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Sukanta Das

Assistant Professor, Department of Veterinary Anatomy, College of Veterinary Sciences and Animal Husbandry, R.K. Nagar Tripura West, India

Kabita Sarma

Veterinary Sciences, Khanapara, Assam Agricultural University, Guwahati, Assam, India

Histomorphological, histochemical and ultrastructural study of sweat and sebaceous gland of Assam hill goat (*Capra hircus*) during pre-ruminant, transitional and ruminant age groups

Sukanta Das and Kabita Sarma

Abstract

The present investigation was conducted in total eighteen numbers of Assam Hill Goat divided in three groups (preruminant (0-3 wks), Transitional (3-8 wks) and ruminant age groups (above 8 wks) with six numbers of animals in each group collected randomly to investigate the histomorphological, histochemical and ultrastructural feature of sweat and sebaceous gland. Histomorphological study showed that both the sebaceous and sweat glands were associated with the hair follicle. sebaceous glands were simple alveolar type and acini was composed of squamous to cuboidal cells with peripheral stem cells and necrotic cells, without nucleus containing sebum, opened in to the hair. Both the mono and bilobbed glands were present and duct was lined by flat cells. Sweat glands were located much below having various shape mostly sac like elongated lined by secretory and supported by spindle shaped myoepithelial cells. The population and activity of both sweat and sebaceous were maximum in ruminant age group. Sweat glandular higher activity were noticed in the transitional and ruminant age groups, characterized by secretory caps. Connective tissue fibres were anchoring the sebaceous and sweat glands. Ultrastructural study showed sebaceous glands were at one side of the follicle opened in to the follicle and sweat gland fundus part was locate at the level of hair bulb. Transmission electron microscopy showed the peripheral stem cells of flat elongated shaped sebaceous gland with round nucleus and indistinct nucleolus abundant lipid droplets and mitochondria, Golgi. Gland was supported by basal lamina and collagen fibres. Flat oval Sweat secretory cells were connected by zonula occludense, supported by myoepithelial cells and connective tissue. The free end containing microvilli. Cells were containing abundant mitochondria, distinct nucleus and ribosomes. Acid phosphatase, alkaline phosphatase, and Adenosine triphosphatase activity were detected in the sweat and sebaceous gland. Sweat gland was strong PAS positive.

Keywords: Assam hill goat, histochemistry, sebaceous gland, sweat gland, ultrastructure

1. Introduction

The Assam Hill Goat, constitutes a major indigenous non-descript variety in the state of Assam, found in Khasi, Naga and Luchai hills of Assam, important meat type animal with high prolificacy from the North Eastern region of India. They are distributed in the hilly terrain of North Cachar hill, Karbi Anglong districts of Assam and also in the adjoining hilly tract of Meghalaya state (Zaman, 2013) [27]. Assam Hill goats are mostly white with occasional black patches on backline and legs. These goats are short legged with small body size. Both buck and does are bearded and have short cylindrical horns which are directed upwards and outwards. Ears are medium in size, horizontally placed with pointed tips. This is an important meat type animal with high prolificacy. These goats are reared mainly for meat. Adult body weight ranges from 15 to 26kg. Age at first kidding ranges from 337 to 447 days. Average litter size is 1.6 (<https://nbagr.icar.gov.in/en/assam-hill/>. National Bureau of Animal Genetic Resources, Karnal, Hariyana, India 2018) [28].

Two different types of skin glands (sweat and sebaceous) are found in the dermis of the skin serving a variety of functions. Sebaceous glands may be simple, branched, or compound alveolar types mostly associated with hair follicle, holocrine in a nature secretes an oily or waxy matter called sebum (Dellmann, 1993) [7]. The sebaceous gland has emerged as a key contributor to many cutaneous functions including physico-chemical barrier function of the skin against constant environmental challenges, neuro-immune modulatory roles and social communication through a complex intercellular communication networks of the integumentary system (Attila G. Szöllösi *et al.* 2018) [1].

Corresponding Author

Sukanta Das

Assistant Professor, Department of Veterinary Anatomy, College of Veterinary Sciences and Animal Husbandry, R.K. Nagar Tripura West, India

The sweat glands are tubular apocrine types, simple coiled tubular or saccular glands that the most extensively developed in the domestic mammals and located throughout most of the skin. The structure of these glands varies considerably among species. (Dellmann 1993) [7]. Domestic animals have apocrine glands at the base of each hair follicle, distribution of glands varied in different animals. Functionally both sebaceous and sweat gland plays a vital role in thermoregulation, body defense, part of delivery system and social communication (Dellman, 1993). The sebaceous gland has emerged as a key contributor to many cutaneous functions including physico-chemical barrier function of the skin against constant environmental challenges, neuro-immune modulatory roles and social communication through a complex intercellular communication networks of the integumentary system (Attila, 2018) [1]. The role of sebum in the impregnation of fur, thermal insulation as well as pheromones production in animals (Pochi, 1977) [20] However the composition of lipids are important in water resistance protection from UV radiation and establishment of commensal bacterial flora of the skin (Pappas, 2009, Dahlhoff, 2016 & Drake, 2008) [21, 8, 9]. So Assam Hill Goat being a local important goat here an attempt is made to find the anatomical feature of the sebaceous and sweat gland as so far there is no elaborate study is recorded till date.

2. Materials and Methods

2.1. Grouping of Animals

The present investigation was conducted in 18 numbers of Assam Hill Goat (*Capra hircus*) divided in three groups (preruminant (0-3 wks), Transitional (3-8 wks) and ruminant age groups (above 8 wks) with six numbers of animals in each group to investigate the histomorphometrical feature of sweat and sebaceous glands.

2.2 Collection of samples: Aseptically 3-4 mm skin samples were collected from the leg region from each age groups and fixed in 10% neutral buffered formalin solution (for at least 72 hours).

