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# Histological and Cytoarchitectural Measurements of Human Epidermis in Different Anatomical Sites of Embryonic, Fetal and Neonatal Iraqi Subjects in Al-Hilla/Iraq Maternity Hospital

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**Abstract**

The integumentary system covers the surface of the embryo (skin) and its specialized skin structures including hair, nails, sweat glands, mammary glands and teeth. During fetal skin development, the epidermis changes from a single layer of ectodermal cells at 7–8 days of gestation into a more apparent stratified, keratinized epithelium at 22–24 weeks. The aim of the study is to identify the histological and cytological changes that take place during fetal, embryonic and neonatal development. Human Fetal samples were obtained from fully informed, consenting patients undergoing spontaneous terminations of pregnancy from emergency department at Al-Hilla maternity. Neonatal samples were obtained from neonates after sudden deaths from maternity wards. Anatomical Sites included abdomen, forehead, back and feet sole. A total of 30 embryos and foetuses aged between 2-6 months and 15 neonates were used for this study. Fresh tissues were sectioned using a freezing cryostat. Tissues were sectioned at 5µm in -24°C and collected on microscopic slides. Slides were allowed to air dry for 30 min prior to hematoxyline and eosin staining. Tissues were also photographed using scanning electron microscopy SEM. Cytological measurements were taken using image j software and data was analysed using graph prism. Various cytological and histological changes takes place during fetal and embryonic development. Our study shows the stages of fair follicule formation as well as number of nucleated layers present at each stage of development and at different anatomical sites.

**Keywords**: Epidermis, Fetal, Embryonic, Postnatal, SEM.

## INTRODUCTION

The human integumentary system is made of thick and thin skin which covers the human body and serves a specific function. Skin appendages ensure a number of critical functions which are essential for human survival. The skin is made of two distinct layers, the epidermis and dermis [1, 3,4,9]. The overlying epidermis contains ,epidermal appendages such as hair follicle, sweat and sebaceous glands. The two major tissue organizations of epithelial (ectoderm, epidermis) and mesenchyme (mesoderm connective tissue, dermis and hypodermis). An extensive population of melanocytes which are derived from the neural crest and sensory nerve endings [3,9]. During fetal skin development, the epidermis changes from a single layer of ectodermal cells at 7–8 days of gestation into a more apparent stratified, keratinized epithelium at 22–24 weeks [5]. Meanwhile, the epithelial cells express different patterns of cytokeratins and proteins of the cornified cell envelope. The thickness of the skin varies in different parts of the body, and the proportions of dermis and epidermis also differ[1,3,8]. Between the scapula, the dermis is especially thick, whereas on the palms of the hands and soles of the feet, the epidermis is thickened. The terms thick skin and thin skin refer to the *thickness of the epidermis* and not to the thickness of the skin as a whole [10]. In thick skin the epidermis is especially well developed, whereas in thin skin it forms a relatively narrow layer. At its junction with the dermis, the epidermis forms numerous ridge like extensions, the epidermal ridges that project into the underlying dermis [2]. Complementary projections of the dermis fit between the epidermal ridges and form the dermal papillae. The surface patterns of the skin, such as the fingerprints, reflect the pattern of the dermal papillae [7]. The epidermis forms the surface layer of the skin and consists of keratinized stratified squamous epithelium. The cells, keratinocytes, undergo an orderly progression of maturation and keratinization to produce a superficial layer of dense, flattened, dead cells at the surface [1,3,9].

## MATERIALS AND METHODS:

**Subjects:** A total of 52 embryos and foetuses aged between 2-6 months were used for this study as follows (2 months n= 5), (3 months n= 5 ), (4 months n= 6 ), ( 5 months n= 9 ) and ( 6 months n= 5 ). neonatal tissues were obtained from fully informed consenting parents a total of 15 neonates were used for this study. All subjects were free from skin diseases or abnormalities, any abnormal or deformed skin was not used in this study.

**Histology:** Fresh tissues were fixed in optimum cutting temperature (OCT) and sectioned using a freezing cryostat at 5µm in -24°C and collected on microscopic slides. Slides were allowed to air dry for 30 min prior to hematoxyline and eosin processing. **S**

**canning electron microscopy :**Fresh tissues of all 4 sites from all ages were embedded in glutaraldehyde then processed with osmium tetroxide and a series of alcohol concentration according to manufacturer protocol before coated with gold and photographed using scanning electron microscope (SEM).

**Measurements**: Cytological measurements were taken using image j software using pixel calibration according to software instructions. Data was analyzed using graph prism.

## RESULTS:

Our results show a several histological and cytological changes that takes place at the age of 2-3 months of gestation these include the formation of A major transition is seen after 4 and 5 months of gestation including the formation of germinative layers. Maturation of all epidermal layers was visible after 6 months of gestation. Number of nucleated layers is increased as well as reduction inter-follicular space between hair follicles. Thickness of the epidermis and number of basal cells increases with gestational age. Several skin appendages were also visible after 3 months however their presence and abundance is dependent on the anatomical site. Presence of horny layer becomes apparent and basal cells increases gradually. A positive correlation was seen between

epidermal thickness and number of basal cell present. SEM micrographs showed a layer of ectodermal cells at early months of gestation however there is a gradual appearance of several layers and some clearly apparent epidermal appendages were seen.

