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The Study of Histological Observations of Ovine Claw Coronary Region and the Architecture Morphology of Arterial Anastomosis of This Area

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Abstract: This study was carried out with the aim of assessing the importance of coronary region for providing a clear comprehensions study into the structure and function of coronary dermal papillae and their role in potentiality of adaptive variations. A total of 30 sheep claws were collected and tissue sections were extracted from the coronet. Following processing and embedding, tissues from each claw were cut and stained to visualize a number of histological aspects. The samples were also evaluated using cast form to detect the arterial blood supply. This study indicated the architecture distribution of blood anastomosis within the foot, where the main anastomosis of blood capillaries was in proximal, distal and caudal part of sheep foot. The presence of such complex arterial anastomosis in the blood vessels suggest that a greater blood flow capacity to the sheep feet. Histologically, the results showed that the coronary region of claw sheep had numerous perioplic and coronary dermis. The dermal papillae were distributed on the coronet region with average number 11/mm^2. The results indicated that average thickness of stratum basale and stratum spinosum was 202.4±3.3 µm, and average stratum corneum was 68.3±4.2 µm, while the whole epidermal tissue started from the basement membrane to the external surface was 270.8±2.5 µm. The data of the current study will be used as a reference literature to treat the pathologically affected sheep feet diseases.

Keywords: Blood Anastomosis, Cast Form, Claws Sheep, Coronary Region, Dermal Papillae.

Koyun Tırnak Koroner Bölgesinin Histolojik İncelemesi ve Arteriyel Anastomozunun Yapısal Morfolojisi

Öz: Bu çalışma, koroner dermal papillaların yapısı ve işlevi hakkında net bir kavrayış çalışması sağlamak için koroner bölgenin önemini ve adaptif varyasyonların potansiyelindeki rolünü değerlendirmek amacıyla yapılmıştır. Toplam 30 koyun ayak tabanı toplandı ve doku bölümleri tırnağın taç kısmından çıkarıldı. Doku takibi ve parafin blokajından sonra, kesitler alındı ve mikroskobik değerlendirmeler için H&E ile boyandı. Örnekler ayrıca arteriyel kan kaynağını tespit etmek için alçı form kullanılarak değerlendirildi. Bu çalışma, kan kılcal damarlarının ana anastomozunun koyun ayağının proksimal, distal ve kaudal kısımlarında olduğu gösterdi. Kan damarlarında bu gibi kompleks arteriyel anastomozların varlığı, koyun ayaklarına daha fazla kan akış kapasitesi olduğunu göstermektedir. Histolojik olarak, sonuçlar koyunlarda ayak tabanının koroner bölgesinin sayısız perioplik ve koroner dermise sahip olduğunu göstermiştir. Dermal papillanın, ortalama sayısı 11/mm^2 olduğu saptandı. Stratum basale ve starum spinosum 202.4 ± 3.3 um ve stratum korneum 68.3 ± 4.2 um olarak ölçüldü. Tüm epidermal dokunun bazal membrandan dış yüzeye uzaklığı 270.8 ± 2.5 um idi. Bu çalışmanın verileri, potansiyel olarak tedavi için yeni kanıtlar sağlayarak, patolojik olarak etkilenen koyun ayak hastalıklarında referans kaynak olarak kullanılabilecektir.

Anahtar Kelimeler: Alçı Formu, Dermal Papilla, Kan Anastomozu, Koroner Bölge, Koyun Ayak Tabanı.

