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HISTOLOGY, HISTOCHEMISTRY AND ULTRASTRUCTURE OF DERMIS IN DEER, GOAT AND SHEEP

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ABSTRACT

Histology, histochemistry and ultrastructure of dermis of skin in deer, goat and sheep was studied using the skin samples collected from spotted deer brought for post mortem at the College of Veterinary & Animal Sciences, Mannuthy, Thrissur zoo and Forest department; and from goat and sheep freshly slaughtered in Meat Technology Unit, Mannuthy. Samples of 1cm^3 were collected for histological and histochemical studies from 27 regions of skin. Specimens for histological techniques were fixed in 10 per cent neutral buffered formalin. Serial sections of 5µm thickness were made. Histochemical staining was done with Alcian blue for carbohydrates, Oil Red 'O' in propylene glycol for lipids and Gomori's method for acid and alkaline phosphatases. Samples of 1 mm^3 size fixed in 2.5 per cent gluteraldehyde were processed for scanning electron microscopy. Histologically, the skin consisted of two layers, *viz*. a superficial epidermis and the deeper dermis, joined to the underlying structures such as muscle and cartilage by the subcutaneous tissue. Dermis had two layers: a superficial, thin papillary layer and a deep, thicker reticular layer. Papillary layer conformed to the contour of the stratum basalis of epidermis. Even though a clear demarcation was absent between papillary and reticular layers of dermis, both could be distinguished by the difference in nature and arrangement of connective tissue fibres, with the reticular layer consisting of large, coarse and loosely interwoven bundles of collagen fibres. Demarcation between dermis and subcutaneous tissue was obscure ventrally, but clear in dorsal regions. Sweat and sebaceous glands, as well as hair follicles were epidermal structures located in the dermis and subcutis. Blood vessels, lymph vessels and nerves traversed the dermis. Connective tissue components of the dermis provide

strength and flexibility to the skin; cushion the body from stress and strain; its blood vessels account for nourishment and waste removal of both dermal and epidermal cells and nerve endings provide the sense of heat and cold making the dermis an important component of the largest sense organ of the body.

Keywords: Deer, dermis, goat, histology, histochemistry, sheep, ultrastructure

INTRODUCTION

Skin of animals consists of two layers, epidermis and dermis. The latter is placed between epidermis-with which it makes up the cutis- and subcutaneous tissue; and primarily consists of dense irregular connective tissue to cushion the body from stress and strain. Dermis, synonymously known as corium, forms the thickest layer of the skin. It is divided into two layers; the superficial area adjacent to the epidermis is called the papillary region and the deep thicker area is known as the reticular dermis. Dermis is tightly connected to the epidermis through a basement membrane. The dermis is made of irregular connective tissue, providing strength and flexibility to the skin. In addition, hair follicles, sweat glands, sebaceous glands, lymphatic vessels, muscle fibers, nerves and blood vessels are present in the dermis. The blood vessels provide nourishment and waste removal for both dermal and epidermal cells and nerve endings provide the sense of heat and cold. Among wild ruminants, deer and its relatives of Cervidae family have especially notable integumentary regions, significant in modes of silent communication within the species, probably through visual and olfactory sensory routes. Since the experimental evidence substantiating such notions is scanty, the present study was undertaken to investigate the structural differences in histology, histochemistry and ultrastructure of dermis of skin in three species of wild and domestic ruminants.

MATERIALS AND METHODS

Histology, histochemistry and ultrastructure of dermis in deer, goat and sheep were explored using skin samples collected from spotted deer brought for post mortem at College of Veterinary and Animal Sciences, Mannuthy, from Thrissur zoo and Forest department; and from goat and sheep freshly slaughtered in Meat Technology Unit, Mannuthy. Samples of $1cm^3$ were collected for histological and histochemical studies from 27 regions of skin, *viz.* muzzle, infraorbital, horn glands, dorsal face, lateral face, ventral face, ear pinna, dorsal neck, lateral neck, ventral neck, dorsal abdomen, lateral abdomen, ventral abdomen, dorsal and palmar skin of forelimb, dorsal and plantar skin of hindlimb, interdigital skin of fore and

