



## **Histological Changes in Rat Fetal Skin Development Between Gestational days 8-14**

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### **ABSTRACT**

Skin is formed by epidermis, dermis and hypodermis and this forma is indicated as embryological Perspective, Epidermis is derived from ectoderm, while dermis and hypodermis. Are originating from meso derma. The present study was designed to follow up the embryonic development of skin in bat at different stage of embryonic Period, (65) Sixty five Rats was included in this study. Twenty two (22) adult male and fourth three (43) adult female. The mating af animal was Performed and the whole females were put under observation to ensure its comes to pregnancy by showing the preserve of reginal plague at day one after meeting. From day eight to tenth of pregnancy. the skin of fetus were examined which indicated the presence of delicate Cells in the epidermis with farces of Keratin. The deeper layers were containing Newly formation of blood vessels without blood, finny hairs, sweat ducts and nerve Connection were Still newly formed which continue to fourteenth day of Pregnancy, The next days of pregnancy indicated Progress growth of epidermal cells and Collagen fibers in the dermis associated with Primordial hair follicles. The conclusion / Between day 8 and 14 of pregnancy the fetus of Rats were developed its skin and continuous growth of the skin layers with its accessories.

## 1. Introduction

The skin is a crucial organ in mammals that consists of three layers, each contributing to the body's protection, temperature regulation, and prevention of water loss, showcasing its multifunctional role beyond just serving as a barrier [1]. In fetal development, the skin's outer layer, known as the epidermis, originates from the ectoderm, one of the primary layers formed during early embryogenesis, which also gives rise to other structures like nerves and skin appendages. Special signals as their guide help the cells progress from the ectoderm and become connected, through differentiation and morphogenesis, resulting in the formation of complex skin layers including the epidermis and its structures like hair follicles [2]. Tissues found deeper down, such as the dermis and subcutis, take origin from the mesoderm layer during the developmental phases, establishing the main key in the matter of structure and operation of dermis during the later developmental stages. Building a basis to know how the skin layers are formed and what are their protective and nourishing properties which are very important for maintaining the skin's health and recovering after the skin is damaged is indeed the fundamental development [3]. Sebaceous glands are the specific type of structures on skins, that secrete sebum (a waxy substance used mainly for moisturizing the skin as well as serving as one of the barriers against microbial attacks) by maintaining the skin barrier as well as the skin microbiome [4]. Rat fetuses take the chronic journey for undergoing the sweat glands progression which is part of their system for cooling down and keeping fluid balance, and this process is gradual and it takes time to be ready for performing these life essential functions. Rat pups born too early can be suffering from their skin development, as during the first day of their life their skin, even including its protective barrier, undergoes the most important changes and also its maturation, which in premature pups may be compromised or altered [5]. Understanding how the skin of newborn rats changes after birth allows us to connect this to how premature birth can affect human skin growth by observing how skin layers and structures form differently from body to different body parts. The study examines how rat skin develops before and after being born too early.

## 2. MATERIALS AND METHODS

The study used 65 rats from Tikrit University's Veterinary College, including 22 males for mating and 43 females, with ages between 10-12 weeks. Rats were kept in plastic cages with specific sizes, covered floors with sawdust, and maintained under healthy conditions with regular cleaning and sterilization. The experiment ensured a controlled environment with 25°C room temperature, alongside a 12-hour light-dark cycle. To study rat mating, firstly made sure the female rats weren't pregnant by keeping them away from males for 25 days, then paired two females with one male as showed in (Fig.1)., and once a vaginal plug was seen, they isolated those females, marking the start of their pregnancy as showed in (Fig.2).



