



Prenatal development of dew claws in buffalo: A histomorphochemical study

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ABSTRACT

The present study was conducted on 12 buffalo foetii obtained from slaughter house. The curved crown rump length (CVRL) of all the foetii was measured and their age was estimated in days. The dew claws of forelimb and hind limb were fixed in 10% neutral buffered formalin and processed by acetone-benzene schedule. The paraffin sections of 5–7 µm thickness were cut and stained with Hematoxylin and Eosin, Masson's trichrome, Periodic acid Schiff, alcian blue and bromphenol blue stains. At 10.0 cm CVRL, the claws comprised mesenchymal cells covered by thin epidermis with varying thickness. With advancing age at 18.0 cm CVRL, their wall was composed of 2 layers of cuboidal cells covered by a thin cornified cell layer. In 28.1 cm CVRL foetus, it was made up of stratum externum, stratum medium, stratum internum, corium and rudimentary phalanx. The stratum internum consisted of a single layer of basal cells with elongated nuclei, which rested upon the basement membrane. The epidermis of dew claws consisted of a single layer of stratum basale, 1–2 layers of stratum spinosum cells, 1–2 layers of stratum granulosum cells and an overlying keratinized layer. The corium was observed between horny lamellae of stratum internum and spongy bone. It contained collagen, reticular and elastic fibers with large number of blood vessels. At 45.5 cm CVRL, the stratum internum was made up of lamellae extending inward from stratum medium. These lamellae appeared as finger like projections at this stage and became branched at 60 cm CVRL onwards. The primary and secondary branches were also observed with the advancement of foetal age. Histochemical study revealed a moderate to strong reaction for acid and neutral mucopolysaccharides and basic proteins.

Key words: Buffalo, Dew claws, Histomorphological study

A dew claw is a vestigial digit on the foot of many mammals, birds, and reptiles. It commonly grows high on the leg so that in digitigrade species, when the animal is standing, it does not make contact with the ground. Hoofed animals walk on the tips of special toes, the hoofs. Cloven-hoofed animals walk on a central pair of hoofs, but many also have an outer pair of dew claws on each foot. These are a little farther up the leg than the main hoofs, and similar in structure to them. In some species (such as cattle) the dew claws are much smaller than the hoofs and never touch the ground. In others (such as pigs and many deer), they are only a little smaller than the hoofs, and may reach the ground in soft conditions or when jumping. Some hoofed animals (such as giraffes and modern horses) have no dew claws.

MATERIALS AND METHODS

The present study was conducted on 12 Indian buffalo foetii ranging from 10 cm CVRL to 75 cm CVRL (125 - 295 days). The samples were collected from non-descript buffaloes slaughtered at slaughter house and from the Veterinary Clinics GADVASU, Ludhiana. The age of foetii

was determined by measuring the CVRL as a curved line in cm using an inelastic thread along the vertebral column between the most anterior parts of frontal bone to the rump at ischiatic tuberosity (Edward 1965). The approximate age of the foetii was calculated as per Soliman (1975).

$$Y = 28.66 + 4.496 X \text{ (CVRL } < 20 \text{ cm)}$$

$$Y = 73.544 + 2.256 X \text{ (CVRL } \geq 20 \text{ cm)}$$

where, Y is the age in days and X is the CVRL in cm.

Immediately after collection, the tissue samples were fixed in 10% neutral buffered formalin (10% NBF) and Bouin's fixatives. After the fixation, the tissues were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968) and the sections of 5–6 µm were obtained on glass slides with the help of manual rotary microtome. These paraffin sections were subjected to Haematoxylin and eosin for routine morphology, Masson's Trichrome for collagen fibres in connective tissue (Luna 1968), Verhoeff's stain for elastic fibers and Gridley's stain for reticular fibers (Sheehan and Hrapchak 1973). For histochemical studies, the sections were stained with PAS and AB PAS for demonstration of neutral and acid mucopolysaccharides.

RESULTS AND DISCUSSION

Histological observations: At 10.0 cm CVRL, the claws

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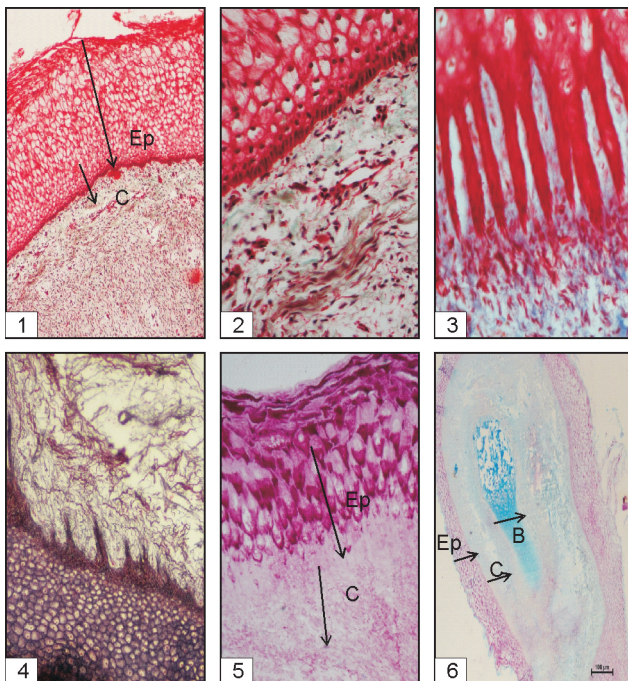
comprised mesenchymal cells covered by thin epidermis with varying thickness. With advancing age at 18.0 cm CVRL, their wall was composed of 2 layers of cuboidal cells covered by a thin cornified cell layer. In 28.1 cm CVRL foetus, it was made up of stratum externum, stratum medium, stratum internum, corium and rudimentary phalanx. The basic histological architecture of dew claws is similar to that of equine hoof (Dellmann and Eurell 1998). The stratum externum was mainly a keratinized layer, stratum medium of stratum spinosum and stratum granulosum and stratum internum of stratum basale and primary laminae extending from the stratum medium. From inwards, the epidermis of dew claws consisted of a single layer of stratum basale, 1–2 layers of stratum spinosum cells, 1–2 layers of stratum granulosum cells and an overlying keratinized layer which was clearly demarcated at this stage. The number and size of these layers in the epidermis increased with the age of the foetus and was 15–20 layered structure at 31.5 cm CVRL (Fig. 1). Stratum

