

Research Article

ROLE OF APOPTOSIS AND HISTOGENESIS FORMATION OF SKIN LITTLE MOUSE DURING GESTATION

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Abstract

Embryonic Differentiation of Skin that consisting of multi-layer tissue which growth depend on the time of gestation with apoptosis and cell proliferation that consider basic biological functions that need to be precisely controlled during embryonic Differentiation. Therefore, we aimed in this study to known Differentiation of skin little mouse during gestation by study histologic structure and Apoptosis during embryo development. Used (15) pregnant female mice, weighing between 25 - 30 g, aged 10 - 12 weeks, the pregnant female we divided according to the days of pregnancy into 3 group equal (15th, 17th and 20th day). And harvested (6 - 8) embryos from each female and chose 2 embryos from each female so the total embryos for each groups are 10. The result of Histological pattern of the skin of mouse embryo at 15th day of gestation show Periderm, Basal layer, Spinous layer, Basement membrane and Dermis with Dermal Condensate. With TUNEL assay for detection of apoptotic cells in mouse skin show as fluorescent light point. And Histological pattern of the skin of mouse embryo at 17th day of gestation show Periderm, Granular layer, Basal layer, Spinous layer, Basement membrane and Dermis with Hair germ. With increase apoptotic cells comparative with group 15th day. While, the Histological pattern of the skin of mouse embryo at 20th day of gestation show Cornified layer, Granular layer, Basal layer, Spinous layer and Basement membrane and Dermis with Hair peg and Dermal papilla. With high significant apoptotic cells comparative with group 15th and 17th. In this study we attempt to clarify the role of apoptosis and histological layer embryonic in development of skin: the epidermis and dermis.

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1. Introduction

The skis protect the body from microorganism infection and dehydration. It also his sensory and thermoregulatory functions. the skin many layers is superficial layer epidermis and deep layer, dermis and subcutaneous layer (Kogut and Bilousova, 2018).

Epidermis layer it is the first layers, initiated from embryonic ectoderm and A deep layer, the dermis, develops from the underlying mesenchyme (Sadler, 2018). The epidermis, is contain basically of keratinocytes, in addition sebaceous glands, follicles, sweat glands that initiated from dermis which contain extracellular matrix with blood and lymph vessels and nerve and separate between layers basement

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membrane. The hypodermis contains basically of adipose tissue that covered muscles and bones (Koster and Roop, 2007). In the mouse, the ectoderm formed at E9.5 day after then generate the epidermis and appendages present (Koster and Roop, 2007; Millar *et al.*, 1999). Then completely basal layers in E15.5, During this period covers the epidermis by protective layer called periderm function of the periderm unclear (M'Boneko and Merker, 1988; Benitah and Frye, 2012) and differentiated almost at E17.5 (M'Boneko and Merker, 1988) and formation hair follicle at E14 (Driskell *et al.*, 2011; Ohyama *et al.*, 2010). Apoptosis, or programmed cell death, is a biological process that occurs in multicellular organisms. During the organ development phase and differentiation (Riwaldt *et al.*, 2017). Therefore, we aimed in this present study to clearer the Role of Apoptosis and Histogenesis formation of skin little mouse during gestation.

2. Material and Methods

Experimental Design

Used (15) pregnant female mice, weighing between 25 - 30 g, aged 10 - 12 weeks, kept the animals under illumination about 10 hrs dark and 14 hrs light in plastic cages, fed with standard pellet diet and fresh tap water. Typically, the mating process between female and male sometime occurs at night, checking for vaginal plug for 8 - 24 hrs, after fertilization separated the females from the male, that represented the first day of pregnancy, about 19-20 days it is gestation period (Danneman *et al.*, 2012).

Samples Collection

The pregnant female we divided according to the days of pregnancy into 3 group equal (15th, 17th and 20th day). The animals euthanized, Dissection of the abdominal wall and harvested (6 - 8) embryos from each female and chose randomly 2 embryos from each female so the total embryos for each groups are 10, to perform this study (Table - 1).