2.3 Histomorphological and Histochemical Examination:

After the proper fixation of the tissues were processed as per the standard technique of procedure (Luna, 1968) [15]. The paraffin blocks were sectioned in Shandon Finesse microtome at 6µm thickness, and the sections were stained with different staining methods as per (Luna, 1968) [15]. Mayer's Haematoxylin and Eosin staining technique for histoarchitecture of hair follicle, Van Gieson's method for collagen fibres, Gomori's method for reticular fibres., Hart's method for elastic fibres, PAS & Alcian Blue stain for acid and neutral mucin. For histochemical parameters, skin samples were collected and preserved in liquid nitrogen at -196 degree centigrade immediately after collection. Said samples were sectioned at 10µm thickness in cryostat microtome (Leitz) maintained at -20 °C. They were temporarily stored at -22 °C, and then treated for histochemical staining with the following methods, Alkaline Phosphatase cobalt method, Acid Phosphatase, Adenosine Tri-Phosphatase.

2.4. Transmission and Scanning electron Microscopy: For the ultra structural study 2-3mm skin samples were collected and immediately fixed in Karnovsky's fluid for four hours, then kept in cacodylate buffer (0.1M). Samples were then

taken to the North Eastern Hill University, Shillong Meghalaya, India, for the further processing as per the standard protocol for TEM and SEM described by NEHU and viewed at various magnification.

(The Research work was carried out as per the approval of the Institutional Animal Ethics Committee, Approval No: 770/ac/CPCSEA/FVSc/AAU/IAEC/16-17/373, Assam Agricultural University: Khanapara, Guwahati-781022)

3. Results

3.1 Histomorphology: The histomorphology of the skin of Assam Hill Goat (*Capra hircus*) in the present investigation revealed that sebaceous and sweat glands were associated with hair follicle in all the age groups of goat (Fig. 1,2). Sebaceous glands were mostly found at the upper part of the dermis in present study at the level the neck above the isthmus bulge below the infundibulum of the follicle (Fig.1,2). The sebaceous glands were of variable shaped like oval, round, pear, monolobed or bilobbed (symmetrical or asymmetrical) and simple alveolar type of gland (Fig:3,4).. Glands were mostly bilobbed with a single or two opening (4,b,e,f). The gland mass was composed of variable sizes of sebocytes depending on the amount of sebum contained in it. Histologically the glandular morphology showed three zones, with variable degree of differentiations. The outermost peripheral zone contains the least differentiated, mitotically active population of sebocytes. These cells grow and move centrally and accumulate lipid droplets, and forms the maturation zone. The central zone of necrosis where sebocytes disintegrate and release their content via holocrine secretion. The peripheral zone were lined by large squamous to cuboidal sebocytes with cells with distinct nucleus are the stem cell with cloudy cytoplasm. This Central zone sebocytes were with pyknotic and disintegrated nuclei and cloudy cytoplasm, vacuolated and then reaches the central necrotic zones characterized by disintegrated cells with loss of nuclei, occasional pyknotic nuclei, and propelled their content into excretory duct (Fig 4). The duct of the sebaceous gland mainly consisted of a simple flat epithelium connected to the hair follicles. In the preruminant age groups of Assam Hill Goat showed a very few numbers of sebaceous and sweat gland with least activity amongst the other three age groups. (Fig:5 a,b,c). In cross section, the sebaceous glands were found at the lower end of the follicle on two or one side (Fig.3,4 b,d). In between the lobes of gland net like network of connective tissue fiber with blood vessels were seen. The sebocytes boundaries are well demarcated. The pouch like opening was observed at the site of opening of sebaceous gland into the hair. (Fig-3 and 4 c)

Sweat glands were located at the lower part of the follicle much below the sebaceous gland (Fig.2,5,6) of elongated sac, round bag, secular to coil shaped found to be least active in preruminant but well developed, with active epithelium in transitional and ruminant age groups in the present study deep into the reticular dermis (Fig.6). The supranuclear portion of the glandular cell was highly eosinophilic. The apocrine sweat glands were lined by flattened to low cuboidal. At some places of sweat gland epithelium secretory caps were seen with eosinophilic cytoplasm (Fig.6). Secretory cells and spindle shaped elongated myoepithelial cell rests on the basement with distinct flat nucleus. The secretory caps that indicated their secretory activity, were observed in the free apical cytoplasm of secretory cells protruded into the lumen. Secretory caps were observed only in the transitional and

ruminant age groups (fig 6). The duct of sweat gland was above the sebaceous gland (Fig. 4).

Abundant collagen, reticular fibres were found anchoring the sweat and sebaceous gland. Elastic fibres were found sebaceous gland, sweat gland in all the age groups. Elastic fibres were distinct around the sweat glands and abundant.

The collagen fibres were the thickest in all the age groups compare to other connective tissue fibres. Network of connective tissue fibres in cross section in net like fashion was observed between the lobes of sebaceous gland (Fig: 7, 8, 9).

