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Gross, Microanatomical and Ultrastructural Studies of Interdigital Gland in Madras Red Sheep (*Ovis aries*)

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ABSTRACT

Background: Madras Red sheep is a well-recognized meat type breed reared only on free range system to yield tasty meat. The aim of this study was to determine the gross, microanatomical and ultrastructural details of the interdigital gland in Madras Red sheep.

Methods: The interdigital glands were removed immediately after slaughter and subjected for gross morphological, histological, immunohistochemical studies and ultrastructural observations.

Result: The results revealed that a well-developed tobacco-pipe shaped interdigital gland was present in all the four limbs which was composed of an orifice, excretory duct, body and bent or flexure. Histologically, wall of the gland was composed of epidermis, dermis and capsule from within outward. Epidermis was made of stratified squamous keratinized epithelium. Dermis was composed of dense irregular connective tissue with sweat, sebaceous glands, arrectores pili muscle and hair follicles were embedded within. The sweat gland appeared as group of tortuous tubules under scanning electron microscope. The oily secretion of the gland helps in maintaining the healthiness of foot during movement of the animal.

Key words: Interdigital gland, Madras red sheep, Microanatomy, Ultrastructure.

INTRODUCTION

Pheromones are produced by the scent glands which plays an important biological role between the conspecific individuals (Konig and Liebich, 2007; Dyce *et al.* 2010 and Corona and levy, 2012). Odoriferous molecules of pheromones secreted in urine, faeces or by modified cutaneous glands can be perceived through the olfactory system of conspecifics (Rekwot *et al.* 2001). In addition to social and sexual communications, Abbasi *et al*. (2009) mentioned that the secretion of interdigital gland has fungicidal and bactericidal effects and offer protection against ultra-violet radiation. In contrast, Hodzic and Zilic (2020) mentioned that the secretion did not have any bactericidal effect.

Pheromone Binding Proteins (PBPs) facilitates the slow release of odorants into the air and thereby preventing the evaporation or degradation of the pheromones. In the ungulate family the scent glands are localized in specific areas, such as inguinal sinus, infraorbital sinus, interdigital gland as pouches or pockets.

The structure of interdigital gland has been described in different exotic sheep breeds (Misk and Misk, 2013), (Demiraslan *et al.* 2014), (Awaad *et al.* 2015), (Mohamed and Adogwa, 2016), (Yilmaz *et al.,* 2017) and also in other mammals *viz*., elephant (Lamps *et al.,* 2001) and fallow deer (Parillo and Diverio, 2009).

Madras Red sheep is an indigenous, meat breed, approved by NBAGR (National Bureau of Animal Genetic Resources) reared mainly in Northern districts of Tamil Nadu, India which has an average adult male body weight of 26.33±0.39 kg. This breed was mainly reared on free range system and formed the livelihood of poor farmers and tribal people (Balasubramanyam *et al.* 2010).

However, the studies related to the interdigital glands in Indian sheep breeds are scanty and little is known about these glands in Madras Red Sheep. Taking into account the importance of these breeds, the present study was aimed at the gross, microanatomical and ultrastructural details of the interdigital gland in Madras Red sheep.

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MATERIALS AND METHODS

Six pairs of fore limbs and hind limbs of adult Madras Red sheep were used in this study. The manus and pes regions were collected from the corporation slaughter house and carefully dissected to collect the interdigital gland, followed by the gross observations were noted.

Radiograph of the gland was taken after evacuating its secretion by massaging, followed by lavaging using saline solution and by injecting an Iohexol contrast agent (Yilmaz *et al.* 2017).

The gland was dissected out and gross morphometric parameters like gland weight, body length, body width, duct length and duct width were measured by using a Vernier caliper. The data was analysed by SPSS 22.0. Tissue pieces were subjected for various histological and histochemical stains (Luna, 1968 and Singh and Sulochana, 1996). Immunohistochemical localization of T lymphocytes (CD3+) was done as per Dharani *et al.* (2020).

Scanning electron microscopic (SEM) study was done as per Karahan *et al.* (2007). The SEM images were observed by a Phenom Pro scanning electron microscope at CATERS facility in Central Leather Research Institute, Chennai.