**Embryonic, Fetal and Neonatal histology**

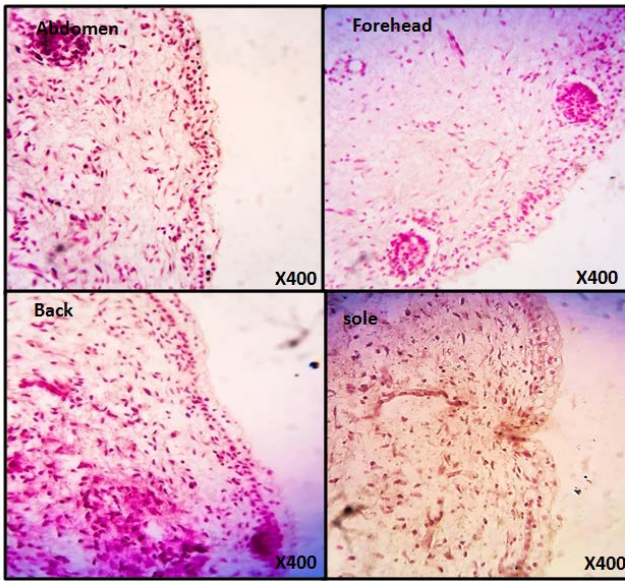


**Figure 1:** Histology of a 2 months old embryo (H&E). **Top left**: Tissue from an abdomen area showing 2 nucleated layers and a simple ectoderm. **Top right:** Tissue of a forehead area showing several nucleated layer representing a periderm. **Lower left:** A tissue from the back area also showing a simple periderm and a germinative layer. **Lower right**: A tissue from a feet sole showing 2-3 nucleated layers and a simple periderm.

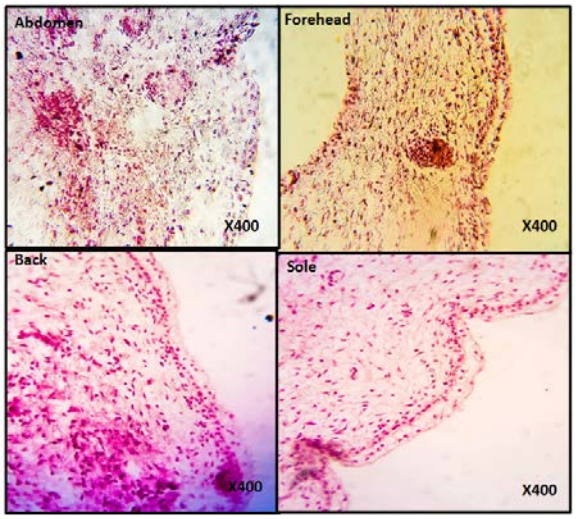


**Figure 2:** Histology of a 3 months old embryo (H&E). **Top left**: Tissue from an abdomen area showing a 3 nucleated layers and a solid ectoderm. **Top right:** Tissue of a feet sole area showing several nucleated layers representing an periderm and a germinative layer. **Lower left:** A tissue from the forehead area also showing a simple periderm and a germinative layer with vascular islands. **Lower right**: A tissue from the back area showing 2-3 nucleated layers and a simple periderm. General germinative (basal) cell repeated division is noticeable.

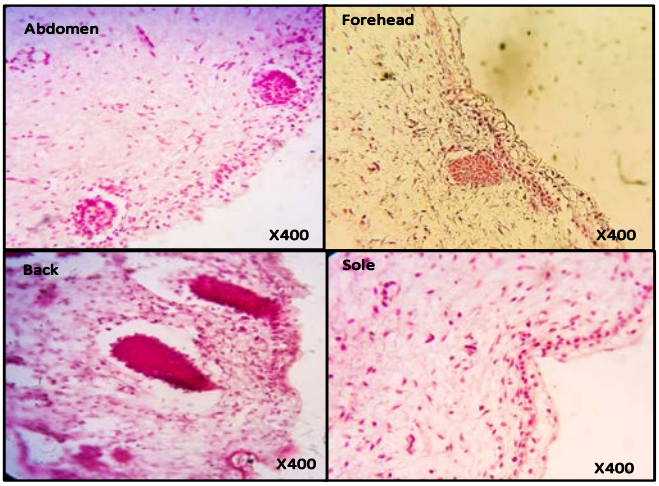


**Figure 3:** Histology of a 4 months old fetus (H&E). **Top left**: Tissue from an forehead area showing a 3-4 nucleated layers and a solid corneium present. **Top right**: Tissue of a feet sole area showing several nucleated layers representing a stratum germinativum. **Lower left**: A tissue from the abdomen area also showing a simple periderm and a germinative layer with vascular islands and what appears to be a cornieum. **Lower right**: A tissue from the back area showing 4-5 nucleated layers and a simple periderm with epidermal hair follicles . General germinative (basal) cell repeated division is noticeable with extension into the epidermal layer. General proliferation generates folds in basement membrane is seen in all tissues.