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INTRODUCTION

The distal extremity of odd-toed and/or even-toed ungulates is protected by a unique structural component called hoof/claw. This has various different segments; each of them has various rates of horn growth and abrasion with different function. (1,2). The external morphology of the hoof or claw wall is indirectly interrelated with the shape and function of the internal parts of the animal foot (3,4). Small ruminants are cloven-footed animals that means the hoof consists of two digits (3), rather than one compact entity similar to that of equine feet (5). These digits are equivalent to the third and fourth fingers of the human hand (6). In general, the claws are termed by their relative position on the animal foot. The claws/hooves have a highly complicated and anastomosis blood supply which is mainly originated from small branches of the dorsal tarsal/metatarsal arteries and common palmar/plantar digital arteries (7,8). In the rear limb, most of the digital arteries are formed from the small branches of common planter digital arteries (6). These digital arteries are extended at the level of second digit to provide blood supply to the coronet and further down to the heel bulbs (5), whereas, there are nearly up 8 to 10 small branches arising distally to supply blood to the sole margin. This morphoorganization of arterial blood supply in animal feet is a highly important in healthy feet, as it is a subtle alteration of the microvascular system in the coronary region could change the amount of keratinous tissue and this leads to change in the structural and functional components of the hoof wall (9). For instance, in equine hoof, it was found that any deffct in the capillary network of the terminal arch and the small arteries arising from it helped in the development of chronic laminitis and/or can be develop to cause decreases in the rates of horn growth. However, up to now, there has been very little published research regarding the importance of blood circulation on the ovine claw coronary region (9,10). The hoof or claw is deemed as the most vital part for weight bearing and/or for movement (11). A comprehensive understanding of morphology of ovine claw coronary region would help in understanding the normal physiology of the conformation of sheep claw and further consequences of overgrowth rate, great wear or trimming claw horn in disease and/or healthy feet (12). It would as well contribute in understanding the role of claw horn in persistence and spread of infectious foot diseases (13,14). Therefore, this study was aimed to provide a complete and clear investigation of anatomical and histological observations of claw sheep coronary region, also to investigate the architecture morphology of such important part in sheep foot this would help to determine the causes, which result in the hoof deformities or possibly eliminate some causes to aid in future management plans.

MATERIALS and METHODS

Ethics Statement

This investigation was performed under the highly strict adherence to the main rules of care and the way of using domestic animals in research, teaching and experiment (15).

Experimental Animals

Sheep hooves were collected from the slaughterhouses one-hour post euthanasia following ethical approval by The College of Veterinary Medicine, University of Diyala. A total of 30 sheep hooves were divided into 20 of them were used for histological sampling and 10 for arterial cast form. Tissues samples of 6-7mm thickness were taken from the dorsal coronet as well as from the quarter regions both medial and lateral, and instantly fixed in 10% neutral buffered formalin for 24 hours. Then all tissue samples were processed by routine methods, embedded in paraffin wax and then sectioned at 5 µm and stained with routine staining (H&E).

Measurements of the epithelial thickness were calculated using calibrated a Fiji J software. Measurements of epidermal layers were performed on each image, measuring the space between basement membrane and cell keratinization, and the space between the basement membrane and the end of hoof wall. These measurements were recorded as mean±SEM using Excel software. Ten sheep hooves were collected for cast form of blood vessels, phosphate buffer saline (PBS) was injected via the medial and/or lateral palmar/plantar common digital arteries to evacuate blood vessels from the blood. Followed by injected with acrylic resin (self-curing repair powder and liquid containing Methyl methacrylate monomer, 4-dimethylamino toluene), extra pressure was applied to ensure the resin reached to fine blood capillaries. After that, all samples were left for 48 hours at 21 °C to reach full solidification, and then the samples were transferred for maceration as follow. Then, each sample was incubated in 40% potassium hydroxide for 72 hours to ensure digestion whole tissues (16). The soft tissue was completely macerated, so the cast was heavily contaminated with macerated tissue. Then the cast was washed with tap water. At the end, it was cleaned gently by washing with fine jet of syringe and then dried by air for examination and taking photograph using digital camera.