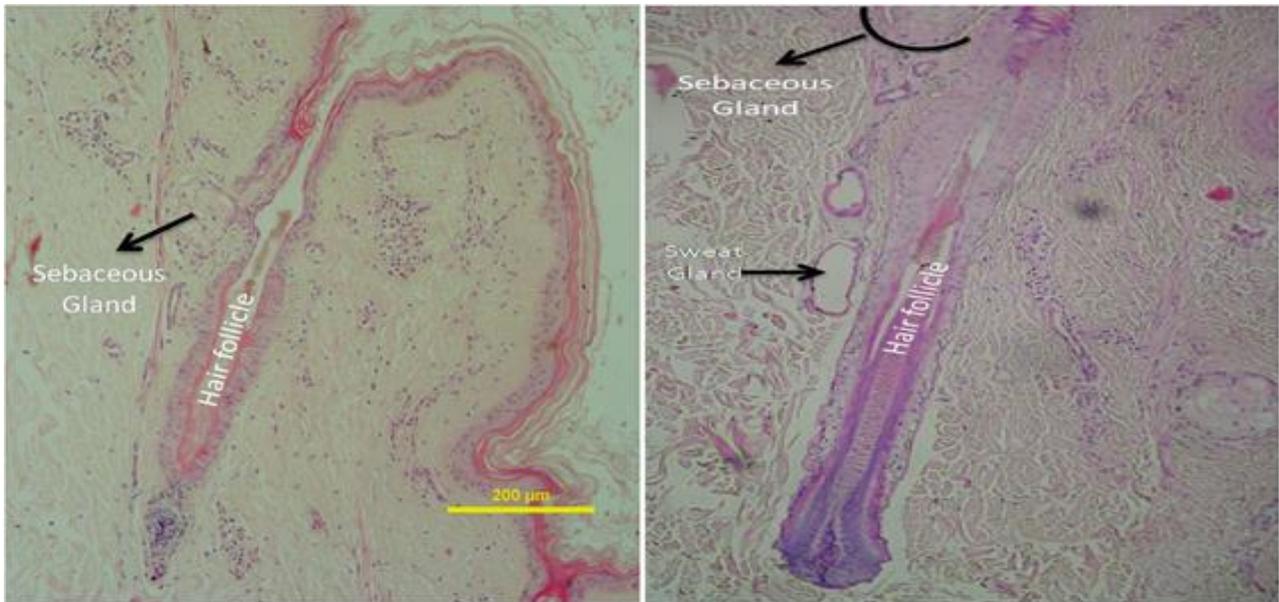


Fig 1, 2: Showing the sebaceous gland and sweat gland associated with hair follicle and the location (H&E at 400X magnification).

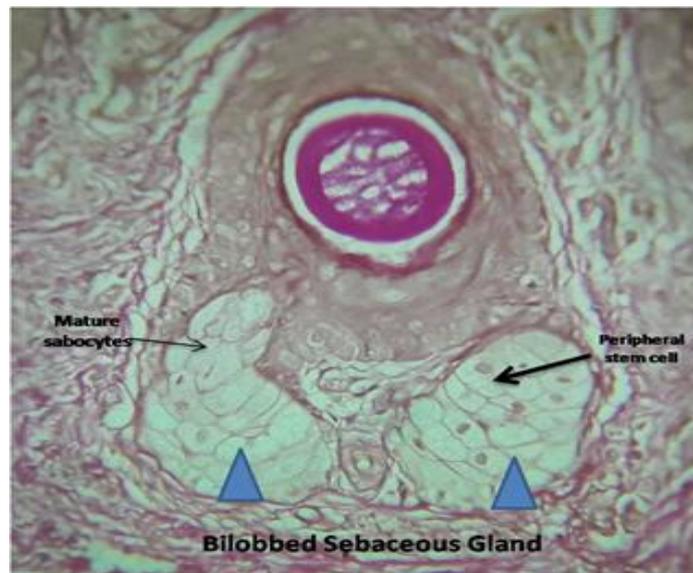


Fig 3: Showing the lobulated alveolar berry like sebaceous gland (Von Geisson at 400X magnification).

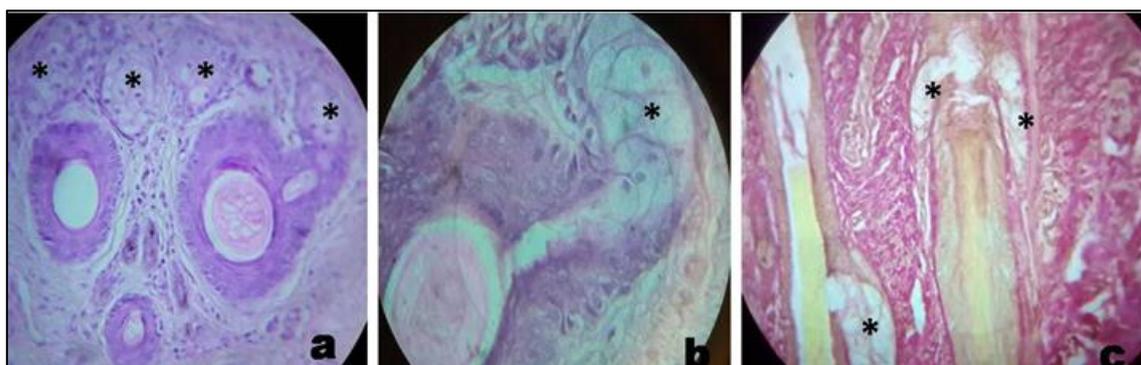


Fig 4: (a,b,c) * Marks are showing the various types of sebaceous glands (bilobbed, monolobbed with single and two opening into hair canal, a and b (H&E), c (Von Geisson) at 400X magnification

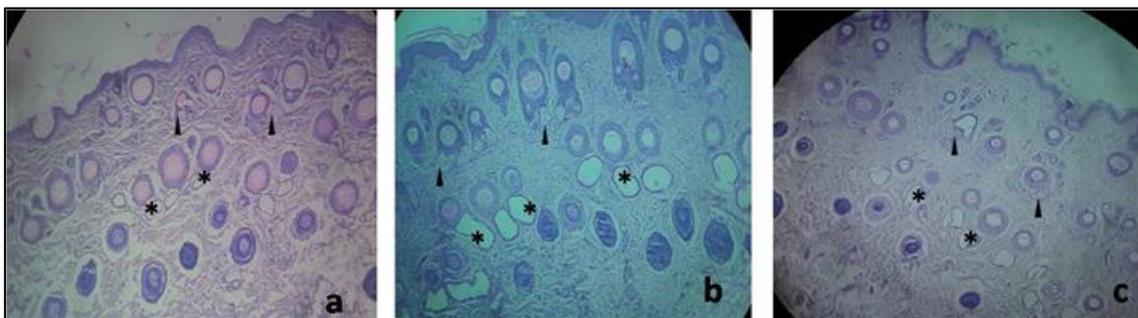


Fig 5: Showing sweat (arrow) and sweat gland (*) during preruminant (a), transitional(b) and ruminant(c) age groups (H&E at 100X Magnification).