This study was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai in the year 2019.

RESULTS AND DISCUSSION

Gross anatomy

In madras red sheep, the interdigital gland was found in all the four limbs and situated between the distal portion of the first phalanx and proximal part of the second phalanx. The gland was surrounded by a pad of adipose tissue and loose connective tissue. The orifice of the gland was observed on the dorsal aspect at the level of pastern joint (Fig 1a). Shape of the gland resembled like a tobacco pipe and comprised of four parts *viz.* orifice, duct, body and a bent or flexure (Fig 1a, b). The body and duct were clearly separated by a bent. A similar observation was made in various other sheep breeds by Misk and Misk (2013), Alexandre-pires *et al.* (2014), Suzer *et al.* (2015), Awaad *et al.* (2015), Mohamed and Adogwa, (2016), Yilmaz *et al.* (2017) and Mobini and Adermanabadi (2017). Whereas, Janicki *et al. (*2003) reported that the interdigital sinus was found only in the hind feet of roebuck and it extended between the half of the proximal phalanx and the distal phalanx. The lumen of the gland filled with whitish dense secretory material with a foul odour as per Yilmaz *et al. (*2017) who also observed the secretory material with foul and rancid odour. Protruding into the interior of the gland numerous wool fibers, which were darker in colour and was observed throughout the gland and protruded out through the orifice which was contrast to Awaad *et al.* (2015) who stated that the dark coloured hairs covered the excretory duct and the distal part of the corpus, while the rest of the corpus contained pale coloured non-pigmented hair.

The mean±SE of various gross parameters *viz.,* gland weight (gm), body length (cm), body width (cm), excretory duct length (cm) and width (cm) of interdigital gland in fore limb and hind limb respectively were recorded as 0.62±0.06, 1.53±0.06, 0.62±0.05, 2.18±0.11, 0.31±0.02 and 0.52±0.05, 1.34±0.08, 0.62±0.02, 1.99±0.08, 0.30±0.02. There was no significant difference observed in the gross parameters between the forelimb and hindlimb, however, the numerical value of the various parameters was found to be higher in forelimb compared to hind limb. The morphometric findings were in contradiction to Abbasi *et al.* (2009) and Mohamed and Adogwa (2016) who reported a significantly longer and wider gland in fore limb than the hind limb. Average weight of the interdigital gland was 0.61±0.13g which was lesser when compared to Kivircik sheep (0.84±0.24g) by Suzer *et al.* (2016). The difference might be due to variation in the average body weight between these two species. Kara *et al.* (2020) observed that the size of the interdigital gland was larger in forefeet when compared to hindfeet in Hasakans Hasmer sheep.

Contrast radiography of the digits confirmed the presence of the interdigital gland in between the digits at the level of first and second phalanges (Fig 1c).

Histology and histochemistry

Wall of the interdigital gland was composed of three clearly distinguishable layers *viz.*, epidermis, dermis and capsule from within outward (Fig 2a). This was due to the fact that interdigital gland was an invagination of the skin during

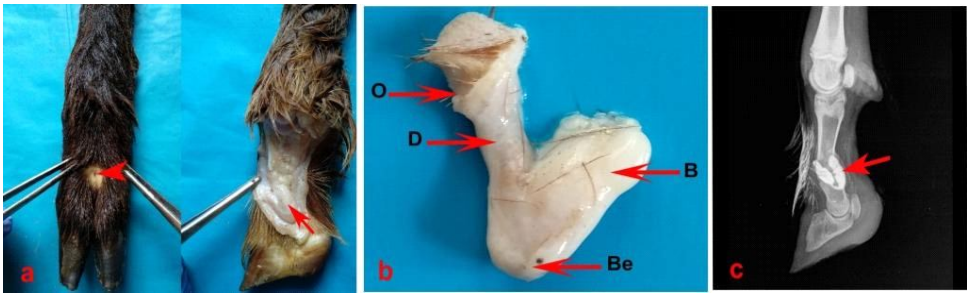

Fig 1: Photograph of the manus of Madras Red sheep showing a) *In-situ* position of the Interdigital gland (arrow) and its opening (arrow head); b) Parts of tobacco-pipe shaped Interdigital gland B - Body, D - Duct, Be - Bent, O - Orifice; c) Medio-lateral contrast radiographic appearance of the Interdigital gland (arrow).

development. However, the melanin pigments observed by Mobini and Adermanabadi (2017) was not observed in the present study which might be due to the different geographical existence.

