10.21608/avmj.2024.299348.1295 Assiut University web-site: www.aun.edu.eg

MORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON THE DEVELOPMENT OF SKIN IN WHITE NEW ZEALAND RABBIT DURING FETAL PERIOD

OMIMA M.M. RASHAD [1]; MARWA M. HUSSEIN [2]; ABDEL-RAZIK H. ABDEL-RAZIK [3] AND SHEHATA M.M. SOLIMAN [1]

[1] Department of Histology, Faculty of Veterinary Medicine, Minia University, El-Minia, Egypt

[2] Department of Cell and Tissues, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.

[3] Department of Histology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt

Received: 1 August 2024; **Accepted:** 5 November 2024

ABSTRACT

The present study aimed to investigate the development of fetal rabbit skin. At the 16th gestational day, the developing skin consisted of a single layer of cuboidal or low columnar cells representing the primitive epidermis and loosely organized mesodermal cells representing the presumptive dermis. On the 18th gestational day, the epidermis was composed of single or double cell layers. Moreover, the uppermost mesodermal cells of the presumptive dermis formed a clear condensation just underneath the presumptive epidermis. On the 20th gestational day, the developing epidermis acquired a clear stratification. The sup-epidermal layer of the dermis became denser with many blood vessels. At the 25th gestational day, the developing epidermis showed more distinct stratification with more elongated and more differentiated hair follicles. Moreover, the primitive sebaceous glands started to develop as an outgrowth from the upper part of the primitive hair follicles. On the 30th gestational day, the epidermis assumed a well-differentiated and relatively mature appearance. No sweat glands were detected in fetal rabbit skin at all studied ages. We concluded that the development of the skin and its appendages in rabbits was largely recognized during fetal life

Key words: Skin; Dermis; Epidermis; Development

INTRODUCTION

Rabbits are one of the most important and popular experimental models in animal medicine, particularly in the fields of experimental dermatology pharmacology, due to their relatively large size and docile nature (White *et al.,* 2002). The skin is considered the largest organ of the animal body, and it can form a strong barrier between the animal body and the external environment (Verma *et al.,* 2003). Rabbit skin illnesses may be the most frequent cause for owners to bring their animals to the veterinarian. These diseases are also prevalent in lab colonies and can compromise the standard and circumstances of research (White *et al.,* 2002). It is possible to see a rabbit or a rodent's general health through its skin. Observing anomalies in the skin and pelage may need dietary or

Corresponding author: Marwa M. Hussein
E-mail address: marwahussien1985@gmail.com
Present address: Department of Cell and Tissues, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

husbandry modifications, or it may result in the identification of another underlying illness (Harvey, 1995). Skin is formed of three layers; the uppermost layer is the epidermis, which consists of a stratified squamous keratinized epithelium. The middle layer is the dermis, and it is formed of dense connective tissue that supports the epidermis. The subcutaneous tissue or hypodermis is the deepest layer, which mostly consists of pads of adipose tissue that attach the skin to the deep fascia or periosteum (Arda *et al.,* 2014). The skin has many different cell types that are derived from embryonic ectoderm, such as keratinocytes, melanocytes, Merkel cells, and neurons. While many other skin cell types are derived from mesoderm, including fibroblasts, endothelial cells, fat cells or adipocytes, and Langerhans cells (Loomis *et al.*, 2008) and (Alshaiji, 2019). In mammals, embryonic skin epithelial cells are pluripotent and can choose between epidermal and hair follicle cell fates. Epithelial progenitor cells in the skin can differentiate into epidermis and the epithelial parts of the skin appendages, including sebaceous glands and hair follicles (Byrne *et al.*, 2003). Even though rabbits are widely utilized as the primary laboratory animal used in experimental human dermatology, they are also the primary model used to investigate the processes of human skin regeneration and healing. The current study aims to provide new insights into the histological and histochemical characteristics of developing skin in fetal White New Zealand rabbits.

MATERIALS AND METHODS

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 25 New Zealand white rabbit fetuses, which were collected on gestational days 16, 18, 20, 25, and 30. 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).

Sampling procedures:

New Zealand rabbit females were submitted to natural mating. The data analysis took into account both the day of mating and the age of the fetuses. Prior to the sacrifice of each pregnant rabbit doe, the females were given anesthesia with 35 mg/kg ketamine and 5 mg/kg xylazine. All precautions for using and dealing with laboratory animals were taken into consideration. Several skin specimens from the dorso-lumbar part of fetuses 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 collagen fibers and muscle fibers, Orcein for identification of elastic fibers and Bromophenol blue for identification of protein inclusions (Kim, 2019).

