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**HISTOGENESIS OF THE CUTANEOUS ENCLOSURE  
OF THE AURICLE OF THE EXTERNAL EAR IN DOGS**  
(With One Table & 13 Figures)

By

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تنسج الغطاء الجلدي لصيوان الأذن في الكلاب

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تم دراسة التغييرات الهستولوجية التي تحدث في جلد صيوان الأذن في أجنة الكلاب التي تتراوح أطوالها ما بين ٧٠ - ٢٦٠ ملليمترًا . وقد تكونت البشرة في الأجنة التي تراوحت أطوالها ما بين ٧٠ - ٨٠ ملليمترًا من نسيج طلائي مركب يغطيه طبقة سطحية . وفي الأجنة التي بلغت أطوالها ١٥٠ ملليمترًا استبدلت الطبقة السطحية بطبقة قرنية . وقد بدأت بشائر الاغماذ الشعرية في جلد صيوان الأذن للأجنة التي تراوحت أطوالها ما بين ٧٠ - ٨٠ ملليمترًا . ثم زادت زيادة مضطردة بتقدم الحمل إلى أن تميزت إلى إغماذ أولية مركزية وإغماذ أولية وحشية في الأجنة التي تراوحت أطوالها ١٥٠ ملليمترًا . وقد بدأ تكوين الغدد الأنثيبية في جلد صيوان الأذن للأجنة التي بلغت أطوالها ١٥٠ ملليمترًا . بينما ظهرت بشائر الغدد الدهنية في الأجنة التي تراوحت أطوالها ١٧٠ ملليمترًا .

**SUMMARY**

The histomorphological changes occurring in the foetal skin covering the auricle of the external ear of dog (Egyptian-land race) ranging from 70 to 260 mm CVR length were studied.

In foetuses of 70-80 mm CVR length, the epidermis was composed of stratified epithelium covered by periderm. The periderm was replaced by layers of stratum corneum in foetuses of 150 mm CVR length.

The analgen of hair follicles appeared in foetuses of 70-80 mm CVR length. These follicles increased in size and depth with the advancement of age. In foetuses of 150 mm CVR length, the hair follicles could be differentiated into primary central follicles and primary lateral follicles. The primordium of the tubular glands could be also seen at this stage. However, the primordia of sebaceous glands were demonstrated in foetuses of 170 mm CVR length. At 220-260 mm CVR length, the tubular glands were differentiated into a duct and an end-piece. The sebaceous glands were designed into a collection of cells on one or both sides of the central primary follicles and showed signs of activity.

## INTRODUCTION

Functionally, skin is a most extraordinary organ. It is of greatest importance in preventing the organism from desiccation, protecting it from the environmental stresses and regulating the temperature of the body (MONTAGNA and PARAKKAL, 1974).

Although the cutaneous structures of the dog had been studied by several authors (WEBB and CALHOUN, 1954; LOVELL, 1955; LOVELL and GETTY, 1957; GREED, 1958; BAKER, 1966; SCHWARZ *et al.* (1979) and MOUSTAFA (1986), data on the prenatal development of the skin covering the auricle of the external ear could not be found in the available literature.

Therefore, the aim of the present work was to study the prenatal development of the epidermis, dermis, hair follicles and skin glands of the auricle of the external ear in the dog.

## MATERIAL and METHODS

The material employed in the present study originated from the external ears of dog fetuses obtained from bitches (Egyptian land race) sacrificed at various periods of gestation. The fetuses were removed shortly after evisceration, and the crown-to-rump (CVR) length was measured to the nearest millimeter (Table 1).

Table (1): Material available for study

No. of fetuses	2	3	2	1	1	2	2	1	1	2	1	1	2	3
CVR(mm)	70	80	130	130	150	155	170	180	180	200	220	220	240	260
Sex	♂	♀	♀	♀	♀	♀	♀	♂	♂	♀	♀	♀	♀	♀

The auricles of the external ears were removed from the fetuses and fixed in 10% neutral buffer formalin and Bouin's fluid. After proper fixation, the material was dehydrated, cleared and embedded in paraffin wax. Serial vertical and horizontal sections were cut at about 7  $\mu$ m and stained with Haematoxylin and Eosin, van Gieson's stain, Crossman's trichrome and PAS technique (DRURY and WALLINGTON, 1980).

