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The Development of the Integumentary System in Dogs

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Abstract

The skin and its appendages are components of the integumentary system. Particularly in dogs, the appendages are: the hair, claws, footpads, and sebaceous, sweat and mammary glands. Few studies report the initial development of these structures in domestic species. The present study aimed to describe the development of the integumentary system during the embryonic and fetal periods in dogs *(Canis familiaris)*. Totally, 9 embryos and 31 fetuses were used for gross and microscopic descriptions. Macroscopically, the skin of concepts in embryonic ages had a translucent aspect, which allowed for the visualization of internal organs with a remarkable presence of blood vessels. The skin appendages were only identified in fetuses. Microscopically, in the embryonic period, the epidermis consisted of a single surface layer of flattened cells denoted the germinativum stratum or germinal layer. Later, a new layer form due the proliferation of keratinocytes to form the periderm. When the epidermis of the fetus was observed. There was more than one cell layer that developed between the germinal and periderm layers. In conclusion, the skin shows little development during the embryonic period, whereas its division into the dermis and epidermis and the formation of several cell layers is pronounced in the fetal period. Similarly, skin appendages developed during the fetal period.

Keywords: Embryo; Fetus; Development; Integumentary; Dogs

Introduction

The integumentary system consists of the skin, hair, corneous appendages and associated glands. This system performs many functions that are important to maintain the homeostasis of the body of an animal. The integumentary system protects the body against external factors from the environment, fluid loss and the entry of harmful substances and invading microorganisms. Additionally, the skin is responsible for regulating body temperature through the sweat glands and blood vessels, and the superficial nerves and their sensory endings allow for sensitivity [1]. For dogs in particular, hair is an important characteristic to determine different species, in addition to skin protection and body temperature regulation (because this species does not have sweat glands, except in the footpads). Finally, for veterinary medicine, skin diseases related to the integument are often observed in clinical practice.

According to Getty [2] and Dyce et al. [3], the skin and its appendages are components of the integumentary system. Particularly in dogs, the appendages are the hair, claws, footpads, and sebaceous, sweat and mammary glands.

Few studies report the initial formation of these structures in domestic species. Süsskind-Schwendi et al. [4] reported the formation of claws in dogs. The skin and its appendages were studied in pigs by Meyer and Görgen [5], by Bragulla [6] in horses, by Bragulla et al. [7] in cats and by Sengel [8] in mice. In general, this system has also been studied in mammals by Hardy [9] and in other vertebrates by Oliveira-Martinez et al. [10], who have focused on the molecular events that surround tissue differentiation.

In dogs, the gestation period is approximately 61 days and is divided into three periods: the egg period (from the 2nd to 17th day), the embryonic period (between the 19th and 35th day) and the fetal period (from the 35th day to birth) [11]. The origins of the systems, including the integumentary system, are formed in the embryonic period. In dogs, development is peculiar because the embryonic period extends for more than half of the gestation, thus differing from most mammalian species, which have a longer fetal period compared to embryonic period [12,13].

It is important to study the development of the integumentary system because its structure can be considered a strong morphological evidence for the classification of animals within phylogenetic systematics, such as the difference between nails, hooves and claws [4]. Although the embryonic and fetal development of dogs have been previously reported [11,14], many aspects related to the embryology and organogenesis of this species remain scarce in the literature. Additionally, studies related to the organogenesis of the integumentary system in dogs can contribute to an understanding of the pathologies related to changes in this system, particularly for diagnosis and treatment. Furthermore, these animals have been used as experimental models for different diseases, including as a homologous model to human [15].

In this regard, studies that contribute to the clarification of dog developmental biology are important. Thus, the present study aimed to describe the development of the integumentary system during the embryonic and fetal periods in dogs (*Canis familiaris*) using macroscopic descriptions and light microscopy.

Materials and Methods

Sample collection

Altogether, 9 embryos and 31 fetuses were used. The samples were obtained from pregnant uteri in spaying campaigns conducted in the

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neighborhood of Rio Pequeno in the city of São Paulo, SP, Brazil. To collect the embryos, the uteri were washed with saline, and to remove the fetuses, an incision was performed in the uterine body until the fetus was exposed and the extra-embryonic membranes and placenta were released.

