Journal of Animal Research: v.7 n.2, p. 377-384. April 2017

DOI: 10.5958/2277-940X.2017.00054.7

Histogenesis of Skin in Early Prenatal Goat (*Capra hircus*)

Prabhakar Kumar*, Ajay Prakash, MM Farooqui, Archana Pathak, Sri Prakash Singh and Varsha Gupta

Department of Veterinary Anatomy, College of Veterinary Science & Animal Husbandry, DUVASU, Mathura, U.P., INDIA

**Corresponding author: P Kumar; Email: drdablu@gmail.com*

Received: 29 Jan., 2017 **Revised:** 22 Feb., 2017 **Accepted:** 28 Feb., 2017

ABSTRACT

The study was conducted on the skin of 12 goat embryo/ foetii aged between 23 to 50 days of gestation. Between 23 to 28 days of gestation the embryos surface epithelium contained a single layer of flat cells. The periderm layer was first noticed in patches in the thoracic limb at 28 days of gestation and appeared in other region of body between 32 to 34 days of gestation. The cells of periderm were flat with spindle shaped dark nuclei while basal layer was made up of cuboidal cells. Distinct continuous periderm was first observed in 40 day old embryo. At this stage, the basal layer contained cuboidal cells while periderm had flattened cells. Discontinuous thin basement membrane was observed at many places. At 42nd day of gestation at some places the epithelium consisted of 3 – 4 layer of cells. The cells of middle layers were polygonal in shape with dark staining eccentrically placed rounded nuclei; these cells were comparatively larger than the cells of basal layer and their nuclei were relatively smaller. Between 46th -50th days of gestation the surface epithelium on the dorsal aspect of foetii was comprised of 2-3 layers only whereas in remaining part considerable variation was noticed in the number of layer. The subepithelial connective tissue was cellular in the early stage in which reticular fibers first appeared at 40 days of gestation and formed network at 44 days of gestation but no collagen or elastic fibers were seen.

Keywords: Histogenesis, epidermis, skin, prenatal, goat

The goat sector contributes about INR 14453 crore to the Indian economy, out of this INR 648 crore from skin which is about 8 percent of total GDP from livestock sector (Chakrabarti *et al.*, 2014). The slaughter by-products, the skin and fibers of goat are utilized as raw material by the leather and textile industries (Ozfiliz *et al.,* 2002). Skin plays many important roles viz. protective mechanical barrier, prevention of loss of moisture from body, thermoregulation, synthesis of Vitamin D. Due to presence of sensory receptors the immediate environment (touch, pressure, heat, cold, pain etc) is experienced by animals through its skin (Dyce *et al.*, 1996). On perusal of literature it is observed that very little attention has been paid on the chronological study of the histogenesis of skin in goat particularly in early prenatal period. Therefore, the present study was designed with the aim that it will not only fill the gap in the literature but also will help the scientific community.

MATERIALS AND METHODS

The present study was conducted on the skin of twelve apparently healthy goat embryo/ foetuses of non descript breed between 23 to 50 days of gestation. Immediately after collection, the weight of the embryo/ foetus was recorded. Approximate age of the embryos (upto 30 days of gestation) were estimated on the basis of foetal measures and phenotypic characteristics given by Njaa (2012) and Anonymous (2008) in goat. The age of the remaining embryos/ foetuses was estimated by using the formula derived by Singh *et al.* (1979) in goat after interpolation of formula of Hugget and Widdas (1951). Upto 44 days of gestation, the whole embryos/ foetus were processed for paraffin embedding technique. After 44 days gestation the foetuses were bisected longitudinally and one part of the foetus was processed for paraffin embedding technique. Five to six micrometer thick sections were stained with hematoxylin and eosin, Wilder's reticulin

stain, Weigert's resorcin fuchsin stain (Luna, 1968) and Mallory's triple stain (Crossman's modification, 1937). Micrometry was done with the help of computerized Leica DM750 microscope. For each micrometric parameter six readings were taken at different places in the same slide. The micrometrical observations data were subjected to statistical analyses by SPSS version 17.0 software for the test of significance (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Surface Epithelium (Epidermis)