## RESULTS

In fetuses of 70-80 mm CVR length, the epidermis of the auricle of the external ear ranged from 54 to 67.5  $\mu$ m in thickness on both the lateral and medial surfaces. It consisted of 7-8 cell layers (Fig. 1). The basal layer was composed of a single row of columnar cells which had acidophilic cytoplasm, large oval vesicular nuclei, distinct cell boundaries and rested on a distinct PAS-positive basal lamina. Mitotic

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division could be observed among these cells (Fig. 2). Few pigment cells were demonstrated between the cells of this layer. The middle layer was consisted of 5-6 polyhedral cell layers. These cells showed clearly defined outlines, less acidophilic cytoplasm and rounded, vesicular, centrally or eccentrically located nuclei. The epidermis was covered by the periderm which was represented by a single layer of flattened cells with rod-shaped, deeply-stained nuclei. PAS-positive materials were clearly demonstrable at the boundaries of the cells of the middle layer. These materials increased in amount towards the upper cell layers.

The dermis could be distinguished into 2 zones; an outer narrow cellular zone and an inner wide zone, which demonstrated few fine collagenous fibers, blood capillaries, wide-lumened blood vessels and various widely distributed connective tissue cells.

The primitive stages of the hair follicles were represented as several localized thickening of the basal layer at various intervals which bulged beyond the deep surface of the epidermis (Fig. 3). They were surrounded by a layer of fibroblasts constituting the primitive hair germ. The rate of development of the hair follicles varied within both surfaces of the auricle. They were more developed at the lateral surface than the medial surface (Fig. 3 & 4).

Towards 130 mm CVR length, the epidermis covering the skin at the medial surface was obviously increased in thickness (108 to 122  $\mu$ m) than at the previous stage. It was differentiated into four layers namely; stratum basale, stratum spinosum, stratum granulosum and periderm (Fig. 5). The stratum basale was composed of cuboidal cells with large oval deeply-stained nuclei. The stratum spinosum consisted of 3-4 polyhedral cell layers at the lateral and 5-7 cell layers at the medial surface of the auricle. The cells showed vacuolated cytoplasm and large rounded, vesicular, centrally or peripherally located nuclei. The stratum granulosum was represented by a single layer of spindle-shaped cells with large oval nuclei. The cytoplasm was relatively paler and contained few amounts of small-sized basophilic granules. The periderm was formed of 1 or 2 cell layers with peripheral acidophilic cytoplasm and flattened deeply-stained nuclei.

The dermis was consisted of fine collagenous fibers and several fibroblasts. The fibrillar elements as well as the fibroblasts were distributed into 2 unequal layers. They were disposed parallel to the epidermis in the outer third of the dermis, while within the deeper two thirds they were found to be relatively fewer and unevenly distributed (Fig. 6).

The primordia of hair follicles increased substantially in amount and were represented by follicle plugs (Fig. 6). The plugs grew obliquely within the dermis with different rates. The cells of the follicle plug were arranged either regularly around the periphery, where they were continued with the stratum basale, or scattered irregularly within the center of the plug.

On reaching 150 mm CVR length, the thickness of the epidermis ranged from 50 to 67.5  $\mu$ m at the lateral surface and 81 to 108  $\mu$ m at the medial surface of

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the auricle. The stratum basale showed numerous pigment cells which were more developed at the lateral surface of the auricle (Fig. 7). The amount of vacuolated cells of the stratum spinosum was obviously fewer than those of the previous age. The stratum granulosum was formed of 2 layers of spindle-shaped cells which showed numerous basophilic granules of different sizes (Fig. 8). The periderm was replaced by 2-3 layers of anucleated flattened cells. These cells possessed highly acidophilic cytoplasm constituting the primary elements of stratum corneum.

The outer configuration of the epidermis was corrugated due to the exits of the hairs at the hair pits.

The changes which occurred into the dermis included a relative increase in the thickness of collagenous fibers. Numerous of these fibers arranged themselves in the dermohypodermal junction and were disposed parallel to the auricular cartilage.

The rate of development and density of the hair follicles varied at both surfaces of the auricle of the external ear. They were more developed and abundant at the lateral surface than at the medial surface. The follicles were distinguished into two varieties namely a large and a small type. The largest follicles formed the elements of the central primary follicles and the smallest follicles formed the elements of the lateral primary follicles. Several of the largest follicles contained well-differentiated keratinized hairs and an outer and an inner root sheath. Some of the largest follicles extended to the deep dermal level and showed hair papilla and hair matrix. Several pigment cells were scattered at the hair matrix (Fig. 7).