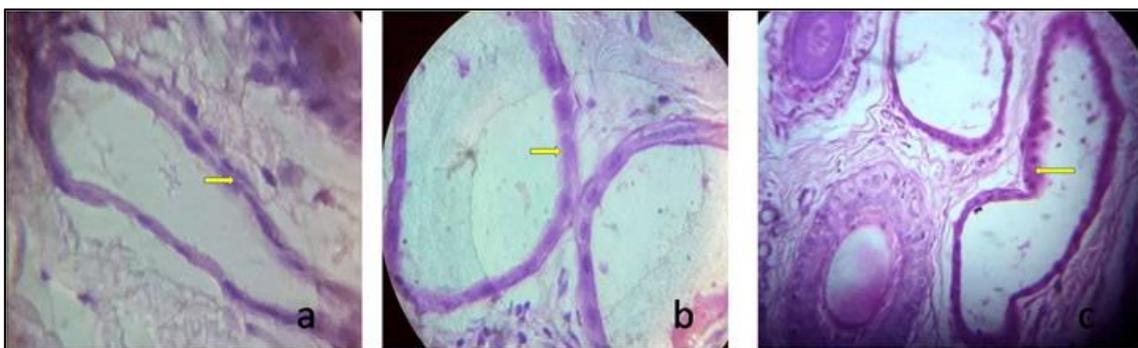


Fig 6: Showing the sweat gland during preruminant, (a) transitional (b) and ruminant (c) age groups with secretory cells (Yellow arrow) and myoepithelial cells and secretory caps (c) H&E 1000X Magnification.

3.2 Histochemistry: Weak to moderate activity was seen for acid phosphatase in the sweat and sebaceous gland in preruminant and transitional age groups. Whereas moderate to strong activity was seen in ruminant age groups animals (Fig. 13 a,b,c) : Moderate to strong activity in the sweat and sebaceous gland was observed in the ruminant age groups for alkaline Phosphatase. In other groups it showed weak to moderate reaction (Fig.14 a,b,c). A moderate to strong

activity also seen in the sweat and sebaceous gland. In preruminant age groups the sweat and sebaceous gland showed very weak reaction whereas in transitional and ruminant age groups sweat and sebaceous gland showed moderate to strong activity for ATPase (Fig. 15 a,b). The sweat gland epithelium showed strong reaction for PAS (Fig.16). In combined PAS- Alcian the capsules of the gland showed a PAS positive activity (Fig.16).

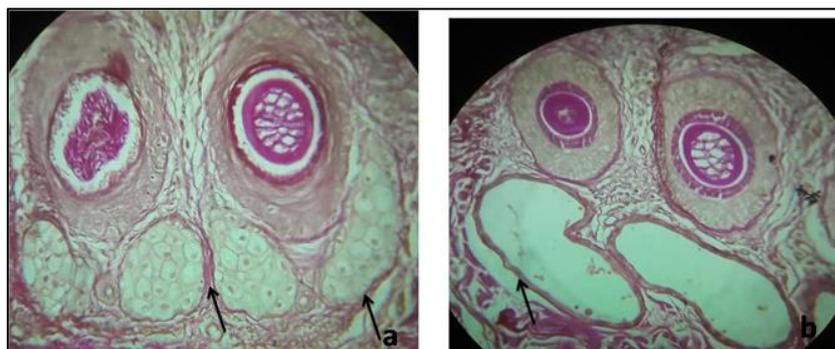


Fig 7: Von Geison stain (collagen fibres around the sebaceous and sweat gland marked arrow) at 400X magnification

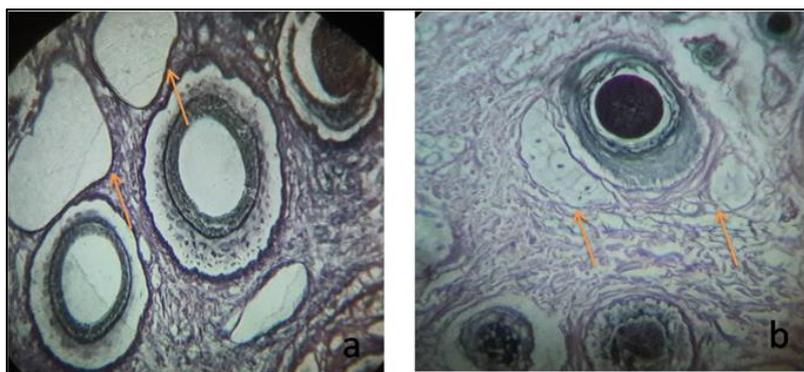


Fig 8: Gomoris Stain (reticular fibres around the sebaceous and sweat gland marked arrow) at 400X

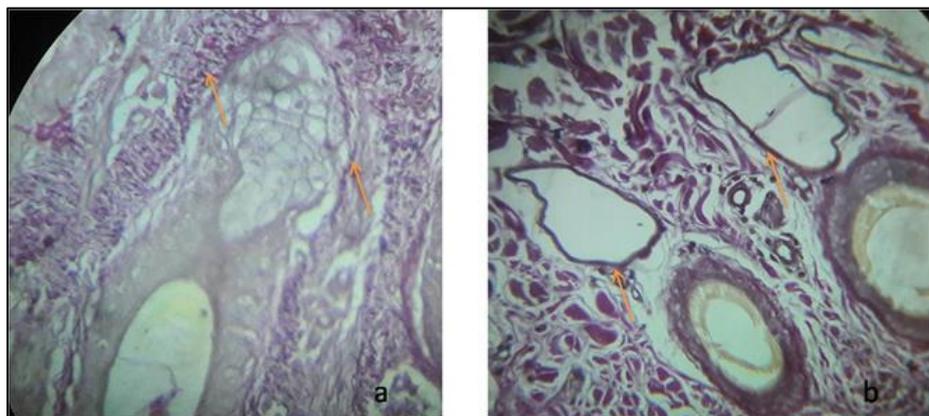


Fig 9: Harts Stain (reticular fibres around the sebaceous and sweat gland marked arrow) at 400X