**Figure 1:** rats in sterilized conditions



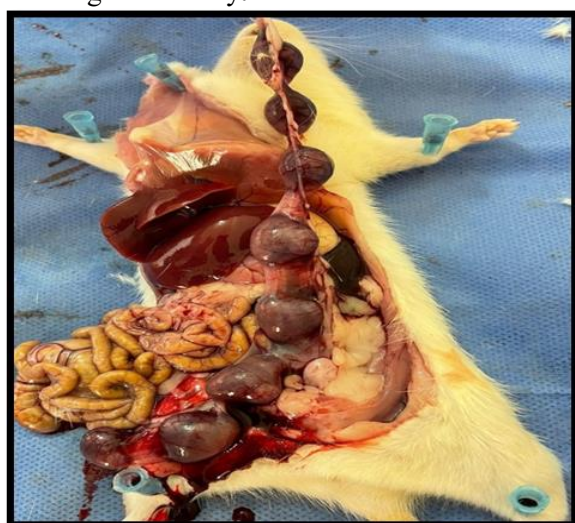
**Figure 2:** Vaginal Plug that forms after the male and female mate

The study observed the mating process, followed by daily checks starting on the 8<sup>th</sup> day of pregnancy until birth on the 14<sup>th</sup> day, and then tested the newborns' tissues on specific days after birth. The experiment used a special model to track and record the growth of fetuses in the womb by measuring the mother's uterus after an autopsy, including any changes during and after pregnancy. 24 rats were divided into groups. Each group had 2 females from day 1 to day 21. After birth, they used one rat per group. They studied the embryos by dissecting them. Each group represented a different day of the study. Starting from the 8<sup>th</sup> day of pregnancy until the 14<sup>th</sup> day. To prepare for dissection, female rats were made to sleep using a piece of cotton soaked in chloroform, a closed box until they stopped moving as seen in (Fig.3).



**Figure 3:** Method of anesthesia using chloroform

A rat was secured on a table and carefully cut open from its lower belly to chest to remove its uterus for tests as shown in (Fig.4).and safe keeping in a special liquid for future study. The sample was first fixed in a mix of formalin, calcium chloride, and water to keep it safe and unchanged for a day.



**Figure 4: Method of dissection of rats**

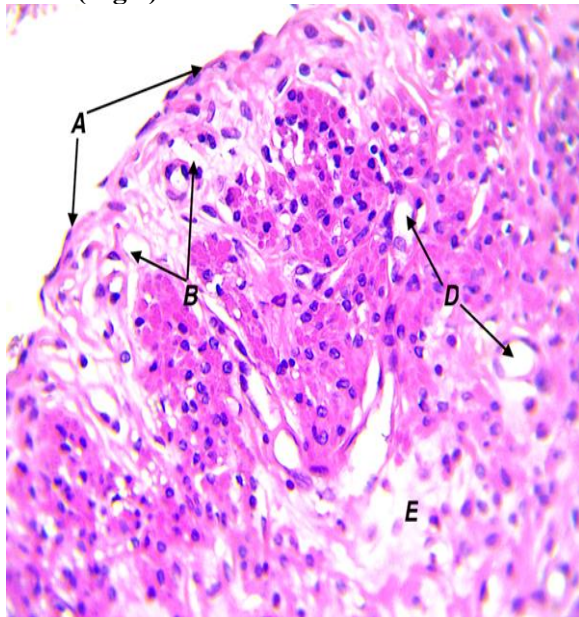
Next, it was washed with a special solution and tap water to remove extra preserving substances, ensuring it stays in a balanced state. Finally, the sample was dehydrated with alcohol at increasing strengths and made clearer with xylene, making it easier to examine. In the infiltration stage, samples were heated in a wax mixture at 60°C twice to prepare them. During embedding, samples were encased in wax within an "L" shaped mold and labeled for identification. For sectioning, thin slices of the sample were cut, flattened in warm water, and placed on slides with a special adhesive. The adhesive used on the slides is made from egg whites, glycerol, and thymol, and slides are dried on a hot plate. The glass slides with tissue samples were cleaned of wax using xylene and then dried, either naturally or in an oven at 40°C. They were then soaked in varying strengths of ethyl alcohol (from 100% to 70%) for 4 minutes each to further clean and prepare them. The slides were stained with hematoxylin to highlight cell nuclei in purple and eosin to color other cell parts red, with washing steps in between to remove excess dye and sharpen the colors. Finally, the slides were treated with alcohol in increasing concentrations and cleared with xylene, making them ready for examination. A glass cover called "Cover Slide" is put on tissue sections with a drop of "D.P.X" to dry them quickly at 40°C, then stored safely. Tissue slices are checked under a special microscope by Olympus (Japan) and photographed with Kress's system, including a digital camera for clear images.