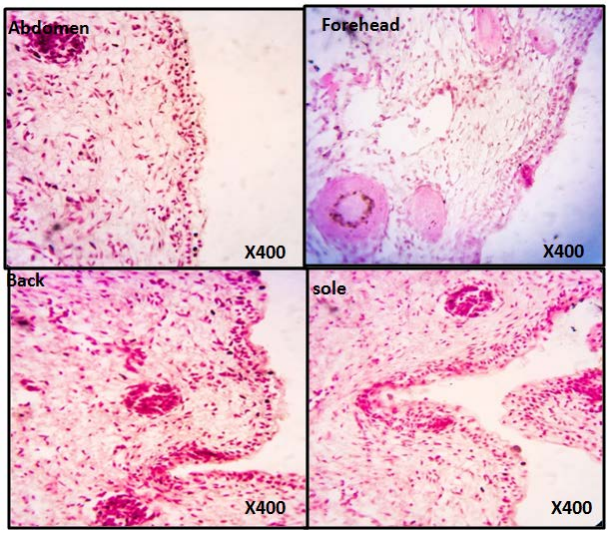


**Figure 4:** histology of a 5 months old fetus (H&E). **Top left**: tissue from a feet sole area showing 4-5 nucleated layers with a corneium present. **Top right**: Tissue of an abdomen area showing several nucleated layers representing a stratum germinativum and several hair pegs growth. **Lower left**: A tissue from the back area also showing a simple periderm and a germinative layer with vascular islands and what appears to be a cornieum. **Lower right**: A tissue from the forehead area showing 4-5 nucleated layers and a simple periderm with epidermal hair follicles . General germinative (basal) cell repeated division is noticeable. General proliferation generates folds in basement membrane is seen in all tissues.

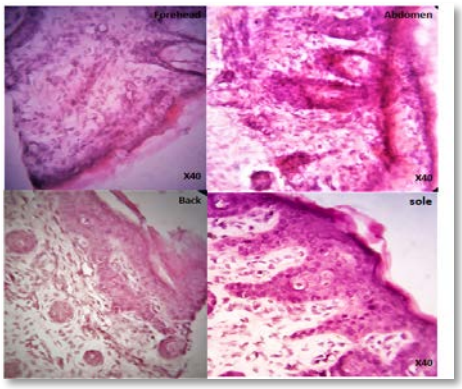


**Figure 5:** Histology of a 6 months old fetus (H&E). **Top left**: Tissue from a feet sole area showing 4-5 nucleated layers with a corneium present. **Top right**: Tissue of an abdomen area showing several nucleated layers representing a stratum germinativum and several hair pegs growth. **Lower left**: A tissue from the back area also showing a simple periderm and a germinative layer with vascular islands and what appears to be a cornieum. **Lower right**: A tissue from the forehead area showing 4-5 nucleated layers and a simple periderm with epidermal hair follicles . General germinative (basal) cell repeated division is noticeable. General proliferation generates folds in basement membrane is seen in all tissues.

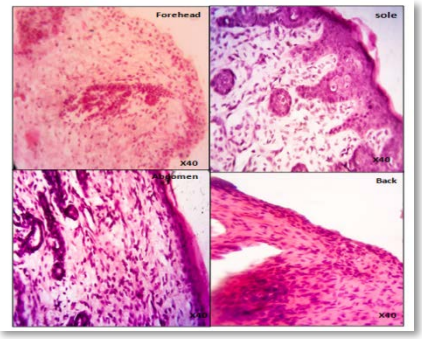


**Figure 6:** Histology of neonatal tissues (H&E). **Top left**: Tissue from the forehead area showing 6 or more nucleated layers with a horny layer present. **Top right**: Tissue of a feet sole area showing several nucleated layers representing a stratum basale and defined epidermal layers and what appears to be sweat glands. **Lower left**: A tissue from the abdomen area also showing a simple horny layer and a defined germinative layer. **Lower right**: A tissue from the back area showing a number of nucleated layers and a simple horny layer with epidermal hair follicles extending into the mesoderm. General germinative (basal) cell repeated division is noticeable. General proliferation generates folds in basement membrane is seen in all tissues.

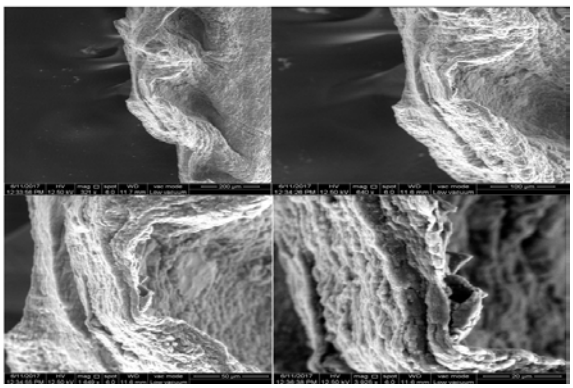

**Figure 7:** Electron micrograph of a 2 month embryo X 640. **Top left:** A micrograph of an abdomen area showing several simple layers of periderm present. **Top right :** A micrograph of forehead area with no defined epidermal features. **Bottom left:** A micrograph of a back area showing some simple layers of periderm. **Bottom right:** A micrograph of a feet sole showing some layers with gradual appearance of cornified cells.