3.3 Ultra structure of Sebaceous and sweat Gland of Assam Hill Goat (*Capra hircus*)

Scanning electron microscopy revealed the presence of sebaceous gland and sweat gland associated with hair follicle (Fig. 10 a,b) The main fundus portion of the sweat gland was found at the lower part of the follicle almost at the level of hair follicular bulb and the duct runs along the follicular length and opens into hair follicle. Few duct of sweat glands were seen at lower part of follicle. Alveolar sebaceous glands were seen at one side of the follicle opened directly into the hair follicle (Fig.10 a,b)

The transmission electron microscopy it was revealed that Sebaceous and sweat glands were in association with hair follicle. The sebaceous gland in the present investigation was surrounded by a connective tissue sheath which consisted mainly of collagenous fibers and fibroblasts. Gland was supported by thin basal lamina. The shape of the sebocytes were flat with round nucleus and inconspicuous nucleolus. Cells were containing numerous lipid droplets, round shaped mitochondria, Golgi bodies and smooth endoplasmic reticulum was the characteristics of the mature cells. The lipid droplets varied greatly in size and number. Lipid droplets

fused with each other and increase in size. The nuclear membrane was dark thick and distinct with attached rough endoplasmic reticulum Very few organelles were visible as the cell was completely filled with lipid droplets in mature cells and few vesicles were seen. Necrotic cells were completely filled with fusing lipid droplets and the nucleus was pyknotic or complete absence of nucleus and cellular demarcation was lost. (Fig 11 a,b). When the basal cell divide one cell remain as basal stem cell and another synthesizes the sebum and remain euchromatic.

In the present investigation the ultra-structure of sweat gland revealed that fundus secretory cell were flat to oval flat linked by a junction complex of zonula occludens. The myoepithelial cell, with myofilaments, around the sweat gland cells. Secretory cells were containing abundance of mitochondria. and ribosomes (Fig 12). The basal duct cells exhibit features common to myoblasts. Microvillis were seen at the free surface of the secretory cells (Fig.12). Gland was supported by the connective tissue. Membrane bound secretory granules were seen at the apical portion of the cell. Secretory cell lies on the basal lamina enclosed by collagen fibers.

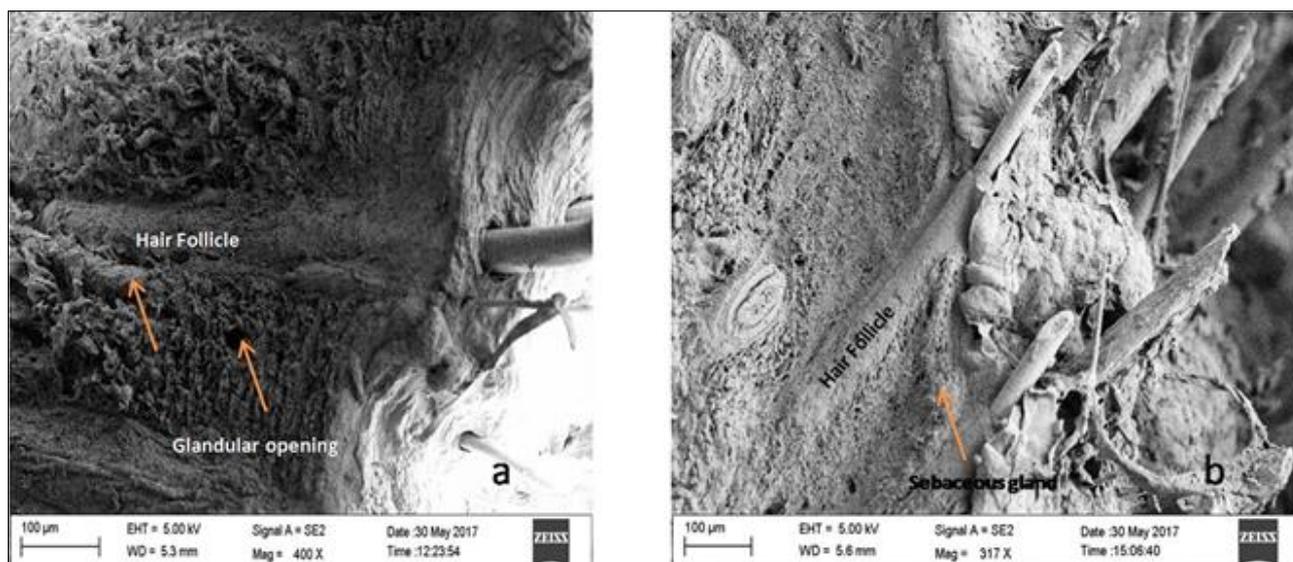


Fig 10: Scanning electron microscopy showing the sebaceous (b) and sweat gland (a) marked arrow)