Table - 1: Samples groups are arranged according to the days of pregnancy

Days of pregnancy	Number of female mice in each group	Number of embryos that were taken randomly
15	5	10
17	5	10
20	5	10
Total	15	30

Then take the body parts of the harvested embryos that contain the skin and processing the histological and TUNEL technology.

Histological Examination

The Specimens were taken from leg and hand including: skin, were fixed immediately after remove by 10 % buffer formaldehyde and washed with tap water after 72 hrs. and to remove the water from tissue passage the tissue by upgrading alcoholic concentration from 70

%, 80 %, 90 % and 100 %. Then remove the buffer formaldehyde solution from the tissue by xylol, after that infiltrated the spacemen by paraffin wax at 58 °C and sectioned at thickness 5 µm by rotary microtome then stained with Hematoxylin and Eosin stain (Luna, 1968).

Detection of Apoptosis by TUNEL Assay

Use TUNEL-based detection Kit from abcam/UK company code abcam 66108, to detect fragmented DNA of apoptotic cells then observed by the Fluorescent microscope that showed as fluorescent light point (Huda, 2014).

3. Result

Description of variable tissue compartments of skin

The developmental organizations of skin little mouse during gestation which seen in

serial transverse sections for exam TUNEL-Assay and Histology.

The histological pattern of the skin at 15th day of gestation

The result of histological section of the skin of mouse embryo at 15th day of gestation showed variable tissues include; Periderm, Basal layer, Spinous layer, Basement membrane and Dermis with Dermal Condensate and by use TUNEL assay detection the apoptotic cells in tissue section as fluorescent light point. The development of layer skin was distinctly (Figure - 1).

