Histological study of the hoof development in sheep

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In order to study of the hoof development in sheep, 16 healthy sheep embryos with different ages immediately after slaughters of their mothers in slaughter house were collected hooges were cut half sagitlly and fixed in 10% buffered formalin. Routine histologic processes were used and 6μ thick paraffin sections were stained by haematoxylin-eosin method and studied under light microscope. The hoof has covered by the real skin at first. The primary changes will be seen on the stratum germinativum of the hoof skin, which caused to formation of the tunica interna of the wall and the papillary layer of the sole and heel. The more supperficial layers were then made by them, so that the tunica media of the wall were made by the epidermis of the corium and upper $\frac{2}{3}$ of the wall. It made up of hard keratin, thus the manufacturing epidermis of this layer were devoided of the stratum granulosum and keratohyalin granules. The tubular layer of the white line were made by the lower $\frac{1}{3}$ epidermis of the wall. The papillay layers of other parts of the hoof were made by the papillary epidermis of the same parts. The last layer were made before birth was the tunica externa. This layer were made by the papillay epidermis of the periople skin.

Key words : Development, Hoof, Sheep, Wall, Periople

Hoof is the keratinized portion of the epidermis arranged in tubules and covers the distal end of the diqit. It is composed of three main parts, the wall, which is the portion visible when the foot is placed on the ground, the sole and a prominent bulb that constitute the part of the ground surface of the hoof. The arrangment of the cornified cells in tubules are mostly spring-like in these parts. This coiled arrangement of the cells helps to dampen the compression of the hoof when it strikes a hard surface, and as a whole this organ has important role in protecting the underlying tissues reducing the surface pressure exerted on them and bearing the weight of the animal.

Investigation for finding the normal structure of this organ especially in solipeds (1, 2, 3, 5, 8) is not new, but with investigation into the sheep hoof. I found that there is little attention into the histological structure of this tissue in the sheep limit data on microscopic structure have been available only on adult ruminents (1, 2, 3) but not on prenatal and perinatal sheep. Therefore I paid special attention to the whole period of development with the purpose of gaining an insight into the structure of the sheep hoof in relation to ontogeny. Present study is concerned with sheep hoof developmental process from the early embryonic life until birth and gives a detailed description of the structures involved, i.e., three layers of the hoof and their differentiation, the pattern appearance of the hoof and a real structure of it right at the birth.

Material and methods

Sixteen healthy sheep embryos of different ages varied from 36 to 140 days were collected. The ages were estimated by measuring the crown - rump length (4). Each limb as well as each hoof was cut in half sagitally and fixed in 10% buffered formalin. Routine histologic processes were used and paraffin sections were prepared with the thickness of 6μm. Slides were stained by haematoxylin - eosin and studies under light microscope.

Results

On the days 36 and 40 of the sheep embryos, limbs were formed as small appendages and consist of masses of mesenchymal cells covered by thin epidermis. No differentiation happened between the different tissues constructing limbs.

on the 46th day, mesenchymal condensation occured at the middle of the foot to form the third phalanx later. The thickness of the epidermis covering the limb was not the same at different part of it, i.e., it was thicker at the foot, where the hoof would be made later, and in the foot it was thicker at the wall. At this time the wall consisted of two layers of cuboidal cells at the stratum germinativum comparing with the other parts of the limb which have just one layer, and 3-4 layers of spinosal cells covered by a thin layer of granulosa cells containing small amount of keratohyalin granules. Finally the wall as well as the whole limb was covered by a thin cornified cell layer.

On the days 50 and 55, cartilagenous model of third phalanx was formed. The stratum germinativum in the epidermis of the