The primordia of tubular glands were demonstrated as cord-like 2 to 3 cells-thick structure which extended from the lower side of the primary central follicles (Fig. 9).

In fetuses of 170 to 200 mm CVR length, the epidermis of the skin covering the medial surface of the auricle decreased slightly in thickness (77 to 101  $\mu$ m) in fetuses of 170 mm CVR length and reached 68 to 95  $\mu$ m in 200 mm CVR length fetuses. The stratum basale was represented by one layer of cuboidal cells with large oval or rounded dark nucleus. The stratum spinosum was composed of 3-5 layers of polyhedral cells. The cells showed acidophilic cytoplasm and large oval or rounded vesicular nuclei, with distinct nucleoli. The amount of the basophilic granules in the cells of stratum granulosum were relatively more than at the previous age. The stratum corneum increased in thickness specially at the lateral surface of the auricle (Fig. 10).

The central primary follicles exhibited a relatively more developed inner root sheath and a hair papilla. Numerous lateral primary follicles were increased both in depth and size where they demonstrated a hair bulb, outer root sheath and the primary elements of hair. The pigment cells increased within the hair bulb more than at the previous age. The lateral primary follicles were relatively small in size and less developed at the medial than at the lateral surface (Fig. 11).

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The primary elements of the sebaceous glands appeared on the ental side of the central primary follicle below the level of the tubular gland buds. They were represented by 1 to 3 polyhedral cells with large, rounded, vesicular, centrally-located nuclei, distinct nucleoli and pale acidophilic cytoplasm (Fig. 10). These buds were invested with one layer of flattened cells derived from the outer root sheath.

In foetuses of 220 to 260 mm CVR length, the epidermis slightly decreased in thickness and reached 40 to 54  $\mu\text{m}$  at the lateral and 61 to 77  $\mu\text{m}$  at the medial surface of the auricle. The stratum basale was composed of one layer of cuboidal cells with acidophilic cytoplasm and large oval vesicular nuclei. The stratum spinosum was consisted of 2-3 polyhedral cell layers. The cells had acidophilic cytoplasm and large oval vesicular nuclei. Several cells of both stratum basale and stratum spinosum were large in size with vacuolated cytoplasm and pyknotic, eccentric, crescent-shaped nuclei. The stratum granulosum was consisted of 2 layers of oval cells containing numerous basophilic granules. These granules were relatively more abundant than at the previous age. The stratum corneum increased in thickness.

The PAS - positive materials were relatively fewer, within the lamina epithelialis, than during the earlier stages of foetal development.

The cellular elements of the dermis decreased relatively in density than at the previous age. The collagenous bundles became obviously thicker and densely arranged within the outer portion of the dermis. These fibrillar elements were more thicker at the medial than at the lateral surface of the auricle.

Some of the lateral primary hair follicles exhibited the primary elements of inner root sheath.

The primordia of the tubular glands of the primary central follicles increased in size and depth and were differentiated into a duct and an end piece (Fig. 12). The primordia of the tubular glands of the lateral follicles were represented by oval outgrowths which bulged beyond the hair follicles and consisted of several basophilic cells with large deeply-stained nuclei. The sebaceous glands increased relatively in size and were represented by a collection of about 5-15 large polyhedral cells which originated from one or both sides of the central primary follicles (Fig. 13). The cytoplasm was foamy and their nuclei were large, rounded and vesicular. The sebaceous glands were, more developed at the lateral than at the medial surface of the auricle.

## DISCUSSION

The present study revealed that the epidermis of auricle of the external ear of 70-80 mm CVR length dog foetuses was composed of stratified epithelium covered by periderm. The periderm not only acts as a protective covering for the developing epidermis but also participates in the exchange of material between the foetus and amniotic fluid (HOYES, 1968 and WOLF, 1967). The periderm was replaced by cells of stratum corneum in dog foetuses of 150 mm CVR length, which increased obviously in full term foetuses. This may constitute a device to withstand the watery nature

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of the surrounding fluid (MULLER and KIRK, 1976). The stratum corneum retards water loss from the inner hydrated layers and prevents the entrance of most toxic agents. It may also protect the animal against the environmental damage and maintains the internal milieu (ROTHBERG *et al.*, 1961).

The present investigation demonstrated that the cells of the stratum basale showed several mitotic activities which decreased with the advancement of age. Like haematopoietic tissue and intestinal mucosa, the epidermis belongs to a class of tissue whose cell production is commensurate with the constant loss (MONTAGNA and PARRAKAL, 1974).