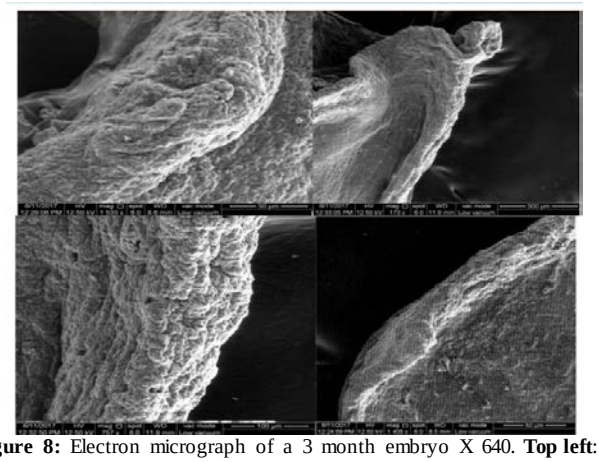

**Figure 8:** Electron micrograph of a 3 month embryo X 640. **Top left**: a micrograph of an abdomen area showing several layers of periderm present. **Top right :** a micrograph of forehead area with no defined epidermal features. **Bottom left:** A micrograph of a back area showing some layers of periderm. **Bottom right:** A micrograph of a feet sole showing some layers with rough surface cornified appearance.

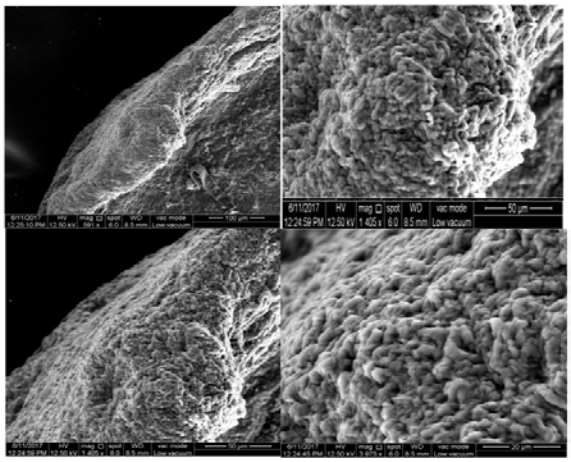

**Figure 9:** Electron micrograph of a 4 month fetus X 640. **Top left**: A micrograph of an abdomen area showing several layers of periderm present. **Top right :** A micrograph of forehead area with no defined epidermal features. **Bottom left:** A micrograph of a back area showing some layers of periderm. **Bottom right:** A micrograph of a feet sole showing some layers with rough surface.

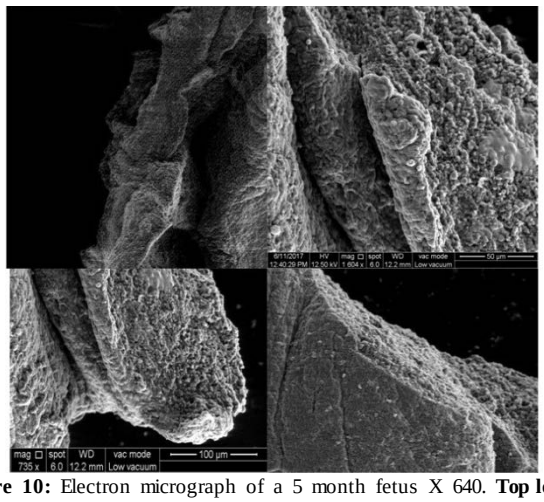

**Figure 10:** Electron micrograph of a 5 month fetus X 640. **Top left**: A micrograph of an abdomen area showing several layers of periderm present. **Top right :** A micrograph of forehead area with no defined epidermal features. **Bottom left:** A micrograph of a back area showing some layers of immature epidermis. **Bottom right:** A micrograph of a feet sole showing some epidermal layers with rough surface.

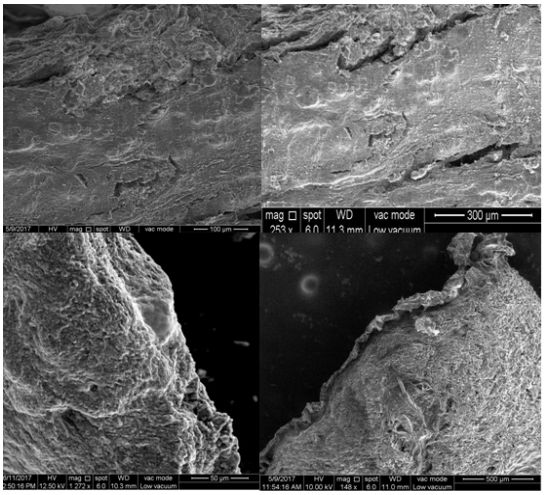

**Figure 11 :** Electron micrograph of a 6 month embryo X 640. **Top left:** a micrograph of an abdomen area showing several layers of epidermis present. **Top right :** A micrograph of forehead area with clear defined epidermal features such as basal layer and horny layer. **Bottom left:** A micrograph of a back area showing some layers of the epidermis. **Bottom right:** A micrograph of a feet sole showing some epidermal layers with rough surface.

