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Article:

Histomorphological features of the skin in neonatal rabbit

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Abstract

The present study aimed to investigate the morphological features of neonatal rabbit skin. The epidermal structure of haired skin was fundamentally the same for all ages. It was comprised of basal, spinous, granular, and corneal layers. Adult skin contained compound hair follicles composed of one primary hair and a collection of secondary hairs surrounded by connective tissue sheath. Different levels of hair follicles were detected in the dermis. There weren't any observed sweat glands in the examined skin samples.

Keywords: Dermis, Epidermis, Hair, Rabbit, Skin.

Introduction

Rabbits are popular model in laboratory animal medicine due to its relatively large size and quiet nature [1]. Skin is the largest organ of the body and consists of a multi-layered structure, which forms a permeable barrier between the body and the outside environment allowing an exchange of heat, air, as well as fluids containing matter of very low molecular weight [2]. The skin consists of two layers epidermis and the dermis. The epidermis contains cells in several stages of differentiation. During the differentiation process, the cells migrate from the basal layer to the surface where they cornify. The dermis consists of connective tissue and contains nerves and blood vessels. The subcutaneous tissue hypodermis is the deepest layer, which mostly consists of pads of adipose tissue that attach the skin to the deep fascia or periosteum [3]. This study provides a description of the histology of normal skin of the New Zealand rabbit allowing researchers to better understand the normal structure as the rabbit is the main model used to study healing and regeneration processes of human skin.

Materials and Methods

Ethical approval

The experimental protocol was approved by the Institutional Review Board of the Ethical Committee of Animal Care and used in scientific research of the faculty of veterinary medicine at Minia University (IRB-FVM-MU-2024-108).

Source of specimens:

Materials employed in the present study were obtained from the laboratory animal's house at the Faculty of Medicine, Assiut University. It consisted of 8 New Zealand White neonatal rabbits that were collected on days 3 and 10 days.

Sampling procedures:

The skin specimens from the dorsolumbar region were dissected carefully and immersed in a fixative consisting of 10% neutral buffered formalin. The fixed specimens were dehydrated in ascending grades of ethyl alcohols cleared in xylene and embedded in paraffin wax. Finally, paraffin

blocks of the processed samples were prepared. Thin sections (5-6 μm thick) were cut, dried in an electrical incubator and stained with Harris's hematoxylin and eosin for detection of the general structure of the skin, Periodic Acid-Schiff (PAS) for detection of mucopolysaccharides, Masson's trichrome for detection of collagenous fibers and muscle fibers and Bromophenol blue for identification of protein inclusions [4].

Semi-thin Sections:

Small skin specimens from the dorsolumbar region were preserved by immersion in Karnovsky's fixative overnight at 4 °C [5]. Karnovsky fixative was prepared from a mixture of 10 mL of 25% paraformaldehyde, 10 mL of 50% glutaraldehyde, 50 mL of phosphate buffer, and 30 mL of distilled water. The samples were post-fixed in 1% osmic acid, 0.1 mol/L phosphate buffer at pH 7.3 for 2h at room temperature. Afterward, the samples were dehydrated in ascending grades of ethanol followed by propylene oxide and embedded in an Araldite-Epon mixture. Semi-thin sections were cut at 1- μm thickness with a Reichert Ultracut (Leica, Germany), stained with toluidine blue, and were finally examined by LeitzDialux 20 Microscope. Photos were taken using a Canon digital camera (Canon PowerShot A 95).

Results

Early postnatal at 3 days old rabbit, the epidermis that formed the uppermost multi-layered compartment of the skin showed a keratinized stratified squamous epithelium with four distinct layers: The basal layer was composed of one row of columnar cells with large rounded nuclei resting on a basal lamina. The spinous layer was composed of two to three rows of irregularly arranged polygonal cells that contained large rounded nuclei. Cells of the granular layer contained keratohyaline granules. The superficial corneal layer was represented by acidophilic flattened cells with no cell boundaries. The cornification of the skin surface was distinct and formed of several layers of keratin plates or flakes (Figure 1 A).