### 3.RESULTS

The histological findings from skin tissue samples taken at different stages of pregnancy. Includes notes on the epidermis, dermis, and associated structures such as hair follicles and sebaceous glands. It indicates the presence of edema and angiogenesis with vessels filled with blood and various cellular components such as fibroblasts and mesenchymal cells. In addition, there are signs of keratinocyte layers, vacuolation in the cytoplasm of epithelial cells, and infiltration of inflammatory cells. These findings suggested a dynamic process of tissue remodeling and development during pregnancy. The eighth day of pregnancy The epidermis contained several rows of epidermal cells with widespread mesenchymal tissue. The basal layer extends into the dermis, forming epidermal papillae. The surface of the epidermis contains keratin filaments, and the dermis appears to

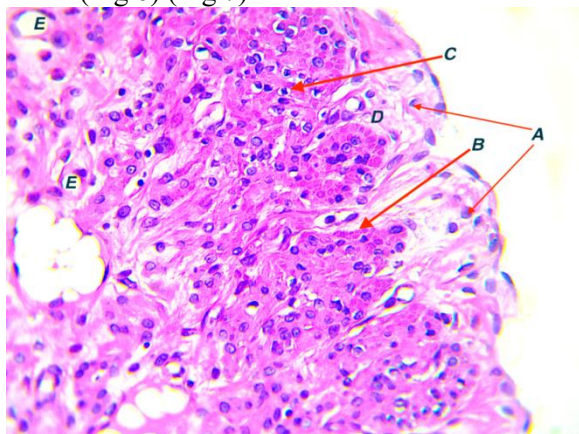


contain a spread of bundles of colloidal fibers. As in (Fig.5).

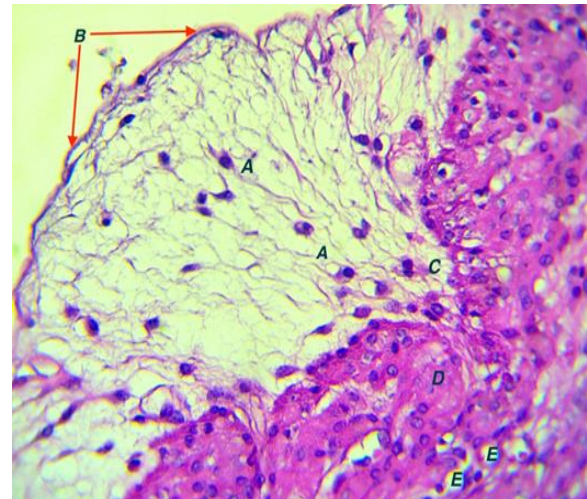


**Figure 5:** (A) Skin epidermis covered with keratin (B) Sweat gland ducts (C) Dermis (E) Mesenchymal tissue(H&EX40).

In the ninth day of pregnancy the epidermis of the skin consists of several rows of pale epithelial cells covered with thin keratin on its surface, and the basal layer of the epidermis is intertwined with the dermis, forming epidermal papillae, from which there is an extension of single nerve fibers connected to the basal layer of the epidermis. The dermis is wide and contains bundles of colloidal fibers with fibroblasts, and the subdermis layer contains blood vessels devoid of blood and is surrounded by small numbers of white blood cells, as shown in the (Fig.6) (Fig.7).