The epidermis was composed of stratified squamous keratinized epithelium and the prominent keratin layer was observed facing towards the lumen (Fig 2b, c). The integrity of keratin layer may help in preventing the invasion of bacteria causing foot lesions.

The dermis was composed of dense irregular connective tissue, hair follicle, apocrine sweat gland, sebaceous gland and arrectores pili muscle (Fig 3a). The connective tissue consisted mainly of collagen fibers (Fig 3b). The sebaceous gland was closely related to the hair follicle which was located in the upper part of the dermis. Sweat glands were of coiled tubular type located deeper in the dermis. The secretory unit of the interdigital gland was lined with simple columnar to high cuboidal epithelium. The lining cells of the secretory acini had eosinophilic cytoplasm with centrally placed spherical nucleus and was surrounded by myoepithelial cells, few elastic fibers and collagen fibers (Fig 4a, b). Eosinophilic secretory material was present within the lumen of the secretory unit. The secretions of apocrine sweat gland, basal membrane of the cells showed positive for PAS (Fig 5a) and Alcian blue (Fig 5b). The different types of histochemical moieties play a major role in the secretory activity of gland (Singh and Roy, 1998). The apocrine sweat gland secretion was PAS negative (Abbasi *et al.* 2009) in Lori's sheep; Alcian blue negative (Parillo and Diverio, 2009) in fallow deer and Hasak and Hasmer sheep (Kara *et al.* 2020), however, the present study revealed that the secretion was PAS and Alcian Blue (pH 2.5) positive.

The outer fibrous capsule was composed of parallel bundles of collagen (Fig 3b) and elastic fibers (Fig 6a). The excretory duct had a similar structure as that of the body

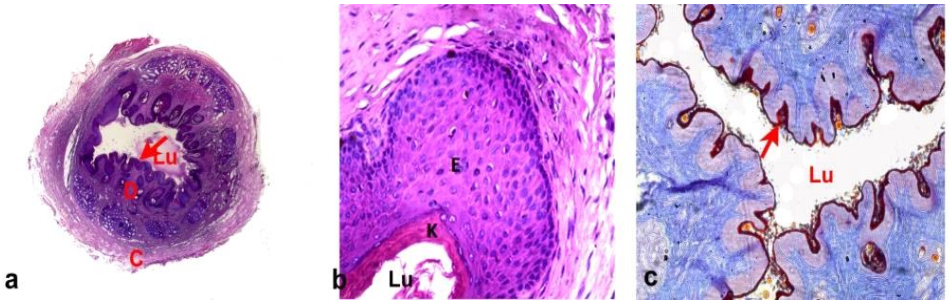

Fig 2: Photomicrograph showing a) Wall of the Interdigital gland. Epidermis (arrow), Dermis (D), Capsule (C) and Lumen (Lu) H&E stain, × 12.5; b) Epidermis (E) made of stratified squamous epithelium with prominent Keratin layer (K), Lumen (Lu); H&E stain, × 400; c) Keratin layer (arrow)in the epithelium and Lumen (Lu) Ayoub-Shklar method, × 50.

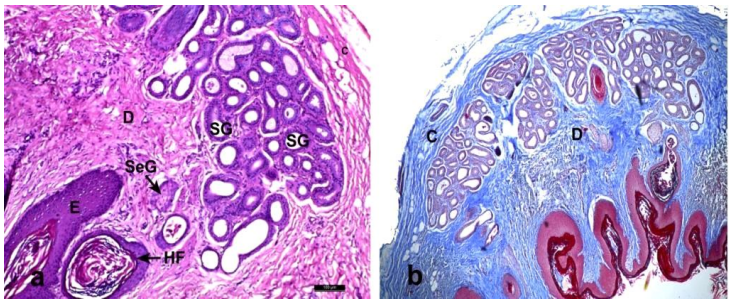

Fig 3: Photomicrograph showing a) Epidermis (E) and Dermis (D) consisted of Sweat gland (SG), Sebaceous gland (SeG), Hair follicle (HF) H&E stain, × 50 b) Distribution of collagen fibers in Dermis (D) and Capsule (C), Masson's trichrome stain × 50.