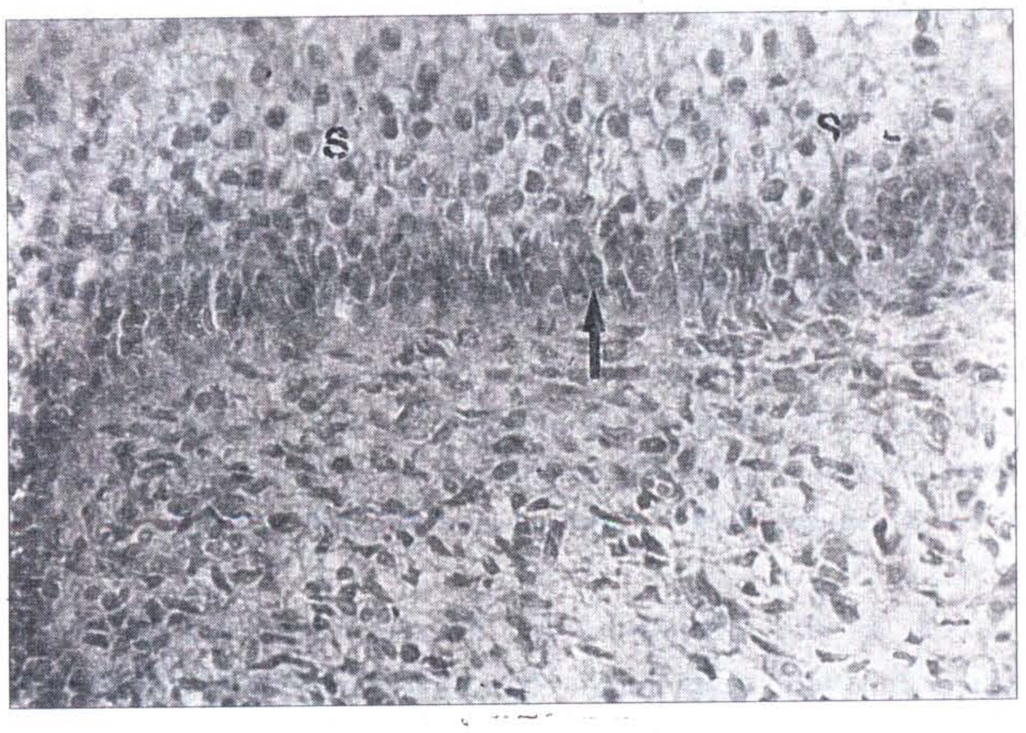

Fig. 1. Microscopic structure of the wall epidermis in the hoof of a 50 day sheep embryo. Note the stratum germinativum with 2-3 condensed cuboidal cell layers (arrow), and the stratum spinosum with 6-8 cell layers (S), H&E ×450.

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wall increased into 2-3 condensed cuboidal cell layers and the stratum spinosum increased into 6-8 cell layers (Fig. 1).

On the day 60, the laminae was formed in the stratum germinativum and its subjacent corium of the wall (Fig. 2). The spinosal cells covering the laminae were highly swelled which caused increase in the thickness of the epidermis of the wall.

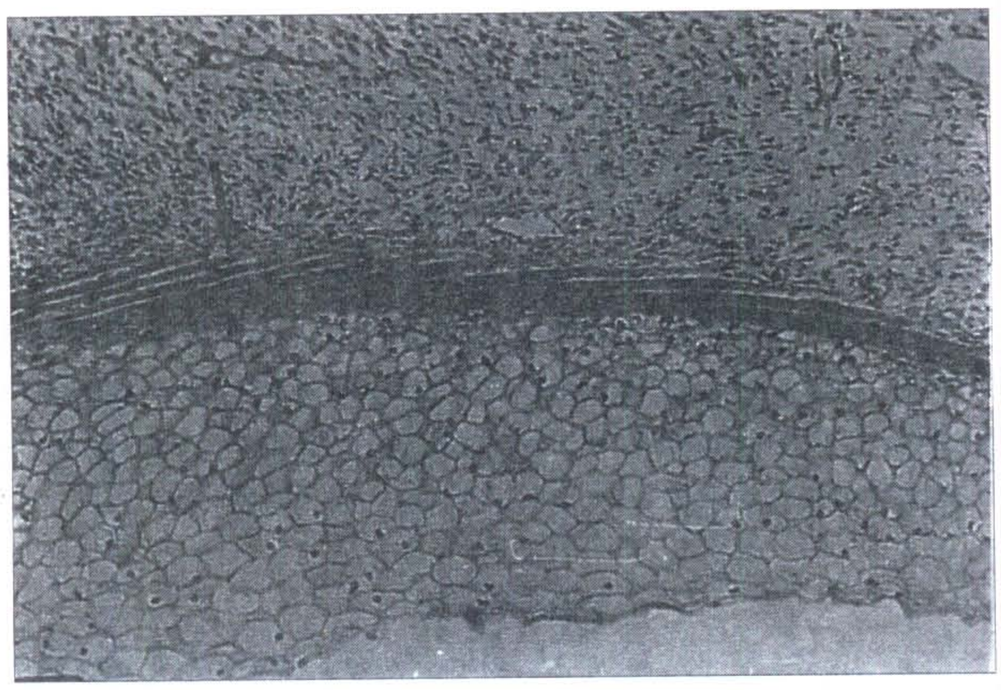

Fig. 2. Histological section demonstrating laminae (arrow) in the epidermis of the wall of the hoof in a 68 day sheep embryo, H&E ×180.

