HISTOLOGICAL STUDIES ON PRENATAL SKIN OF DEVELOPING GADDI SHEEP FOETUS

RAZVI, R., SHUKLA, P., RAJPUT, R. AND PATHAK, V.

Division of Veterinary Anatomy, Himachal Pradesh Krishi Vishvvidlaya, Palampur, 176 061, Himachal Pradesh. E. mail: doctorrahika356@gmail.com, Cell: 09882261153

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Abstract: Histological studies of skin from dorsal regions of neck, thorax and loin of body were carried out on eight healthy Gaddi sheep foeti, which were collected from local slaughter houses of Palampur (H.P). After measurement of crown-rump length (CRL), the age of foeti were determined. Skin tissue samples were collected, fixed (10% neutral buffered formalin), processed, sectioned and finally stained to study their histological developmental changes. Differentiation of epidermis took place from an ectoderm germinal cell layer and rate of differentiation and growth of epidermis was fast in neck dorsal region than other two regions under study. At CRL 16.4 cm appearance of collagen fibers, sweat glands and elastic fibers was also observed. The sebaceous glands were seen at CRL 19.8 cm. At CRL 24.5 increase of collagen fiber synthesis deep in dermis was seen; this process caused the dermis to contain two layers: the superficial layer (papillary layer) and deep layer (reticular layer). All sub-layers of epidermis were developed along with the cells of hair follicles. The number and growth of sweat glands was higher in thorax dorsal region than other two regions under study. The thickness of epidermis and dermis increased with advancement of gestational age. At CRL 42.4 cm, the structure of the skin was complete. The reaction for bound lipids was intense in epidermis and moderate in dermis with Sudan black B stain. Fine reticular fibers were seen around sweat, sebaceous and hair follicles. Strong PAS positive reaction was seen in outer layer and moderate in the inner layer of hair follicles. With the advancement of gestational age the PAS reaction changed from mild to intense and from mild to moderate in epidermis and dermis respectively.

Key words: Skin, Gaddi sheep, Foetus

INTRODUCTION

Skin is the soft outer covering of vertebrates. In mammals, the skin is an organ of the integumentary system made up of multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs and protect the body from pathogens and water loss. It is the largest and the heaviest organ of body by having 16% of the total weight of animal [1,2]. It is also involved in tactile perception and has accessories such as sweat and sebaceous glands [3]. Histologically, skin has three layers: epidermis, dermis and hypodermis [4]. Epidermis is multilayered tissue composed of epithelial cells that originate from ectoderm and dermis, the layer of connective tissue that originates from mesoderm. Under skin, hypoderm or under skin tissue is located and it is made of loose connective tissue. In fact, hypoderm is not accounted as a part of skin, nevertheless it connects the skin as a layer of loose connective tissue to the underlying layers it. This layer is in accordance with superficial fascia [5]. Thickness of the skin and its accessories depends on the species, age, sex and nutritional conditions in adult animals [6]. Foetal skin is quite delicate, develop slowly in uterus and before delivery become...
fully formed. In present contribution various stages of skin development in Gaddi sheep has been brought out.

**MATERIAL AND METHODS**

**Sample collection:** Eight healthy sheep foetii were collected from the local slaughter houses of Palampur (H.P). Sample collection was done immediately after slaughter of animal under hygienic conditions. For age determination, the crown-rump length of foetii were measured and age was calculated using the equation; \( Y = 2.74X + 30.15 \) [7]. Whereas \( Y = \) age of foeti in day and \( X = \) Crown Rump Length in cm. As per CRL, the age so calculated is given in table 1. Foeti skin samples (0.5 to 1 cm long) were taken from dorsal regions of neck, thorax and loin.

**Processing of sample:** The skin samples so collected were fixed in 10% neutral buffered formalin for 48 hrs and than treated with alcohol-benzene series and paraffin embedding was done. The sections of 5-6μm were cut and utilized for routine and special histological staining techniques [8] to study the histomorphology and histochemistry. The histological stains so done were Haematoxylin and Eosin (H&E) stain, Gomori’s reticular method, Periodic acid-schiff’s reaction, Luna stain for mast cells and Masson’s trichrome for collagen [8].

**Sample analysis:** The histo-architecture of epidermis and dermis was observed in different regions and at different stages of gestational age. The development and numbers of sweat glands, sebaceous glands were determined. Moreover, the developmental changes of reticular fibers, collagen fibers and mast cells were analyzed. Overall histological changes of skin were observed in the different gestational ages and regions under study.

**RESULTS**

**Skin structure at CRL 7.0 cm:** The epidermis of skin was present as single layer of simple squamous cells at this stage of gestational age. Differentiation of epidermis took place from an ectoderm germinal cell layer. The thickness of epidermis was similar in all body regions under study. At 7 cm CRL (49.33 days of age), the sections of foetal skin did not reveal the development of any sebaceous or sweat gland or any of their duct system (Fig. 1). Uniform connective tissue was observed in the dermis beneath. The thickness of skin was observed uniform in all regions under study. Mild PAS reaction was observed throughout skin in all regions.

**Skin structure at CRL 9.7 cm:** The gradual differentiation of epidermis from ectodermic layer was observed. Baseline layer cells of epidermis (germinal layer) were more visible because of high mitotic division (Fig. 2). In the derma fine smooth muscles were also observed. The rate of differentiation was observed faster in neck dorsal region compared to other regions under study.

**Skin structure at CRL 11.8 cm:** The epidermal differentiation continued and three layers of epidermis were observed in neck dorsal region. In other regions under study, the two layers of epidermis were clear (rarely 3 layers) at some places. At some places, because of mitosis the germinal layer of epithelium divided and deepens in dermis forming epidermal crests/epidermal pegs. Reticular fibers were observed at these stages which were distributed uniformly throughout the dermis. At few places hair follicles seemed as epidermal cell aggregation protruded towards dermis and distant from each other (Fig. 3).

