IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 11, Issue 2 Ver. II (February 2018), PP 17-23
www.iosrjournals.org

Histogenesis of Collagen and Elastic skin fibers in prenatal goat

Zaidi B., Tripathi M. and Mishra R.P.
[1]*Mata Gujri Mahila Mahavidyalaya (Auto.) Jabalpur, (M.P.) India*
[2]*Rani Durgavati Vishwavidyalaya, Jabalpur, (M.P.) India*
Corresponding Author: Zaidi B., Tripathi M.

Abstract *The study was conducted on 30 goat foetuses (Capra hircus) of all three age groups. As studies revealed Goat skin contains intense network of collagen and elastic fibres making a compact and a peculiar arrangement. After reporting the genesis on histochemical ground , TEM(Transmission Electron Microscope) analysis has been done to get the magnified images of histogenesis. The genesis and growth of skin fibres (collagen, elastic & reticulin) in all three foetal age groups shows a specific pattern in which the fibres originate in specific fetal age and grow up in a peculiar mode up to a precise age of late foetal group, after it build up fully the structural changes can be seen in fibrous tissue with further growth. Advance method carried out with the TEM study in which we find magnificent apparent images of various regions (10 CRL, to 40 CRL samples) and explicate the histological & developing stages of fibres clearly.*

Key words: *Collagen, elastic, Fibroblast, Histogenesis, TEM.*

Date of Submission: 10-02-2018 Date of acceptance: 26-02-2018

I. Introduction

The skin is the largest, dynamic, vital and complex organ of the body (Montagna, 1956; Bal, 1977). The structural intricacies of skin in the course of long history of the animal evolution have assigned it diverse functional roles which are fascinating, yet enigmatic. Foetal skin absorbs water and other solutes during early pregnancy; mammalian skin is most impermeable to water and to most other substances. The site of barrier has been located at stratum corneum (Klingman, 1964). The structure and thickness of the skin is variable as per species, breed, age and sex of domestic animal (Bal, 1977). Dellmann and Brown (1987) reported the thickest skin over the dorsal surface of the body and on the lateral surfaces of the limbs. It is thinnest on the ventral side of the body and medial surface of the limbs. The development of skin and its associated structures has been explored in general in domestic animals, click and man (Shumway, 1949Bloom and Fawcett, 1982 and Arey, 1974). However information on morphometrical differences, histogenesis and histochemical changes of skin fibres in foetal, neonatal and adult goat appears to be mearge. The dermis is composed of three types of skin fibres Collagen, Elastic and Reticular fibers that are present *throughout* the dermis.The papillary layer, the upper layer of the dermis, contains a thin arrangement of collagen fibers along with elastin and reticular components. The lower, reticular layer is thicker and made of thick collagen fascicles intermingled with elastin bundles that are arranged parallel to the surface of the skin.

II. Material And Methods

The present study was conducted in three groups as Group I - 10 fetuses (up to CR length 10 cm), Group II - 10 fetuses (CR length10 to 20 cm) and Group III - 10 fetuses (CR length 20 cm and above) indigenous goat. The skin pieces were obtained from city abattoir and were placed in bag full of ice. The skin samples were collected from different regions of the body of fetal goat- dorsal, ventral, thigh, neck and flank region with the help of razor blade, scissors and forceps. The tissues were fixed in 10% neutral buffered formalin solution for 36 to 48 hours. (Lillie and Fullmer, 1976; and Drury and Wallington, 1980). The tissues were then processed in laboratory by adopting standard methods (Drury and Wallington, 1980) of dehydration, clearing and embedding. They were processed and sectioned using routine histological procedures. The paraffin tissue sections of 3-5 um thickness were stained with van giesons and orcein stain to study collagen and elastic fibers configuration.