On the 68th day, papilation of the epidermis of the toe started. The granulosa cells of the toe were increased in number and there were keratohyalin granules in their cytoplasm that were larger in size at the superficial layers. They were covered by a thick layer of comified cells, the stratum corneum (Fig. 3).

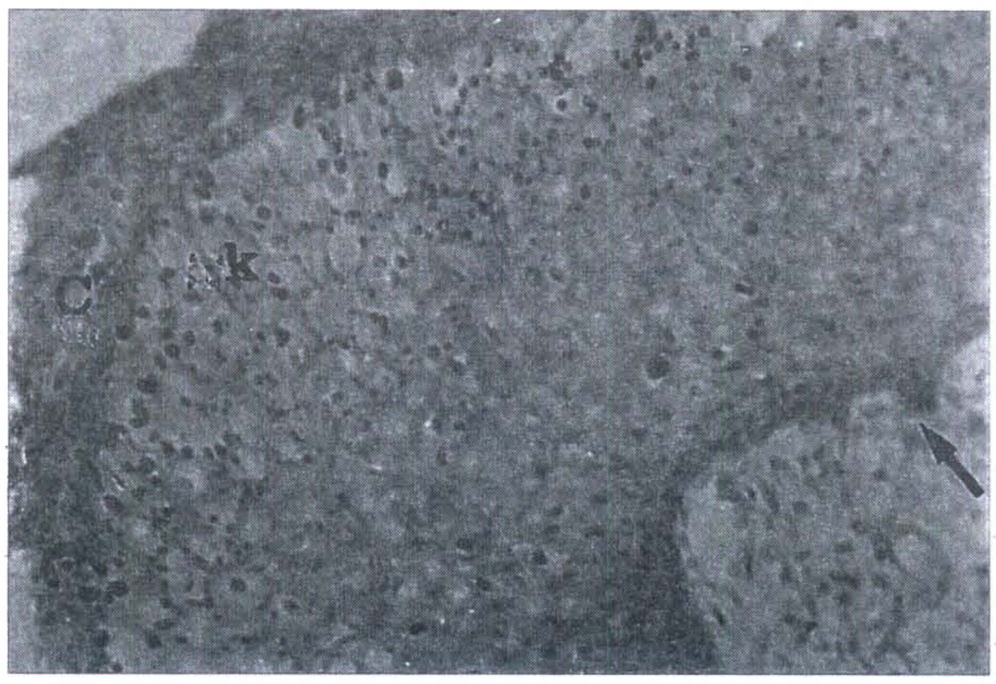

Fig. 3. This is a section from toe epidermis of hoof in a 68 day sheep embryo. Note the papillation in the basal cells (arrow), large amount of keratohyalin granules in the stratum granulosum (K) and a nearly thick stratum corneum (C), H&E ×180.

On the 75th day hair follicles observed for the first time in the whole limb skin except foot, so that the border of the foot was then clearly detectable from the limb skin.

Papilation was started in the epidermis of the coronary region. It's germinal cells were highly divided and made many spinosal celllayers, which moved toward the ground surface. Most of the later cells had lost their nuclei or had picnotic ones. In the laminar part as well as in the toe, the epidermises were highly thick and their sowllen spinosal and granulosal cells were going to make a thick keratinized cell layer on the top of the stratum corneum.

On the 80th day, the germinal epithelium of the periople was become highly thick with more cell layers than the normal skin. The epidermal cells of the coronary region, like those in the laminar part of the wall, were started to produce a thick stratum corneum.

On the 86th and 95th days, keratinization in the epidermal cells of the coronary region were furthur progressed. The germinal epithelium of the sole was become papillated and had increased the number of swollen spinosal and granulosal cell layers which were covered by a thin stratum corneum.