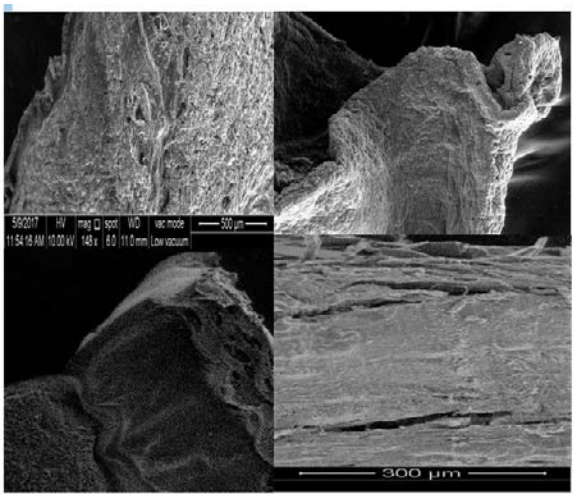


**Figure 12 :** Electron micrograph of a neonate X 640. **Top left** a micrograph of an abdomen area showing several layers of epidermis present. **Top right :** A micrograph of forehead area with no defined epidermal features. **Bottom left:** A micrograph of a back area showing some layers of the epidermis and a horny layer. **Bottom right:** A micrograph of a feet sole showing some layers with rough surface and epidermal layers with dermis transition.

**Histological and cytological measurements**

| Age | Anatomical site | Thickness of epidermis (µm) | Distance between hair follicles (mm) | Inter-follicular space (mm) | No of basal cells in 1 $mm^2$ | No of nucleated layers |
|---|---|---|---|---|---|---|
| 2 M | Abdomen | - | 1.22 ± 0.52 | 1.1 ± 0.13 | 149.8 ± 3.2 | 1.3 ± 0.98 |
| | Sole | - | - | - | 148.13 ± 7.6 | 1.6 ± 0.76 |
| | Forehead | - | 0.91 ± 0.31 | 0.97 ± 0.09 | 105 ± 5.1 | 1.5 ± 0.76 |
| | Back | - | - | - | 117.6 ± 5.1 | 1.7 ± 0.61 |
| 3 M | Abdomen | 1.76 ± 0.11 | 0.83 ± 0.21 | 0.8 ± 0.10 | 159.0 ± 4.2 | 2.3 ± 0.2 |
| | Sole | 2.3 ± 0.21 | - | - | 162.5 ± 7.5 | 3.7 ± 0.15 |
| | Forehead | 2.64 ± 0.27 | 0.91 ± 0.24 | 0.85 ± 0.11 | 127.7 ± 4.9 | 3.2 ± 0.14 |
| | Back | 2.1 ± 0.20 | 1.22 ± 0.51 | 1.1 ± 0.18 | 132.3 ± 2.1 | 3.6 ± 0.15 |
| 4 M | Abdomen | 2.2 ± 0.31 | 0.90 ± 0.31 | 0.90 ± 0.11 | 193.0 ± 4.1 | 3.3 ± 0.16 |
| | Sole | 2.8 ± 0.82 | - | - | 154.0 ± 2.5 | 3.5 ± 0.15 |
| | Forehead | 3.3 ± 1.20 | 0.92 ± 0.19 | 0.85 ± 0.09 | 140.4 ± 2.9 | 3.2 ± 0.17 |
| | Back | 3.0 ± 0.98 | 1.4 ± 0.58 | 1.1 ± 0.17 | 140.9 ± 1.7 | 3.5 ± 0.16 |
| 5 M | Abdomen | 3.4 ± 1.32 | 0.37 ± 0.09 | 0.35 ± 0.06 | 206.0 ± 7.2 | 3.7 ± 0.70 |
| | Sole | 3.5 ± 1.41 | - | - | 185.0 ± 6.5 | 3.8 ± 0.64 |
| | Forehead | 3.2 ± 1.29 | 0.27 ± 0.07 | 0.24 ± 0.07 | 156.0 ± 4.1 | 3.8 ± 0.67 |
| | Back | 3.7 ± 1.39 | 0.82 ± 0.12 | 0.27 ± 0.08 | 148.0 ± 1.9 | 3.6 ± 0.62 |
| 6 M | Abdomen | 5.3 ± 1.11 | 0.18 ± 0.16 | 0.21 ± 0.06 | 193.0 ± 7.7 | 3.8 ± 0.82 |
| | Sole | 4.7 ± 1.21 | - | - | 196.0 ± 6.9 | 3.8 ± 0.71 |
| | Forehead | 5.3 ± 1.42 | 0.14 ± 0.04 | 0.11 ± 0.03 | 222.0 ± 5.1 | 3.9 ± 0.72 |
| | Back | 4.8 ± 1.53 | 0.2 ± 0.13 | 0.13 ± 0.02 | 204.0± 5.3 | 3.9 ± 0.69 |
| Neonatal | Abdomen | 6.5 ± 3.31 | 0.21 ± 0.05 | 0.20 ± 0.04 | 294.0± 10.8 | 8.6 ± 0.30 |
| | Sole | 6.8 ± 3.15 | - | - | 277.0 ± 10.4 | 7.8 ± 0.14 |
| | Forehead | 6.3 ± 3.11 | 0.12 ± 0.07 | 0.12 ± 0.05 | 237.0 ± 7.1 | 8.6 ± 0.37 |
| | Back | 6.5 ± 3.24 | 0.21 ± 0.09 | 0.18 ± 0.02 | 326.0 ± 9.8 | 7.5 ± 0.22 |