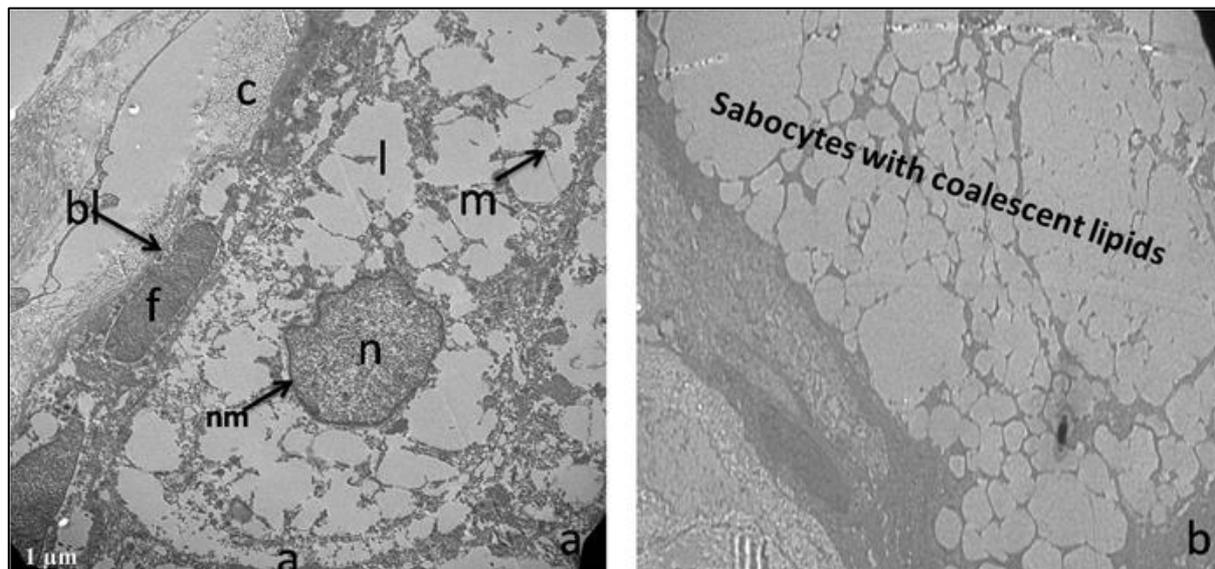


Fig 11: TEM image showing peripheral stem cells of sebocytes with nucleus (n), mitochondria (m), lipid droplets (l), nm (nuclear membrane), supporting connective tissue collagen (c), basal lamina (bl) and fibroblast (f)

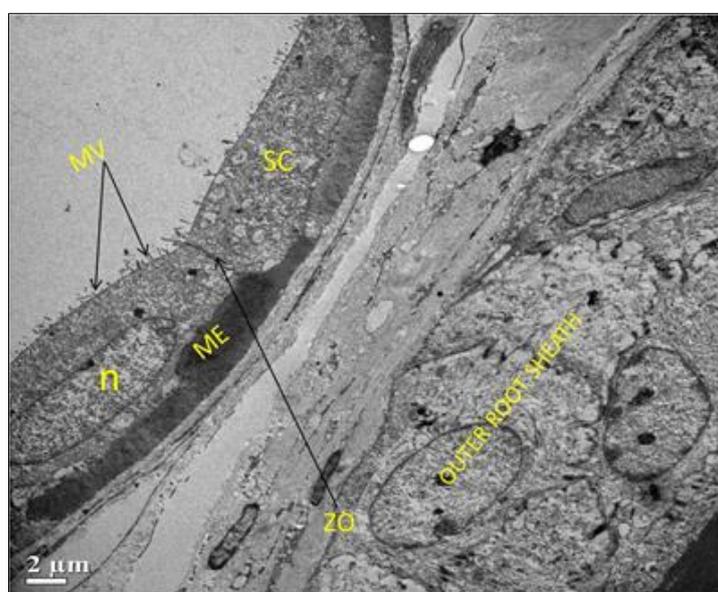


Fig 12: TEM image of sweat gland showing secretory cells (SC), with nucleus (n), microvilli (mv), and myoepithelial cells and outer root sheath of hair follicle (ORS), zonula occludens (ZO)

4. Discussion

In the present investigation the association of sebaceous and sweat glands with hair follicle findings were in consonance with the findings of various authors. (Koul, 1990, Dellman, 1993, Kapadnis, 2004, Pathak, 2012, Bacha, 2012, & Mobini, 2012) [14, 7, 13, 19, 3, 16]. Sebaceous glands were mostly found at the upper part of the dermis in present study which is in support to Mobini, 2012 [16] in Bhaktiari sheep but Dellmann, 1993 [7] has reported that they present only in the deep layer of dermis. The sebaceous glands were monolobed or bilobbed with single or two opening. Similar findings were also reported by Banks, 1993 & Dellman, 1993 [4, 7] in Goat. Sebaceous gland was simple alveolar type gland, looked like many lobed barriers. Dellmann, 1993 [7] reported the sebaceous gland as simple, branched, or compound alveolar in domestic animals, Mobini 2012 [16] in bhaktiari sheep reported branched alveolar type. The sebaceous glands were found at the level of the neck above the isthmus bulge below the infundibulum of the follicle, also reported by Catherin, 2009 [6]. Variable shapes of glands may be round, oval, pear shaped

asymmetrically bilobbed observed. Sebaceous glands were lined by peripheral large squamous to cuboidal sebocytes with cells with distinct nucleus are the stem cell with cloudy cytoplasm were the peripheral zone. Banks, 1993 & Mobini, 2015 [4] reported that sebaceous glands were lined with a simple to stratified cuboidal epithelium. In the present investigation the sebocytes with the cloudy cytoplasm, vacuoles, pyknotic nucleus and fewer organelles details was the characteristics of central zone of the gland. The sebocytes after in this zone get filled with sebum and loss their nuclei and finally reaches the central necrotic zones for propelling their content into excretory duct (Attila, 2018 & Bacha, 2012) [1, 3]. The duct of the sebaceous gland mainly consisted of a simple flat epithelial lining, connected the hair follicles however Dellmann, 1993 [7] has stated that the duct of sebaceous glands lined with stratified squamous epithelium and Mobini, 2012 [16] Iranian Native Sheep reported that the ducts were lined by cuboidal epithelium, this may be due to variation in the breeds of animals.

In the preruminant age groups of Assam Hill Goat showed a very few numbers of sebaceous and sweat gland with least activity amongst the other three age groups (Mobini, 2012)^[16]. The cross sectional profile revealed that the sebaceous glands were found at the lower end of the follicle on two or one side. Nagaraju, 2012^[18] reported that sebaceous glands were present on the either side of the follicle in cattle and in goat in between the hair follicle in cross section.