On the 105th day, the more basal epidermal cells of the coronary region were produced a thin layer of pale keratin toward the ground surface. Formtion of this layer caused separation of the basal epidermal cells from the granulosal and the corneal cells at this regoin (Fig. 4).

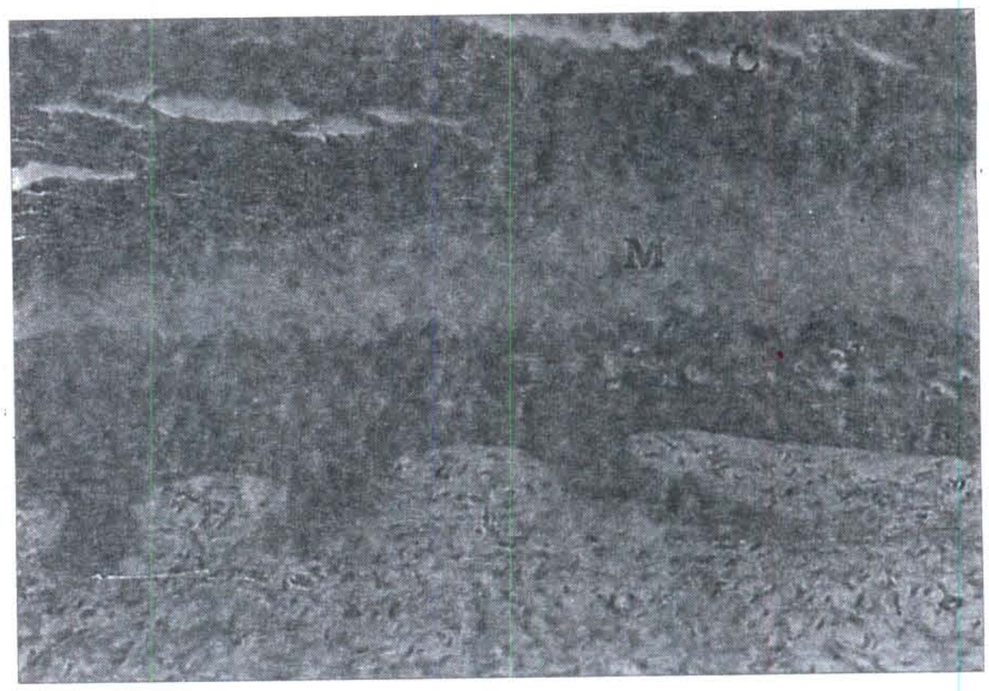

Fig. 4. This is a section from coronary epidermis of hoof in a 105 day sheep embryo. Note the formation of a keratinized layer before the stratum granulosum (M). The color of this keratinize layer is different from the stratum corneum (C), H&E ×180.

It means that the two later cell layers were not involved in the formation of that keratiniyed layer, the middle layer of the wall. The protein components of the keratin in the tunica media seemed to be slightly different from the keratin material of the stratum corneum, by getting different color of haematoxylin-eosin stain. It had pale color compared with the highly basophilic stain of the stratum corneum (Fig. 4). By the formation of this thin keratin layer, the tunica media, in the coronary region, the epidermis of the periople was became papillated and produced more swollen cells projected over the coronet and started to produce comified layer toward the ground surface (Fig. 5). This keratinized layer, the stratum externum (stratum tectonium), will covered the whole parts of the wall later. The perioplic swollen epidermal cells of the bulb were incerased in numbers. They were tended to project over the sole.

On the 110th day, formation of the tunica media of the wall toward the ground surface was furthur progressed. It had caused the same separation of the stratum granulosum and the stratum corneum of near $\frac{2}{3}$ of the upper laminary epidermis of the wall, like