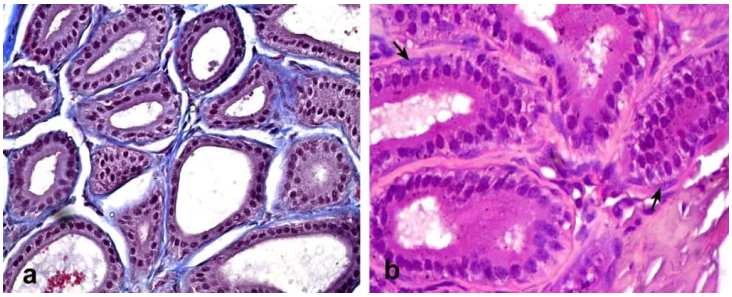

Fig 4: Photomicrograph showing a) Distribution of sweat gland surrounded by collagen fiber bundle Masson's trichrome stain × 400; b) Presence of myoepithelial cells (arrows) in the sweat glands, H&E stain, × 1000.

except that the lumen was found to be smaller. Abbasi *et al.* (2009) reported that the sweat gland was lined by bilayered cuboidal cells which is contrast to the present study. The sebaceous gland, hair follicle and apocrine sweat gland appeared closer to the capsule. The apocrine sweat gland was lined by simple cuboidal epithelium.

Immunohistochemistry

Immuno histochemical staining using anti-CD3+ monoclonal primary antibody revealed the presence of T-lymphocytes in the basal layer of epidermis (Fig 6b) which may help in combating foot lesions in Madras Red sheep to certain extend.

Scanning electron microscopy

The wall of the gland in madras red sheep was composed of epidermis, dermis and capsule from within outward (Fig 7a). Epidermis was composed of stratified squamous keratinized epithelium (Fig 7b) and numerous wool fibers were extended from it (Fig 7c). The wool fibres showed the cuticular pattern (Fig 8a). The sebaceous glands were round bubble-like structures situated closer to the stratified epithelium. The apocrine sweat glands were in the form of coiled tubules surrounded by thick collagen bundles (Fig 8b, 8c) (Karahan *et al.* 2007).

The luminal surface of the gland showed the presence of smaller and larger folds which may help in increasing the storage capacity as per the physiological status of the gland. The foul-smelling secretory material within the lumen may serve as a trail marker (Dyce *et al.* 2010) which imparts odoriferous signals in the social communication between the animals (Aslan *et al.* 2010). This is contradicted by Janicki *et al.* (2003) who postulated that interdigital gland might be involved in secreting lubricating material owing to its location in the interdigital space. Sudden elevation of the temperature leads to impaction of oil gland which is located in the Interdigital space, especially in long wool breeds sheep (Jaber and Mazeg, 2020).

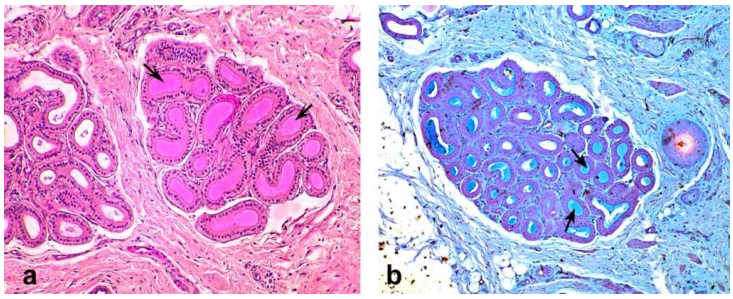

Fig 5: Photomicrograph showing the apocrine sweat gland with its secretion a) Positive for PAS reaction (arrows), PAS × 100; b) Positive reaction (arrows) with Alcian Blue (pH 2.5), Alcian Blue, × 100.