For TEM analysisTissue pieces measured approx 1mm size for fixing. Placed in glass processing vials and closed with plastic caps. Only 50 gram of skin was acquired for each experiment. After cleaning the skin, it was chopped into fine small pieces (0.3 to 0.5 cm) while taken for fixation. For electron microscopy, small segments of tissues are fixed in a solution containing PBS (Phosphate buffer saline) 0.1M in 2.5% glutaraldehyde at pH=7.2 -7.4, the tissues were fixed for 2 hours, followed by postfixation in 1% osmium tetroxide (dissolved in PBS (Phosphate buffer saline) for 2 h. Tissues were dehydrated in an ascending acetone series, 70%, 80%, 90% with three changes of 100% (for 15 minutes each change) and prepared for Transmission

Electron Microscopic study by block making and ultrathin sectioning prepared in ultramicrotome (Ultracut-UCT, Leica) at High Security Animal Disease Laboratory (Jeol JEM-1400), Bhopal.

III. Results

In the foetuses of group I having CR length upto 10.00 cm, formation of the skin fibers and other associated structures such as hair follicles, sebaceous glands or sweat glands were not seen in the subepithelial and deep matrix.

The subepithelial matrix consisted of the darkly stained cells which were stellated, fusiform, elongated or ovoid in shape. These cells were denser in the superficial part in comparison to the deep part. The cells in the deep layer appeared matured as was evident by the staining characteristics. As clear in the samples the connective tissue appeared denser with the advancement of the age of the foetus due to increase in the cell population and synthesis of the fibre protein. The connective tissue fibres appeared to arise at the angles of the fibroblast cells in the deep zone of the matrix ie dermis in group II foetuses (Fig.1&2). Advance method carried out to see its magnifying view in TEM (Transmission Electron Microscope) study of skin fibres of goat (*Capra hircus*) in which we find wonderful apparent images of genesis of collagen and its growth in different age groups and explicate the histological and developing stages of collagen fibres clearly. The study was conducted on all age groups of goat (*Capra hircus)*. As studies revealed the network of collagen fibres is compact and very sturdy in goat skin. In our observation when examined with the TEM, fibre formation which was not evident in the sections of group I foetus was apparent in **group II** fetuses showing The primary structure of collagen fibres (Fig. 8b)

These fibres were predominately of collagenous type and extended in different directions. In later group II These formed close network in the deep part of the dermis. The connective tissue in subepithelial matrix/dermis confirmed general pattern in all the regions of the body. It consisted of connective tissue cells and matrix of the newly formed delicate connective tissue fibres which were less prominent in deep part of the dermis. However, in dorsal region as shown in the sections, the collagen bundles appeared to be more consistent in cellularity and thickness (Fig.3,4). In neck region the fibre arrangement appeared similar to that of the dorsal region but more irregularly oriented (Fig. 5). In ventral region the density of fibres was moderate. However, the collagen appeared condensed at places. In thigh region it appeared similar to dorsal region. However, it was relatively less cellular. In flank region the fibres was relatively uniform and its intensity was regular but less dense. In the deep part of the dermis there was a narrow strip that consisted of collagenous fibres which separated the dermis from the hypodermis by an even uniform space suggesting fragility of the delicate connective tissue between the hypodermis and deep dermis in most of the regions in this group.

The elastic fibres appeared to arise at the angles of the fibroblast cell mainly in superficial and middle zone of dermis (Fig. 6) in group III. It is clear in the TEM studies fibrils first appear in 23CRL of Group III Feotuses (Fig. 8a). Elastin components are produced as the deposition of a small amount about 0.1 um wide in late group III fetuses. These fibres in sub epithelial matrix/dermis confirmed general pattern in all the regions of the body. The matrix here mainly consisted of connective tissue cell and newly formed delicate elastic fibres which were less prominent in deep part of the dermis particularly in initial stages. The intensity of fibers as reported was general in all samples that elastic assimilation was started appearing in neck & dorsal region of the skin (Fig. 7)

In the late group III samples the connective tissue comprising amalgam of the collagen and elastic fibres in all regions of the body, but the collagen fibres predominated in all the regions. Initially the fibres appear as bundle of fine, threadlike subunits of about 15 or 20 nm in diameter. With the development of these collagenous matrix the dermis appeared to be more cellular and condensed in the superficial zone i.e. beneath the epidermis. In later stages (in the fetuses of 30 to 35 CR length, (Fig 3,4) they form close and compact network in the deep part of the dermis particularly in group III. As recorded here the dermal connective tissue was more fibrous with largly developed collagen fibres. The intensity of collagen varied in different regions of the body and was maximum ()and minimum ()in neck and ventral region of the body respectively. The dorsal, flank and thigh regions were in descending order (Table 1).

