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Comparative histomorphometrical study of the lamellae in odd-toed and even-toed ungulate animals

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Abstract

This study aimed to create a hoof lamellae model by combining the histomorphometric data of lamellae from animals with odd and even toes. The comparison study showed that odd-toed horses and donkeys have two unique types of epidermal lamellae (EL) in the innermost layer of the capsular wall, known as primary (PEL) and secondary epidermal lamellae (SEL). In contrast, even-toed cows and camels have only PEL. These lamellae form a complex and complicated dermo-epidermal connection by interlocking with the corresponding primary (PDL) and secondary dermal lamellae (SDL). The PEL were all the same shape, and cells made up the intertubular horn of the stratum medium. Animals with odd toes had SEL. A group of non-cornified basal cells and a central area with partially keratinized cells were composed of these Els. A distinct and straightforward connection between SEL and PEL was shown via connective tissue fibers. In both odd-toed and even-toed animals, the PEL originates from basal cells placed at the proximal extremity of the stratum internum. As the cells migrate towards the outermost edge, they undergo keratinization, contributing to the overall count of keratinized cells in the PEL. The results indicated significant differences in lamellae between those animals with odd-toed and even-toed feet. In addition, the current outcomes deliver a new perspective on the relationship between the architecture of hoof lamellae in animals with odd or even toes and their increased ability for keratin formation, which might explain the development of hoof diseases in the future.

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Introduction

The mammalian distal limb exhibits significant morphological variety in its integumentary appendages, including claws, nails, and hooves. This variation is observed across closely related mammalian species and even within the same family (1-3) asserts that the dermal papillae of growing horse hooves exhibit distinct characteristics compared to those of even-toed cattle or pigs and other comparable digital end organs such as the nail and claw. It has been found that the dermal papillae on dog and cat claws could be better developed on the odd and even toe adaptive angulates such as cattle and/or equine capsular walls (3). Due to evolutionary history, this corresponds to homology theory (1). The hoof compartments, including the sole, are similar to those in pig, sheep, and cow hooves or feline and canine claws (4,5), as well as those in primate nails (6). Digital end structures can be conceptualized as entities capable of adjusting to diverse mechanical forces. Consequently, a cornified layer develops, such as in the distal compartments of mammals (3,7), which offers mechanical strength. The hoof wall's inner surface has a lamellar structure that is 2-3 mm thick, with tubules positioned between the lamellae (8). The capsule wall consists of three distinct layers, namely the externum (periople), the medium (coronet), and the interior (lamellatum). The epidermal lamellae's orientation on the

horsefoot's inner surface is from the proximal to the distal region (9). The equine inner capsular wall is made up of primary and secondary epidermal lamellae (P and SEL) that are interconnected with the dermal lamellae (DL) (9,10). Under the distal phalanx, a digital cushion is essential in a bovine hoof. In cattle feet, a hairless epidermis displays three distinctive cellular stratums: [1] a glossy stratum corneum, [2] the underlying epidermal cell stratums, and [3] the stratum dermis on the posterior surface of the feet. A camel's claw consists of a fold, a wall, and a sole (11). The fore and back digital pads contain the epidermis, dermis, and subcutis. While a large subcutaneous cushion is present in elephants' feet (12), fat and connective tissue make up these cushions. However, the ovine (13) doesn't have digital pads on its feet like the bovine, but elephants do (12). Many animals, including bovine, equine, camel, and swine, have been shown to have complex circulatory systems in their hooves (5,14).

The objective of this study was to conduct a comparative analysis of the histological and architectural characteristics of the stratum internum of the hoof wall. The study also examined the histo-comparative properties of the hoof lamellae in both odd-toed and even-toed ungulates to make a model of the hoof wall.

Materials and methods

Ethical approval

All procedures involving domestic animals in this study were carried out following the strictest standards for animal care and research. The experiment was conducted according to guidelines approved by the College of Veterinary Medicine, University of Diyala Animal Experimentation Ethics Committee, No. VM 202. February 2022. K&R, dated on 1/2/2022.

Sample collection

Hoof samples were collected from the front feet of 40 animals. The animals were categorized into four groups: the first consisted of 10 front feet from horses, and the second consisted of 10 front feet from donkeys. These were intravenously collected post-euthanized with pentobarbital sodium (150 mg/kg body weight). The third group consisted of 10 front feet from cows, and the fourth group consisted of 10 front feet from camels. These were collected from the slaughterhouse. Hooves were collected one-hour post-euthanasia (14). The tissue samples were acquired from the dorsal area. Based on the histological technique, the samples were fixed, routinely processed without decalcification, and stained with H&E (15). The morphometric measurements of lamellae in odd-toed and even-toed ungulate animals were determined by calculating (mean ± SE) the length and width of the PEL and PDL and the SEL and SDL using calibrated Fiji J software and then imported to a Microsoft Excel sheet. Histological sections were photomicrographs using an Olympus microscope with an OMAX digital camera. The statistical analysis of the data was conducted using the Graphpad Prism software.