The dermis could be differentiated into superficial papillary and deep reticular layers. It was observed that the sub-epidermal region of the dermis contained more cellular elements than the rest of the dermis which appeared to contain more fibrous elements of collagen fibers (Figure 1 B).

Different stages of hair follicle morphogenesis with different levels were detected in the dermis. The majority of the hair follicles were in the terminal stage where the hair shafts extended and protruded through the hair canal upon the surface of the epidermis. The hair follicle was surrounded by an intensive collagenous fibrous sheath and

the cells of the outer root sheaths of the hair follicle showed strong positive PAS and bromophenol reactions (Figure 1 C and D).

At 10 days old rabbit, the rabbit skin was fully developed with an extensive hair coat. The Keratin layer is thicker and more prominent (Figure. 2 A, B, and Figure. 3 A, B). Dermis showed more fibrous and less cellular elements relative to the previous stage. The dermis was relatively thicker with prominent connective tissue fibers and fully matured hair structures (Figure 2 C and D). Adult skin contained compound hair follicles composed of one primary hair and a collection of secondary hairs surrounded by connective tissue sheaths. All hairs emerged through the same follicle outlet of the skin surface. The hairs of each compound follicle shared a common lumen and emerged at the skin surface via a single opening. It was observed that the number of hair follicles increased clearly than the previous age with a proportionate decrease in the amount of dermal fibers (Figure 2 B and Figure 3 B).

Hair follicle at the level of infundibulum; relative to the epidermis extending from the sebaceous gland to the epidermal surface consisted of a hair shaft surrounded by hair type keratin and outer root sheath. At the isthmus level, the middle part extending from the sebaceous duct to the bulge of the sebaceous gland consisted of a hair shaft surrounded by an inner root sheath, outer root sheath, and sebaceous gland. The sebaceous gland appeared as a cellular budding from the upper third of the hair follicles. They appeared large faintly stained polyhedral cells with large rounded vesicular nuclei. They were surrounded by a layer of flattened cells with flattened nuclei (Figure 3 A). The keratinized hair at the level of the suprabulber region had central large vacuolated cells that formed the core of the hair shaft or medulla that was surrounded by the cortex followed by the cuticle that was composed of proximally directly keratinized cells. The inner layer of the inner root sheath became differentiated into the distally directed cuticle of the inner root sheath that differentiated into Huxley's layer that showed keratohyaline granules and pyknotic nuclei, Henle's Layer showed complete keratinization. Subsequently the cornified cells of Huxley's and Henle's layer merge and become indistinguishable (Figure 3 C). The hair bulb: a thickening of the proximal end of the hair follicle formed of dermal papilla containing mesenchymal cells, hair matrix cells appeared columnar in shape, arranged in basal lamina enclosing the dermal papilla, outer root sheath formed of keratinocytes and surrounded by fibrous sheath. The hair follicles became elongated and reached the hypodermis composed of loose connective tissue containing telocytes, fat cells, numerous blood vessels, and panniculus carnosus muscle (Figure 3 D). There weren't any observed sweat glands in the examined skin samples.