Fresh collagen fibres are coloreless strands 1 to 100 um thick that usually follow a irregular course without branching in the connective tissue matrix. The birefringence of collagen fibres are simply the parallel arrangement of thinner fibrous components in collagen fibres to make a bundle of closely packed thin fibrils with periodical striations these unit fibrils are called “collagen fibres” as recognizable by light microscopy. Since collagen fibres are flexible and still offer great resistance to any external force.

The intensity of fibers as reported was general in all samples that elastic assimilation was started appearing in neck & dorsal region of the skin . The fibers were found moderate to weak in thigh, flank region & weak in ventral region (Table 2).

In electron microscopic images we observed the fibres arrangement confirm the basic pattern of fibres array or microfibrils interspersed with elongated patches of amorphous material (Fig.10). A common trait seems to apply to all samples that differential distribution of elastic fibres coincide with structurally distinct collagen ie their presence in dermal layer are in prominent intermingling to support the skin movement and elasticity where as in free extremity it blends into a basement membrane. These fibre bundles found appearing in a peculiar structural pattern (Fig.11). In the late Group III fetuses remarkably intense bundles grouped as compact fascicules are reported apparently intermingled with collagenous bundles. These fibers bring into being in abundance between and around hair follicles in the reticular layer. The sweat and the sebaceous glands were reported to be sparsely layered with elastic fibers. In all the sections in light and electron microscopy it is found in its parallel rings that surround the secretary coil of the eccrine sweat glands.

IV. Discussion

Raheeqa et al. (2015) also showed The thickness of epidermis and dermis increased with advancement of gestational age in Bakerwali goat. They reported Histologically, the total thickness of skin was maximum on the neck
dorsal and minimum on thorax ventral region in all age groups as confirmed in the present report

The dermis formed the lower layer of the skin supporting the epithelium. In group I it consisted of subepithelial connective tissue matrix made up of undifferentiated mesenchymal cells and amorphous ground substances. The cellular contents present in the dermal matrix in initial foetal stage in the present study comprised a group of undifferentiated mesenchymal cells, fibroblasts, macrophages and histocytes. The undifferentiated mesenchymal cells appeared multipotential and formed the intercellular fibrous tissue with age. Similar observations have been reported in laboratory mouse by Van Exan and Hardy (1984). They described the development in 4 phases. The first phase was marked by a decrease in the cell density, second phase began with the cytodiffrentiation of mesenchymal cells and was characterized by the appearance of new cells types (immature fibroblasts, mast cells, myoblasts and cells of indeterminate type). During the third phase the dermis undergoes rapid change. In fourth phase fibroblasts become fully undifferentiated, mast cell density was high, there was a marked increase in the number of collagen fibrils in the dermal matrix as confirmed by this research work. This phase continued after birth, as more collagen synthesized.

Histochemical observations did not reveal the presence of connective tissue fibrous in group I. In group II the collagen appeared at the ends of the elongated undifferentiated cells which increased in number and thickness and formed a sort of fibrous reticulum in group II and in early stage of group III. It became denser and formed the fibre bundles group III onward particularly in the deep zone of the dermis. Ham (1979) reported amorphous intercellular substance in human foetus, proportionately less fibrous intercellular substance in new born in comparison to adult is in support of the finding in present study.

