

E-ISSN: 2320-7078 P-ISSN: 2349-6800 JEZS 2019; 7(1): 1559-1566 © 2019 JEZS Received: 09-11-2018 Accepted: 13-12-2018

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Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com



Histomorphometric study of hair follicle of Assam Hill goat during preruminant, transitional and ruminant age groups

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Abstract

The present investigation was conducted in 18 numbers of Assam Hill Goat divided into three groups (preruminant (0-3 weeks), Transitional (3-8 weeks) and ruminant age groups (above 8 weeks) with six numbers of animals in each group to investigate the Histomorphometric feature of hair follicular feature of Assam Hill Goat. Study revealed two types of hair follicle viz. primary and secondary. Primary being associated with the muscle arrector pilli, sweat gland, sebaceous gland (Mono and bi lobbed) with larger diameter than secondary follicle. Secondary follicles were occasionally associated with sebaceous gland (Mostly monolobbed). Primary follicles were arranged in single, trio, or in group. Follicular groups were well defined in ruminant age groups. The longitudinal section of hair follicle showed infundibulum, isthmus, supra bulbar and bulbar region. Isthmus was the thickened outer root sheath with distinct darkly stained nucleated cell. Dermal papilla was well defined cone shaped with dark stained connective tissue cells. Matrix region of the hair follicle showed differentiated and non differentiating zone with cell dividing figure. Melanocytes were found on the basement of dermal papillae. In cross section hair follicle was round in profile with medulla, cortex, cuticle, inner root sheath, outer root sheath and connective tissue sheath. The inner root sheath was keratinized with 2-3 layered flat nucleuses. Outer root sheath was consisted of 3-4 layers of large cell with thin cytoplasm and the outer layer with obliquely directed nucleus. The hair follicle, sweat and sebaceous gland was covered by abundant on collagen, reticular and elastic fiber. Abundance of elastic fibers was found at the insertion site of arractor pilli muscle. Network of connective tissue in form network at the base of hair follicle was observed in cross section. Various micrometrical parameters like length of hair follicle, breadth and length of hair bulb, dermal papilla, diameter of primary follicle was found to be significantly higher during ruminant age groups than preruminant and transitional age groups.

Keywords: hair follicle, Assam hill goat, histomorphology, micrometry

Introduction

Hair follicle is an integral part of mammalian skin act as body's protective barrier against its external environment. The hair follicle is an autonomous mini-organ, which provides an excellent model system for studying the biology of adult stem cells (SCs) (Jaks) ^[1], plays important role in wound healing, secretion of pheromones and an unique sensory apparatus. Hair follicles vary considerably in size and shape, depending on location. It is the only organ in the mammalian body which, for its entire lifetime, undergoes cyclic transformations from the stages of rapid growth (anagen) to apoptosis driven regression (catagen) and back to anagen, via an interspersed period of relative quiescence (telogen). (Paus and Araújo) ^[2, 3]. There is a paucity of literature regarding the anatomical norms of hair follicles of the Assam Hill Goat. So being, Assam Hill Goat, a major indigenous non-descript variety in the state of Assam the present study will provide a base line data for further study.

Materials and Methods

The present investigation was conducted in 18 numbers of Assam Hill Goat (*Capra hircus*) divided into three groups viz. (preruminant (0-3 wks), Transitional (3-8 wks) and ruminant age groups (above 8 wks) with six numbers of animals in each group. Animals were selected from in an around the Guwahati city. Aseptically 3-4 mm skin samples were collected from the leg region from each age groups and fixed in 10% neutral buffered formalin solution and processed as per the standard technique of procedure (Luna ^[4]). The paraffin blocks were sectioned in Shandon Finesse microtome at 6μ m thickness, and the sections were stained with

different staining methods as per Luna ^[4]. Mayer's Haematoxylin and Eosin staining technique for histoarchitexture of hair follicle, Van Gieson's method for collagen fibres, Gomori's method for reticular fibres., Hart's method for elastic fibres, Bielchowsky's method for axis cylinder and dendrites.