RESULTS

At the 16th gestational day, light microscopic investigations revealed that the fetal skin on the dorso-lumbar part was formed of a thin prospective epidermis and a relatively thick underlying prospective dermis. The prospective epidermis was formed of cuboidal or low columnar cells. These cells were rested on a well-defined basement membrane. The underlying prospective dermis was formed of loose mesenchymal tissue and contained abundant, thin-walled blood capillaries **(Fig. 1 A)**.

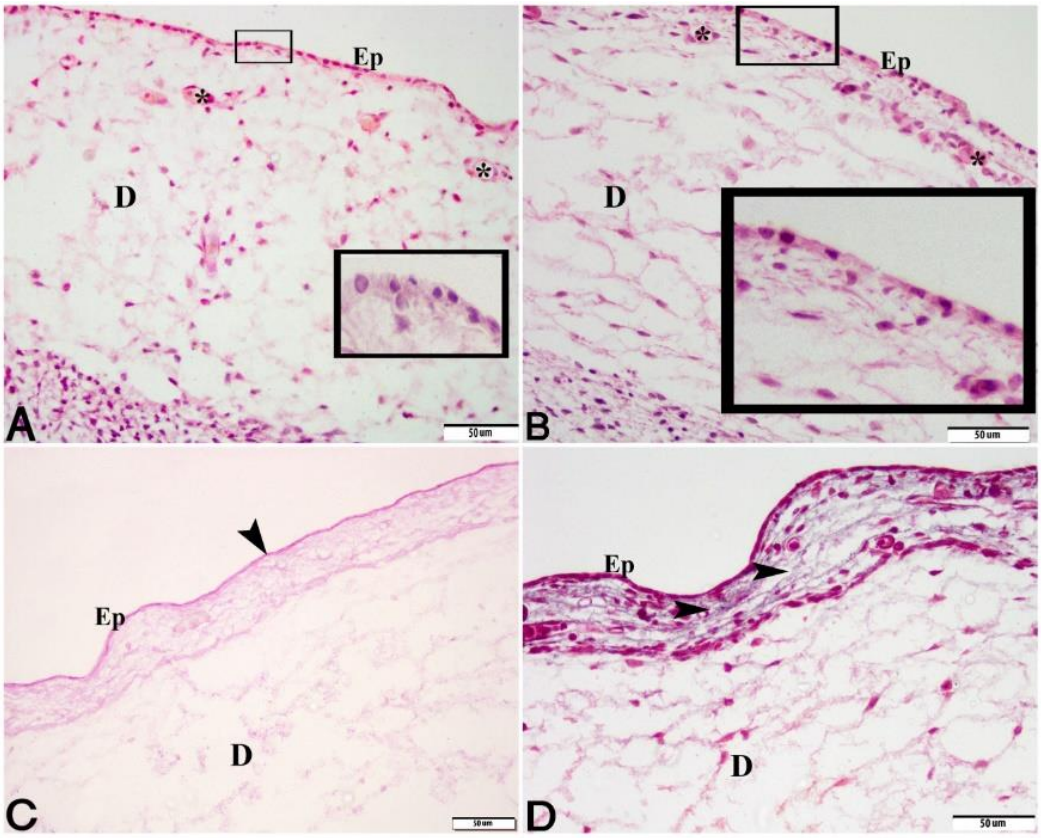

Fig. 1: A: Paraffin section in fetal rabbit skin at the 16th embryonic day shows a developing epidermis (Ep), developing dermis (D), and blood capillaries (*). The magnified square shows the developing epidermis formed of cuboidal or low columnar cells. (Haematoxylin and Eosin). **B:** Paraffin section in fetal rabbit skin at 18th embryonic day, showing epidermis (Ep), dermis (D), and blood capillaries (*). The magnified square shows the developing epidermis formed of a single layer of low cuboidal or flattened cells.(Haematoxylin and Eosin). **C:** Paraffin section in fetal rabbit skin at 18th embryonic day showing; Epidermis (Ep), Dermis (D), and positive PAS reaction of the Epidermis (arrow) (PAS). D: Paraffin section in fetal rabbit skin at 18th embryonic day showing; Epidermis (Ep), Dermis (D), and fine collagen fibers under the Epidermis (arrows) (Masson's trichrome).