Macroscopic analysis

Initially, the embryos and fetuses were measured with a digital caliper (MITUTOYO® model 500-144B) with divisions in millimeters to obtain the crown-rump length (CRL). This measurement is the distance from the nuchal crest to the final sacral vertebra, according to the methodology proposed by Evans and Sack [16].

The embryos and fetuses were then weighed on a precision scale (MARTE AL 500), and the external characteristics were photo-documented with a digital camera (SONY Cybershot 10.1 MP) for further description.

Because the dog breed was undefined, the gestational age in days was estimated by the CRL and external morphological characteristics [12,13,16] (Table 1).

The nomenclature used was established by the International Committee on Veterinary Gross Anatomical Nomenclature [17], International Committee on Veterinary Histological Nomenclature [18] and International Nomenclature Committee on Veterinary Embryological Nomenclature [19].

Light microscopy

For the microscopic analysis, the entire embryos were processed and included, and longitudinal cuts were obtained. During processing of the fetuses, skin fragments were collected in different regions: the dorsal cervical, dorsal lumbar, ventral abdominal and head regions. To demonstrate the formation of skin appendages, the distal portion of the fore- and hindlimbs were separately collected to show the claws and digital pads. Skin fragments were collected from the abdominal region to show the mammary glands, and the nasal planum was collected to demonstrate the tactile hair follicles.

The embryos and tissue fragments collected from the regions of interest were fixed in 10% formalin and dehydrated in an increasing alcohol series (70, 80, 90 and 100%). The fragments were then diaphanized in xylene and embedded in paraffin. After inclusion, the blocks were cut using an automatic microtome (LEICA, RM2165) to obtain 5-µm thick serial sections, which were stained with hematoxylin and eosin [20].

Results

The embryos and fetuses used in this study had the following gestational ages according to the data obtained from the CRL measurements (Table 1): 19, 22, 27, 32, 40 and 44 days.

Macroscopic characteristics

Macroscopically, the skin had a translucent aspect in the fetuses of an embryonic age (19-32 days), which allowed for the visualization of internal organs with a remarkable presence of blood vessels (Figures 1A and 1B). In the fetuses, the skin was less translucent because of increased pigmentation, but the superficial blood vessels remained visible. The skin appendages were only identified in fetuses of fetal age (40-44 days); during the embryonic period, no skin appendages were identified in the macroscopic analysis. In the 44-day-old fetuses, structures such as hair follicles (Figure 1E), mammary glands (Figure 1F), footpads (Figure 1G) and claws (Figures 1C and D) were undergoing formation.

Microscopic descriptions

The microscopic analysis distinguished the different stages of skin formation in the embryos and fetuses. The skin is the largest organ of the body. Initially (at 27 days of gestation) in stage 1, the epidermis consisted of a single surface layer of flattened cells denoted the germinativum stratum or germinal layer (Figures 2A and 2B). A new layer then formed after the proliferation of keratinocytes to form the periderm in stage 2 (Figure 2C). This stage of epidermal development was observed in ages of the embryonic period. These two initial phases were observed in embryos of identical ages, thus indicating that the skin has different stages of development according to the evaluated region of the body. When the epidermis of the 44-day-old fetus was observed, there was more than one cell layer that developed between the germinal and periderm layers, which was denoted an intermediate layer in stage 3 (Figure 2D). In all samples analyzed, the germinal layer was on a single layer of mesenchyme derived from mesoderm.

The initial formation of hair follicles could be observed in the 44-day-old fetus. These follicles consisted of epidermal cells that underwent an invagination process and were located between the mesenchyme of the dermis (Figure 2D).

Gestacional Period	Estimated age (days)	Number of samples	Crown-rump (mean)
Embryonic	19	1	6 mm
	22	2	10 mm
	27	4	16 mm ± 1.41
	32	2	23 mm ± 1.41
Fetal	40	8	49.62 mm ± 2.50
	44	23	68.30 mm ± 4.64

Table 1: Data collected from embryos and fetuses of dogs without breeds defined.