At 23 days gestation the surface epithelium of goat embryo mainly consisted of a single layer of flat cells (Fig. 1). At this stage the average height of the epithelium was 4.42±0.20 µm (Table 1). The nuclei of most cells were flat darkly stained and were located at the centre of the cells (Fig. 1). The averages of vertical and horizontal dimensions of the nuclei of the epithelial cells were 3.75±0.10 and 4.53±0.23 µm, respectively. At most places the junctions between adjacent cells were indistinct and the cells were loosely arranged.

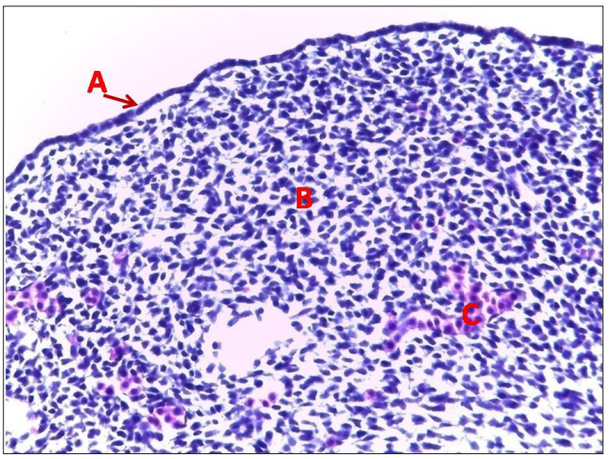

Fig. 1: Photomicrograph of section of 23 day old goat embryo showing single cell layer of epithelial covering (A), undeifferentiated polyhedral cells (B) and hemopoietic blood islands (C). H & E X400

However, at some places compactly arranged cuboidal cells having centrally placed spherical and oval, vesicular nuclei were also encountered; these cells were relatively in close contact to each other and the junctions between the adjacent cells were distinct. At this stage the cytoplasm of the epithelial cells was generally darkly eosinophillic, however, few cells having light eosinophillic cytoplasm were also present. In other embryo of the same gestational age the covering epithelium often consisted of brick shaped cells among the flat cells. The brick shaped cells had centrally placed dark staining large spherical nuclei. Razvi *et al.* (2015) observed single layer of squamous cell in the epidermis of Gaddi sheep at 7.0 cm CRL stage. At 25th and 28th day of gestation the cell shape and the cytological characters of the epithelial cells were almost similar to that of 23rd day old embryo, however, the flat epithelial cells were relatively in close contact with the adjacent cells and the ends of adjacent cells often overlapped (Fig. 2). The average height of epithelial cells and dimensions of their nuclei in these embryo were almost same.

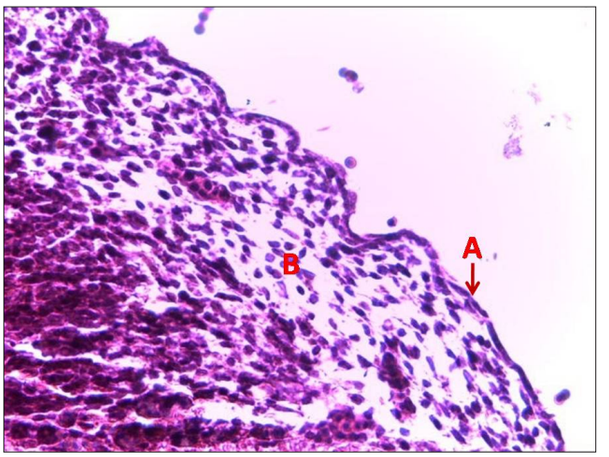

Fig. 2: Photomicrograph of section of 28 day old goat embryo showing single cell layer flattened epithelial cells (A) and subepithelial tissue (B). H & E X200

In 28 days old embryo the thoracic limb bud was visible and the epithelial covering in this area generally consisted of two layers of flat cells, which may represent the basal and periderm layers. At some places in this region, the epithelium consisted of a layer of crowded cells above the basal layer which varied in shape and size (Fig. 3). Mitotic cells were often observed among these crowded cells. This may indicate the process of differentiation of various strata in the epidermis.