**Table 1:** Several histological and cytological measurements taken from all anatomical sites for all ages using calibrated image j software. Epidermal thickness starts to appear at the age of 2 months of gestation and increases in thickness with time. Distance between hair follicles decreases with time as well as inter-follicular space. Number of basal cells starts as a series of single cell layers and then increases with time. Number of nucleated layers increases by one layer after every month of gestation however neonates had an increased number of nucleated layers compared to 6 months of gestation.

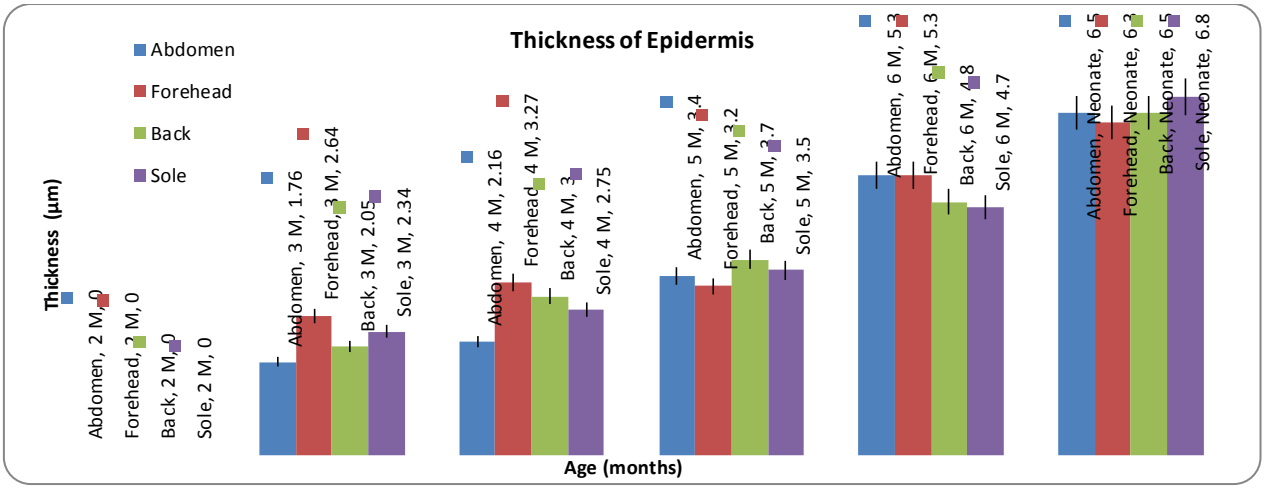


**Figure 13:** graphical comparison between embryonic, fetal and neonate epidermal thickness of all selected anatomical sites showing a gradual increase in epidermal thickness. Epidermal layers starts to appear at 3 months of gestation and increase at a constant rate during development. Statistical analysis shows significance $P < 0.05$ between all values at all ages of gestation and sites.