hind limbs, foot pads, inguinal, preputial (male), scrotal (male), dorsal thorax, perineum and dorsal nasal. Specimens for histological purpose were fixed in 10 per cent neutral buffered formalin (10% NBF), for 48 hours. The fixed specimens were washed, dehydrated and embedded in high melting paraffin (MP 58-60^{O}C). Serial sections of 5µm thickness were made and stained histologically using Haematoxylin and Eosin for routine studies, Gomori's one step trichrome method for collagen muscle fibres and Ayoub Shalkar method for keratin and pre-keratin (Luna, 1968). Histochemical observations were recorded with Alcian blue for carbohydrates, Oil Red 'O' in propylene glycol method for lipids, Gomori's alkaline phosphatase cobalt method and Gomori's method for acid phosphatase (Singh and Sulochana, 1996). Digital images were captured using Leica DM 2000 LED microscope. Samples of 1 mm^3 size were fixed in 2.5 per cent gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h at 4°C and processed for scanning electron microscopy (SEM - Model: JOEL-JSM 5600) as per the standard procedures (Bozzola and Russell, 1998) at Ruska labs, College of Veterinary Science, Hyderabad. Micrometrical parameters were measured using ocular micrometer. Statistical analysis of data was done to find out the relationship between the data collected.

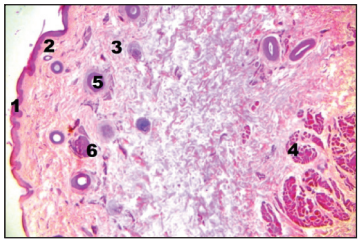
RESULTS AND DISCUSSION

Histology: Histologically, the skin consisted of two layers, *viz.* a superficial epidermis and the deeper dermis, joined to the underlying structures such as muscle and cartilage by the subcutaneous tissue. Sweat and sebaceous glands, as well as hair follicles, were epidermal structures located in the dermis and subcutaneous tissue.

In the three species studied, in all regions, the epidermis was very thin compared to the dermis. Thickness of the dermis varied from a minimum of 275 µm at pinna to maximum 3850 µm at muzzle in deer; 275 µm at pinna to 2338 µm at muzzle in goat and 413 µm at pinna to 1925 µm at lateral neck in sheep respectively. In all the three species studied, muzzle region had the thickest epidermis. Adult deer had the greatest thickness for the region (619 µm) followed by goat (413 µm). Epidermis was thinnest in sheep (234 µm).

Two layers could be distinguished in the dermis. The superficial papillary layer was thin, while the deep reticular layer was thicker (Fig. 1).

Papillary layer: The papillary dermis, the upper layer of dermis, was thickest in the muzzle 413 µm in deer; 234 µm in goat. The papillary layer was made up of fine, closely arranged collagen fibres predominantly (Fig. 2).



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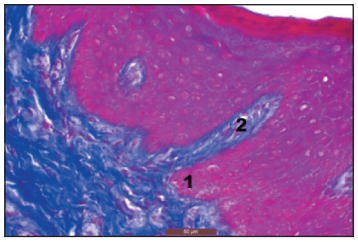

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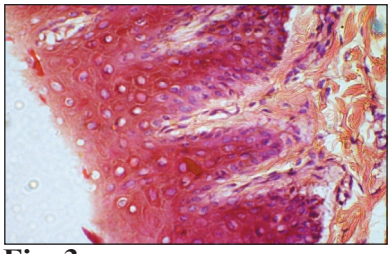

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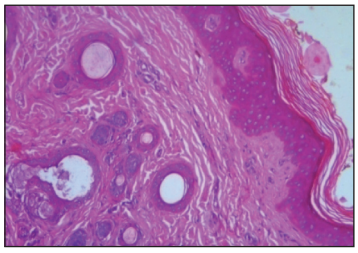

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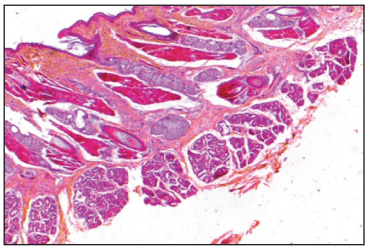