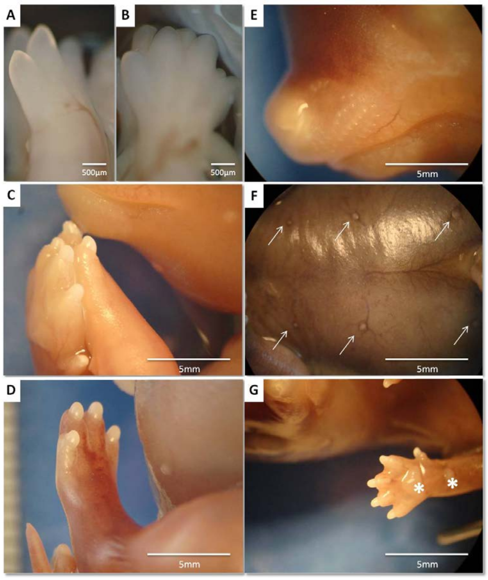

Figure 1: Photos of a dog embryo and fetus showing the formation of skin appendages. The fore- and hind limb of a 32-day-old embryo are shown in A and B, respectively, with digits but no claws. The formation of claws in the fore- and hind limbs of a 44-day-old fetus are shown in C and D, respectively. E, F and G show follicles of tactile hair, mammary glands (arrows) and footpads of the forelimb (asterisks), respectively.

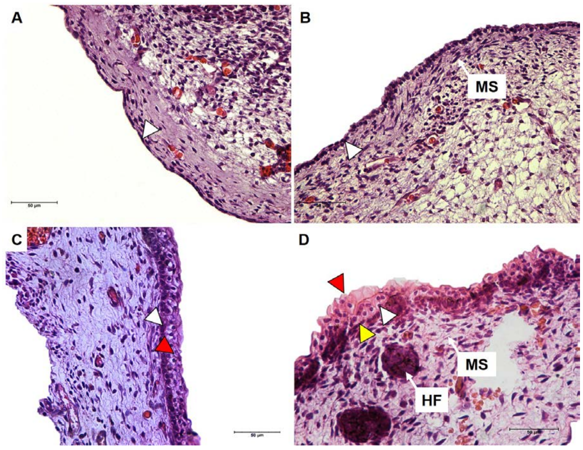

Figure 2: Skin micrographs of a canine embryo at 27 (A and B) and 32 days (C) and a 44-day-old fetus (D) showing the developmental stages of the skin. The white arrow shows the basal layer, the yellow arrow shows the intermediate layer, and the red arrow indicates the periderm. The subjacent mesenchyme (MS) is shown in both ages, and the hair follicle (HF) is only observed in the fetus. Hematoxylin and eosin (HE) staining, 40x magnification.

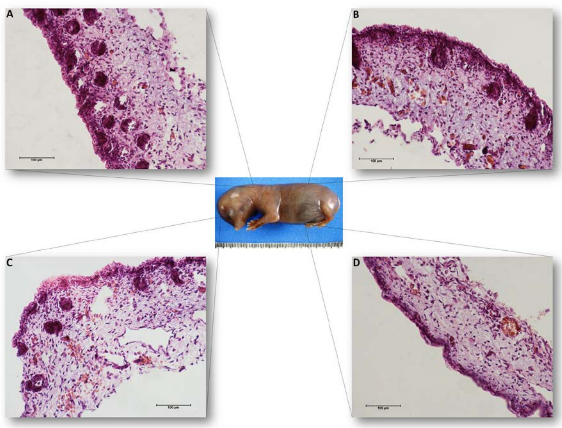

Figure 3: An illustrative scheme of the skin micrographs in different body regions of the 44-day-old canine fetus. (A) the dorsal cervical region; (B) the dorsal lumbar region; (C) the head; and (D) the ventral abdominal region.

At this age, when the skin of different body regions was evaluated, hair follicles occurred in different amounts. In a comparative analysis of the dorsal (cervical and lumbar) and ventral regions and the head, the dorsal region showed a greater number of follicles compared to the head, and the ventral region presented no follicles (Figure 3).

Both macro- and microscopically, all skin appendages were only observed in conceptuses of fetal age (from 40 days of gestation). In these conceptuses, the formation of claws and digital, metacarpal and metatarsal pads, on both the fore- and hindlimbs, could be observed. The mammary glands were present in the abdominal region. The hair follicles were distributed throughout the body, and the formation of tactile hair follicles was observed, particularly in the nasal planum.