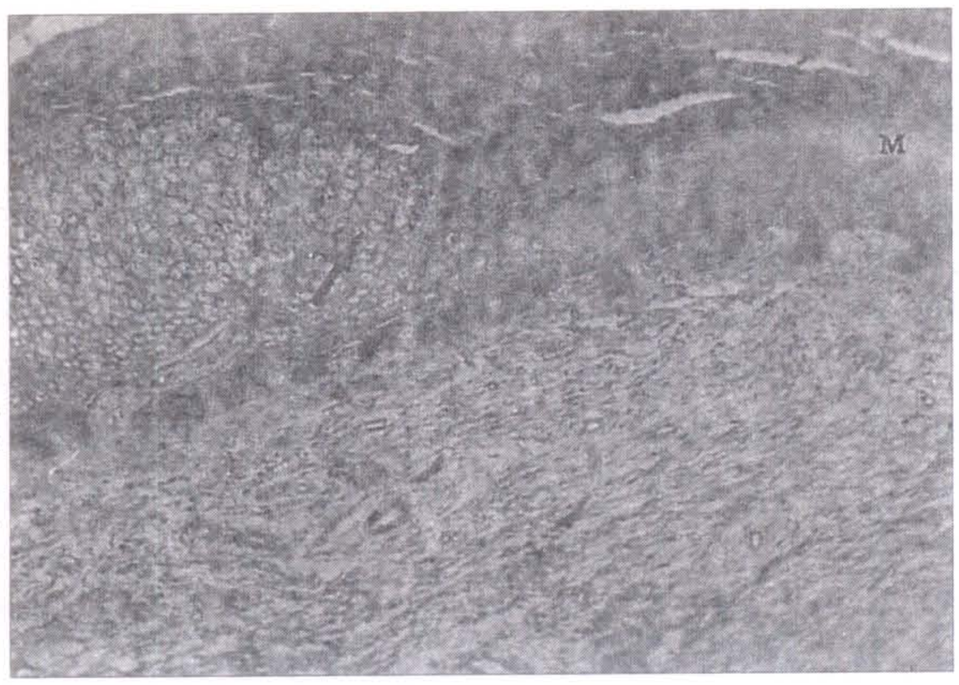

Fig. 5. This is a section from perioplic and coronary epidermis of wall of hoof in a 105 day sheep embryo. The perioplic epidermis contain many swollen spinosal and granulosal cells projected over the coronet (arrow). The tunica media is seen in the coronary epidermis (M), H&E ×90.

in the coronet, from the more basal cell layers (Fig. 6). These basal cell layers of the lamina in accompanied with those in the coronet involved in the formation of the tuncia media. In the coronet, the arrangement of the basal cells for the formation of tubular and in tertubular horns of the tunica media was spring liked. In the epidermis of the sole right after the stratum granulosum, a new layer of swollen cells were formed. By the formation of this new layer, the perioplic epidermis of the bulb strated to produce a keratinized cell layer over the sole.

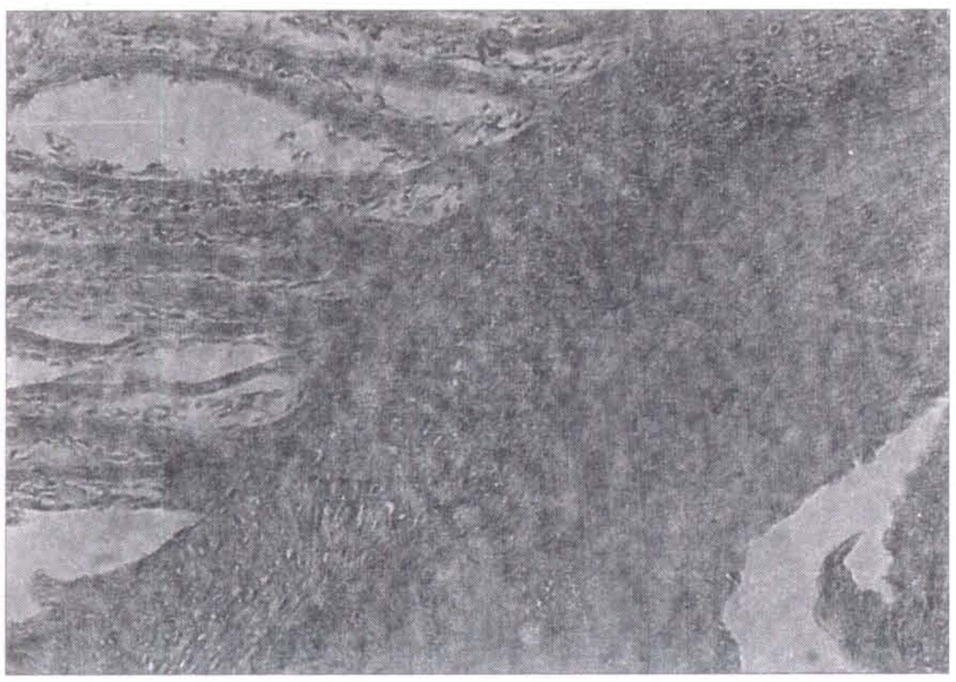

Fig. 6. Microscopic section from wall epidermis of hoof in a 130 day sheep embryo. Note some epidermal cells contain keratohyalin granules (arrow) and some are devoided of them (W). The tunica media (M) and the tunica Externa (E) are cleary observed, H&E ×180.