At the 18th gestational day, the epidermis was limited by a continuous basal lamina and was composed of a single cell layer consisting of cuboidal cells with large rounded or oval nuclei **(Fig. 1 B)**. The epidermis showed a PAS positive reaction **(Fig. 1 C)**. The uppermost mesodermal cells of the dermis became more condensed just underneath the epidermis and contained many wide blood vessels **(Fig. 1 B-C-D)**, and fine collagen fibers **(Fig. 1 C).**

At the 20th gestational day, stratification became clear in some areas of the developing epidermis. The sup-epidermal layer of the dermis became denser with many blood capillaries **(Fig. 2)**. Moreover, 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 and elastic fibers **(Fig. 2 C-D)**. The most distinguishing feature of this stage was the initiation of hair follicle development. The hair follicle appeared as a confined proliferation and condensation of the epidermal basal cells to form a hair bud or placode. Localized aggregations of mesenchymal cells in the dermis were formed beneath each developing bud to develop the future dermal papilla of hair follicles **(Fig. 2 A-C)**. The dermis increased in thickness with a marked increase in collagen and elastic fibers **(Fig. 2 C-D)**. The primitive follicle showed a strong PAS-positive reaction **(Fig. 2 B)**.

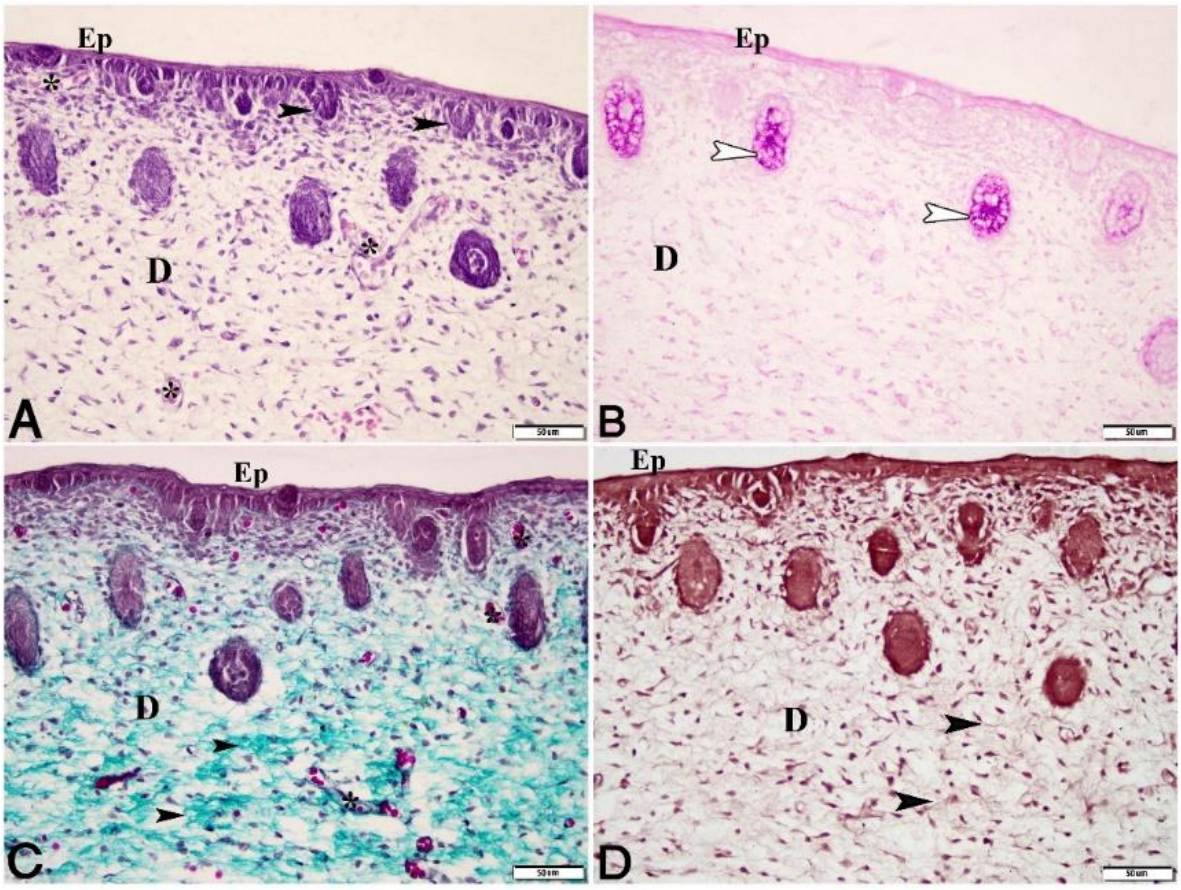

Fig. 2: Paraffin section in fetal rabbit skin at 20th embryonic day, showing **A:** Epidermis (Ep), dermis (D), blood capillaries (*), and hair placode (arrows) (Haematoxylin and Eosin). **B:** Epidermis (Ep), dermis (D), and positive PAS reaction of the hair follicles (arrows) (PAS). **C, D**: Well-recognized collagen and elastic fibers in the dermis of the skin (arrows) (Masson's trichrome and orcein).