In 32 and 34 days old goat embryo the epithelial covering

Table 1: Thickness of epidermis, height of basal cells and dimensions of basal cell nuclei (Mean ± SE) in early prenatal goat

Parameter	Gestational Age (days)											
	23	**25**	**28**	**32**	**34**	**38**	**40**	**42**	**43**	**44**	**46**	**50**
Thickness of epidermis	4.42 ± 0.20^{a} (3.85-5.04)	5.00 ± 0.26 ^{a} (4.18-5.83)	4.96 ± 0.30 ^{a} (3.92-5.73)	10.49±0.34^{bc} (9.23-11.18)	10.19 ± 0.2 ^{b} (9.55-11.33)	11.81 ± 0.45^{c} (10.23-13.18)	13.99 ± 0.77^{d} (11.55-15.91)	19.11 ± 0.38^{e} (17.93-20.29)	18.26 ± 0.33^{e} (17.28-19.26)	23.08 ± 0.75^{f} (20.06-24.88)	23.75 ± 0.51^{f} (21.98-25.39)	26.73 ± 0.77^{g} (24.78-29.08)
Height of basal cells	4.42 ± 0.20^{a} (3.85-5.04)	5.00±0.26^{b} (4.18- 5.83)	4.96 ± 0.3^{a} (3.92- 5.73)	6.34±0.16^{bc} (5.92- 6.89)	7.02 ± 0.17^{cd} (6.22- 7.41)	7.7±0.32^{d} (6.93- 8.82)	8.04±0.22^{e} (7.06- 8.56)	10.69 ± 0.32^{ef} (9.32-11.58)	11.41±0.27^{ef} (10.89-12.61)	11.73 ± 0.30^{ef} (10.49-12.58)	10.85 ± 0.6^{ef} (8.45-12.37)	10.92 ± 0.29^{f} (10.12-11.88)
Dimension of basal cell nuclei	VD 3.75±0.10 (3.46-4.08) HD 4.53±0.23 (3.94-5.52)	VD 4.69±0.15 (4.19- 5.2) HD 6.14±0.14 (5.75-6.69)	VD 4.62±0.22 (4.01- 5.44) HD 6.75±0.19 (5.96-7.32)	5.33±0.13^{ab} (4.93- 5.89)	5.46±0.31^{bc} (4.37- 6.52)	6.16±0.38^{cd} (5.11- 7.22)	6.59±0.22^{e} (5.69- 7.12)	6.52±0.18^{e} (5.93-7.02)	6.45±0.17^{e} (5.82-7.01)	6.61 ± 0.21^{e} (6.01-7.16)	6.64 ± 0.48^{e} (5.29-8.27)	6.72±0.28^{e} (5.93- 7.79)

VD and HD represent the vertical and horizontal dimensions of nuclei flat cells respectively. The nuclei at rest of the stages were rounded and its diameter was measured.

*Mean with different superscripts differ significantly ($p<0.01$) row wise.

was formed by cuboidal cells and often a layer of flat cells (periderm) was observed over the basal layer (Fig. 4). The latter was more conspicuous in 34 day old embryo particularly in the caudal region. Moreover, at 34 days gestation undulations were also found in the surface epithelium. At these stages the epithelial height was almost same and measured as 10.49±0.34 µm and 10.19±0.26 µm, respectively (Table 1).