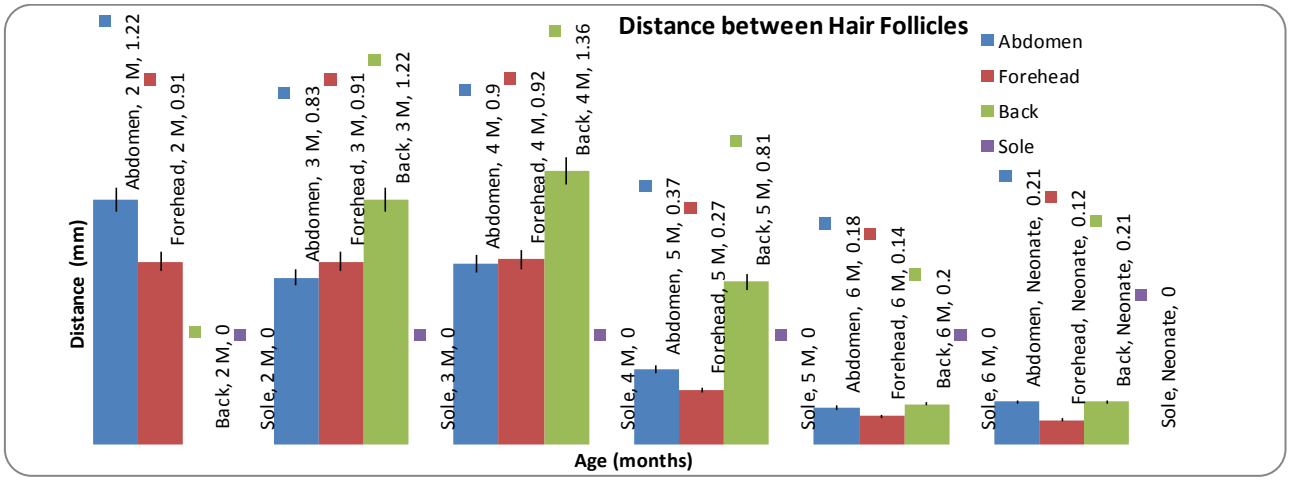


**Figure 14:** Graphical comparison between embryonic, fetal and neonate showing the distance between 2 hair follicles of all selected anatomical sites. An apparent decrease in distance between hair follicles which is expected with increase in gestational period. Sudden appearance of follicles is seen at 3 months in the back area. The distance between hair follicles at 3 months and 4 months increases for the back area which is not common in epidermal development. Statistical analysis shows significance $P < 0.05$ between all values at all ages of gestation and sites expect for 6 months and neonatal where there is no statistical significance.

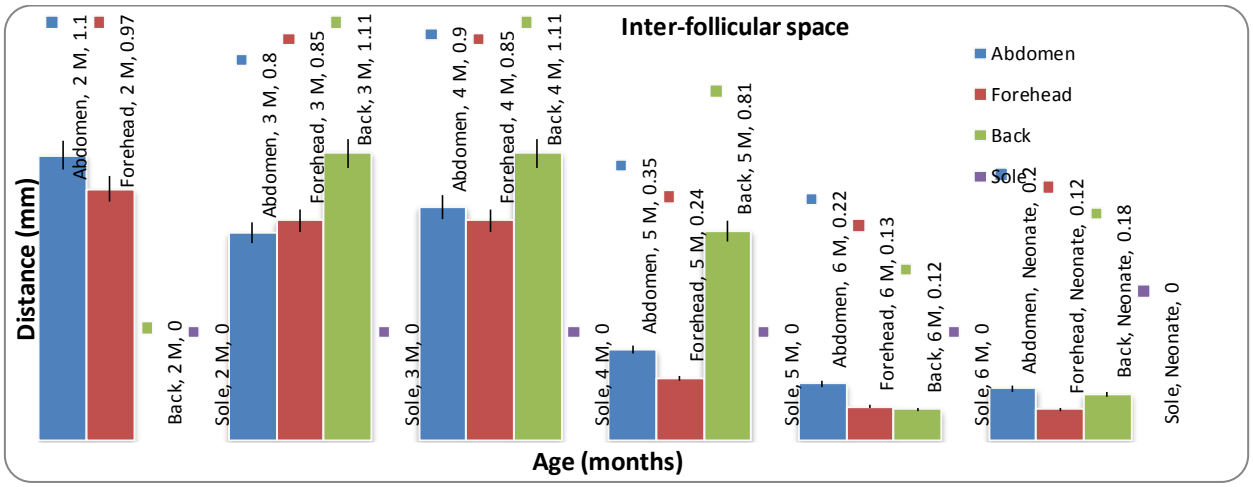


**Figure 15:** Graphical comparison between embryonic, fetal and neonate showing the inter-follicular space between two hair follicles of all selected anatomical sites. An apparent decrease in inter-follicular distance between hair follicles which is expected with an increase in gestational period. No difference in inter-follicular space is observed at 3 months and 4 months for the back area. No appearance of follicles is seen in the feet sole area at all ages. Statistical analysis shows significance $P < 0.05$ between all values at all ages of gestation and sites.