At 25th gestational day, the epidermis became multilayered and showed several invaginations of the primordia of hair follicles **(Fig. 3 A-B)**. The basal layer was composed of one row of columnar cells with large, rounded nuclei resting on clear basal lamina. The superficial layer appeared as acidophilic flattened cells with indistinct cell boundaries **(Fig. 3 B)**. The dermis was distinguished into superficial papillary and deep fibrous layers **(Fig. 3 A-B-C-F)**. It contained more collagen and elastic fibers than previous ages **(Fig. 4 A-B-D).** Different stages of developing hair follicles could be seen. They increased in length and extended deeply within the dermis. They were surrounded by a fibrous sheath derived from the dermis. Positive bromophenol and PAS reactions were recorded in the epithelial cells of the hair follicles **(Fig. 3 E-F)**.

At 30th gestational day, Semithin sections stained with toluidine blue showed that the epidermis could be distinguished into basal layers formed of one row of columnar cells with oval nuclei, the spinous layer composed of two to three rows of irregularly arranged polygonal cells that contained large rounded nuclei, the granular layer appeared as fusiform cells with basophilic granules, and the superficial cells of this layer became fusiform with oval nuclei and indistinct cell boundaries representing the keratin layer **(Fig. 4 G-H)**. The dermis became thicker sheath that could be demarcated **(Fig.4 A)**. The cells of the outer root sheath of the hair follicle and the dermal papillae were more developed **(Fig. 4 A-B)**. The fibrous component of the dermis clearly increased and contained more collagen and elastic fibers **(Fig. 4 C-D)**. The hair follicles