The epidermis in dog foetuses of 70-80 mm CVR length demonstrated PAS-positive materials which decreased in amount as development progressed. Similar findings were observed in the epithelium of the stomach of goat foetuses (KAMEL *et al.*, 1987). The latter authors suggested that, the accumulation of such reactive substances might be required for energy production as well as significant barrier to gasteric mucosa.

The chronological events concerning the epidermal thickness, demonstrated by the present study, showed that the epidermis of the auricle of the external ear ranged from 54 to 67.5  $\mu\text{m}$  in both the medial and lateral surfaces at 70-80 mm CVR length dog foetues. The epidermis then increased in thickness till it reached 108-122 at the medial surface of the auricles of 130 mm CVR long faetues. Towards the end of gestation the epidermis decreased in thickness where it reached 61 - 77  $\mu\text{m}$  at the medial surface and 40 to 54  $\mu\text{m}$  at the lateral surface in full-term foetues. The variations of the epidermal thickness, observed during the intrauterine life in both the medial and the lateral surfaces covering the auricles, could be attributed to the rate of development and density of hair coat and associated skin glands in both surfaces.

As revealed from this investigation the dermis could be distinguished into 2 zones; an outer narrow cellular zone and an inner wide zone in foetues of 70-80mm CVR length. The fibrillar elements as well as the fibroblasts distributed into 2 unequal layers in foetues of 130 mm CVR length, which increased in thickness and number as development progressed. The dermal tissue plays a major role in protection and acts as a barrier to infection. Another important function of the dermis is its role in inductive interaction with the epidermis as well as ion exchange, water binding and fibrillogenesis (MONTAGNA and PARAKKAL, 1974).

The present study revealed that, the rate of development and density of the hair follicles varied at both surfaces of the auricle of the external ear during the different stages of prenatal life. They were more developed and abundant at the lateral surface than at the medial surface. Similar results were obtained by BREAZILE (1976) in domestic animals. MARGOLENA (1959) suggested that, the appearance and differentiation of follicles were dependent on the physiologic and histologic ability of the skin to initiate and support such development and growth. The role

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played by the skin in the regulation of body temperature was attributed to the hair coat as well as the cutaneous vasculature (MULLER and KIRK, 1976).

The primordium of the sebaceous glands could be demonstrated in the present study in 170 mm CVR length dog foetuses and designed into a collection of cells on one or both sides of the central primary follicles which showed signs of activity at 220 mm CVR. Similar findings were observed by FERNANDO (1965) and MULLER and KIRK (1976). The latter authors stated that, these glands began to demonstrate their activity shortly before birth, then reached their final size at a later stage. The sebum secreted by the foetal sebaceous glands smears the foetal skin and prevents chafing injuries from the amnion as the growing foetus becomes progressively confined in its fluid-filled sac (EL-SAKHAWY, 1973). Moreover, the oily secretion produced by sebaceous glands tends to keep the skin soft and pliable by forming a surface emulsion which spreads over the surface of the horny layer to retain moisture and thus maintains proper hydration (MULLER and KIRK, 1976).

Contrary to what was stated by BACKMUND (1904) and FERNANDO (1965) that, the final development and differentiation of the tubular glands occurred postnatally, the present study revealed that, the tubular glands of the auricle were differentiated into a duct and an end-piece at full term foetuses (220-260 mm CVR length).

In conclusion, a complete study of the developmental changes occurring within the skin covering the auricle of the external ear in dogs must involve further investigation both in the early postnatal life and throughout the prepubertal age.