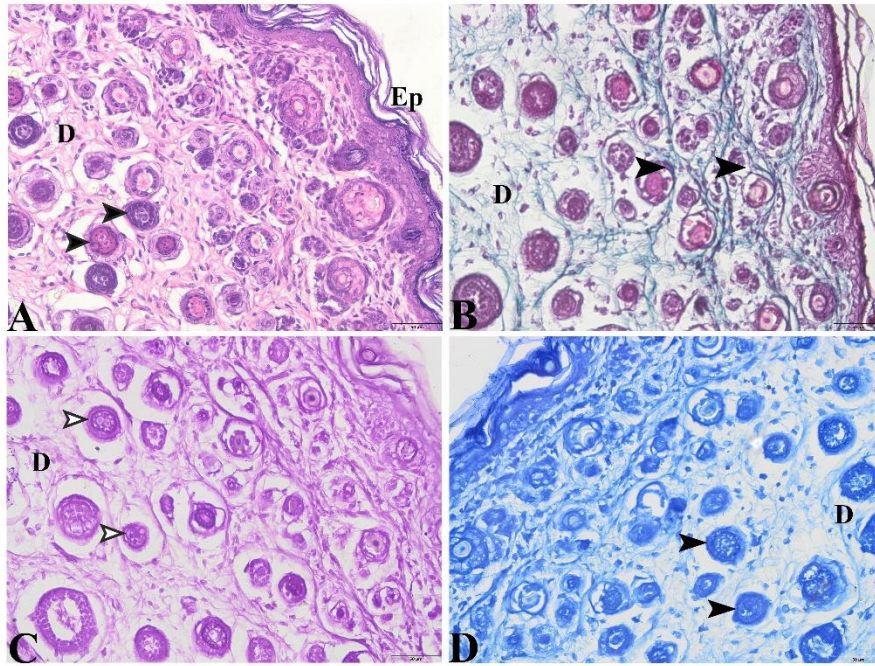


Figure 1: Paraffin section in fetal rabbit skin at 3 days old rabbit showing: **A.** Fetal skin showing Epidermis (Ep) and dermis (D). Notice, high hair follicle density (black arrow heads) (Haematoxylin and Eosin). **B.** Dermis show more prominent collagen fibers (black arrow heads) (Masson's trichrome). **(C, D).** Outer root sheath of the developing hair follicles shows PAS positive reaction (white arrow head) and bromophenol blue positive reaction (black arrow head). (PAS and Bromophenol blue)

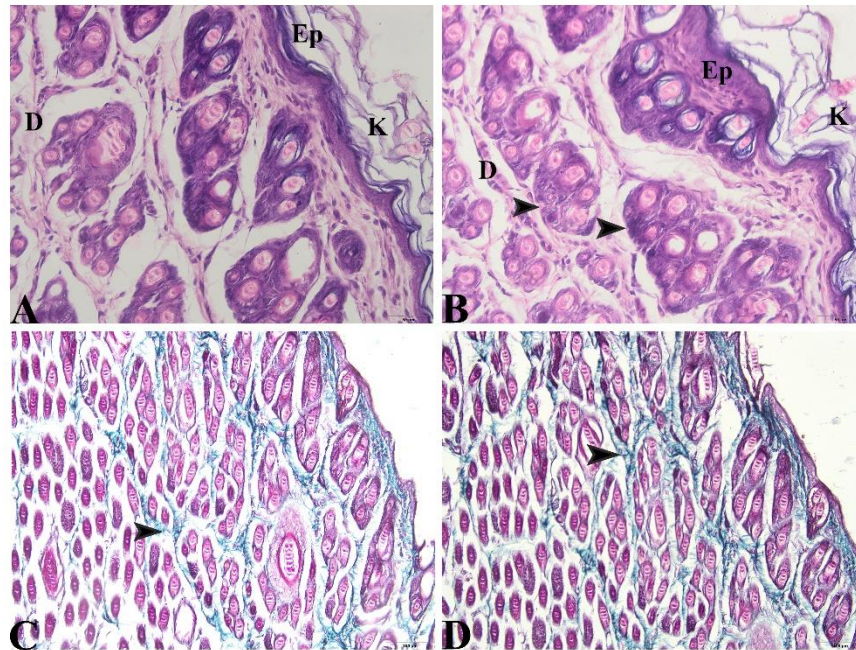


Figure 2: Paraffin section in skin of 10 days old rabbit showing: **(A, B).** Fetal skin showing Epidermis (Ep) and dermis (D). Notice, keratin layer is thicker and more prominent (K). Dermis show more fibrous and less cellular elements relative to previous stage and presence of compound hair follicles (black arrow heads). (Haematoxylin and Eosin). **(C, D).** Prominent connective tissue fibers in the dermis of skin (arrows) (Masson's trichrome).