Moreover similar development was noticed by Meyer and Gorgen (1986) in dermis of foetal porcine skin between 40 days gestational age (40 d-GA) and birth. The first fibroblast arrangement occurs about 47 d-GA, while between 60 to 65 d-GA the early fibre appear. They can be identified as collagen about 67 d-GA. Somewhat later the dermis shows 2 strata.

The other fibre components appeared in group II onward. Elastic fibres were not demonstrated even upto late foetal stages of group II. With the advancement of age the fibre components of the dermis increased and differentiated to support the associated structure of the skin. The statement has similarity with the finding of Arey (1974) in man and Meyer and Gorgen (1986) in foeatl porcine skin. Arey (1974) reported in man that collagen fibres emerged in third month and elastic fibres in the sixth month of development. The first elastic fibres were not demonstrated before 100 d-GA. A clear increase in fibrocyte number occours about 70-80 d-GA, the next one at birth. Only gradually does a distinction between the compact dermis and loose subcutaneous tissue become recognizable. Razvi et al. (2015) report differs from the findings in *Capra hircus* in which he found, the appearance of elastic fibres along with collagen at 16.4 CR length in Gaddi Sheep foetal stages. Collagen incredibly increases at 24.5 CR length in deep dermis.

PHOTOGRAPHS
GROUP (II)

Fig 1. Photomicrograph – cross section of skin of foetal goat, (group II) CR length 15 cm, dorsal, V G, x 40 showing weak collagen synthesis in dermis

Fig 2. Photomicrograph – cross section of skin of foetal goat, (group II) CR length 15 cm, ventral, V G, x 40 showing weak collagen synthesis in dermis

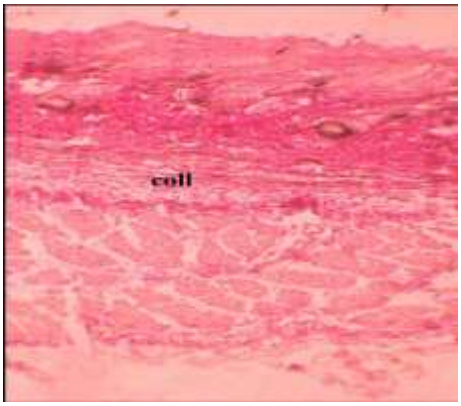

Fig 3. Photomicrograph – cross section of skin of foetal goat, (group III) CR length 28 cm , ventral, V G, x 40 showing moderate to intense collagen in dermis

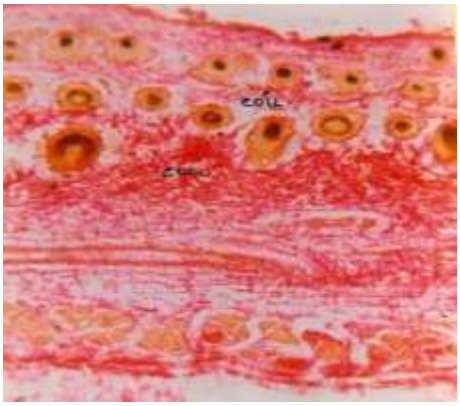

Fig 4. Photomicrograph – cross section of skin of foetal goat, (group III), dorsal, V G x 100 showing moderate to intense collagen in dermis

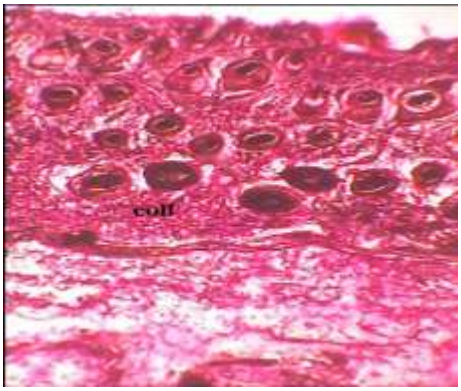

Fig 5. Photomicrograph – cross section of skin of foetal goat, (group III) CR length 36 cm , neck, V G, x 10 showing moderate to intense collagen in dermis