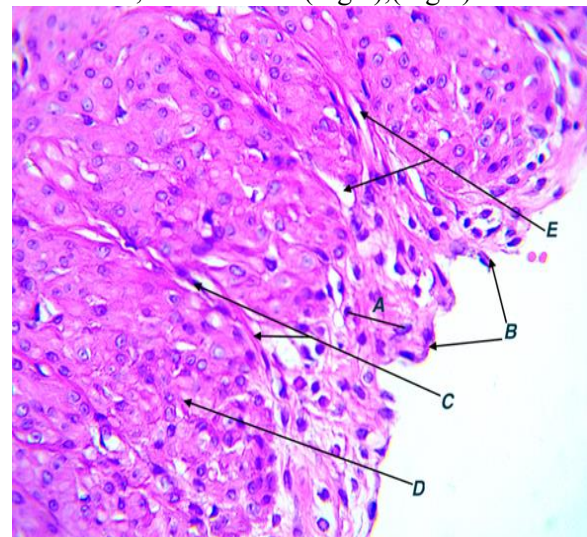


**Figure 6:** (A) Skin epidermis composed of more than one row of epithelial cells (B) Epidermal papillae (C) Dermis (D) Sweat canaliculi (E) Capillary blood vessels(H&EX40).



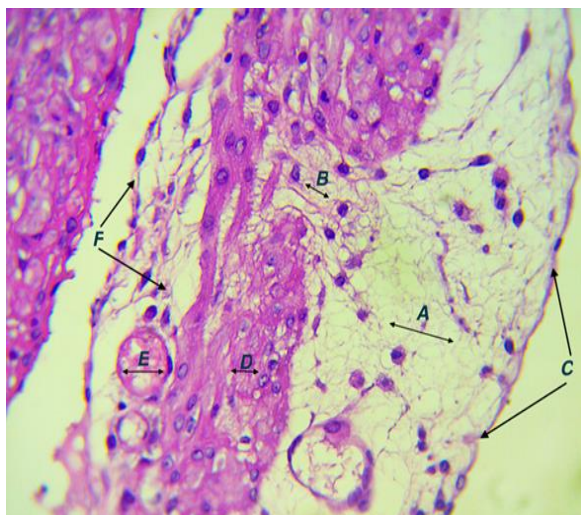
**Figure 7:** (A) Skin epidermis, which contains epidermal cells and mesenchymal cells. (B) Keratin filaments On the surface of the epidermis (C) epidermal papillae (D) dermis (E) hair follicles (H&EX40)

On the tenth day of pregnancy, the skin tissue contained an epidermis composed of several rows of epithelial cells with a number of surface ridges of the epidermis and a row of basal cells intermingled with the dermis with nerve fibers extending from the depth of the dermis towards the row of basal cells. The dermis contained bundles of colloidal fibers and in it. Fibroblasts, capillary blood vessels, and sweat ducts were also found, as shown in (Fig.8),(Fig.9).



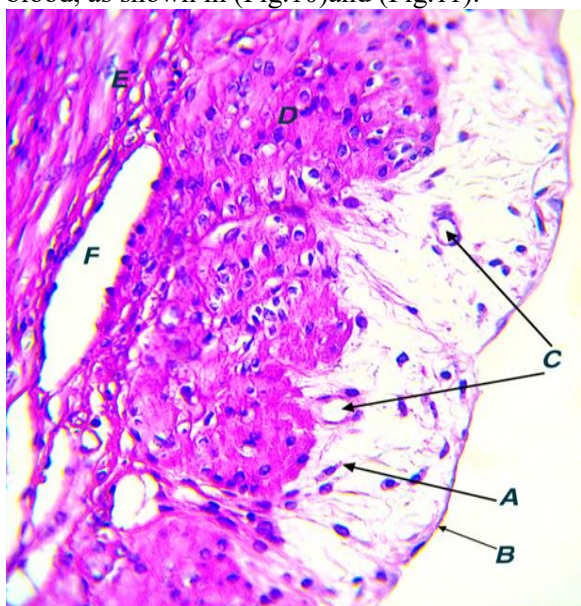
**Figure 8:** (A) Skin tissue, the epidermis of the skin is composed of several rows of epithelial cells (B) lining the surface of the epidermis (C) Nerve fibers connected to the basal row of epidermal cells (D) Dermis, which contains bundles of colloid fibers with fibroblasts (E) Sweat gland ducts (H&EX40)