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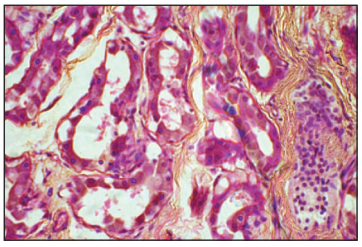

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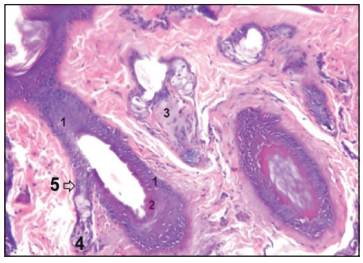

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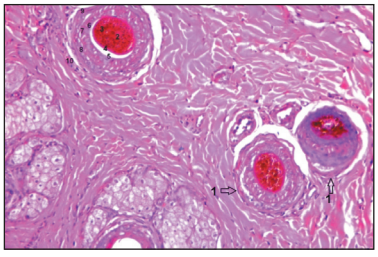

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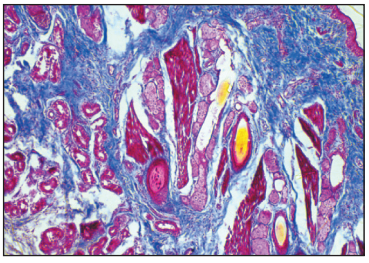

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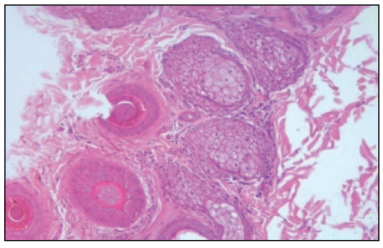

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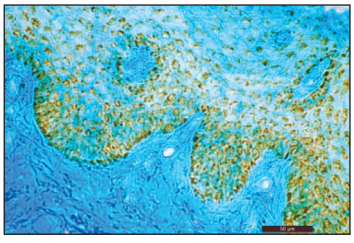

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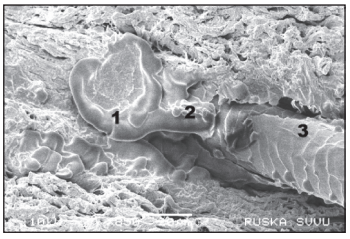

Fig. 12.

Fig. 1. Skin over infraorbital region in 2-days old Cross bred Malabari Goat. H & E. x 50

1. Epidermis 2. Papillary (upper part of) dermis 3. Reticular (lower part of) dermis 4. Muscle in hypodermis 5. Hair follicle 6. Sebaceous glands

Fig. 2. Muzzle region in adult goat. Gomori's one step Trichrome x 400

1. Rete peg 2. Dermal papillae

Fig. 3. Interdigital region, forelimb in foetal deer. Ayoub Shalkar method for keratin and pre-keratin x 400

Fig. 4. Dorsal aspect of hind limb showing broad and compound dermal paillae. Adult female goat. H & E x100

Fig. 5. Dorsal Neck showing apocrine sweat glands. Foetal deer. Keratin x 40

Fig. 6. Interdigital Region of Hind Limb. Foetal deer. Gomori's one step Trichrome x 400

Fig. 7. Interdigital Region. Adult female goat. H & E x100

1. Outer root sheath 2. Inner root sheath 3.Telogen germinative units(TGU)
4. Sebaceous gland 5. Duct of sebaceous gland

Fig. 8. Horn Gland. Two days-old female goat. H & E x 200

1. Catagen 2. Medulla 3. Cortex 4. Cuticle of hair 5. Cuticle of IRS
6. Huxley's layer 7. Huxley's layer 8. Outer root sheath
9. Glassy membrane (arrows) 10. Connective tissue sheath

Fig. 9. Interdigital region showing eccrine sweat glands, Hind limb in foetal deer. Gomori's one step Trichrome x 100