basale was comprised tall columnar cells with oval and deeply basophilic nuclei. According to Mueller (2003), this is first epidermal layer responsible for reproduction of epidermal cells and is most active in the areas where most of the horny claw is formed. The spinous cells were polyhedral in shape with marginal rounded nuclei. The intranuclear vacuolization was very typical in the spinous cells, but the quantity of the vacuoles varied (Fig. 2). In present study, there was no vacuolization observed in the basal layer of dew claw, which was reported in canine claw (Muller *et al.* 1993). In stratum granulosum, the cells had a cuboidal form with round nuclei located in the centre or at the periphery of the cells. The number and size of intranuclear vacuoles increased in the stratum spinosum, but the location and form of the vacuoles is the same as in the granulosa cell layer. The stratum corneum was made up of flat cornified epidermal cells. The keratinized layer comprised eosinophilic, fibrillar keratin with a gradual transition to a clear central core of fibrillar, refractile keratin with occasional linear streaks of increased eosinophilia assumed to represent less mature keratin. Similar findings were reported in the canine claws (Muller *et al.* 1993).

At 45.5 cm CVRL, the stratum internum was made up of lamellae extending inward from stratum medium (Fig. 3). These lamellae appeared as finger like projections at this stage and became branched at 60 cm CVRL onwards. The primary and secondary branches were also observed with the advancement of foetal age. The lamellar corium had dermal lamellae which interlock with the epidermal lamellae. The corium provides the hoof with nourishment and its dense matrix of connective tissue connects the basement membrane of the dermal- epidermal junction to the periosteal surface of the rudimentary phalanx (Pollitt 1994). Some of the blood vessels were also observed in these lamellae. Similarly, Hirschberg *et al.* (2001) identified both the pododermal papillary and dermal blood vessels in the bovine dew claws of juvenile and adult animals.

The vascular corium or dermis was observed between epidermal lamellae of stratum internum and spongy bone. It contained dense fibrous collagen fibres along with reticular and elastic fibres, large number of blood vessels (Fig. 4), which increased with the age of foetus. In the spongy bone, hyaline cartilage was observed with endochondral ossification of rudimentary digits. Similar structure of subjacent in the canine claws was observed by Muller *et al.* (1993).

Histochemical observations: Histochemical study revealed a moderate to strong reaction for acid and neutral mucopolysaccharides in the epidermis. A strong PAS-positive reaction was observed in the different layers of epidermis however the corium showed a weak to moderate activity (Fig. 5). The intensity of PAS reaction decreased from outer to innermost layer. The activity of AB/PAS reaction was found to be more activity in the corium, subdermal layer and epidermal lamellae. A moderate to strong reaction of Alcian Blue was observed in the keratinized layer and a strong to intense reaction in the



Figs. 1–6. 1. 31.5 cm CVRL buffalo foetus showing different layers of epidermis (Ep) and corium (C) in dew claw. Haematoxylin and eosin stain. $\times 100$. 2. Higher magnification of Fig. 1 showing tall columnar cells with oval and basophilic nuclei in Stratum basale, polyhedral cells with marginal nuclei in Stratum spinosum and vascular corium. Haematoxylin and eosin stain. $\times 400$. 3. 45.5 cm CVRL buffalo foetus showing primary lamellae extending inward from stratum medium towards stratum internum. Haematoxylin and eosin stain. $\times 400$. 4. 45.5 cm CVRL buffalo foetus showing dense fibrous collagen fibres, reticular and elastic fibres and blood vessels in the corium. Gridley's stain. $\times 100$. 5. 31.5 cm CVRL buffalo foetus showing PAS reaction in different layers of epidermis (Ep) and corium (C) in dew claw. Periodic acid Schiff stain. $\times 400$. 6. 31.5 cm CVRL buffalo foetus showing AB/PAS reaction in different layers of epidermis (Ep), corium (C) and spongy bone (B) in dew claw. Alcian Blue/Periodic acid Schiff stain. $\times 40$.

spongy bone of dew claws. Therefore, a reverse pattern of localization of acid mucopolysaccharides was observed than that of neutral mucopolysaccharides in different layer of dew claws. The reaction of Alcian blue indicated the presence of both the sulphated and carboxylated acid mucopolysaccharides and sulphated and carboxylated sialomucins (glycoproteins). This showed that chondroitin sulphate B and hyaluronic acid are major components of stratum corneum and hyaline cartilage of dew claws. Hashimoto *et al.* (1992) also observed weak lectin binding capacity of the intercellular matrix in the stratum corneum of the epidermis in equine hoof which may be due to the loss of desmosomes during keratinization. The deposition of sudanophilic lipids and basic proteins was found moderate to strong in different components of dew claws in buffalo foetus. The reaction of Sudan Black B and bromphenol blue indicated the qualitative status of lipids and basic proteins present in the cellular components.

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