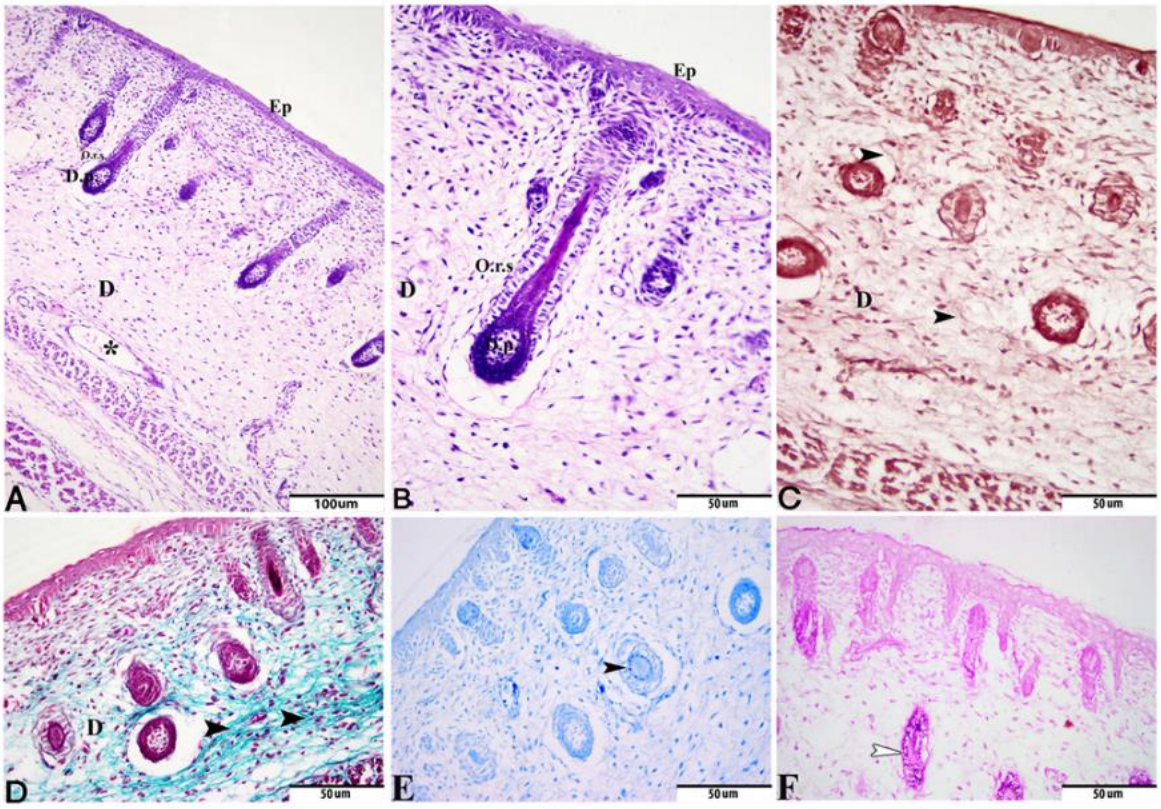

Fig. 3: Paraffin section in fetal rabbit skin at 25th gestational showing **A:** Epidermis (Ep), dermis (D), blood capillaries (*), outer root sheath (Ors) and dermal papillae (Dp) of the hair follicle (Haematoxylin and Eosin). **B:** Higher magnification of the hair follicle showing the outer root sheath (Ors) and dermal papillae (Dp) (Haematoxylin and Eosin). **C, D:** Well-recognized elastic and collagen fibers in the dermis of the skin (arrows) (C: Masson's trichrome and D: orcein). **E.F:** Positive bromophenol and PAS reaction of the hair follicles (arrows) (E: Bromophenol blue and F: PAS).

increased greatly in number and depth. They became almost fully differentiated, where the external root showed strong positive bromophenol and PAS reaction **(Fig.4 E-F)**. At this stage, the sebaceous glands appeared as small lightly stained cellular budding from the upper third of the growing follicles **(Fig.4 A-D)**.

DISCUSSION

The present work investigated the skin developmental events of New Zealand rabbit skin during prenatal life. In the current investigation, at the 16th gestational day, the fetal skin on the dorso-lumbar part is represented by a thin primitive epidermis and thick primitive underlying dermis. The epidermis was constituted of one layer of cuboidal cells with large rounded or oval nuclei. The dermis appeared as a loose arrangement of mesenchymal cells containing numerous, thin-walled blood capillaries. On the contrary (Sayed-Ahmed and Elnasharty, 2014) recorded that the epidermis at the 15th gestational day was composed of a double layer. It could be differentiated into a basal layer of cuboidal or low columnar cells and a superficial irregular or globular-shaped cell layer (periderm). He mentioned that the underlying connective tissue of the presumptive dermis appeared as a loose arrangement of cells without any exacting pattern of arrangement. Inoue and Mihara (2004) reported that in rat fetuses at the 11th and 12th gestational day, the epidermis consists of one to two layers of cells, but the periderm cells and basal cells of the epidermis were arranged irregularly and

could hardly be identified. They added that on the 13th gestational day, the periderm cells and the basal cells of the epidermis were easily identified, and both cells assumed a flattened shape.