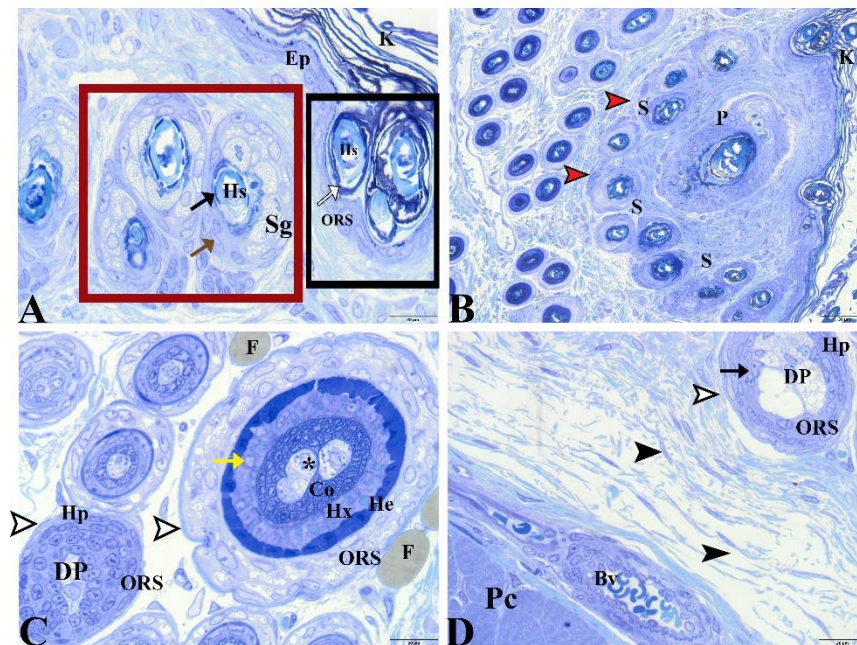


Figure 3: Semi thin section stained by toluidine blue in fetal rabbit skin at 10th postnatal day showing, epidermis (Ep), dermis (D) and hypodermis. The epidermis (Ep) is well distinguished and keratin layer (K) is thick and formed of several layers keratin plates or flakes. -Notice, hair follicle at level of infundibulum (Black Square) consists of hair shaft (Hs) surrounded by hair type keratin (white arrow) and outer root sheath (ORS). -Hair follicle at level of isthmus (red square) consists of hair shaft surrounded by inner root sheath (black arrow head), outer root sheath (brown arrow head) and sebaceous gland (Sg). **B.** Compound hair follicle formed of large primary follicle (P) surrounded by multiple smaller secondary follicles (S) enveloped by connective tissue sheath (red arrow heads). **C.** Hair follicle at level of suprabulber region formed of hair medulla (*) and hair cortex (Co) surrounded by inner root sheath that differentiated into Huxley's layer (Hx) show keratohyaline granules and pyknotic nuclei (yellow arrow), Henle's Layer (He) show complete keratinization, outer root sheath (ORS) and fibrous sheath (white arrow head). **D.** Hypodermis is composed of loose connective tissue containing telocytes (black arrow heads), and fat cells (F) numerous blood vessels (By) and panniculus carnosus muscle (Pc). Notice, Hair bulb (Hb) is formed of dermal papilla (Dp) containing mesenchymal cells, hair matrix (black arrow) appeared columnar in shape, arranged in basal lamina enclosing the dermal papilla. Outer root sheath (ORS) formed of keratinocytes and surrounded by fibrous sheath (white arrow head).

Discussion

The present work investigated the normal histological structure of the rabbit's thin skin. In early postnatal 3 days old rabbits, the epidermis showed a keratinized stratified squamous epithelium with four distinct layers, the basal layer was composed of one row of columnar cells with large rounded nuclei resting on basal lamina. The spinous layer was composed of two to three rows of irregularly arranged polygonal cells that contained large rounded nuclei. Cells of the stratum granulosum contained basophilic granules. The superficial stratum corneum was represented by acidophilic flattened cells with no cell boundaries. The cornification of the skin surface was distinct with clear folds of different heights. Similar observations were obtained in rabbits [1], in ferrets [6] [3], and in humans [7]. In the present study we observed that the dermis could be differentiated into superficial papillary and deep reticular layers the sub-epidermal region of the dermis contained more cellular elements than the rest of the dermis which appeared to contain more fibrous elements of collagen fibers same findings were observed by Martin in the ferret [6]. He also added that the superficial papillary layer and

the deep reticular layers of the dermis were comprised of irregular collagenous tissue. The collagen fibers of the papillary layer were smaller in diameter and more loosely organized.