Fig. 10. Adult female goat showing sebaceous glands. Muzzle Region. H& E x100

Fig. 11. Muzzle region in adult male sheep. Alcian blue x 400

Fig. 12. Dorsal abdomen. Six years-old female deer. SEM x 850

1. Branched tubule of sebaceous gland 2. Duct 3. Hair

Epidermal projections into the dermis formed the rete pegs (Fig. 2). In turn, the fine fibres of papillary layer interdigitated into the stratum basalis of epidermis and conformed to the contour of the stratum basalis of the epidermis. Thus, papillary elevations of the dermis into epidermis *viz.,* the dermal papillae, containing blood vessels and nerves also showed a similar pattern. The epidermis was entirely devoid of blood vessels and received nourishment from the capillaries of the papillary dermis through these projections.

Extremities like face and limbs *viz.* muzzle, dorsal part of limb, interdigital and infraorbital regions had abundant and deep rete pegs and dermal papillae than the neck and trunk regions (Fig. 3) indicating increased vascularity according to the need in these areas. Highest dermal papillae could be seen in the muzzle (Fig. 2). Compound dermal papillae could be observed in the dorsal part of hindlimb (Fig. 4) and infraorbital regions.

Unlike the epidermis, papillary dermis was highly vascular and presented

large number of capillary loops in the dermal papillae (Fig. 2). Fibroblasts and macrophages were the predominant cell types in the papillary layer. The observation was in accordance with those of Ross and Pawlina (2011), who also opined that the layer is named for these finger-like projections, which extended toward the epidermis and contained either terminal networks of blood capillaries or tactile Meissner's corpuscles.

Eventhough a clear demarcation was absent between the papillary and reticular layers of the dermis, both could be distinguished from each other because of the difference in the nature and arrangement of connective tissue fibres (Fig. 1, 4).

Reticular layer: Reticular layer consisted of large, coarse and loosely interwoven bundles of collagen fibres. It receives its name from the arrangement of collagenous, elastic, and reticular fibers that weave throughout it.

In the reticular layer, bundles of collagen fibres were arranged mostly parallel to the skin surface (Fig. 4). A few perpendicularly directed fibres could be traced down to the subcutaneous layer. These bundles ran parallel to the hair follicles and contributed to the formation of the interlobular septa that separated the subcutaneous adipose layer into numerous lobules. In addition to the parallel fibres, alternate layers of collagen fibres were also observed at an angle to the former. In the papillary layer, they were thinner. Towards the deeper aspect, size of the collagen bundles in the reticular layer greatly reduced and they were seen as small, thin bundles.

According to Ross and Pawlina (2011), the orientation of collagen fibers within the reticular dermis creates lines of tension called Langer's lines, which are of some relevance in surgery and wound healing.

In deer, thickness of the reticular dermis varied from 234 µm at pinna to 3438 µm at muzzle; whereas in the goat the values were 275 µm and 2338 µm; and in the sheep those being 275 µm and 1444 µm, respectively. The reticular layer was thickest in the muzzle region in deer and goat. But in sheep, lateral neck presented more thickness of 1650 µm. Average thickness of the reticular layer was about eight to eleven times more than that of the papillary layer in this region in deer, where as in goat and sheep it was only three to four times more.

The separation between the papillary and reticular layers was more distinct in the dorsal and lateral abdomen regions. In the dorsal aspect of the body,

the dermis was clearly demarcated from the subcutaneous fat also (Fig. 5). But a clear demarcation lacked between the dermis and subcutaneous tissue in the ventral neck region and lateral and ventral abdominal regions.

Blood vessels, lymph vessels and nerves traversed the dermis (Fig. 6). Small and medium-sized arteries predominated in the reticular layer. The tunica media of these arteries was composed of smooth muscle fibres. Numerous blood vessels were noticed in the reticular dermis and near the sweat glands. Most of them formed the arteriovenous anastomoses or glomi. Glomi were most numerous in the muzzle region.

The cellular elements were less abundant in the reticular layer when compared to the papillary dermis. These were found to be associated with the compound tubular sweat glands. Large number of receptors and nerve bundles could be noticed in the dermis.