Different parameters of histo-micrometry were recorded on Haematoxylin and Eosin stained sections by means of standard methods of micrometry using Nikon E 200 camera mounted microscope and Image Pro Express Ver-2.0 Software (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 dated 30.07.2016, Assam Agricultural University: Khanapara, Guwahati-781022)

Results and Discussion

The present investigation of hair follicle of the Assam Hill Goat (Capra hircus) revealed the presence of two types of hair follicle viz; primary and secondary. Primary being the larger in diameter than secondary and located at the deeper part of the dermis at an oblique angle by arrector pilli muscle in association with sebaceous, sweat gland in all groups (Fig:1a,b,c, 2,a,b,c, 3,a,b,c). Findings were in consonance with Koul^[5] in non-woolly goat, Dellman^[6] in small ruminants, Moradi ^[7] in Raini goat, Kapadnis ^[8] in Osamnabadi goat, Pathak^[9] in Chegu goat, and Bacha^[10] in goat, Fozi^[11] in Raieni Cashmere Goat, Mobini^[12] in Iranian Bhaktiari sheep. Whereas in sheep the hair follicle were straight reported by Bacha^[10]. Mobini^[13] in Lori- Bakhtiari sheep. Though Mobini ^[12] found the superficial secondary hair follicles in association with arrector pili muscle, sebaceous glands, and the primary follicles without sebaceous glands in Iranian Bhaktiari sheep.

The secondary follicles were mostly located at the upper part of dermis in scattered or in group and devoid of all the accessory structure but occasionally associated with monolobed or bilobbed sebaceous gland (Fig: 4, a,b,c). Similar findings were also reported by Banks^[14] & Dellman ^[6] in Goat. Renani ^[15] reported the absence of sweat gland was the distinguishing structure differentiating secondary follicles in Iranian Cashmere goat. Pathak ^[9] in Chegu goat reported that secondary follicles were arranged in clusters which varies from place to place. Fozi ^[11] in Raieni Cashmere goat reported that secondary follicles were associated with monolobed sebaceous gland. Mobini ^[13]. In Lori-Bakhtiari sheep. The secondary follicles in the present investigations were devoid of medulla (Fig: 6) Finding was supported by Kapadnis ^[8] in Osamnabadi goat and Mobini ^[12] in Iranian Bhaktiari sheep.

Primary follicles of Assam Hill Goat were arranged either singly, paired, trio, in linear arrangement or as a compound follicular unit as also reported by Moradi ^[7] in Raini goat, Fozi ^[11] in Raini cashmere goat (Fig 5,a,b,c,d). Compound hair follicles were not well defined during preruminant age groups (Fig1a, 4a). In transitional and ruminant age groups compound follicles with 2-3 primary follicle and 2-3 numbers of secondary follicle and 2-3 primary follicle and 3-4 numbers of secondary follicle mostly respectively (Fig: 3a. 4c 5c,d). Dellman ^[6] in small ruminant and Khan and Talukdar ^[16] reported 3 primary follicles were associated with 3-6 secondary follicle in Black Bengal goat. Kapadnis ^[8] reported 3 primary follicles associated with 3 to 5 secondary hair follicles in Osamnabadi goat. Nagaraju ^[17] reported single row of primary follicle associated with 2-3 secondary follicle in goat. So this arrangement of follicle can be specific criteria of Assam Hill Goat.

The cross sections of hair follicle in all the three age groups of the Assam Hill Goat at various level of hair follicles were observed for zone of protein synthesis, zone of differentiation, zone of keratinization, zone of elongation zone. Zone of protein synthesis at dermal papillae characterized with active dermal papilla with distinct nucleus and melanocytes attached to basement with dark pigments. (a), zone of differentiation with differentiating cell and forming the various cell lineages of the hair follicle (b), zone of keratinization with all the differentiated cell lineages and keratinized cortex and medulla) (c) and zone of elongation zone was with elongated keratinized hair follicle (d). (Fig:8,9 and 10).

At cross section, the hair follicles were found to be consisted of medulla, cortex, cuticle, inner root sheath, outer root sheath, connective tissue sheath from inner to outer in all the age groups of Assam Hill Goat (Fig:7 and 8c,9c,10c). Findings were in accordance with Dellman^[6] in small ruminants, and Banks^[14] in goat, Kapadnis^[8] in Osamnabadi goat, Razvi^[18].

The eusinophilic Inner root sheath was consisted of 2-3 layers of cell (Fig.11), findings were in accordance with Dellman^[6] and Banks^[14] in goat and Kapadnis^[8] in Osamnabadi Goat. Keratinization of inner root sheath ended at the height of sebaceous gland opening. (Fig: 11c) Henley's layer of inner root sheath was a single layer of flattened nucleus. The inner root sheath immediately below the entrance of the sebaceous gland of the hair follicles were corrugated and formed circular folds (Fig:11c) findings were in agreement with Dellman^[6] in small ruminant. Outer root sheath continued with epidermis (Fig.13) similar was also reported by Banks ^[14] in small ruminant. Outer root sheath was composed of 3-4 cell layers with large round to oval nuclei with thin cytoplasm and the outer marginal cells were obliquely directed. (Fig.7). The thickness and cellularity of outer root sheath varies with the level of follicle along the length. It is single layered at the level of bulb higher up composed of multi layered cells (Fig:12).Similar observations were also recorded by Steen [19] in human.