On the 120th day, the thickness of the tunica media of the wall had increased. When it reached to $\frac{1}{3}$ lower parts of the wall and the white line, caused the separation of the stratum corneum from the underlying epidermal cell layers (Fig. 6). The epidermal cells of these regions, except their stratum corneum, involved in the formation of the tubular layer of the white line. This layer was composed of many, not completely keratinized, swollen cells. The thickness of the newly formed layer in the sole were highly increased. Coveration of the granulosal and corneal cells of the perioplic epidermis of the bulb over the sole were furthur progresses, therefore we can see two distinct granulosal and corneal cell layers in the sole. The more basal on was belonged to the sole epidermis and involved in the formation of the tubular layer of the sole and the next one was belonged to the perioplic epidermis of the heel and involved in the formation of a keratinized cell layer covered th sole the stratum extemum (Fig. 7).

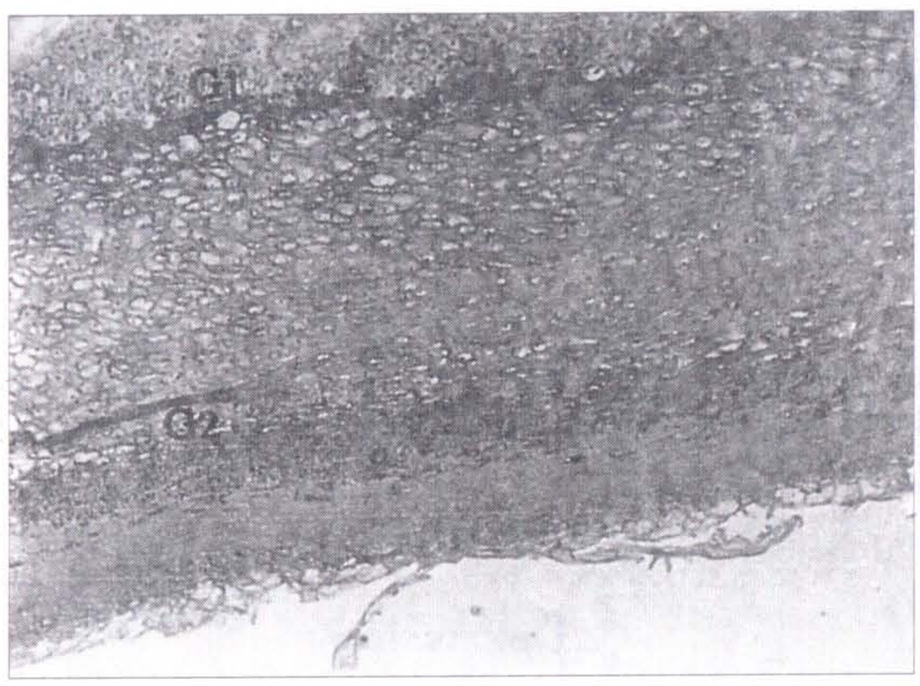

Fig. 7. This is a section from sole epidermis of hoof in a 130 day sheep embryo. There are two distinct layer of the stratum granulosum this part. The first one (G1) belong to the sole epidermis and involve in the formation of the tubular layer of the sole. The second one (G2) belong to the perioplic epidermis and involve in the formation of the tunica externa which cover the sole, H&E ×90.

On the 130th day, the keratinized tubular layer was formed in the heel (Fig. 8) and on the 140th day, the thickness of the tubular layer was highly increased in the heel and tubular and intertubular organization were formed. It was highly thicker than the tunica media of the wall.