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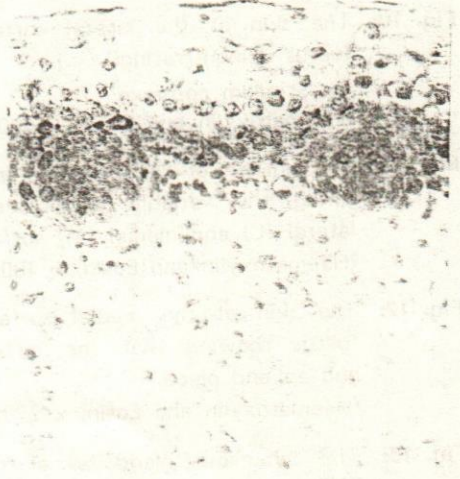
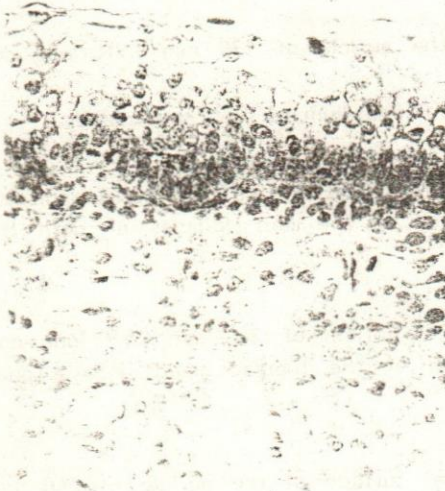
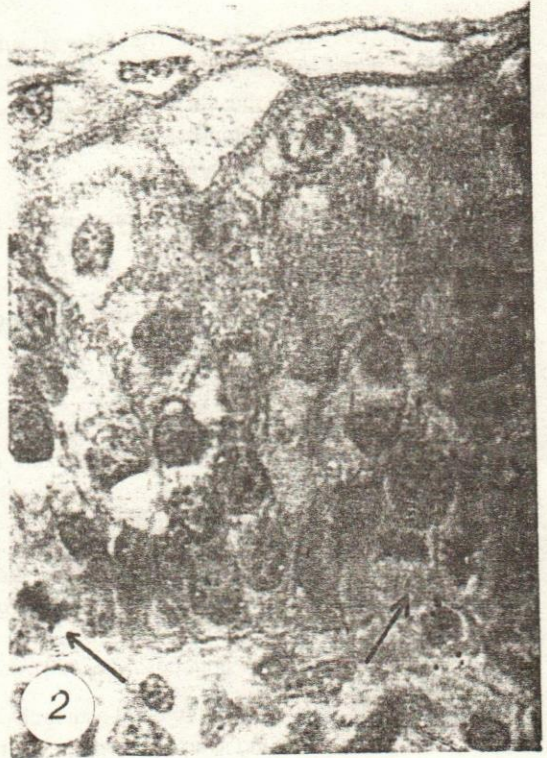
### LEGEND OF FIGURES

- Fig. 1:** The auricle of the external ear of a dog foetus of 70 mm CVR length showing:  
 E<sub>1</sub> and E<sub>2</sub>: Epidermis at the medial and lateral surface respectively.  
 D : Dermis. C: Primordium of the auricular cartilae.  
 (Haematoxylin and Eosin, x 250).
- Fig. 2:** Epidermis of the auricle of a dog foetus of 70 mm CVR length showing several mitotic division at the basal layer (arrows).  
 Haematoxylin and Eosin, x 1000).
- Fig. 3,4:** Skin covering the auricle of the external ear of a dog foetus of 70 mm CVR length at the medial (3) and lateral (4) surfaces showing the primordium of hair follicles.  
 (Haematoxylin and Eosin, x 400).



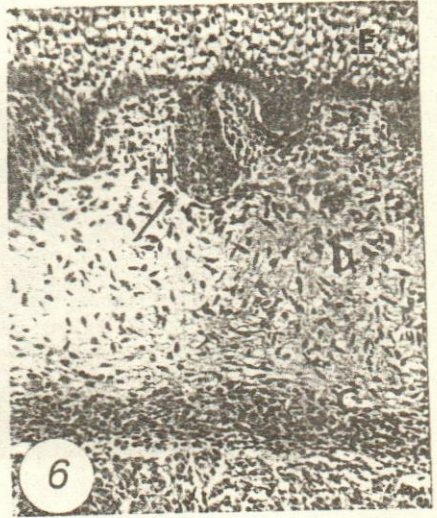
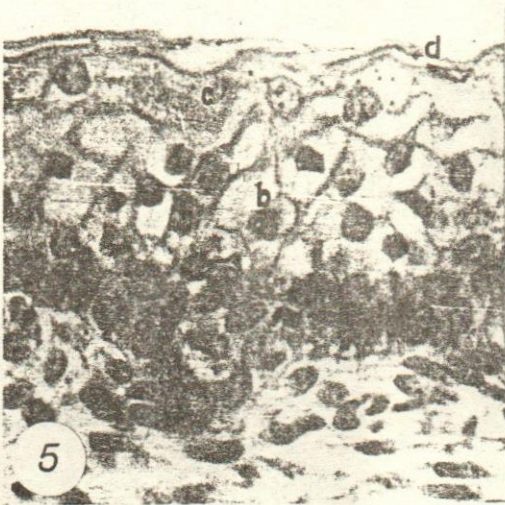


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