Results

Hoof lamellae of the odd-toed animals

An examination of odd-toed feet showed that they encompassed PEL and SEL. The lamellae interdigitated with the DL, creating an intricate and tangled connection between the dermis and epidermis. The SEL consisted of vital progenitor cells called basal epidermal (be) and a set of suprabasal (sb) cells that were non-cornified (Figure 1). The suprabasal cells were located in the central region of the SEL, as well as in the space between the bases of two lamellae (Figure 1). The nuclei of these cells exhibited similarity to the nuclei of the germinal basal cells. The PEL appeared to be one continuous layer, with cells making up the intertubular horn of the inner stratum medium. In this instance, the secondary layers of the epidermal tissue seemed to immediately link to the primary layers through their suprabasal cells (Figure 1), which were flat and aligned with the primary layers. The laminae join the lamellar corium, forming an intricate bridge between the epidermis and dermis via connective tissue fibers. This connective tissue attached the keratinized capsular wall to the beneath dermal tissue (Figure 1). The stratum corneum included the rigid keratin of the PEL. They were situated near the apex of the dermal laminae, deep within the coronary groove. As the stratum medium lengthens, the cells keratinize and migrate towards the hoof's ground surface. The stratum germinativum makes up the cellular SEL. Each SEL's basal stratum rests on the connective tissue of its corresponding SDL, generating an interdigitation (Figure 1). The keratinized PEL appeared attached to the sidewalls of the stratum spinosum, which made up the central core of each SEL. The structure of the lamellae showed that the basal germinal cells of the SEL divide evenly along their length at the same rate as the descending growth of the horny PL. The PEL appeared to slide distally across the more rigid SEL to facilitate growth.

The cells in the uppermost PL of the stratum internum (si) exhibited less keratinization than those in the remaining lamellae. Additionally, the cells located near the stratum medium (sm) (Figure 1 A and B) had the highest level of keratinization, whereas the cells situated deeper within the stratum displayed the lowest level of keratinization. The PEL consisted entirely of keratinized cells, which gradually spaced apart as they underwent keratinization. Subsequent histological examination revealed that the innermost part of the wall had fully formed the PEL. When cells in the PEL undergo outward pressure, they undergo keratinization and shape alterations that closely resemble those in the intertubular keratinization of the innermost stratum medium. The keratinization of all PEL revealed that they were

typically complete by mid-wall. The PEL cells next to the SEL cells had large villous projections that went into neighboring cells of the SEL. These projections formed fully formed lamellae with a mid-wall. In the horizontal section of the proximal part of the PEL, many cells in the second cell population were more clearly columnar. In some places, these cells have a pseudostratified columnar arrangement. Most had dermal villous protrusions (Figure 1).

Hoof lamellae of the even-toed animals

The findings of the current investigation on bovine and camel foot lamellae indicated that the stratum internum, the innermost layer of the hoof wall, exclusively consists of PEL. The lamellae tightly interweave with the DL, forming a complex and complicated dermo-epidermal connection (Figure 1). The DL exhibited a robust connection with the EL and the basal membrane. Furthermore, the EL had a curved shape at its ends. The dermal connective tissue was associated with an intricate network of interconnected fibrils. The PEL consisted of cells of the intertubular horn of the inner stratum medium. The cells in the uppermost PL of the stratum internum exhibited lower keratinization levels than those in the other lamellae, indicating that the keratinization process occurred continuously over a certain distance. In both odd-toed and even-toed ungulate lamellae, the cells next to the stratum medium displayed the most prominent keratinization, whereas the cells located deeper within the stratum showed the least degree of keratinization. The keratinization process in the PEL closely resembled that of the intertubular horn in the inner stratum medium. The basal cells of the mid-wall lamellae were mostly columnar, but due to the shape of the lamella, some cells had to be wedge-shaped. The basal cells had a comparable structure to those in the stratum medium. Nevertheless, the nuclei of several basal cells in the lamellae exhibited indentations (Figure 1).

The mean±SEM for each sample is presented in Table 1, demonstrating the morphometric data of the hoof lamellae in both odd-toed and even-toed ungulates. The findings showed that the mean length of PEL was 2177.7±0.92 µm in horses, 2032.2±0.90 µm in donkeys, 343.7±0.62 µm in camels, and 886.4±0.98 µm in cows. The mean width was 168.6±0.93 µm in horses, 245.1±0.96 µm in donkeys, 90.7±0.59 µm in camels, and 35.2±0.32 µm in cows. The results also indicated significant differences in lamellae between animals with odd-toed and even-toed feet. There were also substantial differences in lamellae between horses and donkeys and between cows and camels (Table 1).