**Skin structure at CRL 16.8 cm:** At this CRL, gradual structural development was observed in hair follicles and appearance of collagen fibers, elastic fibers and sweat glands was also observed (Figs. 4, 5). The elastic fibers were very thin and observed only around the blood vessels. As the gestational age processed the PAS reaction became intense in epidermis and moderate in dermis.

**Skin structure at CRL 19.8 cm:** The thickness of skin was more in neck dorsal followed by thorax and neck dorsal. Increase of smooth muscles and blood capillaries was observed. The sebaceous glands appeared at this stage of gestation and further development of hair follicles was observed (Figs. 6, 7).
Fig 1. Skin section at CRL 7.0 cm showing epidermis (E) and dermis (D). HE X 100. Fig 2. Skin section at CRL 9.7 cm showing epidermis (E) and dermis (D). HE X 200. Fig 3. Skin structure at CRL 11.8 showing epidermis (E), dermis (D), hypodermis (H) and hair follicles (HF). HEX 200. Fig 4 and 5. Skin section showing developing hair follicles (HF) and sweat glands (SG) respectively. Fig 6. Presence of sebaceous glands (SED) near hair follicle (HF) in skin section at CRL 19.8 cm.
**Skin structure at CRL 24.5 cm:** At this stage of gestational age the appearance of sebaceous was observed originating from the hair follicles. Increase of collagen fiber synthesis deep in dermis was seen; this process caused the dermis to contain two layers: the superficial layer (papillary layer) and deep layer (reticular layer) (Fig. 8). All sub-layers of epidermis were developed along with the cells of hair follicles. Keratinization of epidermis was also observed at this stage.

**Skin structure at CRL 33.5 cm:** At this stage of gestational age, almost all components of skin appeared. Sweat glands were found in minute clusters. Sebaceous glands were associated with hair follicles (Fig. 9). Arrectore pilli muscles were visible. The number and growth of sweat glands was higher in thorax dorsal region than other two regions under study. The thickness of epidermis and dermis increased with advancement of gestational age. Hair follicles were seen more developed in their form (Fig. 10).

**Skin structure at CRL 42.4 cm:** At this CRL, the structure of the skin was complete (Fig. 11). Hair follicles appeared in single, double rows. Cortex and medulla of hair follicles were observed (Fig. 12) and accumulation of melanocytes was noticeable in the cortex of hair follicles. Mast cells were seen scattered in dermis of skin at this stage of gestational age. Thickness of skin was maximum in neck dorsal followed by thorax and loin dorsal region. Number of hair follicles was maximum in neck as compared to other two regions so studied but the difference was non-significant. The number of sebaceous glands was minimum in loin dorsal and maximum in neck dorsal with significant difference.

The reaction for bound lipids was intense in epidermis and moderate in dermis with Sudan black B stain. Fine reticular fibers were seen around sweat, sebaceous and hair follicles. Strong PAS positive reaction was seen in outer layer and moderate in the inner layer of hair follicles.

**DISCUSSION**

The results led us to a presumption that the epidermis of the skin is developed from single layer. It changed from simple squamous to non keratinized stratified squamous and at CRL 24.5cm it changed to keratinized stratified layer. Sengal [9] stated that epidermis of embryo is covered with a single layer of ectoderm and continuously by cells dividing; a layer of epidermis appears on it. At the beginning of 2nd month, by division of germinal layer some classes of interstitial cells appear between two layers and at the last one-third of pregnancy, keratinized layer of epidermis appear. Same findings were reported by Shahrooz [2] in sheep feotii.

At the 3rd month of foetal life, basal layer penetrated deep in skin. The cells change their shape and size to bigger cells. Few of the cells change their form to hair follicles and sweat glands. Researchers showed that hair’s sprout showed itself as an accumulation of epidermis cells which penetrated to the dermis beneath [10]. Sweat glands usually develop before sebaceous glands and the sebaceous glands developed from hair follicles which coincided with findings reported by Shumer [10]. Histochemical study of sweat gland showed that its function is not related to age of animal, but it is mainly affected by weather [11].

This study revealed presence of collagen fibers at CRL 16.8 cm of foetal life and this finding coincided with Shahrooz [2] who reported presence of these fibers in the first half of third month (during 64-75 days) of foetal age in sheep.

Formation of reticular fibers and differentiation of dermis into two layers began at the end of second month of foetal age in sheep [2] and the presence of mast cell was observed in the 5th month of gestational age [12]. The increase in skin thickness was faster at neck dorsal region and development of maximum number of sweat glands was maximum in thorax dorsal regions. The structure of the skin completed in 5th month of gestational age [2].

**REFERENCES**

Fig. 7. Skin section at CRL 19.8 cm showing developing hair follicles (HF). HE X 200. Fig 8. Skin section at CRL 24.5 cm showing papillary dermis (PD) and reticular dermis (RD), sebaceous glands (SEB), sweat glands(SG) and hair follicles (HF) HE X 200. Fig 9. Opening of sebaceous glands (SEB) in hair follicles (HF) at CRL 33.5cm. HE X 400. Fig 10. Showing hair follicles at CRL 33.5. HE X 200. Fig 11. Skin section showing presence of all its components, hair follicles(HF), epidermis (E), keratin(K), reticular(RD) and papillary dermis (PDs), arrector pili muscles(AP), sweat (SD) and sebaceous glands (SEB). HE X 100. Fig 12. Skin structure showing developed hair follicle's cortex (C) and medulla (M in dermis (D). HE X 200.