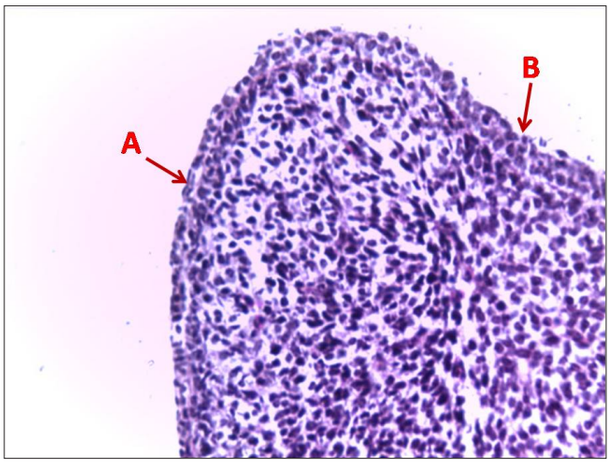

Fig. 3: Photomicrograph of section of 28 day old goat embryo showing double cell layer of flattened epithelial cell (A) and crowded epithelial cells (B) in thoracic limb bud region. H & E X400

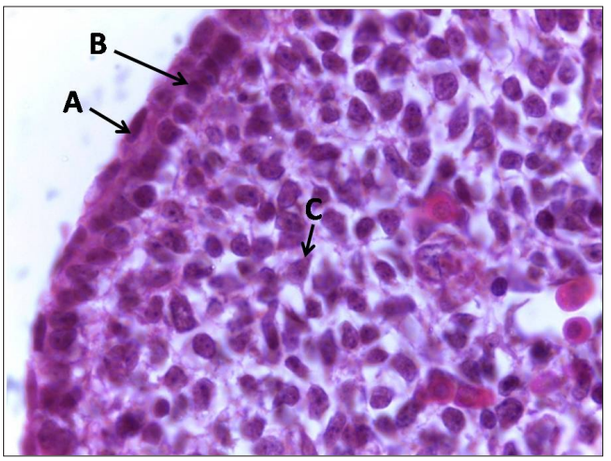

Fig. 4: Photomicrograph of section of 32 day old goat embryo showing flat periderm cells (A), cuboidal basal cells (B) and mesenchymal cell (C). H & E X1000

The above finding indicated that the periderm began to appear first in the thoracic limb bud region and then over the other areas of goat embryo. Distinct continuous periderm layer over the basal layer was observed in goat embryo at 40 days gestation where the epithelium at all places was clearly divisible into two layers. The basal layer was formed by the cuboidal cells with darkly eosinophillic cytoplasm and darkly stained rounded nuclei. The periderm consisted of flattened cells having flat nuclei and eosinophillic cytoplasm. The junctions between adjacent cells of periderm were not clearly demarcated. Below the basal layer at many places sporadic thin eosinophillic basement membrane was found at this stage. The average thickness of epithelium, height of basal cells and diameter of the nuclei of basal cells were found to be 13.99±0.77µm, 8.04±0.22 µm and 6.59±0.21 µm, respectively. Lin Ji-Mao *et al.* (2008) in the skin of goat foetus at 9 week of gestation mentioned that the epidermis was madeup of two layers viz. the surface and the germinative layer.

Researchers have reported the presence of periderm and basal layer in the epidermis of human foetus at second month of gestation (Horstmann, 1957; Serri and Montagn, 1961; Arey, 1974; Copenhaver *et al.*, 1967; Holbrooke, 1983 and Mc Geady *et al.,* 2006) in domestic animals and stated that the cells of the periderm were flattened whereas, the basal layer was composed of cuboidal cells. At 42 days gestation the epidermis of goat foetus showed wide variation in its structural characteristics. At several places it had basal and periderm layers as found at 40 days gestation. However, at some places it was formed by three layers as an intermediate layer was observed between basal and periderm layers. The intermediate layer consisted of large, polygonal lightly stained cells having dark spherical or oval eccentric nuclei. At some other places, particularly where periderm was not visible, the epidermis consisted of a layer of crowded polyhedral and cuboidal vacuolated cells above the basal layer (Fig. 5).