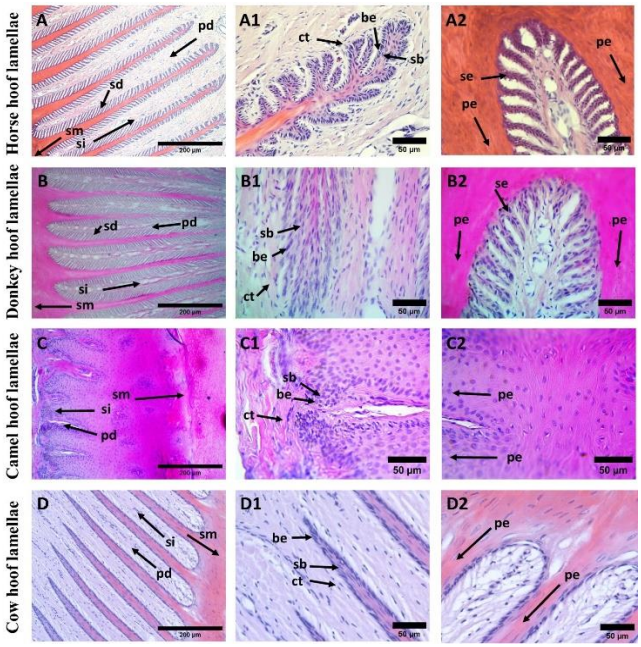

Figure 1: H&E-stained photomicrographs depict the connection between the primary and secondary dermal lamellae and the primary and secondary epidermal lamellae and their interconnectedness. A, A1, and A2 represent images of horse hoof lamellae. B, B1, and B2 represent images of donkey hoof lamellae. C, C1, and C2 represent images of camel hoof lamellae. D, D1, and D2 represent images of cow hoof lamellae. As it showed, (pd) PDL, (sd) SDL, (be) indicated basal epidermal cells, (sb) suprabasal, (ct) connective tissue, (si) stratum internium, (sm) stratum medium, and (pe) and (se) referred to PEL and SEL, respectively. The scale bar in images A, B, C, and D indicates 200µm, whereas images A1, A2, B1, B2, C1, C2, D1, and D2 have a scale bar of 50µm.

Table 1: Morphometric data of the lamellae in odd-toed and even-toed ungulate animals

Variable	Horse hoof lamella	Donkey hoof lamella	Camel hoof lamella	Cow hoof lamella
PEL (length µm)	2177.7±0.92	2032.2±0.90	343.7±0.62*	886.4±0.98*
PEL (width µm)	168.6±0.93	245.1±0.96	90.7±0.59*	35.2±0.32*
PDL (length µm)	2096.8±0.95	2035.2±0.86	325.5±0.97*	894.7±0.63*
PDL (width µm)	263.1±0.63	261.8±0.93	46.08±0.40*	102.9±0.78*
SEL (length µm)	63.9±0.22	123.4±0.34*	-	-
SEL (width µm)	16.02±0.23	20.47±0.12	-	-
SDL (length µm)	63.3±0.62	116.01±0.18*	-	-
SDL (width µm)	30.2±0.30	15.4±0.22*	-	-

- indicated that no SEL or SDL were found on each camel or cow foot. * Indicated a significant difference at $P<0.05$ between the lamellae of animals with odd-toed and even-toed feet.