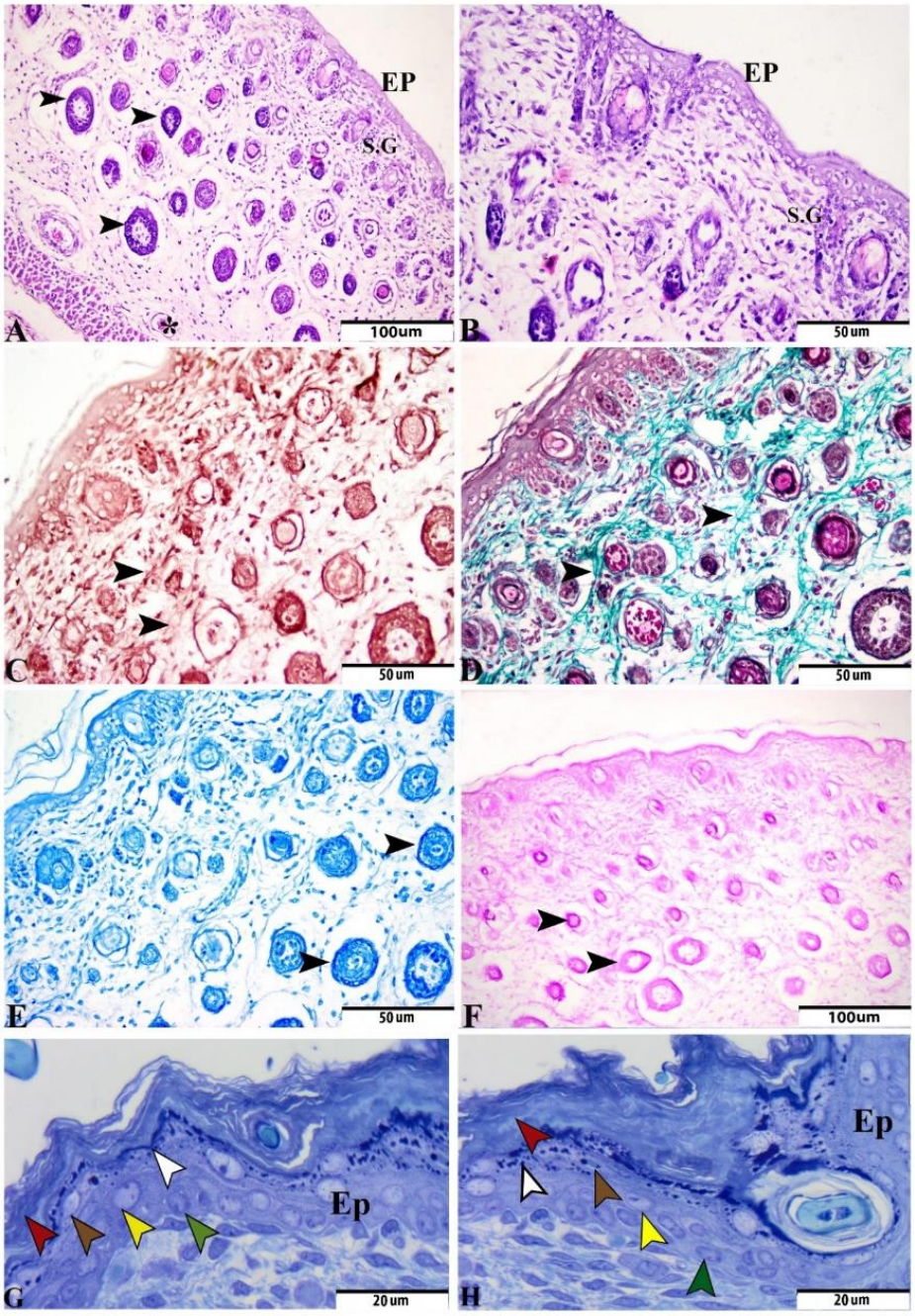

Fig. 4: Paraffin section in fetal rabbit skin at 30th gestational day, showing **A:** Epidermis (Ep), dermis (D), hair follicles (arrows), and sebaceous gland (Haematoxylin and Eosin). **B:** Higher magnification showing the epidermal multi-layers (Ep) and sebaceous gland (SG) (Haematoxylin and Eosin). **C, D:** Well-recognized elastic and collagen fibers in the dermis of the skin (arrows) (Masson's trichrome and orcein). **E, F:** Positive bromophenol and PAS reaction of the hair follicles (arrows) (E: Bromophenol blue and F: PAS). **G, H:** Semithin section stained by toluidine blue showing: The epidermis (Ep) is well distinguished into basal cell layer (green arrowhead), spinous cell layer (yellow arrowhead), granular cell layer (brown arrowhead), and keratin layer (red arrowhead). Notice, that keratohyaline granules (white arrowheads) are seen in cells of the granular layer.

The present investigation showed that at the 18th gestational day, the epidermis was limited by a well-defined basal lamina, and it was composed of one cell layer of cuboidal cells with large rounded or oval nuclei. The superficial mesodermal cells of the dermis became more condensed underneath the epidermis with many wide blood vessels and fine collagen fibers. Similar observations were obtained at the 19th gestational day by Sayed-Ahmed and Elnasharty, (2014). In the present study, at the 20th gestational day, stratification became clear in some areas of the developing epidermis, even though the single-layered epidermis existed in many regions. The sup-epidermal layer of the dermis became denser with many blood vessels. Furthermore, 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 and elastic fibers. The most characteristic feature of this stage was the appearance of a hair bud or placode. The hair bud appeared as ridge-like thickening noted in localized regions as a result of propagation and elongation of the basal cells, and aggregation of mesenchymal cells of the dermis beneath each developing bud, forming the future dermal papilla of the hair follicle. The primitive follicle showed a strong PAS-positive reaction. Similar findings were reported by (Maruyama *et al.,* 1988) and by (Sayed-Ahmed and Elnasharty, 2014) at the 19th gestational day. Schmidt-Ullrich and Paus, (2005) mentioned that the construction of hair follicles during fetal life needs a sequence of successive epithelium-mesenchyme interactions, which are initiated by a dermal message from the mesenchyme, resulting in the formation of hair placodes. When placodes are formed, they start to direct epithelial messages to the mesenchymal tissue, leading to proliferation and condensation of mesenchymal cells to form some mesenchyme condensations, to form the dermal papilla. A second dermal message is reversal. It is sent from the dermal condensate to the epithelium, resulting in the proliferation and growth of epithelial. (Schmidt-Ullrich and Paus, 2005) and (Fuchs, 2008) found that, at approximately the 14.5th gestational day of mouse development, mesenchyme-epithelial interactions lead to the appearance of the first wave of hair placodes, which are recognized as multiple small epidermal invaginations into the dermis. Once commenced, these placodes proliferate and grow continuously to form the hair germs at the 15.5th gestational day, and then hair pegs at the 16.5th – 17.5th gestational day.