The nuclei of the cells of this layer were rounded and varied in location and were either vesicular or darkly staining. Many of these were located very close to the upper margin of the cells and often projected from the surface of the embryo. At few places the epidermis was made by four layers, all the layers above basal layer were madeup of polyhedral cells with either dark staining or vesicular eccentrically placed rounded nuclei. The cells of these layer were comparatively larger than the cells of

basal layer and nuclei were relatively smaller in size. The margin of cells were distinct and contained thin rim of eosinophillic cytoplasm, few of the cells were vacuolated. These cell layers represented early differentiation of stratum spinosum.

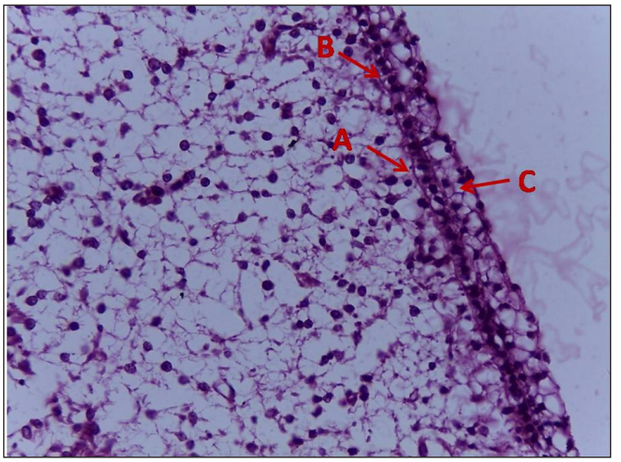

Fig. 5: Photomicrograph of section of 42 day old goat embryo showing basement membrane (A), stratum basale (B) and polyhedral vacuolated cells (C). H & E X400

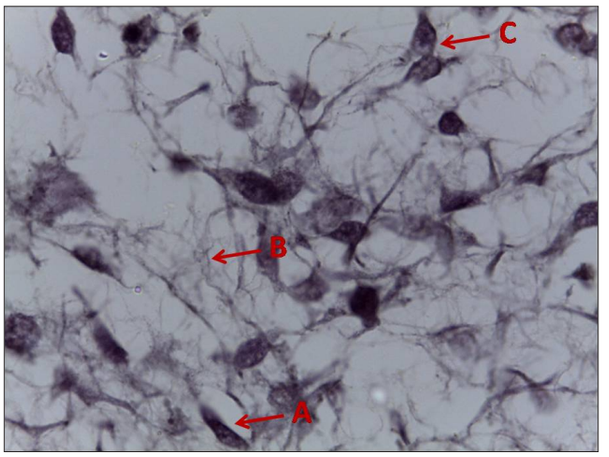

Fig. 6: Photomicrograph of section of 44 day old goat foetus showing fibroblast (A), reticular fibers (B) and mesenchymal cell (C) in dermis. Wilder's Reticular Method X1000

However, according to McGeady *et al.* (2006) in domestic animals the stratum spinosum first appearsed close to mid pregnancy. Contrary to the present study the appearance of the intermediate layer was observed at 10th week of gestation in goat (Lin Ji-Mao *et al.*, 2008), at 10 to 11 week gestation in sheep (Hejazi *et al.*, 2013) and 3rd to 4th month of gestation in human foetuses (Horstmann. 1957; Arey, 1974). The average thickness of epidermis at 42 days gestation was 19.11±0.38 µm. The average height and nuclear diameter of the cells of basal layer were found to be 10.69±0.32 and 6.52±0.18 µm, respectively.

