AJMAS
https://journal.utripoli.edu.ly/index.php/Alqalam/index eISSN 2707-7179

*Original article*

# Histological Study of Skin Growth in Albino Mice During Embryonic Life and After Birth

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**ARTICLE INFO**

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***Received****: 15-04-2023*
***Accepted****: 11-05-2023*
***Published****: 15-05-2023*

***Keywords****. Histological Study, Skin Growth, Albino Mice, Embryonic Life.*



**ABSTRACT**

***Background and aims****. According to importance the skin, because epidermis and its appendages provide a protective barrier that keeps microbes out and essential body fluids in. It receives daily assaults, including harmful ultraviolet radiation from the sun, and scratches and wounds. To do this, it depends on stem cells, which reside in the adult hair follicle, sebaceous gland and epidermis for the purpose of maintaining tissue homeostasis, regenerating hair and repairing the epidermis after injury. The aim of our studies was to focus on skin structure during development in fetal life and after birth.* ***Methods****. The development of the skin of mice was investigated during the embryonic life and after birth, using 64 fetuses ranging in age from 10 days to 21 days, In addition to After birth mice (age one week). The first sign of the skin development was indicated in 10 days' mice embryos as single layer of squamous to cuboidal cells. This epithelium merged directly without basal lamina with surrounding undifferentiated mesenchymal cells.* ***Results****. The early embryonic formation of the skin of the head and neck of mice was at the age (10 days), the skin appears as a single layer of flat cells with oval nuclei, and with age the layer of epithelial cells increases in thickness. In embryos between 18 and 21 years of age, primordial hair follicles begin to emerge as a central density in the stratum basale of the stratum corneum, after birth, the primary follicle shows a hair canal, many of which contain hair. Some of these hairs have fully formed and come out from the surface, and the lower third of these follicles has become more coiled.* ***Conclusion****. Our study confirmed that the basal layer of the epidermis remains morphologically uniform. In the first stage, as the follicles develop, the skin becomes thicker, mainly due to an increase in the thickness of the dermis. Subsequent hair follicle differentiation and maturation is largely dependent on signals from the dermal papilla*

***Cite this article.*** *Abdelsalam F, Moftah H, Elgrabawy S. Histological Study of Skin Growth in Albino Mice During Embryonic Life and After Birth. Alq J Med App Sci. 2023;6(1):240-245. https://doi.org/10.5281/zenodo.7938663*

## INTRODUCTION

The skin is an important organ in animals in general and mammals in particular. The tissue structure and stages of development of skin growth have been of interest to very few researchers. The mammalian skin epidermis together with its derivative appendages, such as hair follicles, sebaceous glands and sweat glands, plays pivotal functions in protecting the organism from dehydration and environmental insults, as well as in regulating the body temperature [1] [2]

The granular layer and the cornified layer are successively formed by the differentiation, maturation, and migration of spinous cells. Transglutaminase and Involucrin are expressed by the granular layer, while Filaggrin and Loricrin are

expressed by the cornified layer [3]. The embryonic ectoderm, which also gives rise to the nervous system, is the source of the epidermis. Ectodermal cells choose between epidermal and neural fates shortly after gastrulation [4]. Adult and embryonic epidermis stratify using some of the same mechanisms. The creation of the basement membrane, which divides the dermis from the epidermis and supplies the basal cells of the epidermis with extracellular matrix (ECM) proteins and growth factors, occurs concurrently with the establishment of the embryonic basal layer. Basal layer cells must escape the basement membrane beneath in order to differentiate and stratify. Hemidesmosomes and focal adhesions allow basal cells to adhere to the basement membrane. [5].

Only the skin of an embryo has an intermediate layer, which is a temporary layer. The intermediate layer's post-mitotic spinous cells are produced through cell division and maturation [6]. Signals from the dermal papilla play a major role in the differentiation and development of subsequent hair follicles [7]. In mouse embryos, sweat gland germs emerge as invaginations of epidermal basal cells at E17.5, shortly before birth. During postnatal day P1–P5, sweat gland germs develop into single long ducts extending deeply into the dermis, with coiled glands at the tip [8].

Mesenchymal cells from the dorsal back skin dermis are derived from dermomyotome, in which Wnt signaling specifies their fate [9]. As these mesenchymal cells proliferate across the skin, their interactions with the epithelium above them result in the development of hair placodes, which are minute epidermal invaginations into the dermis underneath. Early cues from the mesenchyme influence the placement of placodes and define their commitment, according to groundbreaking research on mesenchymal-epithelial tissue recombination in chicks and mice [10].