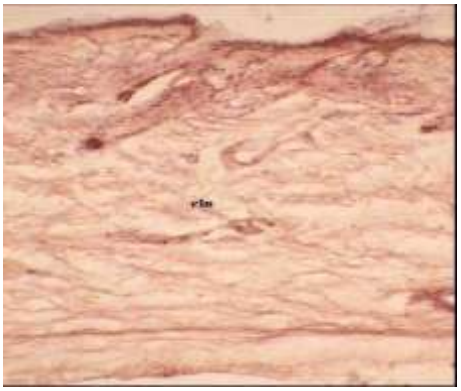

Fig 6. Photomicrograph – cross section of skin of Foetal goat (Group III) early stage, Flank, orcein stain Showing, eln-elastic fibres.

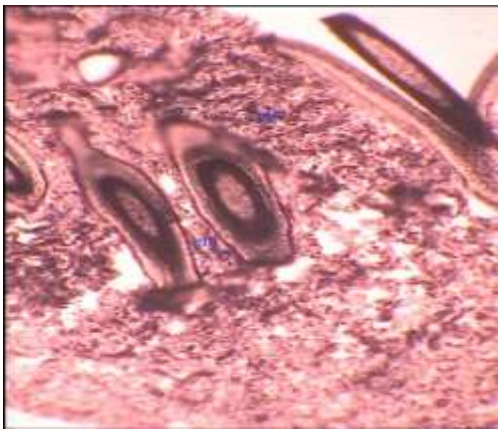

Fig 7. Photomicrograph – cross section of skin of Foetal goat (Group III) late stage, neck, orcein stain Showing hr-hair, eln-elastic fibres**.**

V. Tem Analysis

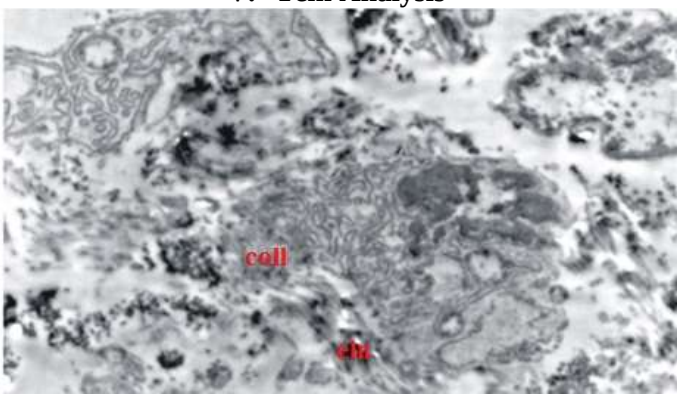

Fig 8 a. Electron micrograph – cross section of skin of fetal goat (23 CR length, group III), ventral region showing

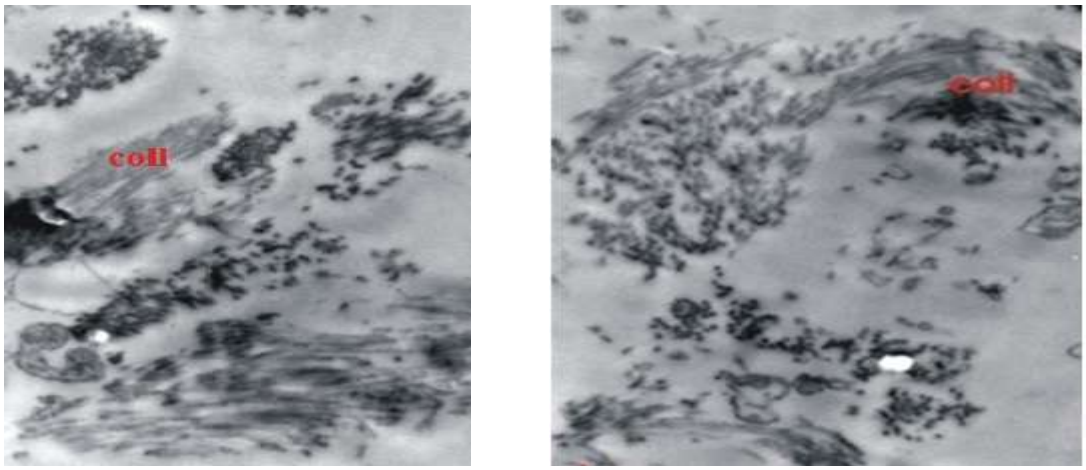

Fig 8 b. Electron micrograph – cross section of skin of fetal goat (19 CR length, group III), dorsal region showing