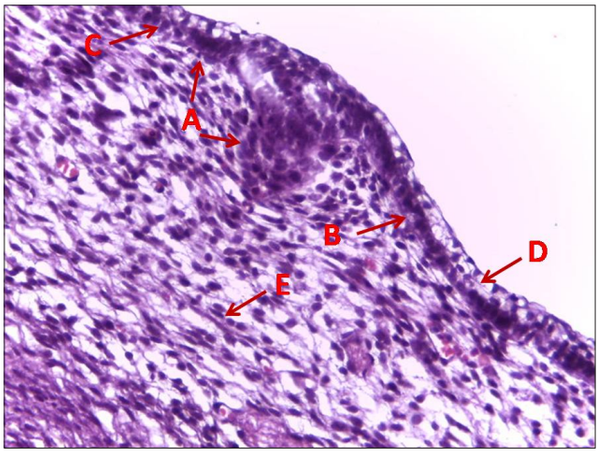

Fig. 7: Photomicrograph of section of 46 day old goat foetus showing early stages of hair follicle (A), basement membrane (B), basal cell (C) and spinosal cell (D) and fibroblast (E). H & E X400

In 43 and 44 days goat foetus the epidermal cell layers varied in number from two to four, however, at some places patches of more than four layers were also observed and their average thicknesses of epidermis at these stages were 18.26±0.33 µm and 23.08±0.75 µm, respectively. Compactly arranged uniform cuboidal cells of basal layer contained centrally placed large nuclei and dark eosinophillic rim of cytoplasm. The cells of the other layers (stratum spinosum) varied in shape and size. These were usually polygonal and ovoid in shape and were relatively larger than the cells of basal layer. Generally the size of cells increased from deeper to surface layer due to which the compactness of cells appeared to be decreased from deeper to surface layer. Vacuolation was not usually observed in the cells of basal layer but in the other layers it was evident; the vacuolation was more pronounced in the cells of the upper (surface) layer than the cells of deeper layers. The cells of stratum spinosum in Merino sheep foetuses were arranged in three to four layers and became flatter as they the approached surface (Lyne, 1957).

At 46th and 50th day of gestation the number of cell layers in the epidermis varied in different body regions of the foetuses. On the dorsal aspect and in the head region the number of cell layers was two to three as the stratum spinosum consisted of one to two cell layers. In ventral part of the foetuses, the number of cell layers was maximum where these counted three to four. The cytological characters of basal and spinosal layers resembled to 44 days gestation. At 46 days gestation discontinuous basement membrane was observed beneath the basal layer of epidermis which appeared at almost all the places at 50 days of gestation. In 46 days old goat foetus, the basal layer of head region had some condensed areas which consisted of crowded and relatively elongated and narrowed basal cells. These condensed areas invaginated the underlying mesenchyme (Fig. 7).

These finding indicated the initiation of hair follicle formation. Hejazi *et al.* (2013) in sheep foetus observed the appearance of hair follicle at 9th week of gestation where as Chaurasia *et al.* (2009) observed first appearance of anlage of hair follicle in the teat skin of goat foetus at 65 days of gestation. The average thickness of epidermis, height of basal cells and diameter of nuclei of the basal cells were found to be 23.75±0.51 µm, 10.84±0.59 µm and 6.64±0.48 µm at 46 days and 26.73±0.77 µm, 10.92±0.29 µm and 6.72±0.28 µm at 50 days, respectively (Table 1). Tripathi *et al*. (1996) in goat foetii reported that upto 10 cm CRL stage the epidermis had an average thickness of 9.22±0.46 µm. Micrometrical data in the present study revealed that the thickness of epidermis significantly increased with the advancement of gestational age, the height of basal cells increased updo 44th days of gestation and the dimensions of basal cell nuclei increased upto 40th day.

Subepithelial tissue (Dermis)

The sub epithelial tissue in the goat embryo between 23rd to 28th days of gestation was densely cellular with very little amount of ground substance. It chiefly consisted of undifferentiated, polymorphic, irregularly arranged mesenchymal cells (Fig. 1). The cytoplasm of these cells was basophilic. The nuclei of most cells were spherical in shape and contained darkly stained chromatin material. The cells had cytoplasmic processes of varying size on their surface. Processes usually were in contact with the same processes of the adjacent cells; the latter formed dense irregular network which supported the lightly eosinophillic scanty ground substance. Often unevenly scattered developing blood cells were found in the subepithelial tissue. In the deeper part of subepithelial tissue some hemopoietic blood islands were also observed (Fig. 1). At 32nd and 34th day of gestation the structure of subepithelial tissue was almost similar to the previous stage but the amount of ground substance was relatively more (Fig. 4). Tripathi *et al.* (1997) in goat reported that in early stages of development the dermis was primarily cellular and was comprised of mesenchymal cells upt 10 cm CRL stage. Bhayani *et al.* (1992) in buffalo foetus reported that the dermis contained only mesenchymal cells between 74 -105 days of gestation.