The development of tactile hair follicles in all of phases could be observed through the histology of the nasal planum. Hair formation begins with the proliferation of the basal layer, which results in the hair bud (Figure 4A). The hair bulb is formed with the extension of the bud toward the adjacent mesenchyme (Figure 4B). In the third stage, the hair bulb becomes elongated and its deepest portion undergoes invagination of mesenchymal cells, which then constituted the dermal papilla (Figure 4C). After the dermal papillae were formed, a greater presence of mesenchymal cells was observed in association with the epidermal cells from the hair follicles (Figure 4D). The hair shaft formation from epidermal cells was then observed, and an extensive proliferation of mesenchymal cells occurred around the hair follicle (Figures 4E and 4F). It is noteworthy that the follicles did not synchronously develop, and different stages of follicular development could be observed in this region. The formation of sebaceous glands associated with the hair follicles was not observed in this developmental phase.

In the histological analysis of the distal portion of the limbs, the formation of claws and digital pads could be observed (Figure 5A). The footpad was characterized by a strong presence of mesenchymal cells externally bound by a layer of epidermal cells. The dermis had an intimate association with the epidermis and a larger number of cells and some cellular matrix accumulated. In this region, the number of blood vessels was much higher when compared to other regions of the limb (Figure 5C). The identical arrangement pattern of the mesenchymal cells was observed in the formation of the claws. There was a greater accumulation of mesenchymal cells in the contact area between the basal layer of the epidermis and dermis cells, thus forming the papillary body. In the epidermis, a basal layer was forming, and it was thicker compared to other regions of the body. A similar event occurs with the intermediate and surface layers because of the evident keratinization process that occurs in these cells (Figure 5B). The formation of the mammary glands was also identified by the microscopic analysis. The proliferation of the epidermis area, generating the breast bud (Figure 6A), was the initial sign. The mammary papilla originated as the bud grew toward the mesenchyme, and it was initially only composed of epithelial cells (Figure 6B).

Discussion

The integumentary system is one of the first to differentiate in all mammals. The ectoderm differentiates into the epidermis, and

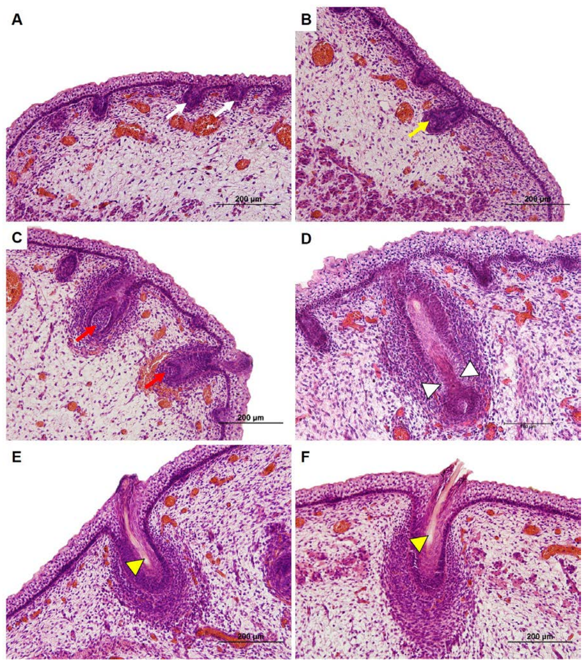

Figure 4: A skin micrograph of the nasal plan in the 44-day-old fetus showing the development of tactile hair. (A) hair bud; (B) hair bulb; (C) dermal papilla; (D) proliferation of epithelial cells toward the surface; and (E, F) formation of the hair shaft.