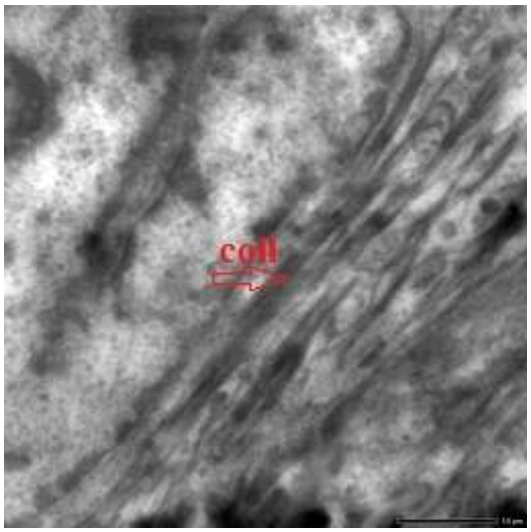

Fig. 9: Electron micrograph – cross section of skin of fetal goat (35 CR length, group III), dorsal region.

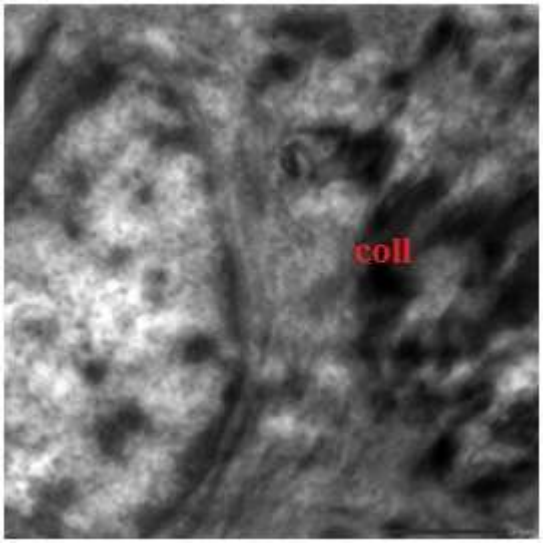

Fig. 10: Electron micrograph – cross section of skin of fetal goat (35 CR length, group III), ventral region.

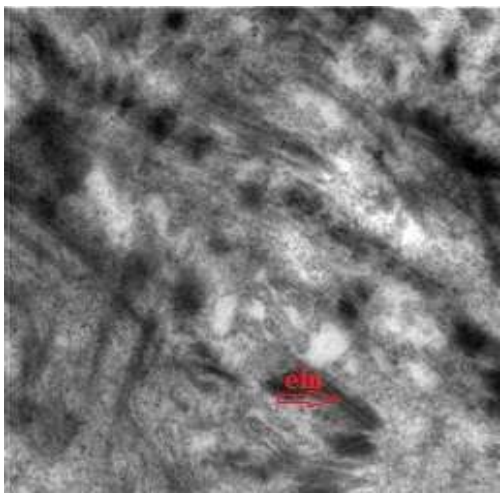

Fig. 10: Electron micrograph –cross section of skin of fetal goat (35 CR length, group III), dorsal region.