At 38th day gestation, the subepithelial tissue was divisible into the superficial and deep zones. Both zones were composed of cells and ground substance. Mesenchymal cell along with few fibroblasts constituted the cellular component of the zones. The superficial zone was comparatively dense cellular and had less ground substance as compared to the deep zone. In the superficial zone the long axis of some of the mesenchymal cells were oriented parallel to epithelial layer. At 40th day gestation, the occurrence of fibroblasts in the subepithelial tissue was relatively more as compared to 38 days old embryo. However, thin eosinophillic extensions at ends of fibroblasts were also visible but there was no sign of fiber formation. Meyer and Gorgen (1986) reported first appearance of fibroblast at 47 days of gestation in foetal porcine skin.

In 42 and 44 days old goat foetuses, the occurrence of fibroblasts were relatively more than the 40 days gestation where several such cells were observed in the dermal tissue, however, mesenchymal cells were still predominant cell type. Thin isolated sparsely arranged reticular fibers were observed in both superficial and deep zones of subepithelial connective tissue. Occasionally, at few places, particularly in the deep zone sporadic network of fine reticular fibers were also observed (Fig. 6). In addition to this at many places fine reticular fibers were present below the epithelial basal lamina. The fibers were relatively more distinct in 44 days old foetus than the 42 days stage. The appearance of reticular fibers in the dermis was reported between 55-60 days of gestation in sheep (Sharooz and Ahmadi, 2005), at 16.8 cm CRL stage

in Gaddi sheep foeti (Razvi *et al.*, 2015), and at 73 days of gestation in buffalo (Panchal *et al.*, 1999).

By 46th day of gestation the fibroblast predominated over mesenchymal cells in the dermal tissue and beneath the epithelium majority of these cells were oriented as having their long axes parallel to the surface epithelium. The mesenchymal cells along with few fibroblasts were concentrated below the condensed cellular epithelial mass forming the initial stages of hair follicles (Fig. 7). These cells also surrounded the initial invagination of developing hair follicles in the dermis. Paus *et al.*, (1999) in rat embryo observed that in the hair follicle development the mesenchymal cells aggregated immediately below the condensed epidermis. The authors also stated that the aggregation of mesenchymal cells below the epidermis indicated the location of the new hair follicle. In 46 days old foetus at several places network of reticular fibers was observed in the deep zone of dermal tissue where as in the superficial zone the fibers were still present individually. The reticular lamina in the discontinuous basement membrane became more distinctin this stage than the previous one. Congested blood vessels were observed and some of them contained few hemopoietic cells. Free erythrocytes were found in the differentiating dermal tissue. At 50th days of gestation the reticular fibers were relatively coarser than 46th day stage. Collagen and elastic fibers were absent in the dermal tissue of goat embryo/ foetus upto 50th days of gestation. These fibers were even lacked in the wall of blood vessels in the dermal tissue.

REFERENCES

Anonymous 2008. Stages of goat fetal development. Available at: www.thekebun.wordpress.com/2008/09/28 / stages-of-goat-fetal-deve. (Accessed: 8 December 2016).

Arey, L.B. 1974. Developmental anatomy. 7th Edn., Saunders, Philadelphia.

Carlson, B.M. 1981. Patten's *Foundations of Embryology*. McGraw-Hill, New York.