The present study revealed that at 25th gestational day, the multilayered epidermis became more prominent and showed several invaginations of the developing hair follicles. The basal layer was composed of one row of columnar cells with large, rounded nuclei resting on clear PAS-positive basal lamina. The superficial layer is represented by acidophilic flattened cells with indistinct cell boundaries. Sayed, Ahmed and Elnasharty (2014) stated in fatal rabbits that at the 28th gestational day, a relatively well-differentiated epidermis formed of keratinized stratified squamous epithelium is recognized. In rat fetuses, (Inoue and Mihara, 2004) reported that, on the 19th gestational day, granular and superficial horny cell layers of the epidermis were well-differentiated, indicating that the process of normal keratinization had been initiated. In the present study, different stages of hair follicle development could be noticed at the 25th gestational day, where they increased in length and started a deep growth within the dermis. The hair follicles became surrounded by a fibrous sheath, which originated from the dermis. The same findings were reported by (Maruyama *et al.,* 1988).

In the present study, at the 30th gestational day, the epidermis was composed of keratinized stratified squamous epithelium and assumed a fully mature appearance. The dermis became thicker and the dermal papillae were well-developed. The fibrous component of the dermis clearly increased

and contained more collagen and elastic fibers. The hair follicles increased greatly in number and depth. They became nearly fully differentiated where the outer root sheath could be demarcated. The cells of the outer root sheath of the hair follicle showed strong positive bromophenol and PAS reactions. (Abdou *et al.*, 2002) suggested that the reaction of PAS-positive mucopoly-saccharides in the follicle outer root sheath might be due to enhanced energy requirements for active cellular proliferation. They assumed that the carbohydrates in the outer root sheath were the source of energy for protein synthesis during hair growth. They added that the outer root sheath possessed high amounts of proteins, as demonstrated by the bromophenol blue stain. At this stage, the sebaceous glands appeared as small lightly stained cellular protrusions from the upper third of the growing follicles. The same findings were reported by (Sayed-Ahmed and Elnasharty, 2014) in rabbits and by (Fuchs, 2008) in mouse fetuses. In the human fetus, (Thiboutot, 2004) noted that sebaceous glands begin to differentiate in the 13^{th} to 16^{th} week of pregnancy, and they are recognized as small cellular bulges on the upper part of developing hair follicles. (Niemann, 2009) reported that the sebaceous gland develops as an outgrowth from the outer root sheath of the developing hair follicle. He added that the undifferentiated sebocytes develop from peripheral basal cells and then move centrally to become fully differentiated sebocytes. In the present study, no sweat glands were detected in fetal rabbit skin at all studied ages. Thus, rabbits may use different methods for thermoregulation. Heat dissipation in non-sweating animals, such as rabbits, is accomplished by breathing more quickly to maximize the amount of high moisture that evaporates through the respiratory air. Furthermore, rabbits' ears and nasal mucosa are important for thermoregulation (Marai and Habeeb, 1994).

CONCLUSION

Finally, we concluded that the development of the skin and its appendages in rabbits was largely recognized during fetal life. The epidermal and mesenchyme interaction might relate directly to the initiation of hair follicle development. Extensive fur covering and the absence of the sweat gland of rabbits should be considered during intensive rabbit production to avoid heat stress upon rabbit thermoregulation mechanisms.

REFERENCES

Abdou, A.S.; Ei-Ganaieny; M.; Khattab, F.; Hekal, S.J.J.O.A. and Production, P. (2002): seasonal changes of the histology and histochemistery of the hair follicles of baladi goats in egyptian desert areas. 27**,** 3721-3736.