Discussion

The connection between the fully keratinized cells of the SEL and the partially keratinized cells of the PEL showed strong and well-established bonds. This indicates that structures similar to the SEL may have a crucial function in enhancing the connection between the dermo-epidermal interaction tissues and the third phalanx's periosteum surface (16,17). This implies that the exterior structure of the hoof capsule is linked to the function and form of the interior parts of the capsular wall hoof (14-18). Also brought attention to the significance of the relationship between the form of the hoof and the pattern of architecture of hoof lamellae. For example, the growth and division of the SEL contribute to applying force on the attachment of the third phalanx (19,20). Additionally, the hoof lamellae, which are weight-bearing tissues, can have different shapes depending on the biomechanical force applied. This is because the length and branching of secondary epidermal lamellae affect the mechanical force on the distal phalanx's attachment (20). Wang (21) suggests that the keratin structure has a morphology that endorses the development of vast strain-transition interfaces. Furthermore, it has been confirmed that the tubules existing in the structure considerably impact the growth of twisting cracks. The lamellae were produced by the organization of basal cells displaying a downward and inward inclination, whereas the keratinization process occurred within a limited spatial extent (19). In the early stages of development, keratinocytes located close to the basal layer are probable, whereas those situated at a greater distance or beyond the basal layer are likely to be in the later stages of differentiation. The observed pattern of cell migration and differentiation aligns with the findings of Eurell (4), which indicate that epidermal basal cells undergo distinct differentiation processes when they migrate over a significant distance and traverse a substantial stratum spinosum. According to Aughey (22), after complete keratinization, the cells undergo a loss of their nuclei, resulting in the formation of the stratum corneum within the intertubular horn of the stratum medium. Consequently, the cells located in the outer layer of the stratum internum, known as the primary lamellae, had a lower degree of keratinization. Cells closer to the stratum medium exhibited higher keratinization, whereas cells located further inside the stratum medium showed a lower degree of keratinization. Rouse (23) stated that the process of cell division followed by cornification is similar to the creation of hard keratin seen in many structures such as quills, wool, hairs, nails, hooves, and claws. Within the middle layer, keratinization was observed throughout, and the PEL was often fully formed. The findings are probably connected to the nature and process of hoof growth, suggesting that modifications in the dermal papillae and/or dermal lamellae of the dermal tissues aim to enhance surface attachment inside the inner hoof (20). DL were vascularized connective tissues encompassing arteries and veins. This link might facilitate communication between the epidermal cells and the blood vessels in the dermis's inner layer (14). Therefore, any malfunction in this link results in discomfort and lameness in the foot (23). The suspensory system and supporting components are essential for the bovine and horse foot (24-30). The study on the even-toed angulate revealed that the basal cells in the stratum internum's primary lamellae had lower keratinization levels than those located farther from the basal cells. The germinal basal cells placed at the tip of the stratum internum are responsible for forming the PL (31,32). The PEL was composed solely of keratinized cells that became increasingly separated as they underwent keratinization (33-36). The findings above are expected to be linked to the attributes and mechanisms of hoof development (37,38). According to Al-Agele (14), the dermal papillae and/or lamellae modifications of the dermis are designed to improve the connection between the surface and the inner hoof and/or claw (39-41).

Conclusions

The morphometric data of PEL and SEL in odd-toed animals and the presence only of PEL in even-toed animals, along with the differences in the architectural morphology of each type of lamellae, provide new evidence regarding this variable configuration and interdigitation between dermo-epidermal interconnection. These data add further knowledge to the framework exploring the role of this architectural design in hoof morphology. However, these results, while still in their infancy, might explain the development of hoof diseases in the future.

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Conflict of interest

There are no conflicts of interest.

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دراسة مقارنة نسجية مترية للصفائح في الحيوانات ذوات الحوافر من ذوات الأصابع الفردية والزوجية

رمزي عبد الغفور العجيلي

فرع التشريح والأنسجة، كلية الطب البيطري، جامعة ديالى، ديالى، العراق

الخلاصة

إن الهدف من هذه الدراسة هو إنشاء دراسة نوعية لصفائح الحافر من خلال المقارنة بين البيانات المورفولوجية النسجية للصفائح من الحيوانات ذات أصابع القدم الفردية والزوجية. أظهرت الدراسة المقارنة أن الخيول والحمير ذات الأصبع الواحد لديها نوعان من صفائح البشرة في الطبقة الداخلية لجدار الحافر، والمعروفة باسم صفائح البشرة الأولية والثانوية، على النقيض من ذلك، فإن الأبقار والجمال ذات الأصابع الزوجية لا تحتوي إلا على صفائح أولية. تشكل هذه الصفائح اتصالًا جلديًا معقدًا من خلال التشابك مع الصفائح الجلدية الأولية والثانوية المقابلة. إن صفائح البشرة الأولية جميعها متشابه من حيث الشكل، وخلاياها تشارك في تشكل الأنابيب المتقرنة للطبقة الوسطية للحافر. بينت الدراسة إن الحيوانات ذات الأصابع الفردية لديها صفائح جلدية ثانوية. وتتكون هذه الأخيرة من مجموعة من الخلايا القاعدية غير المتقرنة وتجاورها منطقة مركزية ذات خلايا متقرنة جزئيا. كما أظهرت النتائج إن هنالك اتصال واضح ومباشر بين صفائح البشرة الثانوية والأولية من خلال ارتباطهما مع الليفات الدقيقة. في كلا النوعين من الحيوانات ذات الأصابع الفردية والزوجية، إن تشكل الصفائح الأولية يكون من الخلايا القاعدية الموضوعة في الطرف القريب من الطبقة الباطنية. إن هجرة الخلايا نحو الحافة الخارجية يخضعها للتقرن، مما يساهم في العدد الإجمالي للخلايا المتقرنة في صفائح البشرة الأولية. أشارت النتائج أيضًا إلى وجود فروق ذات دلالة إحصائية في الصفائح بين صفائح الحيوانات ذات الأقدام الفردية والزوجية الأصابع. بينت النتائج الحالية منظورًا جديدًا حول العلاقة بين بنية صفائح الحوافر في الحيوانات ذات الأصابع الفردية أو الزوجية وقدرتها المتزايدة على تكوين الكيراتين، مما قد يفسر تطور أمراض الحافر في المستقبل.