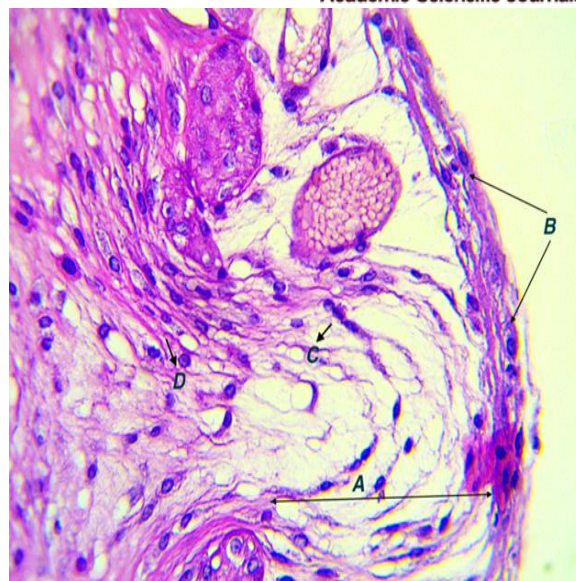


**Figure 9:** (A) Skin tissue, the skin epidermis is composed of epithelial cells and mesenchymal tissue (B) Epidermal papilla (C) Keratin filaments (D) Dermis, which contains bundles of colloidal fibers (E) Sub dermis, which contains small blood vessels (F) Macrophage cells (H&EX40)

On the eleventh day of pregnancy, the epidermis of the skin is composed of mesenchymal and epithelial tissue with openings of the sweat ducts and the surface of the epidermis is covered with keratinous filaments. The dermis is composed of bundles of colloidal fibers and contains hair follicles. The subdermis layer contained large blood vessels devoid of blood, as shown in (Fig.10) and (Fig.11).

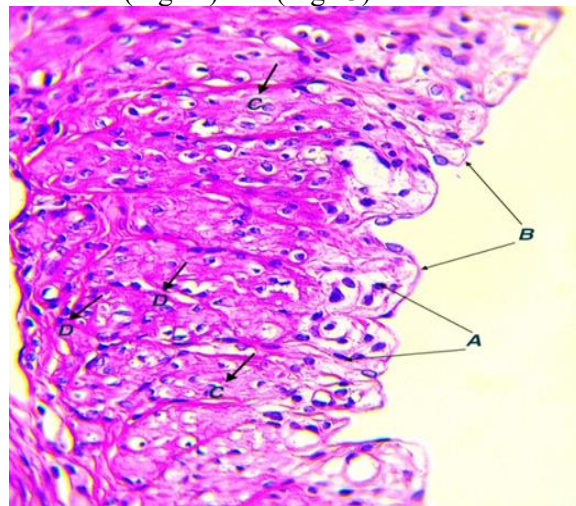


**Figure 10:** (A) Skin tissue, skin epidermis, which contains epithelial cells, mesenchymal cells and tissue (B) Keratin filaments (C) Sweat canals (D) Dermis (E) Hair follicles (F) Subdermal blood vessels (H&EX40)



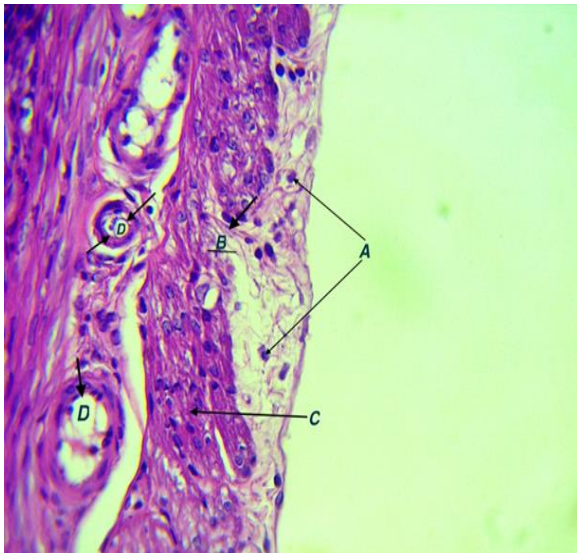
**Figure 11:** (A) Skin epidermis composed of mesenchymal tissue (B) Surface epithelial cells with keratin filaments (C) Nerve fibers (D) Epidermal papilla (H&EX40)