Sweat glands were located at the lower part of the follicle much below the sebaceous gland of elongated sac, round bag, secular to coil shaped found to be least active in preruminant but well developed, with active epithelium in transitional and ruminant age groups in the present study. Similar observations were also recorded by Bacha, 2012^[3] but Razvi, 2015^[23] reported highest density of sweat glands in neonates than adult. Mobini, 2012^[16] reported that the shape of sweat glands were simple coiled saccular in Iranian Bhaktiari native sheep breed. Pathak, 2012^[19] reported that the sweat glands were coiled and open at the neck of the follicle just above the sebaceous gland in Chegu goat. Nagaraju, 2012^[18] reported the presence of saccular coiled sweat glands at the base of the hair follicle with a highly eosinophilic cytoplasm in goat and in cattle the sweat glands were secular, coiled with a large lumen lined with cuboidal epithelium. Aughey and Frye 2001 & Dellmann 1993)^[2, 7] reported simple saccular or tubular sweat gland in domestic animal, while Mobini, 2012^[16] in bhaktiari sheep reported as simple coiled saccular located mostly were mostly below the sebaceous glands,

The apocrine sweat glands were lined by flattened to low cuboidal but Bacha, 2012^[3] reported the epithelium as flattened to columnar in goat. At some places of sweat gland epithelium secretory caps were seen with eosinophilic cytoplasm. Two types of cells were seen secretory cells and spindle shaped elongated myoepithelial cell rests on the basement with distinct flat nucleus (Bacha, 2012 & Wilke 2007)^[3, 26]. Though Wilke, 2007^[26] reported that the typical secretory cells were columnar. The secretory caps that indicated their secretory activity, were observed in the free apical cytoplasm of secretory cells protruded into the lumen (Dellman, 1993 & Wilke, 2007)^[7, 26]. Secretory caps were observed only in the transitional and ruminant age groups. Mobini, 2012^[16] in bhaktiari sheep also reported that Secretory caps in the sweat glands were existed only in the skin of young adult sheep and more. Height of the epithelium was varied from animal to animal and location to location and age which may be depending on the activity of the gland findings were in accordance with Mobini (2012)^[16] in Iranian Bhaktiari sheep Dellman, 1993 in goat also reported that the shape of the epithelium depends on the glandular activity.

Abundant collagen, reticular fibres were found anchoring the sweat and sebaceous gland. Elastic fibres were found sebaceous gland, sweat gland in all the age groups. Elastic fibres were distinct around the sweat glands (Razvi, 2015)^[23]. The collagen fibres were the thickest in all the age groups compare to other connective tissue fibres. Similar findings

were also recorded by Dellman, 1993) in small ruminants and Mobini, 2012^[16] in Iranian Bhaktiari sheep.

Scanning electron microscopy revealed the presence of sebaceous gland and sweat gland associated with hair follicle. The main fundus portion of the sweat gland was found at the lower part of the follicle almost at the level of hair follicular bulb and the duct runs along the follicular length and opens into hair follicle. Few duct of sweat glands were seen at lower part of follicle. Alveolar sebaceous glands were seen at one side of the follicle opened directly into the hair follicle.

The transmission electron microscopy it was revealed that Sebaceous and sweat glands were in association with hair follicle. The sebaceous gland in the present investigation was surrounded by a connective tissue sheath which consisted mainly of collagenous fibres and fibroblasts also reported by Jenkinson, 1985^[12] in domestic animals. Gland was supported by thin basal lamina which is in accordance with Jenkinson, 1985^[12] in domestic animals, Fred Hossler, 2014^[10] in human. The shape of the sebocytes were flat with round nucleus and inconspicuous nucleolus. Cells were containing numerous lipid droplets, round shaped mitochondria, Golgi bodies and smooth endoplasmic reticulum was the characteristics of the mature cells. The lipid droplets varied greatly in size and number. Lipid droplets fused with each other and increase in size also reported by Fred Hossler, 2014^[10]. The nuclear membrane was dark thick and distinct with attached rough endoplasmic reticulum Very few organelles were visible as the cell was completely filled with lipid droplets in mature cells and few vesicles were seen. Necrotic cells were completely filled with fusing lipid droplets and the nucleus was pyknotic or complete absence of nucleus and cellular demarcation was lost. Similar findings were also reported by, Jenkinson, 1985^[12] in Domestic animals Taha, 1988^[25] in camel. Fred Hossler, 2014^[10] reported that the sebaceous gland was the outgrowth of outer root sheath located just above the insertion of the arrector pilli muscle. When the basal cell divide one cell remain as basal stem cell and another synthesizes the sebum and remain euchromatic.

In the present investigation the ultra structure of sweat gland revealed that fundus secretory cell were flat to oval flat linked by a junction complex of zonula occludens. The myoepithelial cell, with myofilaments, around the sweat gland cells. Secretory cells were containing abundance of mitochondria. and ribosomes. Findings were in consonance with Montgomery, 1982^[17] in goat. Wilke 2007 reported that the typical secretory cells were columnar. The basal duct cells exhibit features common to myoblasts. Microvilli were seen at the free surface of the secretory cells also reported by Montgomery, 1982^[17] in goat he also reported the presence of Dovetailing of lateral microvilli but in the present investigation it was not found. Gland was supported by the connective tissue. Membrane bound secretory granules were seen at the apical portion of the cell. Secretory cell lies on the basal lamina enclosed by collagen fibres.

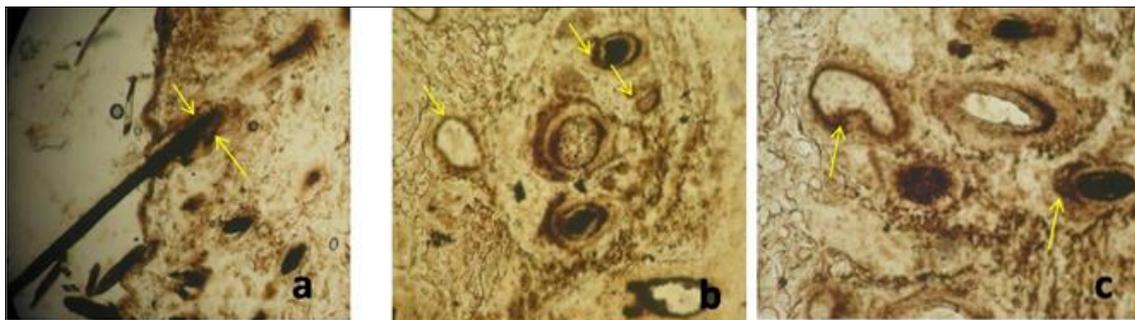


Fig 13: Acid phosphatase activity (Arrow) in prerinant (a), transitional (b) and ruminant(c) age groups.