Longitudinal section hair follicle showed well defined infundibulum (Fig.13), isthmus and bulber neck region and bulb region (Fig: 14a). Bulge region was thickened outer root sheath with distinct and darkly stained with large nucleus where the arrector pilli muscle inserts (Fig: 14a, b,c). (Torkaman) ^[20]. The cells of bulge region showed different morphology other than the outer root sheath cells (Fig: 15 c). Steen ^[19] reported the existence of label retaining cells at the bulge in a undifferentiated form in human. The alveolar sebaceous glands lined with cuboidal large cell were found at the level isthmus bulge of the follicle mostly bi lobbed with a single or two opening. (15 a,b, c,d,e,f). 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: 1,4,5).

The cone shaped dermal papillae was characterized by dark stained elongated nuclei (Fig 12.) and reticular fibers network and connective tissue cells. (Bacha) ^[10] in small ruminant and Mecklenburg ^[21] in goat .

Matrix region of the hair bulb in the present study showed the undifferentiated zone and differentiated zone (Fig.12, 16). Most of the cells around the dermal papillae and adjacent area was showing cell dividing figure with distinct nucleus (Fig:16) findings were supported by Steen ^[19] in human. The

dark coloured melanocytes were found at the basement margin of the dermal papillae (Fig. 12, 16).

Few sebaceous glands were found to be monolobbed in both primary and secondary follicle in the present study (Fig: 6). Which was in agreement with Fozi ^[11] Raini Cashmere Goat. Sweat glands were located at the lower part of the follicle much below the sebaceous gland (Fig.17) of various shape found to be least active in preruminant but well developed, with active epithelium during transitional and ruminant age groups with secretory caps (Fig.1a,2a,3a,4,15a,17). Nagaraju ^[17]. Reported the presence of saccular coiled sweat glands at the base of the hair follicle with a highly eusinophilic cytoplasm in goat

The connective tissue sheaths of the hair follicle were composed of abundance of collagen and reticular fibers with elastic fibers in all the three age groups. (Fig 18, 19, 20). Findings were in agreement with Nagaraju^[17] in goat.

Collagen, elastic and reticular fibers were found to be run along the length of the arrector pilli muscle. Elastic fibers were also found at the insertion site of arrector pilli muscle. (Fig 18, 19, 20). and around the compound hair follicle unit, sebaceous gland, sweat gland in all the age groups (Fig 18, 19, 20) similar findings were also recorded by Dellman^[6] in small ruminants and Mobini^[12] in Iranian Bhaktiari sheep. Extensive reticular fibers network were seen arround the follicle, glands, and arrector pilli muscle and dermal papilla core as well as found to run with the arrector pilli muscle. The bases of the follicle were found to be anchored with fine network of reticular fibers in all the age groups in the present study. (Fig 18, 19, 20) Nagaraju ^[17] reported Reticular fibers were uniformly distributed surrounding the hair follicle in goat and cattle. Razvi ^[18] in reported the presence of reticular fiber network around the follicle forming a busket Bakerwali Goat.

Abundance of fine elastic fibers was seen running parallel to the direction of the arrector pilli muscle and particularly at the insertion site particularly at the bulge region (Fig.20). Also reported by Dellman ^[6] in small ruminant, Razvi ^[18] in Bakerwali Goat. Thin nerve fibers were found around the sweat gland, sebaceous gland and hair follicle at the base and at the insertion of the arrector pilli muscle. Nagaraju ^[17] reported abundance of nerve fibers in goat at the root of the hair follicle.

Micrometry of the various compartment of the hair follicle

The mean primary follicular depth in the present investigation during preruminant, transitional and ruminant age groups were not significant (Table-1). But the values were higher during ruminant age groups compare to preruminant age groups. Mobini ^[22] reported the primary follicle depth is 580-1193.33 μ . Shabir ^[23] reported the minimum and maximum depth of primary follicles (784.33 and 1935.0 μ , respectively) Madras red sheep, Kapadnis ^[8] reported that the depth of primary and secondary follicles were 41±3.56 and 36±2.90 micrometer respectively in Osamnabadi goat.