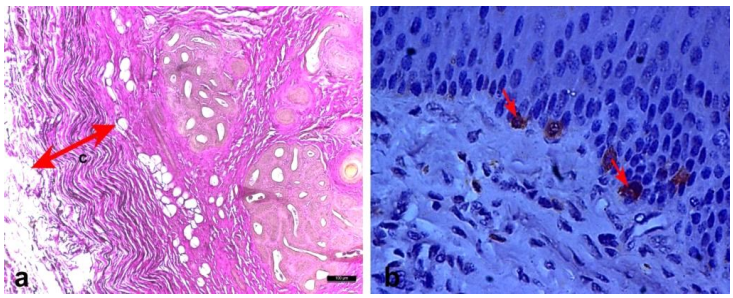

Fig 6: Photomicrograph showing the a) Distribution of elastic fibers in the capsule (C), Weighert's method × 50; b) Distribution of T-lymphocytes (arrows) in stratum basale by using CD3+ MAb × 400.

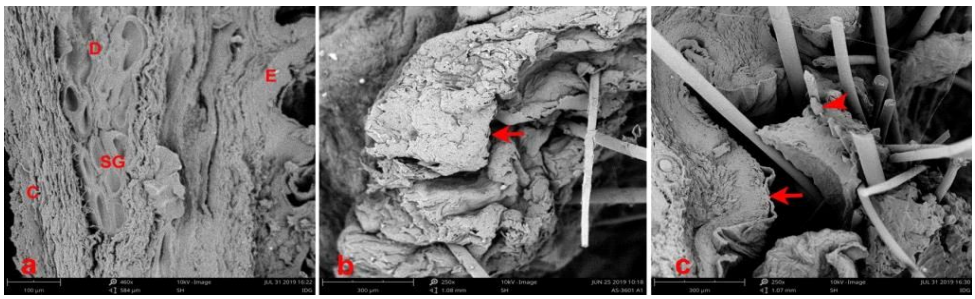

Fig 7: Scanning electron micrograph of Interdigital gland showing the a) Epidermis (E), Sweat gland (SG) within the Dermis (D) and Capsule (C) Bar = 100 µm b) Stratified squamous epithelium (arrow) Bar = 300 µm; c) Epithelial folds (arrow) with wool fibres projecting into the lumen (arrow head). Bar = 300 µm.

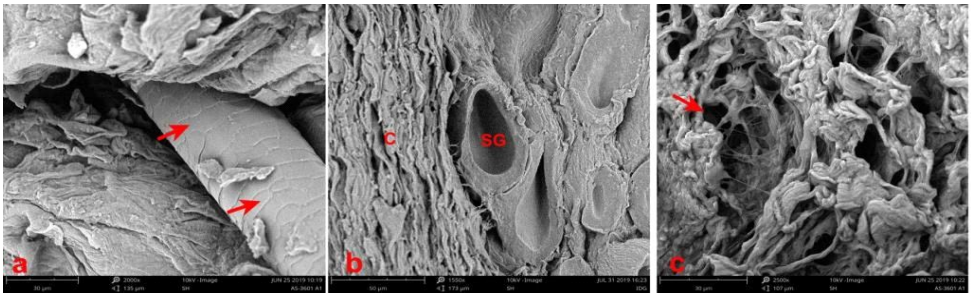

Fig 8: Scanning electron micrograph of Interdigital gland showing a) Cuticular pattern of wool fibre (arrows). Bar = 30 µm b) Apocrine sweat gland (SG) abuts to capsule (C) Bar = 50 µm c) Collagen fibers in the capsule Bar =30 µm.

CONCLUSION

The present study identified that the gross, histological and scanning electron microscopic findings of Interdigital gland in Madras Red sheep. Histologically, wall of the gland was composed of epidermis, dermis and capsule from within outwards. The prominent keratin layer of epidermis and oily secretion of sebaceous gland within the dermis helps in providing moist, intact interdigital skin. In addition, aggregation of lymphocytes in stratum germinativum offer local immune resistance. Scanning electron microscopy revealed the presence of various folds towards the luminal surface. The secretion of the gland perceived by the vomeronasal organ of conspecifics resulted in the trailing behaviour in sheep.

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