On the twelfth day of pregnancy, the skin contained multi-rowed epidermis, and the surface of the epidermis had many zigzags covered with strands of keratin. Nuclei of dark-pigmented skin epithelial cells. The dermis contains many bundles of colloidal fibers and contains protozoan hair follicles with the presence of nerve fibers extending from the base of the dermis to the base of the epidermis as shown in (Fig.12) and (Fig.13).



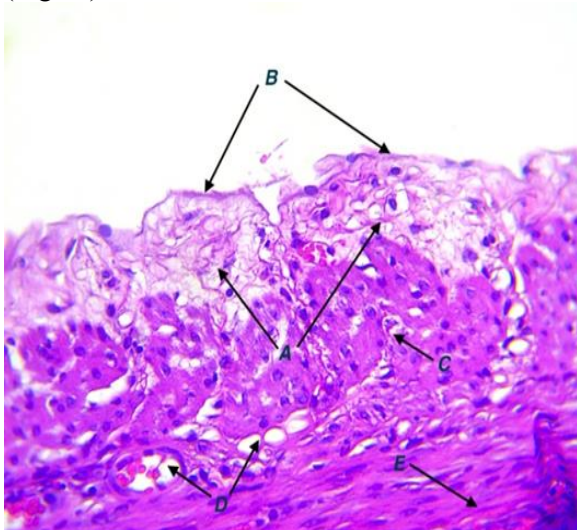
**Figure 12:** (A) The epidermis of the skin is composed of several rows of epithelial cells (B) Keratin filaments (C) The dermis is composed of bundles of colloidal fibers (D) Hair follicles (H&EX40)



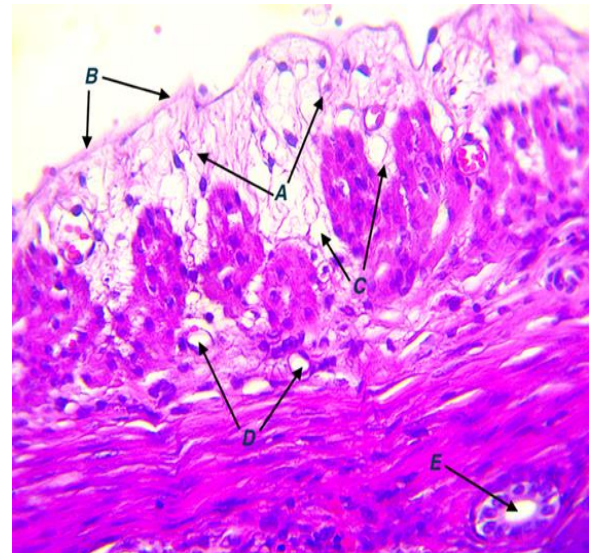


**Figure 13:** (A) Skin epidermis composed of epithelium and mesenchymal cells (B) Human papilla (C) Dermis (D) Hair follicles (H&E X40)

On the thirteenth day of pregnancy, the epidermis of the skin is wide, composed of several rows of epithelial cells, and the surface of the epidermis contains keratinized filaments, describing the basal cells of the epidermis, which overlap in several places with the dermis, composed of several epidermal papillae. The dermis contains bundles of colloidal fibers and contains capillary blood vessels, and some of them are larger blood vessels that contain red blood cells. The subdermis layer contains smooth muscle fibers as shown in (Fig.14).and (Fig.15).

