Capsule (C) surrounds the gland (H and E, ×10)

secretions were PAS-negative. However, when we stained the tissues with Alcian blue, they exposed a positive affinity to this stain as they became blue in appearance.

DISCUSSION

Several studies have shown that the interdigital gland is a type cutaneous gland which like other skin appendages originates as result of the invagination of the ectoderm into underlying mesenchyme (Sivachelvan *et al*., 1992). In sheep, this gland is fully formed by the 90-100 days of prenatal life (Karahan *et al*., 2007). In sagittal sections interdigital gland appears as a curved tube-tobacco pipe in its shaped (Getty, 1975). In some species, glandular secreted material is inside a pouch with a hair-covered excreting duct, from which secretion trickles (Atoji *et al*., 1988), but in

other species this gland is merely a form of skin fold (Janicki *et al*., 2003). In agreement with previous researches, we demonstrated here that in Lori's sheep the interdigital glands are found in all 4 limbs and their shapes are generally pipe-shaped and within their opening ducts a group of wool fibers embedded in glandular secretions is observed. The shape and size of interdigital gland in the roebuck is similar to what is observed in Japanese serow and sheep, with the size of an average hazelnut (Janicki *et al*., 2003). In the goat, there is a primitive type of interdigital sinus (Karahan *et al*., 2007). In Asian elephant (Elephas maximus) the interdigital glands resemble to human merocrine glands were detected within the reticular dermis and their secretions are transferred directly to skin surface (Lamps *et al*., 2001). Sheep and Mufflon have well developed interdigital glands on all 4 legs, while the Roe deer has the glands only on the hind feet (Nickel *et al*., 1981). It has been reported that in sheep, there are interdigital glands measuring approximately 2.5-3 cm in length and 6-7 mm in thickness (Getty, 1975). In Japanese serow, these glands are 2.5-4 cm long and 1.4-2 cm wide (Atoji *et al*., 1988). Average dimension of interdigital glands in Lori's sheep presented here is 15.80×7.6×7.71 mm. The length of excretory duct in this sheep is 26.06 mm, while this parameter has been reported 18-20 mm in case of a sheep (Nickel *et al*., 1981) and 10 mm for a Japanese serow (Ajoji *et al*., 1988). The diameter of opening duct has been reported 2-4 mm for sheep (Nickel *et al*., 1981), while in current study, we measured as 4.99 mm for Lori's sheep. It has been documented that in a Japanese serow the interdigital glands in fore legs are larger than the once observed in hind legs while, there is no a sex-related variation in terms of the size of the glands (Atoji *et al*., 1988). In the current study, we also demonstrated that the size of interdigtial glands in fore limbs is bigger than hind limb's glands, but we found a sex-related difference in respect to the gland size as males showed higher values ($p<0.05$) in all dimensions (except for the lumen diameter) than females. Moreover, we provided the evidence that in breeding season the size of the glands are significantly larger ($p<0.05$) in comparison to non-breeding season, which is in concurrent with what observed previously in the roebuck (Janicki *et al*., 2003). Unlike, the roebuck that the connective tissue does not form a capsule around the interdigital gland and lacks any sweet glands, in sheep the gland is located inside the connective tissue, surrounded by a capsule and also has sweat glands (Janicki *et al*., 2003). Generally, it has been shown that the wall of the interdigital gland in sheep has 3 layers: epidermis, dermis and fibrous capsule. Stratified squamous epithelium with a prominent keratin layer faced the lumen. The inner surface is similar to the skin surface with several wool fibers projecting into the lumen. The dermis contains common skin structures including sebaceous glands, hair follicles, arrector pili muscles and apocrine glands. The fibrous capsule is composed mainly of dense connective tissue, constituting the outermost layer of the wall (Karahan *et al*., 2007). There is also a report that states that the color of the gland skin is lighter than normal skin with fine and colorless fibers whose follicles receive secretions from several sebaceous glands (Getty, 1975). In accord to previous studies, we here demonstrated that in Lori's sheep there is a fibrous capsule around the gland and we also found sweet glands among the covering connective tissue. Moreover, we noticed that these glands occupy a deeper positions compared to more superficial sebaceous glands. However, in this study, we showed that in cases that the sheep skin has colored fibers, the fibers seen in the gland epithelium are colored as well, a result that is contrary to findings of Getty (1975) in sheep. In general, it is believed that these fibers prevent total and immediate discharge of secretion of the interdigital gland and upon, which the secretion of the gland gradually seeps. It has been previously reported that the lumen of the gland is filled with a dense secretory material and a number of fibers embedded in the luminal content (Karahan *et al*., 2007). Our results here confirm this and we demonstrated that the lumen contained secretions of apocrine glands, sebaceous glands and along with a group of wool fibers and desquamated epithelial cells. There are evidences that in a type of Japanese serow the secretions of interdigital glands contain large amounts of glycosaminoglycans along with different kinds of carbohydrates. The main constituents of the materials secreted by interdigital glands are volatile elements and hydrocarbons (Atoji *et al*., 1988). In the deer these secretory component are yellowish and cheesy material emit a foul and rancid odor. The movement of lifting, gathering, sagging and interspacing of hoofs mechanically discharges the gland. In a survey, 46 different volatile compounds have been identified in secretions of this gland in the deer. Interestingly, 5 of these compounds occurred in much greater concentrations in dominant bucks than in subordinate bucks. What this may mean to deer is not known, but it is interesting to speculate that pawing at scrape sites may leave a scent specific to dominant bucks (Wood, 1998). Owing to the location of the opening duct, there is a possibility that this opening is injured by hays and straws and consequently causs chronic infections and inflammation (Karahan *et al*., 2007). In young lambs, it is possible to removed surgically this gland to prevent from any possible infection and lameness (Sivachelvan *et al*.,

1992). Sebaceous glands are a kind of simple alveolar holocrine glands that are lined by epithelial cells and the sebum produced by these cells is discharged by arrector pili muscle contraction (Trautmann and Fiebtger, 2002). In present study, these glands were observed at the side of the hair follicle in the skin that lines the lumen of the interdigital glands. These glands were various in size and their cells showed different levels of activity. Staining of tissues with Alcian blue confirmed that glycosaminoglycans compounds exist in the sebaceous glands as well as the surrounding connective tissues. Moreover, we here demonstrated that apocrine sweet glands, adjacent to fibrous capsule, consist of a belt like complex encircling the wall of interdigiral gland. There are also, some myoepithelial cells around these apocrine glands and through their contraction help gland discharge.

CONCLUSION

The interdigital gland not only plays a role in lubrication and scent communication but has also a fungicidal and a bactericidal activity that helps disinfect the skin, keeps it elastic and protects it from UV radiation. Therefore, more works are needed be carried out in order to identify the chemicals in the secretions of the gland in Lori sheep as well as other Artiodactyls species.

ACKNOWLEDGEMENT

The authors would like to thank research Department of Lorestan University for financial support.

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