RESULTS

Cast form of blood vessels clearly showed the architecture distribution of blood anastomosis within the sheep foot. The main anastomosis of blood capillaries was observed in proximal, distal and caudal part of sheep foot as well as inside foot (Figure 1). The dorsal artery of the third phalanx appeared branches and anastomosis proximally forming coronary circumflex artery and distally circumflex artery of the sole, whereas the caudal surface appeared vascularized by arterial branches anastomose and nourished the coronet and extended further distally to nourish the digital cushion of the heels (Figure 1). The sole of the heel part was observed vascularized by small arterial branches of terminal arch and sole margin arteries.

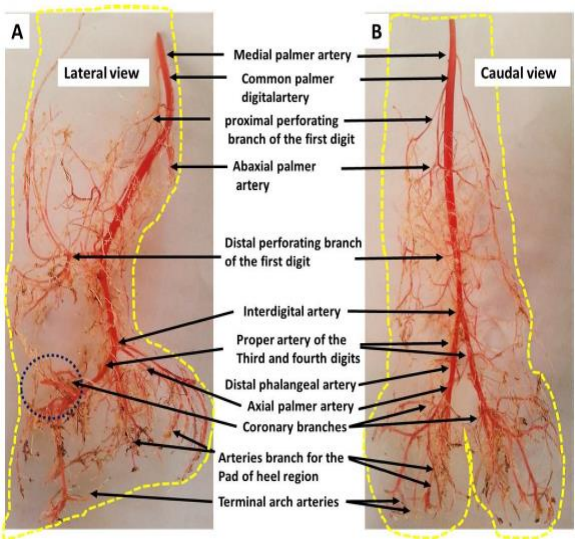

Figure 1. Photographs illustrating cast form of arterial blood supply of sheep foot. A) Shows lateral view of cast form. B) Illustrates distribution and the outlines of cast blood vessels on caudal view.
Şekil 1. Koyun ayağının arteriyel kan akışının döküm şeklini gösteren fotoğraflar. A) Döküm formunun yandan görünüşünü göstermektedir. B) Kaudal görünüm kan damarlarının dağılımını ve dökme ana hatlarını göstermektedir.

The results obtained from histological examinations revealed that the capsular wall had three distinct layers: stratum externum, stratum medium and stratum interum respectively. These findings showed that the stratum medium constituted the main bulk of the capsular wall and comprised from finger shape projections organized parallel and oriented vertically from the surface of interconnection between dermal and epidermal tissues of the coronet toward the ground surface (Figure 2). The dermal area of the periople and/or coronary regions showed to be formed from mesh dense matrix of connective tissue including nerves, arteries and veins, whereas the epidermal layer appeared to be comprised mostly from the stratified squamous keratinized epithelium and was observed continuous with the skin leg and appeared consisted from four expected layers (Figure 2).