The length of follicle was significantly higher in ruminant age group (1083.33 \pm 69.12 μ (Table-1) than the other groups. Length of hair follicular bulb were significantly lower during preruminant (Table-1).

Follicular bulb breadth was significantly high during ruminant age groups (125.83 \pm 4.36 μ (Table-1). Mecklenburg ^[21] stated that the hair bulb diameter of primary hair follicle in sheep

and goat ranges from 180-300 micrometer.

Diameter of primary follicle was significantly higher in ruminant age groups $(140.00\pm12.909 \ \mu$ (Table-1) than preruminant and transitional age groups but the diameter of secondary follicle did not show much significant difference.

Length of dermal papillae was significantly higher in ruminant age group (95.166±6.300 μ (Table-1) than the other groups. Breadth of dermal papillae was significantly higher in transitional and ruminant age groups than preruminant age group. The mean breadth of dermal papillae in the present investigation during preruminant, transitional and ruminant age groups were 21.500±1.460 μ , 29.16±1.108 μ and 32.83±2.072 μ respectively.

Primary follicular density did not show much significance. The mean primary follicular density in the present investigation during pre ruminant, transitional and ruminant age groups were 9.66 $\pm 0.66 \ \mu$, 8.50 ± 0.61 and 8.33 ± 0.802 (Table-1). Secondary follicular density showed significant higher values duirng preruminant age groups than transitional and ruminant age groups. Total follicular density/mm² was significantly higher during pre ruminant (Table-1) age groups than other. Various author reported the follicular density Pathak^[9] reported 30.66±9.68 follicle/mm square in Chegu goat. Kapadnis^[8] 44.30±7.30 with a range of 18-86 in osamnabadi goat. Renani [15] reported that the mean primary, secondary and total primary plus secondary follicle density of Cashmere goat breeds was 2.8±0.1, 30.6±0.4 and 33.4±0.5 per square millimetre. Koul ^[5] reported total follicles per square mm for Black Bengal, Jamnapari, Barbari and Sirohi goats were 16.83 +/- 1.39, 15.86 +/- 1.08, 17.66 +/- 1.41 and 13.19 +/- 1.41 respectively. Mobini ^[24] in adult Iranian native sheep reported that the mean total follicle density per square mm in the various skin regions was 6.25-9.03.

Secondary and primary follicular ration did not show much significant difference. The mean secondary and primary follicular ratio in the present investigation during preruminant, transitional and ruminant age groups were 1.552 ± 0.07 , 1.33 ± 0.084 and 1.55 ± 0.198 respectively. Various author reported the secondary and primary follicular ratios. Koul ^[5] reported secondary/primary follicle ratios (S/P) for Black Bengal, Jamnapari, Barbari and Sirohi goats were 1.57 +/- 0.21, 1.15 +/- 0.16, 1.61 +/- 0.21 and 2.04 +/- 0.21, respectively. Pathak ^[9] reported 18:9 in Chegu goat. Kapadnis ^[8] reported 2.72 \pm 0.64 in Osamnabadi Goat.

Summary and conclusion:

The detailed histomporhological studies of hair follicle with its associated structures of Assam Hill Goat can be used as a baseline study for the researcher and scientist. The basic histomorphological features can also be used for histological identification of Assam Hill Goat. The basic anatomical details of hair follicle of Assam Hill Goat being the local important breed can be used for future research work in the field of stem cells, experimental model studies.

Acknowledgement

The Authors duly acknowledged the Head of the Department of Anatomy & Histology, College of Veterinary Science, Assam Agricultural University, Khanapar, Guwhati Assam, India for providing all the facilities of laboratory. The author would like to thank the Dean, DPGS of faculty of Veterinary Science, Khanpara, AAU, Khanapara, Guwahati, Assam.