Hair follicles: Hair roots were situated in tubular pockets as the hair follicles in the epidermis and extended into the dermis. Hair follicle was composed of four parts, *viz.* hair papilla, hair matrix, inner root sheath and outer root sheath. Hair papilla was the part of dermis encapsulated by the hair matrix cells that formed a structure called the hair bulb.

Different stages of hair follicles were noticed in the dermis, *viz.* anagen, catagen or telogen. Anagen follicles in the deep dermis bore mitotically active cells in the hair bulb; had inner and outer root sheaths, with no signs of apoptosis in the outer root sheath. Catagen showed regressive type of cells; thickening of the basal membrane or apoptotic cells. Telogen were follicles with central wrinkling of the hair canal known as tricholemal keratinization; their hair papilla was reduced to a ball of cells below the capsule of the hair matrix cells of the bulb. Telogen germinative units (TGU) and fibrous tracts were also observed. TGUs were identified in the deep dermis as clusters of epidermal cells with peripheral palisade with no central keratinization (Fig. 7). Fibrous tracts remaining in lower region when follicles are in the catagen phase were characterized as epithelial cells amidst thickened and concentric collagen fibers with increased vascularization in deep dermis and hypodermis (Fig. 8).

Arrectores pilorum: Arrectores pilorum muscle was a small bundle of smooth muscle fibres that inserted obliquely in the connective tissue sheath of the hair follicle (Fig. 8). It was attached to the papillary layer of the dermis, with the outer end extending towards the epidermis.

Glands: Apocrine sweat glands were located mostly in the deeper dermis and in the subcutaneous tissue (Fig. 5). Eccrine sweat glands were embedded in the reticular layer of the dermis and were not associated with the hair follicles (Fig. 9).

Sebaceous glands appeared as large, lobulated, sac-like structures associated with the hair follicles. All these were embedded in the dermis and did not extend into the subcutaneous tissue. The secretory units consisted of a solid mass of epidermal cells, enclosed by a connective tissue sheath that blended with surrounding connective tissue of the dermis (Fig. 10).

Subcutaneoustissue: Subcutaneous tissue also showed fibroblasts, lymphoid aggregations, lymphatics, large blood vessels and nerve bundles. The collagen fibre bundles from reticular dermis extended down to the subcutaneous tissue (Fig. 1).

Histochemistry: In the dermis, the ground substance was positive for alcian blue (Fig. 11) indicating the presence of carbohydrates. Dermis, exhibited diffused positive reaction for Oil red 'O' showing the limited presence of lipids. Even though acid phosphatase activity was meagre, alkaline phosphatase was observed in hair follicles, blood capillaries and eccrine sweat glands of dermis similar to the reports by Mier and Rennes (1982) indicating the regenerating processes progressing in the tissues mentioned.

Scanning Electron Microscopy (SEM): Scanning electron microscopy revealed sebaceous glands associated with hair (Fig. 12). Elastic fibres were seen as bundles of branching fibres in the dermis under SEM and sweat glands were also observed in the deep dermis.

To conclude, the dermis with its papillary layer formed primary and secondary dermal ridges or papillae, corresponding to the epidermal ridges or rete pegs in the present study. By this feature, blood vessels in the dermal papillae nourish all hair follicles and bring nutrients and oxygen to the lower layers of epidermal cells. According to Marks and Miller (2006), the pattern of ridges they produce on the skin surface are partly genetically determined features that develop prenatally. They remain substantially unaltered (except in size) throughout life, and therefore determine the patterns of wrinkles, *viz.* finger prints in human beings and probably muzzle prints in animals, making them useful in certain functions of individual identification. Banks (1981) opined that with age, the dermal papillae tend to flatten and sometimes increase in number. It is also reported that papillae play a pivotal role in hair formation,

growth and cycling. Moreover, as opined by James *et al.* (2005), the dermal papillae and rete pegs greatly increase the surface area between the dermis and epidermis. Since the main function of the dermis is to support the epidermis, the increased area of contact greatly upsurges the exchange of oxygen, nutrients and waste products between these two layers. Additionally, the increase in surface area strengthens the junction between the dermal and epidermal layers; thereby preventing the separation from each other.

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