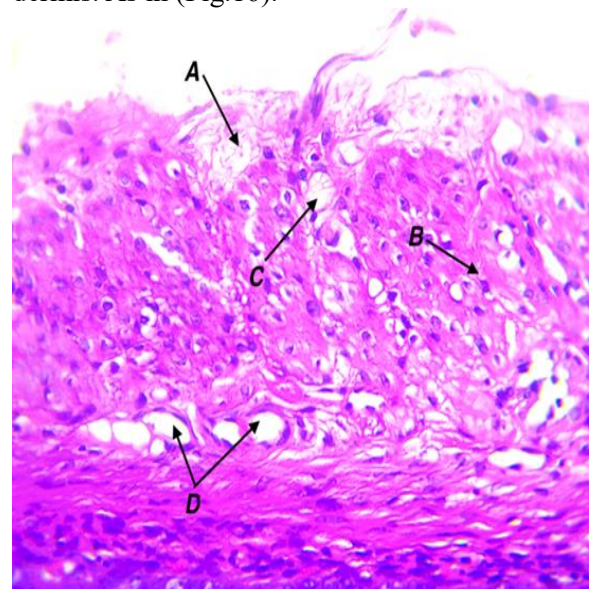


**Figure 14:** (A) Rows of epidermal cells (B) Keratin filaments (C) Bundles of colloidal fibers in the dermis (D) Blood vessels (E) Subdermis layer containing smooth muscle fibers(H&E X40)



**Figure 15:** (A) Skin epidermis composed of soft epithelial and mesenchymal tissue (B) Keratin filaments (C) Epidermal papillae (D) Capillary blood vessels (E) Subdermal sweat gland duct (H&E X40)

On the fourteenth day of pregnancy, the epidermis contains a row of epithelial cells closely packed together, accompanied by mesenchymal cells, with a larger number of mesenchymal cells spreading throughout the dermis. As in (Fig.16).



**Figure 16:** (A) Skin epidermis (B) dermis (C) vein canalicula (D) capillary blood vessels

#### 4.DISCUSSION

The study included collecting skin samples from rat fetuses at different developmental stages, before full birth. These samples are then analyzed using histological techniques to examine structural changes and maturation of the skin and its associated structures over time.

The results of the study contribute to construct an organized source for light and transmission electron microscopic structure of (pre- postnatal) developing rat skin hoping to help future researches on skin during these ages [6].

On the eighth and ninth days of pregnancy tissues which are covered by skin begin to develop ever deeper cell layers together with tissue spots that are located in the surrounding area. The development of the fetus and vital organ being a sign of the healthy growth of the fetus can be an indicator of the fact. The reason behind the growing amount of the epidermal receptors and the nerve fibers responsible for sensation and nutrition would be the formation of skin papillae and blood vessels which are the basics for blood circulation later. This is agreeable with Rooster man, et al. (2006) who also realized that the opening, and growth of the nerve fibers and papillae is particularly more evident during the early phases of pregnancy [7] The survey of skin histology (skin samples) in the laboratory on rats showed that sensory nerve fibers are physically close to the Langerhans cells, which play a vital role in formation of the neural elements. The research had joint opinions with Hu et al. ,( 2018) , who thought that this connection is important while shedding light on the skin sensory functions, allowing the subject to start responding to the changes in the outside environment. Furthermore, have we known that skin is the one of the important structures that built in the eight and ninth days in the prenatal life and that plays the major roles in sensing and feeding? Vascular endothelial growth factor also helps the body in this stage for healing as well as being a skin developmental factor by creating angiogenesis [8]. From the tenth to thirteenth day of pregnancy, the upper skin layer, the epidermis, undergoes considerable development while it is literally riddled with the ectodermal signal of the formation of the fetus itself along with vital organs. These signs demonstrate healthy functioning of the skin's primary functions which include protection, senses, sweating, and hair growth. This seems to be in accord with a study done by the Hu et al where they gave the account of the rat fetus's skin showing rapid development from the tenth to the thirteenth day, which is also probably associated with the continual appearance of its sensory abilities that is a highly important and significant milestone during the pregnancy. Sensory functions, developed in the early life of the fetus, serve as precursors to those that are required to perceive and react to stimuli outside

the skin later. The sensitivity of the skin to a variety of factors like pressure, temperature and chemicals is one of the main components of its defensive function that can keep the fetus safe by enabling the response to potentially dangerous conditions. In the beginning, the fetal skin conceals all sensations of touch, pain, and temperature. Due to the fact the nerve endings and receptors in the skin begin the development in the period of the gestation, this stage of the development gets the basis of the skin of the fetus in the sensory system [9]