Once the placode has formed, downstream signaling events drive the down growth and maturation of the hair follicle. A myriad of changes take place during follicle morphogenesis, as exemplified by the differences between the transcriptional profile of placode cells and their epidermal counterparts [11]. A progenitor population of cells is required for sebaceous gland homeostasis because they produce a steady stream of proliferating, differentiating, and ultimately dead cells that are shed through the hair canal. The gland is encircled by the same basement membrane that marks the mesenchymal-epithelial barrier. Not surprisingly, the sebaceous gland cells that are attached to the inner surface of this membrane share many of the features of epidermal keratinocytes [12,13].

The activity of particular receptors on various epidermis-resident cells, including keratinocytes, Merkel cells (MCs), and free nerve terminals, mediates the sensory function of the skin [14]. MCs are neuroendocrine cells that can be found in hairy and glabrous skin. They are clustered in touch-sensitive areas called touch domes [15]. Thermoregulation in the epidermis is controlled mainly by the sweat glands [16]. The epidermis that formed the uppermost multi-layered compartment of the skin showed a keratinized stratified squamous epithelium with four distinct layers: stratum corneum, stratum granulosum, stratum spinosum, and stratum basale, which rested on the basement membrane. Different stages of hair follicle morphogenesis 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 and were associated with well-developed sebaceous glands in the dermis [17]. Hence, the aim of our studies was to focus on skin structure during development in fetal life and after birth.

## METHODS

### *Study design and setting*

Skin samples of 64 white albino mice embryos ranging from 10 days up to 21 days old were used in this study. In addition to after birth mice (age one week). They were taken from the frontal neck and thorax.

### *Data collection procedure*

After determining the age of the fetus, the female was slaughtered, the abdominal cavity was opened and the placenta was removed, and the embryos were removed. The ages of the fetuses and their number were recorded, and some mothers were left until the birth was completed, then the born mice were left to grow and samples were taken from them at different ages. The samples were fixed to the following tissue stabilizers in 10% neutral Buffered formalin, Bouin's, Zenker's and Susa fluids. Whole embryos, ranging in age from 10 days to 21 days. In addition to After birth mice (age one week) were placed in the stabilizer then they were dehydrated cleared, embedded in paraffin wax. The paraffin sections of 4-6 µm thick were prepared and stained with hematoxylin and eosin, Crossman's trichrome stain, cyan stain, Giemsa stain for dyeing vaginal swabs.

## RESULTS

In the early embryonic formation of the skin in the head and neck of mice at the age (10 days), the skin appears as a single layer of flattened cells with oval nuclei, in other areas the epithelial cells vary from cuboidal to polyhedral with oval or round nuclei the cytoplasm was acidophilic as illustrated in (fig.1).

At an advanced age of 12 days, the layer of epithelial cells increases in thickness (fig.2). At day 15 it consists of two layers of cells, the superficial layer of cells consists of small flattened cells with dark elongated or oval nuclei, while the basal cells are cuboidal or columnar with large oval basal nuclei (fig.3). At 16-day age of fetal life, an intermediate layer of polyhedral cells with large spherical nuclei appear between the basal and superficial layers. In fetuses between the ages of 18-21, a primitive hair follicle begins to appear as a central thickening in the layer stratum basale (fig.4).
Then it invaginated in the dermis, which constituted the follicular plugs, epidermal invagination extends in some areas to the outer third of the dermis. The cells of the follicular plug are either neatly arranged in the periphery and continued with the stratum basale or scattered irregularly within the center of the plug. They are surrounded condensation of two or three layers of fibroblasts. This invagination increases in depth as the dermis consists of fibroblast cells that condensed around the formed follicle (fig.5). After birth, at one Week of age, the thickness of the epidermis increases in, and an additional fourth layer of cells appears beneath the periderm with pyknotic nuclei representing the primordial of the stratum corneum (fig.6).
Several hair follicles extended obliquely to the deep dermal level. Some of the largest follicles showed hair papilla, hair matrix, inner and outer root sheath as well as primordium of the sebaceous gland in the form of a few large vacuolated cells especially in the frontal region of skin, neck, ear pinna (fig.7). The cellular elements of the dermis slightly decreased than that in the previous stage while the fibrillar elements increased. at the end of this stage, the trio-groups could be demonstrated in all of the examined regions. These were composed of two small lateral follicles, one on each side of the central primary follicle (fig 8).
The primary follicle shows hair canal, many of them contained hairs. Some of these hairs were fully formed, emerged from the surface and the lower third of these follicles became more coiled. The arector pili muscle was first observed as a simple bundle of developed smooth muscle fibers extend from the hair follicle to the area below the epidermis (fig.9), this muscle progressed rapidly and became well developed.