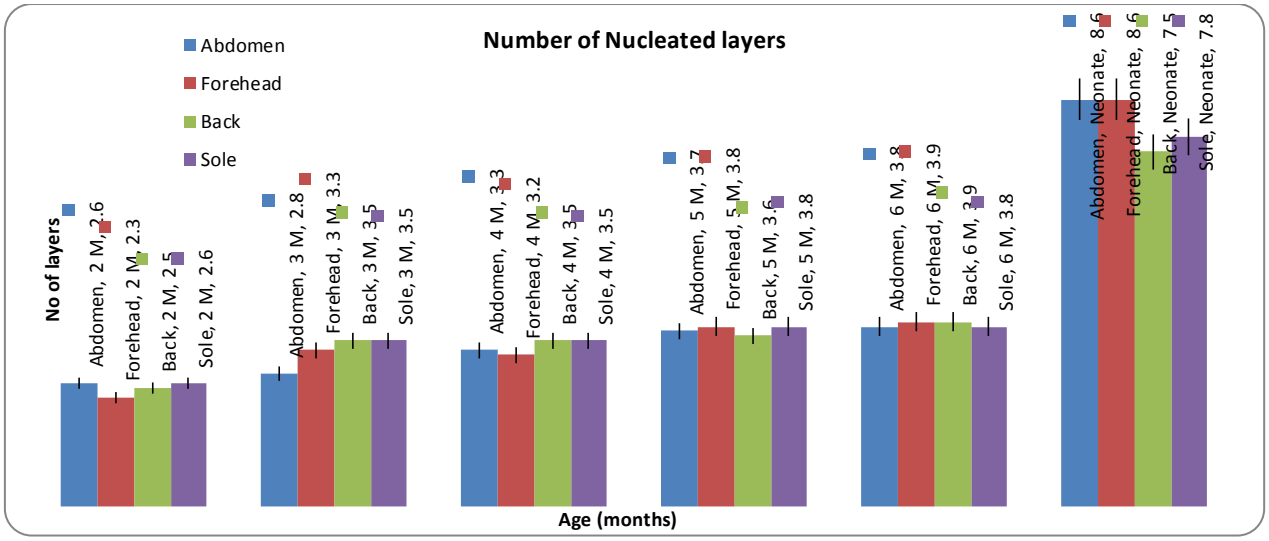


**Figure 16:** Graphical comparison between embryonic fetal and neonates showing the number of nucleated layers present in all ages and selected anatomical sites. An apparent increase in the number of nucleated layers in all ages and sites which is expected with an increase in gestational period due to the transition from a periderm to a mature epidermis. Statistical analysis shows significance $P < 0.05$ between all values at all ages of gestation and sites.

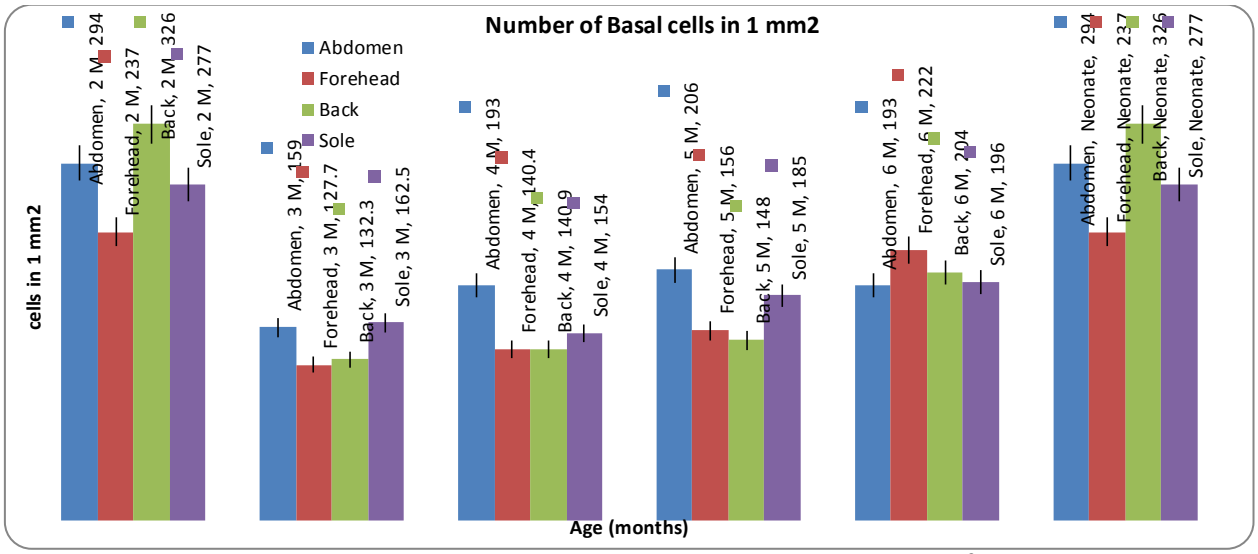


**Figure 17:** Graphical comparison between embryonic, fetal and neonate showing the Number of Basal cells in 1 $mm^2$ in all ages and selected anatomical sites. An overall increase in the number of nucleated layers in all ages and sites which is expected with an increase in gestational period due to the transition from a periderm to a mature epidermis and the formation of the basal layer. Statistical analysis shows significance $P < 0.05$ between all values at all ages of gestation and sites. No statistical significance is seen between 4 and 6 months for the abdomen area.

## DISCUSSION:

The epidermal thickness increases at the age of 3 months of gestation due to gradual formation of the epidermal layers. No epidermal layers or boundaries is seen at 2 months of gestation. The distance between hair follicles at 3 months and 4 months increases for the back area which is not common for integumentary system development. However it decreases in all other anatomical sites. Statistical analysis shows significance $P < 0.05$ between all values at all ages of gestation and sites expect for 6 months and neonatal where there is no statistical significance for the distance between hair follicles. An apparent decrease in inter-follicular distance between hair follicles which is expected with an increase in gestational period. No difference in inter-follicular space is observed at 3 months and 4 months for the back area. No appearance of follicles is seen in the feet sole area at all ages. An apparent increase in the number of nucleated layers in all ages and sites which is expected with an increase in gestational period due to the transition from a periderm to a mature epidermis. During fetal development the number of basal cells tends to increase in all sites at 2 moths. The back area tends to have the highest number of basal cells at 2 months however this decreases after 2 months of gestation but appears to increase in neonatal back.

## CONCLUSION:

This study shows in cytological and histological methods the presence of major changes that takes place during fetal, embryonic and neonatal integumentary system development. Certain histological measurements such as the number of basal cell present and number of nucleated layers vary depending on the anatomical site and age. This can support the scientific evidence that cytological and histological changes will vary depending on the activity of the anatomical site.

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