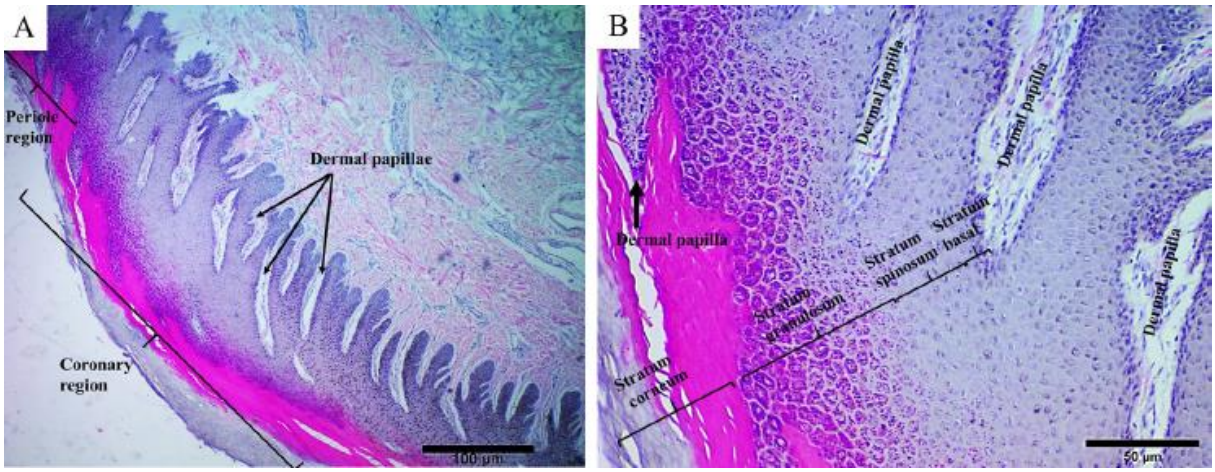

Figure 2. A) Photomicrograph image shows claw coronary region. B) Photomicrograph image shows the fourth layers of epidermal tissue of claw coronary region. These were appeared in the first layer (stratum basale), second layer (stratum spinosum), third layer (stratum granulosum) and fourth layer (stratum corneum). (H&E stain; (A) bar= 100 µm; (B) bar =50 µm).

Şekil 2. A) Fotomikrograf görüntüsü pençe koroner bölgesini göstermektedir. B) Fotomikrograf görüntüsü pençe koroner bölgesinin epidermal dokusunun dördüncü katmanlarını göstermektedir. Bunlar birinci tabakada (stratum basale), ikinci tabakada (stratum spinosum), üçüncü tabakada (stratum granulosum) ve dördüncü tabakada (stratum corneum) görüldü. (H&E stain; (A) bar= 100µm; (B) bar =50 µm).

The stratum basale was appeared as a thin deepest layer consisted from a double set of cuboidal to columnar cells, these cells were placed and arranged along the basement membrane. The long axis of their nucleus was observed directed at an acute angle to the keratinous layer (Figure 3). In addition, it was observed a black pigment of melanocytes (Figure 3) covered some of the basal cells. The second layer (stratum spinosum) was composed of numbers of polyhedral cells, these cells were appeared tightly adhered to each other and seemed to be more flattened toward the third and fourth external layer (stratum corneum). The third layer (stratum granulosum) was observed had granules in their cytoplasm and their nucleus ranged from oval to spherical shape, whereas the outermost layer (stratum corneum) was appeared as dead keratinocytes, which had either lost most or completely of the cells their nuclei toward the keratinous layer (Figure 3).

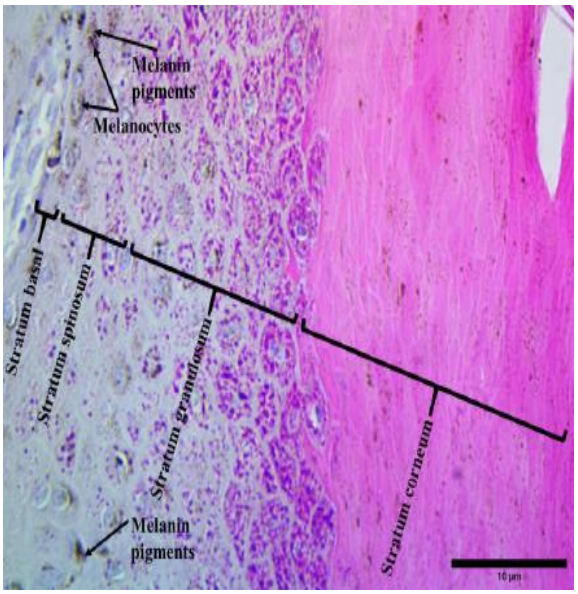

Figure 3. Photomicrograph image shows the fourth layers of epidermal tissue of claw coronary region at high magnification. Melanocytes were appeared at basal, spinosum and granulosum layers. (H&E stain; bar= 10 µm).

Şekil 3. Fotomikrograf görüntüsü yüksek büyütmede pençe koroner bölgesinin epidermal dokusunun dördüncü katmanlarını göstermektedir. Bazal, spinosum ve granulosum tabakalarında melanositler görüldü. (H&E stain; bar= 10 µm).