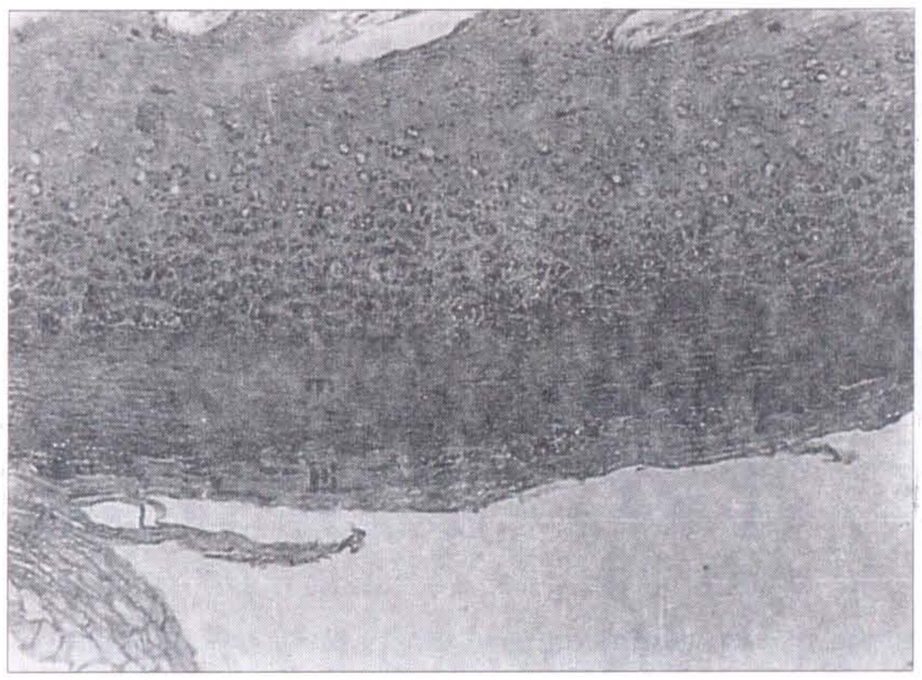

Fig. 8. Microscopic section from heel epidermis of hoof in a 130 day sheep embryo. The tubular layer is keratinized (T) and the tunica externa (E) is covered it, H&E ×180.

In spite of the high thickness of the epidermal cells of the

tubular layer in the sole they were not completely filled with keratin (Fig. 9). Final keratinization of this part may formed after birth.

Fig. 9. This is a microscopic section from the tubular layer of the sole epidermis in a 140 day sheep embryo. Note the cells in the tubular layer are not completely filled with keratin (arrow), H&E ×180.

Discussion

Hoof is one of the specialized organ of the integumentary system suited for weight bearing as well as protecting the foot from suffering by the surface injuries.

Most parts of the hoof are formed of cornified material or keratin arranged in tubes (2). It is composed of epithelial cells which are more or less completely keratinized except in its deepest part the stratum germinativum, here the cells have not undergone cornification and by their proliferation maintain the growth of the hoof (5).

Structurally the hoof composed of three parts, the wall, the sole and the bulb. The wall consists of three layers. The external layer or tunica externa comprises the periople and the stratum tectorium or strtum externum. It is continuous with the epidermis of the skin above and extends downward a variable distance. Usually it forms a distinct band except at the heels where it is much wider and covers the entire surface. The stratum externum is a thin layer of horny scales which gives the outer surface of the wall below the periople. The middle layer or the stratum medium forms the bulk of the wall and is the densest part of the hoof. Its horn tubes run in a parallel direction from the coronary to the basal border (5).

The laminar layer or the stratum inernum consists of nontubular horn elongated at a slow pace " perpendicular " to the stratum medium (2). In the ruminant, it consists of primary laminae only (2). The sole and the bulb consist of tubular and intertubular horn and the underlying papillae. The horn material is softer than the wall (2). The ground or basal border of the hoof comes to contact with the ground surface and connect the wall to the sole is called with zone.

Histologically the hoof is a tightly bounded stratified squamus epithelium with different arrangement in its germinal cells (laminar in the wall, papillar in the other parts) covered by two layers of, keratin. Keratin is the transformation product of the germinal cell differentiation (2). Cells undergoing keratinization become enucleated, flattened, and filled with a secretory product, keratin.

The protecins of the epidermis under go an extensive differentiation program as the proliferative basal cell passes upward through the spinous and granular layers, and terminates as a dead stratum corneum cell (6). Keratin is a physically tough, insoluble protein and is particularly thick over some regione of the body, subject to high friction forces, e.g., palmar - plantar surfaces (7). Soft keratin characterizes the majority of the epidermis and hard keratin is typical of the skin appendages, feathers, parts of hair, hooves, etc (2).