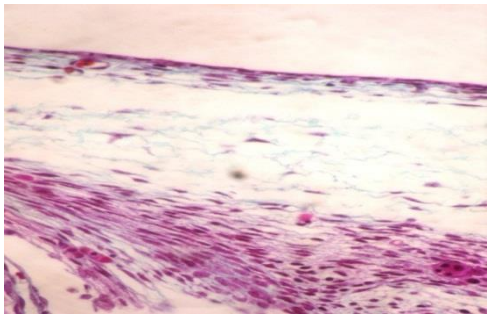

***Figure 1. skin of 10 days old mice embryo showing single layered epidermis of flat cells with oval nuclei. Crossman's trichrome stain X400.***

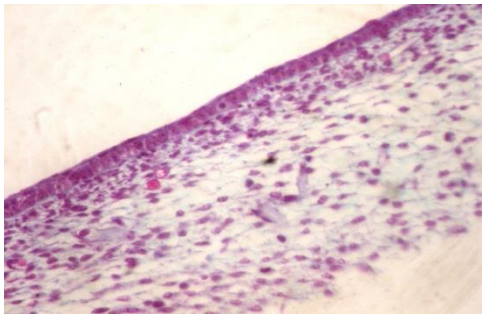

***Figure 2. Skin of 12 days old mice embryo showing single layered epidermis of cuboidal cells with large oval nuclei. Crossman's trichrome stain X400.***

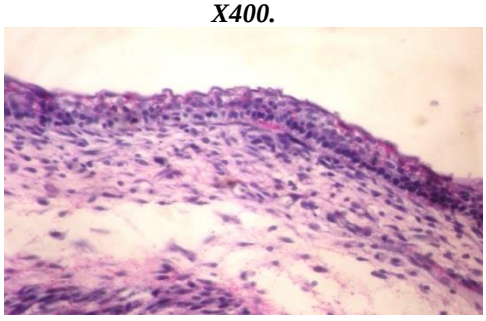

***Figure 3. Skin of 15 days old mice embryo showing basal columnar cells and superficial flattened cells (the epidermis) notice: thin intermediate layer of polyhedral cells in between the basal cells and epidermis. PAS X400.***

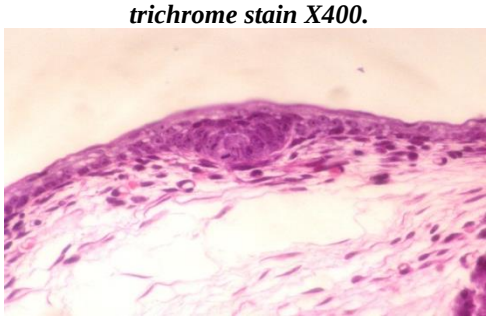

***Figure 4. Skin of 18 Days old mice embryo showing localized thickening in the stratum basal accompanied by its invagination in to dermis to form follicle plug. H&E X400.***

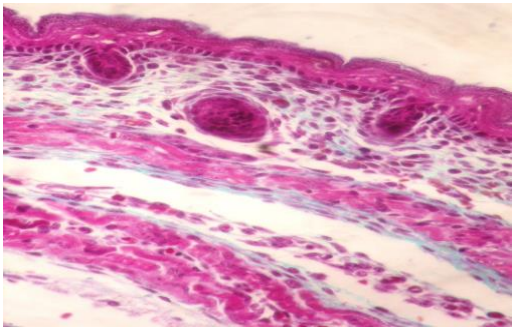

*Figure 5. Skin of 21 Days old mice embryo showing more extension of the follicle plugs in to dermis. The cells were arranged regularly around the periphery and irregularly within the center of the plug. Crossmon 's X400*

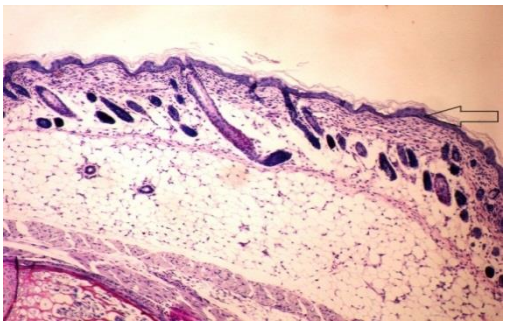

*Figure 6. Skin of After birth, at one-week age, mice showing thickness (Arrow) of the dermis and sub cutis and slightly keratinized hair extended along the dermis. PAS X400*

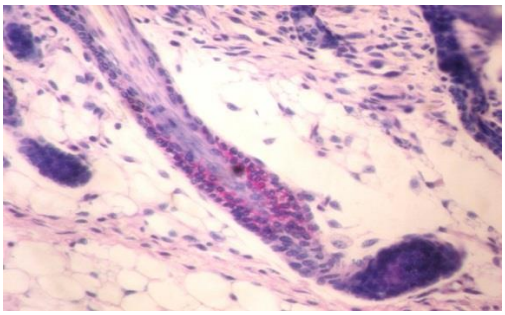

*Figure 7. Skin of frontal region of After birth, at one-week age, mice embryo showing enlarged hair bulb as well as sebaceous glands. cyan stain X1000*