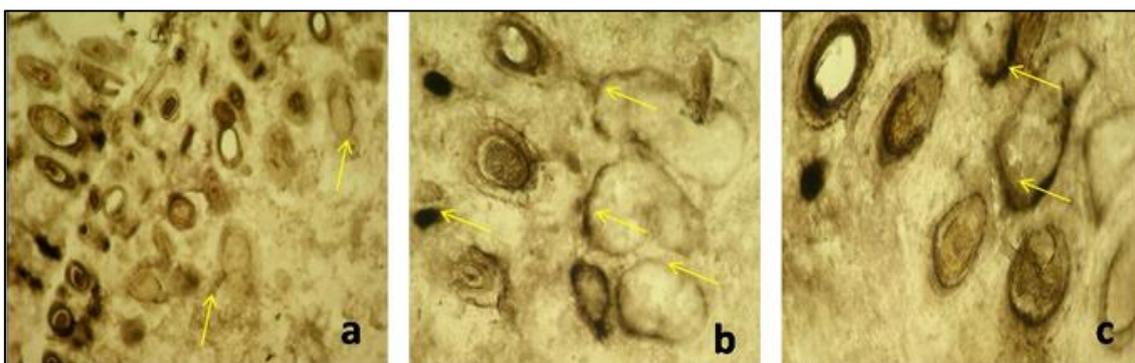


Fig 14: Alkaline Phosphatase activity in prerinant (a), transitional (b) and ruminant(c) age groups.

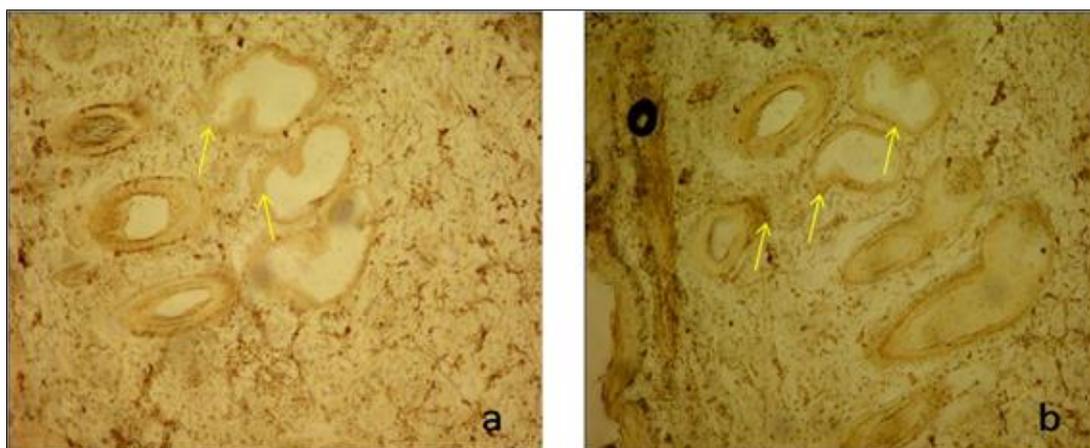


Fig 15: Adenosine Tri phosphatase activity in transitional (a) and ruminant (b) age groups

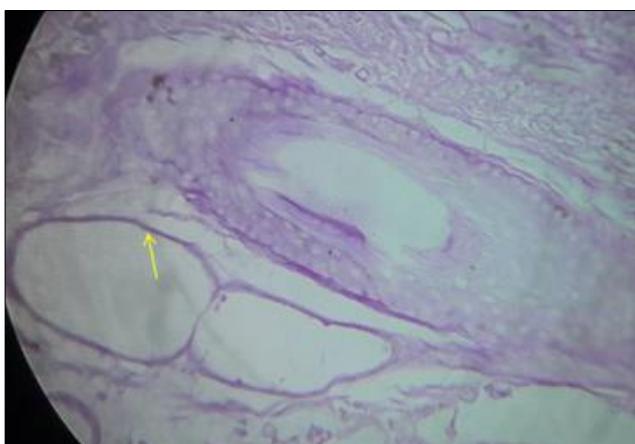


Fig 16: Showing the PAS activity of the sweat gland

strong activity was seen in ruminant age groups animals. Moderate to strong activity in the sweat and sebaceous gland was observed in the ruminant age groups for alkaline Phosphatase. In other groups it showed weak to moderate reaction Handjiski, 1994 ^[11] reported the Sebaceous gland showed follicular cycle dependant activity. A moderate to strong activity also seen in the sweat and sebaceous gland (Saga, 1995) ^[24] reported the alkaline phosphatase activity in the sweat gland of human. In prerinant age groups the sweat and sebaceous gland showed very weak reaction whereas in transitional and ruminant age groups sweat and sebaceous gland showed moderate to strong activity for ATPase. The sweat gland epithelium showed strong reaction for PAS. The glandular secretions of sebaceous glands showed mild PAS reaction. Bhayani, 2005 ^[5] reported that capsules of the sebaceous glands were highly PAS positive in Patanwadi sheep. Razvi, 2015 ^[23] reported that sebaceous glands with intense and its capsule showed mild PAS positive reaction in Bakerwali goat.

5. Histochemistry: Weak to moderate activity was seen for acid phosphatase in the sweat and sebaceous gland in prerinant and transitional age groups. Whereas moderate to

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7. Conflict of Interest Statement: The authors have no conflict of interest

8. Data availability statement: The data that supports the findings of the study are available from the corresponding author upon reasonable request.

ORCID: Sukanta das <https://orcid.org/0000-0003-1468-2594>

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