In the embryonic life hooves as well as other organs formed gradually. Before the formation of the hoof, the whole limb was covered by a normal skin contained keratinized epidermis. For the formation of the hoof the stratum germinativum of its epidermis, in concern with the occurence of alteration in the structure, was the first layer became laminar with just primary laminae in the laminar part of the wall and papillar in the other parts. For the formation of the wall, papillary epidermis of the coronary region in acompany with the near $\frac{2}{3}$ of the upper parts of the laminary epidermis of the wall started producting cornified cells. Investigators believe that the middle layer of the wall is typically hard keratin and devoid of a stratum granulosum and stratum lucidum in the horse (2). My results showed the same in such a manner that the epidermal cells of the coronary and $\frac{2}{3}$ of the upper laminar part were devoid of keratohyalin granules as well as the stratum granulosum. Their keratin was orange in color near the epidermal cells gradually converted to a pale acidophilic color further. Lack of keratohyalin granules in these parts may be used for clear detection of perioplic epidermis from the coronet and the laminar epidermis that participates in white zone keratin production, from this layer involved in the formation of the wall.

The tubular layer of the other parts of the hoof was made from the papillary epidermis of those parts which all contained keratohyalin granules as well as the stratum granulosum. Keratin was highly acidophil in the bulb and the white zone but not in the sole.

In the sole, although there were more than 3-4 granular cell layers in its epidermis, keratinization in the tubular layer was not completed even at the birth and the cells were not filled completely with keratin. May be that is why the newborns lean on the toe and bulb more than the sole.

The last layer which formed in the hoof was the tunica externa. Authors believe that it is a soft keratin extend over the wall and bulb in the horse (2). My results showed the stratum externum as a distinct layer of soft keratin formed from the perioplic epidermis and covered the entire part of the hoof. It has basophilic color compare with the highly acidophilic of the tunica media and the tobular layer.

مطالعه بافت‌شناسی تکامل سم در گوسفند

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بمنظور مطالعه ریزبینی مراحل تشکیل سم در گوسفند تعداد ۱۶ جنین سالم گوسفند با سنین مختلف بلافاصله پس از ذبح مادرانشان در کشتارگاه جمع‌آوری گردید. سپس برشی سهمی میانی به هر یک از سمها داده شده و نمونه‌های بلافاصله در بافر فرمالین ۱۰ درصد قرار داده شد. پس از انجام روشهای معمول آزمایشگاهی، مقاطع پارافینی به ضخامت ۶ میکرون تهیه و با هماتوکسیلین - ائوزین رنگ‌آمیزی و با میکروسکوپ نوری مورد مطالعه قرار گرفت. نتایج حاصله نشان داد که سم ابتدا پوستی مشابه پوست سایر نواحی بدن دارد. سپس اولین تغییر در لایه زاینده اپیدرم پوست این ناحیه حادث می‌شود. این تغییر منجر به تشکیل لایه داخلی دیواره و پاپیلای کف و پاشنه می‌گردد. سپس لایه‌های مذبور شروع به ایجاد لایه‌های سطحی‌تر سم را می‌نمایند، بطوری که اپیدرم لایه‌ای تاج و $\frac{2}{3}$ بالایی دیوار تشکیل لایه میانی دیوار را می‌دهند. این لایه از جنس کراتین سخت است و اپیدرم سازنده این لایه فاقد لایه گرانولوزا و دانه‌های کراتوهیالین است. لایه توبولار خط سفید از اپیدرم $\frac{1}{3}$ پایینی دیوار تشکیل می‌گردد. اپیدرم در این ناحیه واجد لایه گرانولوزا و بالطبع دانه‌های کراتوهیالن است. لایه توبولار بقیه قسمتهای سم از اپیدرم پاپیلار همان نواحی تشکیل می‌شود. آخرین لایه‌ای که قبل از تولد بوجود می‌آید لایه خارجی است که از یک لایه بافت شاخی نرم تشکیل شده و بطور کامل سطح سم را می‌پوشاند. این لایه از اپیدرم پاپیلار پوست تاج ساخته می‌شود.

واژه‌های کلیدی : تکامل، سم، گوسفند، دیوار، پریوپل

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