Alshaiji, J. (2019): The Embryogenesis of the Skin. Pediatric Dermatologic Surgery.

Arda, O.; Göksügür, N. and Tüzün, Y. (2014): Basic histological structure and functions of facial skin. Clinics in Dermatology, 32**,** 3-13.

Byrne, C.; Hardman, M. and Nield, K. (2003): Covering the limb–formation of the integument. Journal of anatomy, 202**,** 113-123.

Fuchs, E. (2008): Skin stem cells: rising to the surface. The Journal of cell biology, 180**,** 273-284.

Harvey, C. (1995): Rabbit and rodent skin diseases. Seminars in Avian and Exotic Pet Medicine, 4**,** 195-204.

Inoue, T. and Mihara, M. (2004): Sequential observation of morphogenesis of cutaneous nerve fibers in fetal rat skin: an immunohistochemical and electron microscopic study. Journal of investigative dermatology, 123**,** 763-770.

Loomis, C.A.; Koss, T. and Chu, D. (2008): Fetal skin development. Eichenfield LF, Frieden IJ, Esterly NB. Neonatal dermatology, Saunders Elsevier health sciences, 2**,** 1-17.

Marai, F. and Habeeb, A.J.E.J.O.R.S. (1994): Thermoregulation in rabbits. 4.

Maruyama, T.; Yoshizuka, M. and Fujimoto, S. (1988): Light and electron microscopy of fetal rabbit skin with special reference to the role of mesenchymal cells in epidermal differentiation. Cells Tissues Organs, 133**,** 143-155.

Niemann, C.J.D.-E. (2009): Differentiation of the sebaceous gland. 1**,** 64-67.

S Kim, S. (2019): Bancroft's Theory and Practice of histological techniques eighth edition/S. Kim Suvarna. Elsevier Limited.

Sayed-Ahmed, A. and Elnasharty, M.A. (2014): Pre and postnatal development of the rabbit thin skin. Glob Vet, 13**,** 622-632.

Schmidt-Ullrich, R. and Paus, R. (2005): Molecular principles of hair follicle induction and morphogenesis. Bioessays, 27**,** 247-261.

Thiboutot, D.J.J.I.D. (2004): Regulation of human sebaceous glands. 123**,** 1-12.

Verma, D.D.; Verma, S.; Blume, G. and Fahr, A. (2003): Particle size of liposomes influences dermal delivery of substances into skin. International Journal of Pharmaceutics, 258**,** 141-151.

White, S.D.; Bourdeau, P.J. and Meredith, A. (2002): Dermatologic problems of rabbits. Seminars in Avian and Exotic Pet Medicine,. Elsevier, 141-150.

دراسات مورفولوجية ونسيجية كيميائية حول تطور الجلد في الأرنب الأبيض النيوزيلندي أثناء فترة ما قبل الولادة

شحاتة محمد محمد سليمان ، عبد الرازق هاشم عبد الرازق ، مروة محمد حسين ، اميمة محمد محمد رشاد

Email: marwahussien1985@gmail.com Assiut University web-site: www.aun.edu.eg

استهدفت الدراسة الحالية فحص تطور جلود اجنة الأرانب النيوزيلاندية. في اليوم السادس عشر من الحمل كان الجلد النامي يتكون من طبقة واحدة من الخلايا المكعبة او العمادية التي تمثل البشرة الاولية وطبقة غير منتظمة من خلايا الميزانشيمية والتي مثلت طبقة الادمة المستقبلية. في اليوم الثامن عشر من الحمل كانت طبقة البشرة متكونة من طبقة واحدة او طبقتين من الخلايا. في اليوم العشرين من الحمل اكتسبت طبقة البشرة النامية تقسيما طبقيا واضحا, واصبحت منطقة الادمة اسفل البشرة مباشرة أكثر كثافة وتحمل العديد من الأوعية الدموية. في اليوم الخامس و العشرين من الحمل أظهرت البشرة النامية طبقات أكثر تميزًا مع بصيلات شعر أكثر استطالة وتمايزًا. علاوة على ذلك، بدأت الغدد الدهنية البدائية في التطور من الجزء العلوي من بصيلات الشعر النامية. في اليوم الثلاثون من الحمل أظهرت طبقة البشرة تمايزًا جيدًا ومظهرناضج نسبيا. لم يتم اكتشاف أي غدد عرقية في جلد أجنة الأرانب في جميع الأعمار المدروسة.