Measurements of the epithelial thickness were documented in (Table 1) and (Figure 4) as mean±SEM calculated for each sample. The results indicated that average stratum basale and stratum spinosum and granulosum was 202.4±3.3 µm, and average stratum corneum was 68.3±4.2 µm, while the whole epidermal tissue started from the basement membrane to the external surface was 270.8±2.5 µm (Figure 4). Further analysis for the number of dermal papillae of coronary region on sheep claw revealed that the average number in the current study was found to be 11/mm^2 (Figure 4).

Table 1. Measurements of epithelial thickness at claw coronary region.
Tablo 1. Pençe koroner bölgesinde epitel kalınlığı ölçümleri.

Epidermal layers at claw coronary region	Epithelial thickness in µm
Stratum basale, spinosum and granulosum	202.4±3.3
Stratum corneum	68.3±4.2
Whole epidermal tissue	270.8±2.5

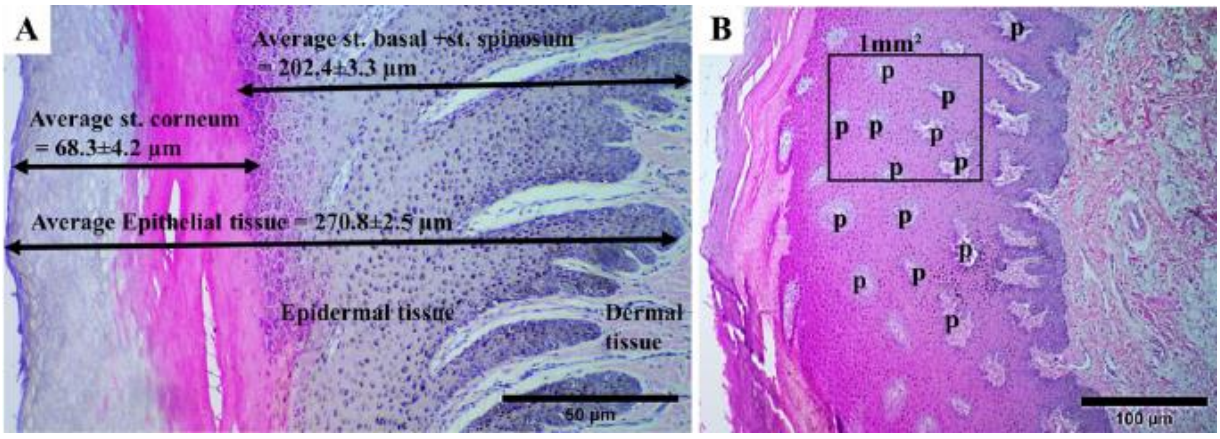

Figure 4. A) Photomicrographs illustrate measurements of epithelial coronary thickness were measured the average thickness of the basal and spinosum layers, corneum and lucidum respectively. B) Photomicrograph shows the number of (p) dermal papillae. Dermal papillae in each cross section of images were counted per surface area. (mm^2). (H&E stain; (A) bar= 50 µm; (B) bar =100µm).
Şekil 4. A) Fotomikrograflar, epitel koroner kalınlığının ölçümlerini sırasıyla bazal ve spinosum tabakalarının, korneum ve lucidumun ortalama kalınlıklarının ölçüldüğünü göstermektedir. B) Fotomikrograf dermal papilla sayısını (p) gösterir. Görüntülerin her bir kesitindeki dermal papillalar, yüzey alanı başına sayıldı. (mm^2). (H&E stain; (A) bar= 50 µm; (B) bar =100µm).