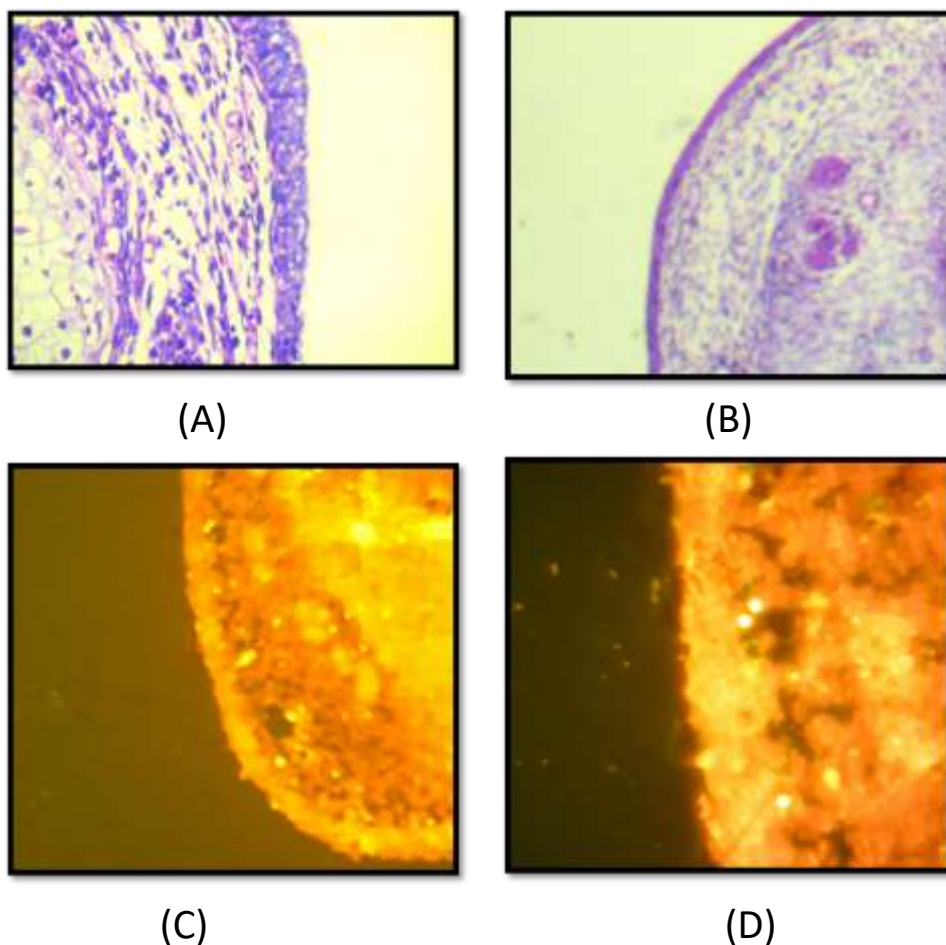


Figure – 1: Histological pattern of the skin of mouse embryo at 15th day of gestation(A&B) show Periderm, Basal layer, Spinous layer, Basement membrane and Dermis with Dermal Condensate (C&D) TUNEL assay for detection of apoptotic cells in mouse Show of apoptotic cells in mouse skin as fluorescent light point

The histological pattern of the skin at 17th day of gestation

The result of the histological pattern of the skin of mouse embryo at 17th day of

gestation show thickness skin due to generation other layers which Periderm, Granular layer, Basal layer, Spinous layer, Basement membrane and Dermis with Hair germ (Figure - 2).