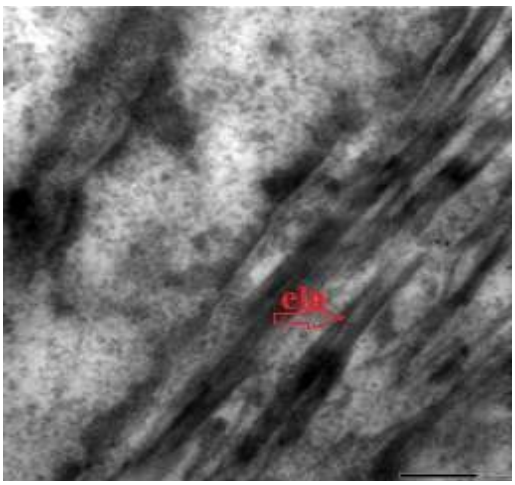

Fig. 11: Electron micrograph – cross section of skin of fetal goat (35 CR length, group III), ventral region.

Table 1. Analysis of cutaneous collagen fibres

	Neck	Dorsal	Thigh	Ventral	Flank
Group I	-	-	-	-	-
Group II	+	+	+	-	-
Group III	+++	+++	++	+	+

Table 2. Analysis of cutaneous elastic fibres

	Neck	Dorsal	Thigh	Ventral	Flank
Group I	-	-	-	-	-
Group II	-	-	-	-	-
Group III	++	++	+	++	+

References

[1]. Arey, L.B. (1974). "Developmental Anatomy. A text Book and Laboratory Manual of Embryology." 7th ed. W.B.Saunders Company, Philadelphie and London, 439-453.

[2]. Bal, H.S. (1977). "The Skin, In: Duke's Physioligy of Domestic Animals." 9th ed. Melvin J. Swenson Ed. Comstock Publishing Associates, A division of Cornell University Press/Ithaca and London, 493-503.

[3]. Bloom, W. and Fawcett, D.W. (1982). "A Textbook of Histology". 10th ed. W.B. Saunders Company, Philadelphie, London, Toranto, Igakcu – Shion/Sounders Tokyo, 563-597.

[4]. Dellmann, H.D. and Brown E.M. (1987). "Textbook of Veterinary Histology." 3rd ed. Lea and Febiger, Philadelphia, 382-415.

[5]. Drury, R.A.B. and Wallington, E. A. (1980). Carleton's Histological Technique. 5th ed. Oxford University Press. *Oxford New York*, Toronto 519.

[6]. Ham, A.W. (1979). Histology, 8th ed. J.B. Lippincott Company Philadelphia and Toronto, 613-644.

[7]. Klingman, A.M. (1964). "Biology of stratum corneum." In W.Montagna and W.C. Lobitz, Jr. Eds. The Epicermis, Academic Press, New York, 387-430.

[8]. Lillie, R.D. and Fullmer, H.M. (1976) Histopathologic technique and Practical Histochemistry. 4th ed. MnGraw-Hill Book Company, A Blakiston Publication, p. 942.

[9]. Montagna, W. (1956). The Structure and Function of Skin. Academic Press Inc, New York, p. 337.

[10]. Meyer W. and Gorgen S. (1986). Some observation on dermis development in foetal porcine skin. *Anat Anaz*. 161(4): 297-307.

[11]. Razvi R., Suri S., Sarma K., & Sharma R., (2015). "Histomorphological and histochemical studies on the different layers of skin of Bakerwali goat". *Journal of Applied Animal Research*, 43(2): 208–213.

[12]. Shumway, W. (1949). "Introduction to Vertebrate Embryology." 4th ed. John Wiley and Sons INC New York, Champan and Hall Limited and London, 213-216.

[13]. Tripathi, M., Zaidi B. and Sarkhel S. (2013). Age related changes in elastic fiber configuration in skin of fetal, neonatal & adult goat *(Capra hircus). Inter J Multidiscipl Res Acad (IJMRA)* 3(7): 562-569.

[14]. Van Exan, R.J. and Hardy, M.H. (1984). The differentiation of the Dermis in the laboratory mouse. *The American J of Anat*, 169: 149-164.

Zaidi B. "Histogenesis of Collagen and Elastic skin fibers in prenatal goat ." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.2 (2018): 17-23.