In this study, we observed different stages of hair follicles with different levels in the dermis. The majority of the hair follicles were in the terminal stage where the hair shafts extended and protruded through the hair canal upon the surface of the epidermis. The hair follicle was surrounded by an intensive collagenous fibrous sheath and the cells of the outer and inner root sheaths of the hair follicle showed strong positive PAS reaction. Positive bromophenol reaction also appeared within the hair follicles.

Hairs exhibit a common structural organization and chemical synthesis in all mammals, with relatively small differences among species. Hair is the holocrine secretion of the dermal papilla and consists of three distinct cell lineages, namely the cuticle, cortex, and medulla. The hair follicle is an independent, unique skin appendage. Hair follicle components derive from two populations of different embryological origin; the epithelial components are the hair matrix, inner and outer root sheath, and hair

shaft and the dermal components are the dermal papilla and dermal fibro collagenous sheath [8].

At 10 days old rabbit, the rabbit skin was fully developed with an extensive hair coat. The dermis was relatively thicker with fully matured hair structures. It was observed that the number of hair follicles increased than the previous age with a proportionate decrease in the amount of dermal fibers. The sebaceous gland appeared as a cellular budding from the upper third of the hair follicles. They appeared large faintly stained polyhedral cells with large rounded vesicular nuclei. They were surrounded by a layer of flattened cells with flattened nuclei. Smith [9] reported that the sebaceous glands were usually found in association with a hair follicle, which, together, was referred as a pilosebaceous unit. It was located in association with the upper portion of the hair follicle, where it was not affected by the hair cycle. Although a majority of sebaceous glands are part of a pilosebaceous unit, some glands can be found without an associated hair follicle. In general, sebum is deposited on the hair inside the follicle and is conveyed up to the surface of the skin along the hair shaft. Sebum lubricates and protects the hair and skin and prevents drying and irritation of membranes. Sebum lipids possibly play a role in waterproofing, and antibacterial sebum lipids may inhibit infections of the skin.

The present study revealed that adult skin contained compound hair follicles composed of one primary hair and a collection of secondary hairs surrounded by connective tissue sheaths. All hairs emerged through the same follicle outlet of the skin surface. The hairs of each compound follicle shared a common lumen and emerged at the skin surface via a single opening. The same findings were observed by Martin [6] in ferrets.

Welle [10] mentioned that, in most animals with compound follicles, each compound group is composed of a central large primary hair follicle surrounded by variable numbers of smaller secondary follicles. On the human scalp, it is common that two to three hair shafts share one infundibulum and protrude from the same orifice.

In this study, the dermis showed aggregation of telocytes with its long process. Telocyte is a novel interstitial cell that is characterized by oval or rounded cell bodies and extends thin long cytoplasmic processes that are connected to each other forming a characteristic network.

Arafat [11] suggests that telocytes play an important role in the regeneration and repair process by increasing the efficiency of fibroblasts and collagen fiber.

Conclusion

Finally, we concluded that the epidermal structure of haired skin was fundamentally the same for all ages. Adult

skin contained compound hair follicles composed of one primary hair and a collection of secondary hairs surrounded by connective tissue sheath. Different levels of hair follicles were detected in the dermis. There weren't any observed sweat glands in the examined skin samples.

Authors' contribution

S.M.M designed the study. A.H.A and M.M.H contributed data collection. M.M.H. and O.M.M. contributed data analysis and figures design, conceived and prepared the draft. S.M.M, A.H.A and M.M.H reviewed the draft. All the authors read and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

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