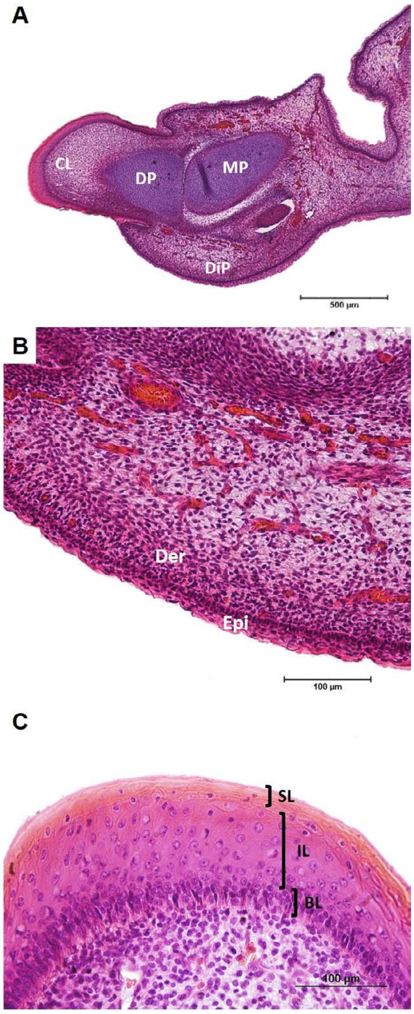

Figure 5: A micrograph of the hind limb digit in the 44-day-old fetus showing the development of the claw and digital pad. (A) the complete digit structure; (B) details of the claw formation; and (C) details of the digital pad. CL: claw; DP: Distal Phalanx; MP: Middle Phalanx; DiP: Digital Pad; SL: Surface Layer; IL: Intermediate Layer; BL: Basal Layer; Epi: Epidermis; and Der: Dermis.

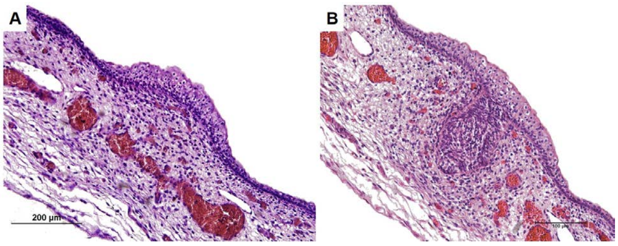

Figure 6: A micrograph of the mammary gland in the 44-day-old fetus. (A) the formation of the mammary bud; and (B) the formation of the mammary papilla.

the lateral somatic mesoderm and dermatome differentiate into mesenchyme [12,13,21]. These cell layers undergo growth and differentiation processes during the embryonic and fetal periods. Sengel [8] described the differentiation of the epidermis as seven layers or strata, germinal, granular, spinous, translucent, cornified and periderm, during the intrauterine lives of mice. The periderm is lost with the development of other layers. The intermediate layer observed here differentiates into all layers following the basal or germinal layer. In our study, the intermediate layer remained, which suggests that this differentiation occurred at a later time in the fetal period.

The development of the hair follicle has been described by several authors, such as Hardy [9] in mice, and in several embryology and histology books and atlases [12,13]. Our study suggests that the formation of hair in dogs is similar to what occurs in other previously described mammals, such as pigs [5]. Similar to all embryogenesis, hair follicle development requires an interaction between the two main tissues. The morphology of hair follicle formation as described herein is only possible because of the cell signaling between epidermal and mesenchymal cells, which have an inductive role on one another through the production and release of messenger molecules [9,10].

In mammals, there are three types of keratin structures in the digits: the nail, hoof and claw [22]. Nails are morphologically well described, whereas there are only a few descriptions of claws, which occur in carnivores. In dogs, the formation of claws has four periods of differentiation, as described by Süsskind-Schwendi et al. [4]. Initially, the connective tissue of the distal section of the digit differentiates, from which the claw will be formed; specific sections of this structure are then formed. With differentiation, the claw begins to show five different segments, and finally, the last period occurs postnatally. In the present research, we observed that such periods begin their differentiation in the fetal period with the possible identification of structures similar to those described in the second period. This result suggests that the final differentiation of the claw in dogs occurs in the final fetal period followed by the final post-natal period.

Mammary gland development was described by Fletcher and Weber [23] in mammals, and according to our results, an identical developmental pattern occurs in dogs. Similar to other components of this system, morphological changes result from induction processes. In mammary glands, the ectoderm induces the proliferation of the subjacent mesoderm, which induces the proliferation of epithelial cells, which undergo invagination to form the gland bud.

An identical developmental pattern of the skin and appendages observed in dogs was also reported for cats. Similar to our study, Knospe [24] described an increase in skin thickness, a formation of hair follicles, and the keratinization of the claws during the early fetal period. Such similarities in the differentiation of these structures can be considered further evidence of the close phylogenetic relationship between these carnivorous species.

Conclusions

According to the characteristics obtained, we conclude that, in dogs, the integumentary system is one of the first to be formed with the differentiation of the skin. The skin shows little development during the embryonic period, whereas its division into the dermis and epidermis and the formation of several cell layers is pronounced in the fetal period. Similarly, skin appendages developed during the fetal period. Sebaceous and sweat glands were not observed in our study, which suggested that they were formed during the late fetal period.

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