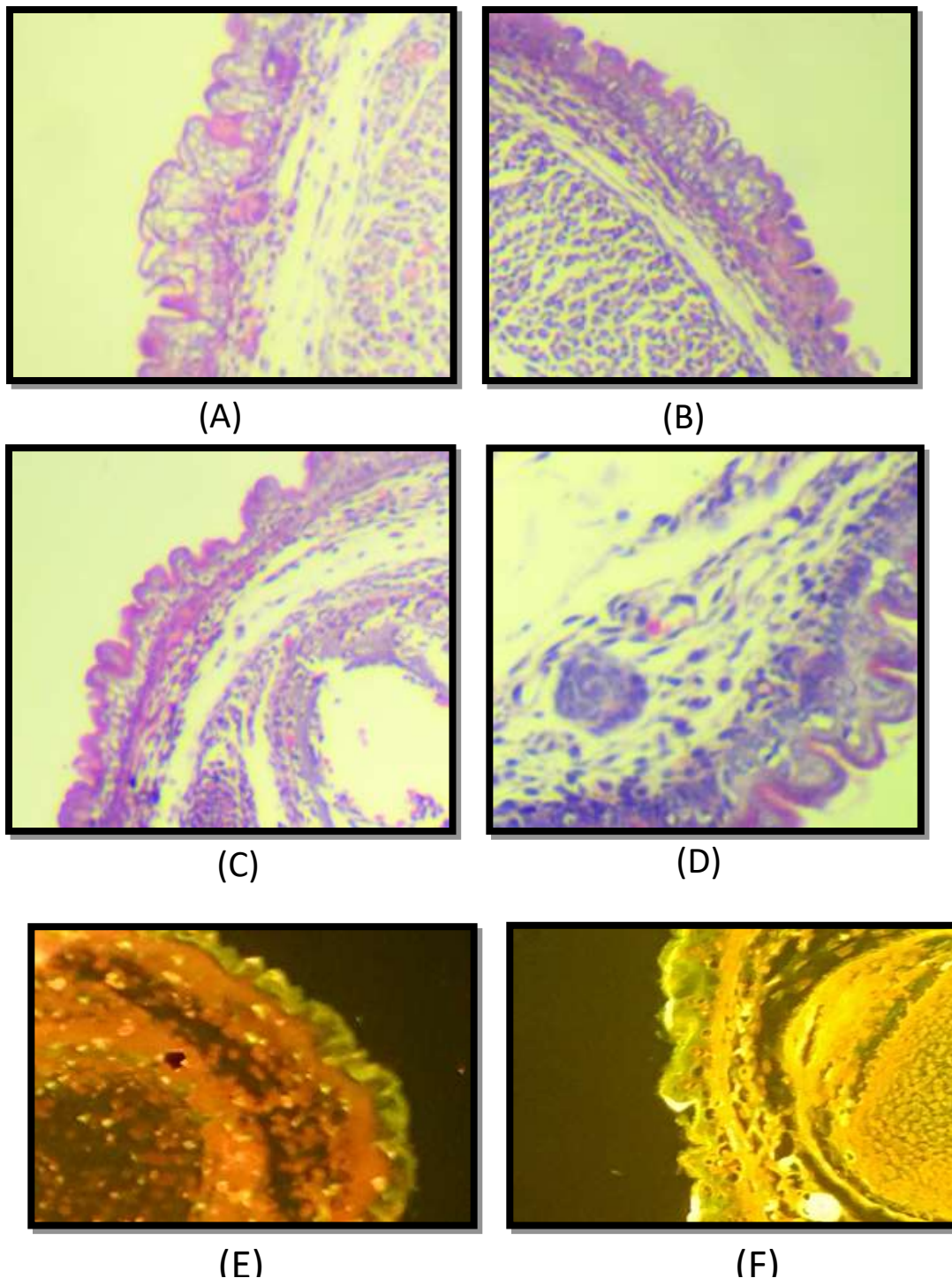


Figure – 2: Histological pattern of the skin of mouse embryo at 17th day of gestation(A,B,C &D) show Periderm, Granular layer, Basal layer, Spinous layer, Basement membrane and Dermis with Hair germ.(E&F) TUNEL assay for detection of apoptotic cells in mouse Show of Apoptotic cells in mouse skin as fluorescent light point