Chakrabarti, A., Kumari, R., Dayal, S. and Dey. A. 2014. Goat Farming- best source of income for rural farmers. Krishi Seva. Available at: http://www.krishisewa.com/articles/livestock/406-goatfarming.html (Accessed: 8 December 2016).

Chaurasia, S., Panchal, K.M., Vyas, Y.L and Desai, M.C. 2009. Histomorphological study on skin and its adnexa on the mammary glands of prenatal non-descript goat. *Indian J. Vet. Anat.*, **21**: 40-42.

Copenhaver, W.M., Bunge, R.P. and Bunge, M.B. 1975. Bailey's textbook of histology. 16th Edn., The Williams & Wilkins Company, Baltimore.

Crossman, G.A. 1937. A modification of Mallory's connective tissue stain with discussion of principles involved. *Anat. Rec.,* **69**: 33-38.

Dyce, K.M., Sack, W.O. and Wensing, C.J.G. 1996. Text Book of Veterinary Anatomy. 2nd Edn., WB Saunders.

Hejazi, S., Yaghoubi, S., Delghandi, M. and Javid, F. 2013. Histogenesis study of skin in sheep. *Life Sci. J.*, **10**: 194-198.

Horstmann, E. 1957. Die Haut. In Handbuch der mikroscopischen. Anatomie des Menschen, **3** (1): 1-276. Ed. W. von Mollendorf. Springer, Berlin.

Hugget, A. St. G. and Widdas, W.F. 1951. The relationship between mammalian foetal weight and conception age. *J. Physiol.,* **114:** 306-317.

Luna, L. G. 1968. Manual of Histological Staining Methods of the Armed Forces Institute of pathology. 3rd Edn., McGraw Hill Book Company, New York, USA.

Lyne, A.G. 1957. The development of the epidermis and hair canals in the merino sheep foetus. *Aust. J. Biol. Sci.,* **10**: 390–397.

McGeady, T.A., Quinn, P.J., FitzPatrick, E.S., Ryan, M.T. and Cahalan, S. 2006. Veterinary Embryology, 1st Edn., Blackwell, Oxford.

Njaa, B.L. 2012. Kirkbride's Diagnosis of Abortion and Neonatal Loss in Animals. 4th Edn., John Wiley and Sons, Ltd., USA.

Ozfiliz, N., Balikçier, M., Erdost, H. and Zik, B. 2002. Histological and morphometric features of the skin of native and hybrid sheep. *Turk. J. Vet. Anim. Sci.,* **26**: 429-438.

Paus R, Muller-Rover S, Van Der Veen C, Maurer M, Eichmuller S, Ling G, Hofmann U, Foitzik K, Mecklenburg L, Handjiski B. 1999. A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis. *J. Invest. Dermatol.*, **113**: 523-532.

Razvi, R., Shukla, P., Rajput, R. and Pathak, V. 2015. Histological studies on prenatal skin of developing Gaddi sheep foetus. *J. Cell Tissue Res.,* **15**: 5329-5334.

Serri, F. and Montagna, W. 1961. The structure and function of the epidermis. *Pediat. Clins N. Am.*, **8**: 917-941.

Singh, Y., Sharma, D.N. and Dhingra, L.D. 1979. Morphogenesis of the testis in goat. *Indian J. Anim. Sci.,* **49**: 925-931.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods. 9th Edn., Oxford and IBH Publishing Company, New Delhi.

Tripathi, M. 1994. Pre and postnatal studies on morphometry morphogenesis and histogenesis of skin in Goat (*Capra hircus*). PhD Thesis, JNKVV, Jabalpur (MP).

Tripathi, M., Malik, M.R. and Shrivastava, A.M. 1996. Histomorphology of skin in prenatal and neonatal goats. *Indian J. Anim. Sci.,* **66**: 1012-1014.

Tripathi, M., Malik, M.R., Parmar, M.L. and Taluja, J.S. 1997. Histomorphogenesis of dermis in prenatal and neonatal goats (*Capra hircus*). *Indian J. Anim. Sci.,* **67**: 752-754.