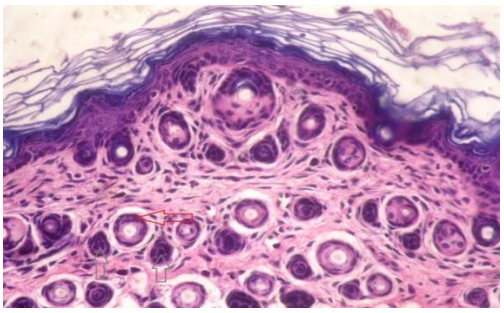

*Figure 8. Skin of neck of After birth, at one-week age, mice showing tri-group of hair follicles composed of two small lateral (Grey Arrows) follicles and central primary one (Red Arrow). PAS X400.*

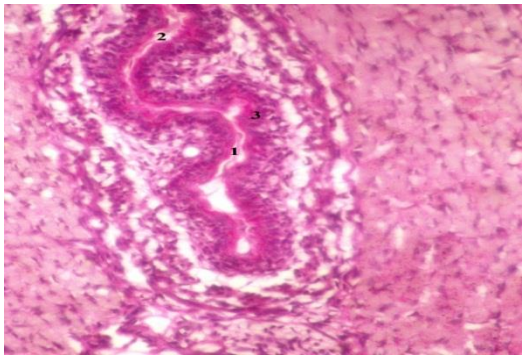


*Figure 9. Skin of neonatal mice, at two-week age, showing: hair structure (1), hair canal (2), more coiled lower third of the hair follicle (3). H&E X1000.*

## DISCUSSION

The findings of our study suggested that early embryonic formation of the skin of mice at the age (10 days), the skin appears as a single layer of flattened cells with oval nuclei, in other areas the epithelial cells vary from cuboidal to polyhedral with oval or round nuclei A similar finding was reported by Elsaid and Faheem [17].

The epidermal basal layer remains morphologically uniform. In stage 1, the basal cells form a visible hair placode which is the primordial of a hair follicle, and dermal fibroblasts begin to aggregate under the placode [18]. As the follicles develop, the skin became much thicker, chiefly due to an increase in the thickness of the dermis. This increased thickness of epidermis during the period of follicle activity in mice is similar to that described in rabbit. The present study revealed that, a primitive hair follicles begin to appear at 18 days old mice embryo.

As a primitive hair germ or plug, another study showed that the hair follicle in rabbits begins to appear at fetal age of 19 days [19]. The onset of primitive hair germ in other species was observed at 50-60 mm CURL dog fetuses [20]. And recorded in sheep embryos around the 78th day [21]. From these previous results, the appearance and

differentiation of the hair follicles may depend on the physiological and histological ability of the skin. Also this difference Could be attributed to the time of gestation period in different domestic animals. Subsequent hair follicle differentiation and maturation largely depend on signals from the dermal papilla [7]. In our study, sebaceous glands began to develop postnataly at 1-5 days. Similar findings were recorded at 45cm CVRL camel fetuses [22]. At 78$^{th}$ days old dog fetuses [21]. In humans, sebaceous glands develop around weeks 13-14 of gestation [18].

In our study, sebaceous glands began to develop near the end of embryogenesis and mature after birth and we agreed on this with Nimann and Horsley's study at the year 2012 [18]. The sebaceous gland forms at the upper part of hair follicle [18] and secretes sebum to Lubricate and keep the waterproof property of hair in Mammals [18] They release oils in to the hair canal for lubrication and protection against bacterial infections [12] [13].

In our study, mice embryo sweet glands germs emerge as invaginations of epidermal basal cells at embryo 17 day. during postnatal day (1-5) sweet gland germ develop in to single long duct extending deeply into the dermis with coiled glands at the tip. This is consistent with the study of Kunisada et al., 2009 [8]. In humans, the sweet glands start to develop during week 13-14 of gestation and mature at about week 24 [23]. Some studies also confirmed that fetal skin wounds have the unique ability to fully regenerate injured skin and heal without scarring [24].

## CONCLUSION

Given the importance of the skin, further studies are recommended, because our research proved that the epidermis's basal layer still maintains its homogeneous morphology. The skin thickens in the initial stage when the follicles grow, primarily as a result of an increase in dermis thickness. Dermal papilla signals play a major role in the differentiation and development of later hair follicles.

### *Conflict of Interest*

There are no financial, personal, or professional conflicts of interest to declare.

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