The Histological pattern of the skin at 20th day of Gestation

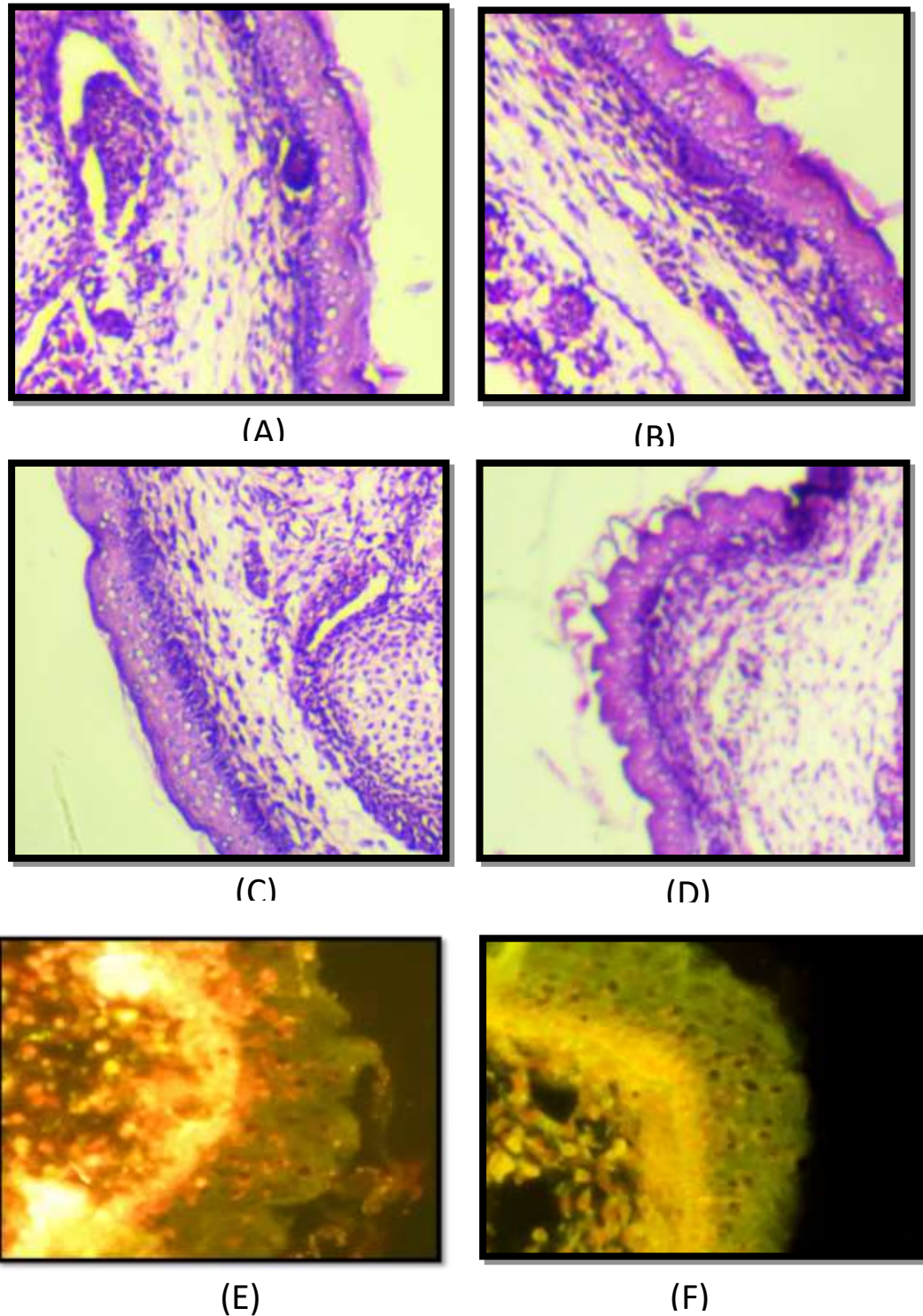


Figure – 3: Histological pattern of the skin of mouse embryo at 20th day of gestation show Cornified layer, Granular layer, Basal layer, Spinous layer and Basement membrane and Dermis with Hair pegand Dermal papilla. (E&F) TUNEL assay for detection of apoptotic cells in mouse Show of apoptotic cells in mouse skin as fluorescent light point

The Histological pattern of the skin of mouse embryo at 20th day of gestation show the skin more thickness and consist of Cornified layer, Granular layer, Basal layer, Spinous layer and Basement membrane and Dermis with Hair peg and Dermal papilla. With high significant apoptotic cells comparative with group 15th and 17th group.

4. Discussion

The result of this study showed the Histological pattern of the skin of mouse embryo at 15th day of gestation show Periderm, Basal layer, Spinous layer, Basement membrane and Dermis with Dermal Condensatem with detection of apoptotic cells and at 17th day of gestation show Periderm, Granular layer, Basal layer, Spinous layer, Basement membrane and Dermis with Hair germ. With increase apoptotic cells comparative with group 15th day. While, at 20th day of gestation show Cornified, Granular, Basal, Spinous layer and Basement membrane and Dermis with Hair pegand Dermal papilla. With high significant apoptotic cells comparative with group 15th and 17th. And this result agreement with researcher that reported. During embryogenesis, the epidermis initiated from ectoderm, that contain on a single-layered epithelium that responsible excretion K8/K18 cytokeratins and rise to the epidermis at E9.5 days in mice, The basal layer, keratinocytes proliferate and contribute to the formation of a transient layer of endoderm-like cells called periderm, between the periderm and basal layer initiated intermediate layer due to Continuous cells division within the basal layer, that responsible rise spinous layer by proliferation and expressing K1/K10. And the spinous layer in to new layers are granular layer and the cornified layer and this structure similar time formation with our study (Kogut and Bilousova, 2019). In mice, as models for differentiation and development skin, there are two sebaceous glands and four different types of hair, this development hair type from the hair follicle that rise with embryonic epidermis by three phases cytodifferentiation, hair follicle organogenesis,

hair placode (Schmidt Ullrich and Paus, 2005; Jones *et al.*, 1994).

5. Conclusion

In this study we attempted to clarify the embryonic development of skin mice and structure depend on the time, The skin constitutes a reservoir epidermis dermis, in addition tissue and gland other tissue that origin of stem cells with recorded contributed the apoptosis in differentiation of skin.

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