Moreover, this research was in consistent with Wolff et al., 2008, the considered the start of sweating as well as hair growth in rat fetuses during the tenth to thirteenth day of pregnancy as an important sign of healthy fetal development. The increased specialization of functions arising from the developing plume of the skin brings many important temperature-regulating and protective advantages. Second to the appearance of sweat glands and hair germs, the process gets even more complicated with the skin cells developing in the epidermis while extending into the dermis [10].

Cutaneous developments during the 14th day of pregnancy reflect the continued growth and increasing complexity of the skin and dermis. The development of epithelial and mesenchymal cells, along with the formation of fibroblasts and blood vessels, indicates the development of basic skin functions such as protection, sensation, and sweating. These developments are an indicator of the healthy growth of the fetus and the development of vital organs. This is consistent with a study conducted by Gibson et al., 1983, where they found that the differentiation of mesenchymal cells into fibroblasts during the critical days of pregnancy in mice contributes significantly to the structural integrity and elasticity of the skin. Fibronectin, a high-molecular-weight glycoprotein, plays a pivotal role in this differentiation process. Its distribution during embryonic rat skin development has been carefully studied, revealing that fibroblasts synthesize and organize fibronectin into a fibrous matrix that supports cell adhesion and proliferation, and is essential for the structural formation of the skin [11]. In addition to a study conducted by Solarte David et al., 2022, who reported that the development of blood vessels from mesenchymal cells during the fourteenth and fifteenth days of pregnancy in mice is a crucial step in ensuring skin function and health. This vascular development is facilitated by the

proliferation and migration of mesenchymal cells, which differentiate into endothelial cells that form blood vessels [12].

## 5.CONCLUSION

between gestational days 8 and 14, there are histological features, in rat fetal skin development. These changes contribute to the overall development and maturation of the skin during this period.

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## التغيرات النسيجية في نمو جلد جنين الجرذ بين أيام الحمل 8-14

محمد صالح<sup>1</sup> , بدر ختلان حميد<sup>2</sup>فرع التشريح والأنسجة، كلية الطب البيطري، تكريت، تكريت، العراق<sup>1</sup>

## الملخص

يتكون الجلد من البشرة والأدمة وتحت الجلد، ويشار إلى هذا الشكل على أنه منظور جنيني، فالبشرة مشتقة من الأديم الظاهر، في حين أن الأدمة وتحت الجلد تنشأ من الأدمة المتوسطة. صممت الدراسة الحالية لمتابعة التطور الجنيني للجلد في الجرذان في مرحلة مختلفة من التطور الجنيني، لذا تم تضمين خمسة وستون (65) جرذاً في هذه الدراسة. اثنان وعشرون (22) ذكراً وبالغاً وثلاثة وأربعون (43) أنثى. تم إجراء التزاوج ووضع الإناث كلها تحت المراقبة للتأكد من وصولها إلى الحمل من خلال إظهار السداده المهبلي في اليوم الأول بعد التزاوج. من اليوم الثامن إلى اليوم العاشر من الحمل. تم فحص جلد الجنين مما يدل على وجود خلايا دقيقة في البشرة بها آثار من الكيراتين. كانت الطبقات العميقة تحتوي على تكوين أوعية دموية جديدة بدون دم وشعيرات دقيقة وقنوات عرقية واتصالات عصبية كانت لا تزال تتشكل حديثاً وتستمر حتى اليوم الرابع عشر من الحمل، وتشير الأيام التالية من الحمل إلى نمو متقدم لخلايا البشرة وألياف الكولاجين في الأدمة. المرتبطة ببصيلات الشعر البدائية. بين اليوم الثامن والرابع عشر من الحمل تطور جلد جنين الجرذان ونمو مستمر لطبقات الجلد وملحقاتها

الكلمات المفتاحية: البشرة، الأدمة، الفئران، الجنين، دراسة نسيجية.