DISCUSSION and CONCLUSION

Analysis obtained from the cast resin for arterial blood supply revealed the presence of a complex arterial anastomosis in the blood vessels suggest that a greater blood flow capacity to the sheep feet; consequently, any defect in such complex arterial anastomosis of sheep feet may develop to ischemic necrosis (17,18). In addition, it was reported that these vascular anastomoses are capable to withdraw almost 50% of the entire limb blood flow, and hence can be engaged in ischemia due to vasoconstriction and blood flow diversion (19). Cast form method enables following the blood vessels via the clear distribution of the capillary network. This method also showed the architecture distribution of blood anastomosis within the sheep foot. These results exhibit the main anastomosis of blood capillaries in proximal, distal and caudal part of sheep foot, which appears in Figure 1. These outcomes are contrary to that of (5,20), as they describe the blood supply of equine feet, however, our study showed similarity

with blood supply of caprine feet and with some differences with bovine feet (5). Furthermore, this study demonstrated the importance of morpho-organization of arterial anastomosis in ovine feet provide further support insights into the hypothesis that the arterial anastomosis of terminal arch and its branches are protected by the mesh of bony canal, as it is reported by other researcher in equine feet (4).

The first set of analyses examined coronary tissue stained with the routine stains H&E revealed the presence of three distinct layers: stratum externum represented by the periople, whereas stratum medium exhibited the main bulk of claw wall and finally the stratum internum showed the lamellae. The stratum medium was found comprised from finger shape projections organized parallel and oriented vertically from the surface of interconnection between dermal and epidermal tissues of the coronet toward the ground surface as expected from previous investigations (21-23). Furthermore, at the stratum externum and stratum medium showed many perioplic and coronary dermis were invaginated and papillated under the epidermis in a precise orderly way, which appeared to have a finger like projections extended and protruded deeply in epidermal tissue, and these papillary projections called dermal papillae. Comparison of these findings with those of other studies (24) confirms the complexity of mammalian integumentary structure that is found by the interdigitaion of dermal papillae. Additionally, the ultimate forms of the capsular wall are generally defined by the configuration of the interface between dermal and epidermal tissue, which is determined in hoof wall by dermal papillae (25). In this study was observed that the core of each papilla contained connective tissue and blood vessels. It can thus be suggested that microanastomoses of these vessels are entered each papilla to supply epidermal tissue. The H&E stain exhibited the dermal and epidermal tissues in contrasting colors in pink and purple respectively, and highlighted the basement membrane border in a purple color. This finding is consistent with recent study done by Al-Agele (26) on equine coronary dermal papillae.

The dermal area of the periople and/or coronary regions showed to be formed from mesh dense matrix of connective tissue including nerves, arteries and veins, as expected from previous observations (27). In contrast, the epidermal layer appeared to be comprised mostly from the stratified squamous keratinized epithelium and was observed continuous with the skin leg and appeared consisted from four expected layers. This finding is consistent with that of (28). According to these results, we can infer that the keratinous layer was directed simultaneously at an angle from the dermo-epidermal junction of dermal papillae toward the ground surface (26).

The results of measurements of the epithelial thickness indicated that average stratum basale, spinosum and granulosum was 202.4±3.3 µm, and average stratum corneum was 68.3±4.2 µm, while the whole epidermal tissue started from the basement membrane to the external surface was 270.8±2.5 µm. It can therefore be assumed that the thickness of the epidermal layers dependent on the cornification of keratinocytes (29). Further analysis for the number of dermal papillae of coronary region on sheep claw revealed that the average number in the current study was found to be 11/mm² and this was less than reported in horses, which showed 21/mm² (26), and slightly less in cattle that reported 13/mm² (30). The interpretations of these findings suggest the requirement for additional examination into the biophysical causes such as increases and/or decreases of growth rate of sheep claw. More analyses of the mechanobiological influences involved in determining the morphology of the claw wall are required and, particularly, the modifications which appear in lame sheep which might be consistent with the anatomy and has substantial consequences for claw function (31,32).

In conclusion, the biological properties of the claw wall have been measured sufficiently to enable an adequate material model providing new two

understandings into the normal compared with pathologically affected sheep feet, providing new evidence for potential treatment.

Conflict of interest

The authors declare that they have no conflict of interest.

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