Cable 1: Micrometrical observations of various	parameters of hair follicle (Mean±SE)	of Assam Hill Goat (<i>Capra Hircus</i>)

	Preruminant	Transitional	Ruminant
Parameters of hair follicle	Mean±SE	Mean±SE	Mean±SE
Depth of hair follicle	$610.00 \pm 73.93 \mu$	756.66± 62.27 μ	831.16±51.41 μ
Length of hair follicle	813.33 ^a ±23.04 μ	898.33 ^a ±32.80 μ	$1083.33^{b} \pm 69.12 \ \mu$
Length of hair follicular bulb	$180.33^{a} \pm 7.007 \ \mu$	225.83 ^b ±9.86 μ	253.50 ^b ±13.02 μ
Breadth of hair follicular bulb	100.83 ^a ±4.90 μ	110.00 ^{ab} ±3.65 μ	125.83 ^b ±4.36 μ
Primary follicle diameter	$82.25^{a} \pm 4.44 \mu$	106.75 ^{ac} ±7.49 μ	140.00 ^b ±12.909 μ
Secondary follicle diameter	27.50 [*] ±2.816 μ	29.083*±2.161 μ	30.33*± 1.382 μ
Length of Dermal papillae	$57.833^{a} \pm 4.475 \ \mu$	74.50ª±2.929 μ	95.166 ^b ±6.300 μ
Breadth of dermal Papillae	$21.500^{a} \pm 1.460 \ \mu$	29.16 ^b ±1.108 μ	32.83 ^b ±2.072 μ
Primary follicle density /mm ²	9.66 ±0.66 μ	8.50±0.61 μ	8.333±0.802 μ
Secondary follicle density /mm ²	$14.833^{a} \pm 0.703 \ \mu$	11.167 ^b ±0.60 μ	12.16 ^b ±0.47 μ
Total Follicular density/mm ²	$24.500^{a} \pm 1.25 \ \mu$	19.667 ^ь ±1.11 μ	20.50 ^b ±0.957 μ
Seconday/primary follicle	1.552±0.07	1.33±0.084	1.55±0.198
Aeans within the same row in each item within each group carrying different superscripts are significantly different at (p<0.05),			

Graphical representation of various parameters of hair follicle of Assam Hill Goat during Preruminant (P), Transitional (T) and Ruminant (R)



De= depth of hair follicle, Le= length of hair follicle, Lb= length of hair bulb, Bb= breadth of hair bulb, Pd= diamter of primary follicle, Sd= diamter of secondary hair follicle, LDP= length of dermal papillae, BDP= breadth of dermal papillae, PFD= primary follicle density, SDF= secondary follicular density, TFD= total follicular density, SF= secondary and primary follicle ratio.



Fig 1: (a, b, c) Preruminant

Fig 2: (a, b, c) Transitional

Fig 3: (a, b, c) Ruminant



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Fig 4: photograph showing the distribution of follicles in the dermis a (preruminant), Transitional (b) and c (ruminant) at 100X H& E



Fig 5: photograph showing the random (a, prerumiant, 100X) Group (Transitional (b at 100X) and Trio (c,d at 100X)H& E



Fig 6: photograph showing the secondary follicle devoid of medulla and primary follicle with lattice of medulla pattern X 1000 H& E



Fig 7: photograph showing the various layers of hair follicle at longitudinal and cross section X 1000 H& E



Fig 8: (a, b, c, d) Preruminant

Fig 9: (a, b, c, d) Transitional



Fig 8, 9, 10 (a, b, c, d): showing the various functional zones of hair follicle a (dermal papille), b (differentiating zone), c (keratinizing zone), d (elongation zone) at 1000X H&E; co=cortex, m= medulla, irs=inner root sheath, ors= outer root sheath, cts = connective tissue sheath.



Fig 11: photograph showing the eusinophillic irs with flat nuclei (a) and irs discontinued after opening of sebaceous gland (c) at 1000x h & E



Fig 12: photograph showing the various layers of hair follicle, dermal pappilae with dark stained nucleus, and glassy membrane (gm) at 1000x h & E

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Fig 13: photograph showing infundibulum region of hair follicle at 400x h & E



Fig 14: photograph showing isthmus, supra bulber, bulber bulge region of hair follicle at a, b400x and c (1000X) h&E



Fig 15: photograph showing the sebaceous gland in preruminant (a, b), transitional (cd), and ruminant (ef) age groups at 1000X H&E



Fig 16: photograph showing the buber region with differentiating and non differentiating region at 1000X H&E



Fig 17: photograph showing the sweat gland in preruminant (a), transitional (b), and cruminant age (Showing secretory caps) groups at 1000X H&E



Fig 18: photograph showing the distribution of collagen fibers associated with hair follicle400X Von Geissons stain



Fig 19: photograph showing the distribution of reticular fibers associated with hair follicle400X Von Geissons stain



Fig 20: photograph showing the distribution of elastic fibers associated with hair follicle400X Von Geissons stain

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