Micro-Morphological Studies on the Epidermis, Hair Follicles and Skin Glands of Prenatal Balami Sheep.

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ABSTRACT
Micro morphological studies on the epidermis, hair follicles and skin glands of prenatal balami sheep breed was undertaken. The specimens were obtained from fetuses collected from Maiduguri Abattoir. Approximate age of each of the fetuses was determined using physical features, crown-rump length, body weight and physiological horizons. Four age groups namely 4- 8 weeks, 9-13 weeks, 14-17 weeks and 19-20 weeks were used. Skin specimens were processed and stained using Haematoxylin and Eosin technique for microscopic examination. It showed that epithelium formed about 3-4 layers with flattened cells and developing primary hair follicles were seen in rows surrounded by rudimentary sebaceous glands and sweat glands 4-8 weeks. At 9-13 weeks, the epithelium was stratified with 4-6 distinct layers. Hair follicular plugs seemed to penetrate into the dermis from basal epithelial layer. At 14-17 weeks, the epithelium was many cell layered and keratinization of hair was also evident. At 19-20 weeks, smooth muscles were seen in small bundles adjoining sebaceous glands. Secondary hair follicles were seen at this stage in small groups adjoining primary follicles. It was concluded from the present study that developmental changes of the epithelium, hair follicles, sweat glands and sebaceous glands in Nigerian balami tropical breeds followed the same sequence and time as those of the temperate breeds. This suggested that these structural developmental changes are influenced genetically and not by environmental factors.

Key words: *Epidermis, Hair Follicles, Skin Glands, Balami sheep*

INTRODUCTION

Sheep are known all over the world for the provision of animal proteins and wool (Mahgoub *et al.*, 2010). Skin that covers the body makes up 7 -12% live weight, besides its biological and immunological importance. It fibers represent and interesting biological material which is also utilized in the textile industry (Pourlis and Christoulopoulos, 2008).

Skin is the heaviest organ of the body and is made up of epidermis and dermis (Sajjad *et al.*, 2013). The epidemics consists mainly of multilayer of keratinocytes (Kocamis and Aslan, 2004) while the dermis which is a connective tissue that supports epidermis is divided into a superficial papillary layer and a deep reticular layer (Dellman, 1993; Kurtdede, 2002 and Mobini, 2012).

Histologists who demonstrated the cellular organization of tissues had helped us to appreciate the structures and functions of skin appendages (Widelitz *et al.*, 1997). Two categories of skin appendages exist, the ones that protrude out of the body surface are hairs, feather scale, nail, claw etc which provide a variety of functions to individuals ranging from environmental

protection to ritual mating displays. The ones that invigilate into the body are the sebaceous gland, sweat and mammary glands etc, which provide specialized physiological functions to individual, ranging from environmental adaptation to child rearing (Widelitz *et al.,* 1997). The skin glands which are derived from interactions of epidermis and dermis within the skin are located close to hair follicle in the papillary layer (Kurdede, 2002). Hair is an appendage of the skin that grows out of the hair follicle (Dellmann, 1993) and can be divided into primary and secondary hairs (Mobini, 2012).

In Nigeria, meat is a common source of animal proteins; other sources of meat include cattle, goat and camel etc. Balami breed is found mostly in the Northern part of Nigeria especially in Borno state. It is a big, predominantly white coat colored sheep with convex facial profile. The ears are large and droopy. The tail is thin and long. The males have prominent horns while the females are polled. It is the largest breed of sheep in Nigeria.

The prenatal histological study of the skin glands and hair coat in man and laboratory animals have been investigated (Serri and Ceremele, 1979 and Militzer 1982). In sheep (Sajjad *et al.*, 2013; Shahrooz and Ahadi, 2005 and Ryder, 1956). In goat (Tripathi and Zaidi, 2013). In cattle (Aslan *et al.,* 2004). In camel (Nagani, 2011) and in Pigs (Meyer and Gorgen, 1985).

Histological study of skin in both tropical and temperate breeds of cattle have been undertaken (Ahmed *et al.,* 1985). Similar findings in sheep and goat of the temperate breeds had also been carried out (Boyd, 1979). However histological study on the pre natal skin of the Balami tropical breed in Nigeria is scanty. This type of study will not only show the inherent nature of these local breed but will also assist in age determination of the fetus. The present study was therefore undertaken the observe the histological changes in the skin of the prenatal Balami tropical breed and compare with the temperate breeds and see whether the changes follow the same sequence and time.

MATERIALS AND METHODS

Fifteen (15) Balami embryos and fetuses of both sexes with ages ranging from 4- 20 weeks were obtained from Maiduguri abattoir and used for this study.

Approximate age of each fetus was determined using the crown-rump length, body weight and physical horizons (Boyd, 1979).

Skin specimens $3cm^2$ for histological study were taken from the neck, rump, flank, forearm, shoulder and hip regions of each sheep and were fixed in 10% formation solution using a properly labeled specimen bottles.

The tissues were processed in the laboratory by adopting standard methods of dehydration, cleaning and embedding. The paraffin tissue (Longitudinal and Transverse) sections of 5 – 10 microns thickness were stained using Harri's haematoxylin and Eosin stain (Andrews, 1998).

Slides were examined under light microscope x 40 and photomicrographs were obtained using Olympus Vanox Microscope.

RESULTS:

The histomorphological changes observed in the skin of the abdomen and limb regions of each of the fetus were similar in sequence to those observed in the poll regions. The fetuses for this study were divided into four groups according to their crown rump lengths and the gestation periods.

Table 1: Showing the number of fetuses.

S/NO	Breed	Weight (g)	C – R Length (cm)	Age (weeks)	Sex
1.	Balami	14.52	8	5-6	M
2.	Balami	32.2	10.4	6-7	M
3.	Balami	66.2	12.5	8-9	F
4.	Balami	69.1	13.4	8-9	F
5.	Balami	71.6	14.5	9-10	M
6.	Balami	114.7	15.2	11-12	M
7.	Balami	190.2	21.4	13-14	F
8.	Balami	420.3	23.2	13-14	F
9.	Balami	425.2	26.5	14.15	M
10.	Balami	1200	29.5	14-15	F
11.	Balami	1020	35.4	15-16	M
12.	Balami	1700	40.3	17-18	F

Group I (5.5 – 12.0cm C – RL; 4 – 8 weeks of gestation).
The epithelial cells became 3-4 layers, with flattened cells. The basal layer was cuboidal while the upper layers became more rounded and large. All the cells were nucleated. Developing hair follicles were observed in rows surrounded by rudimentary sebaceous and sweat glands.

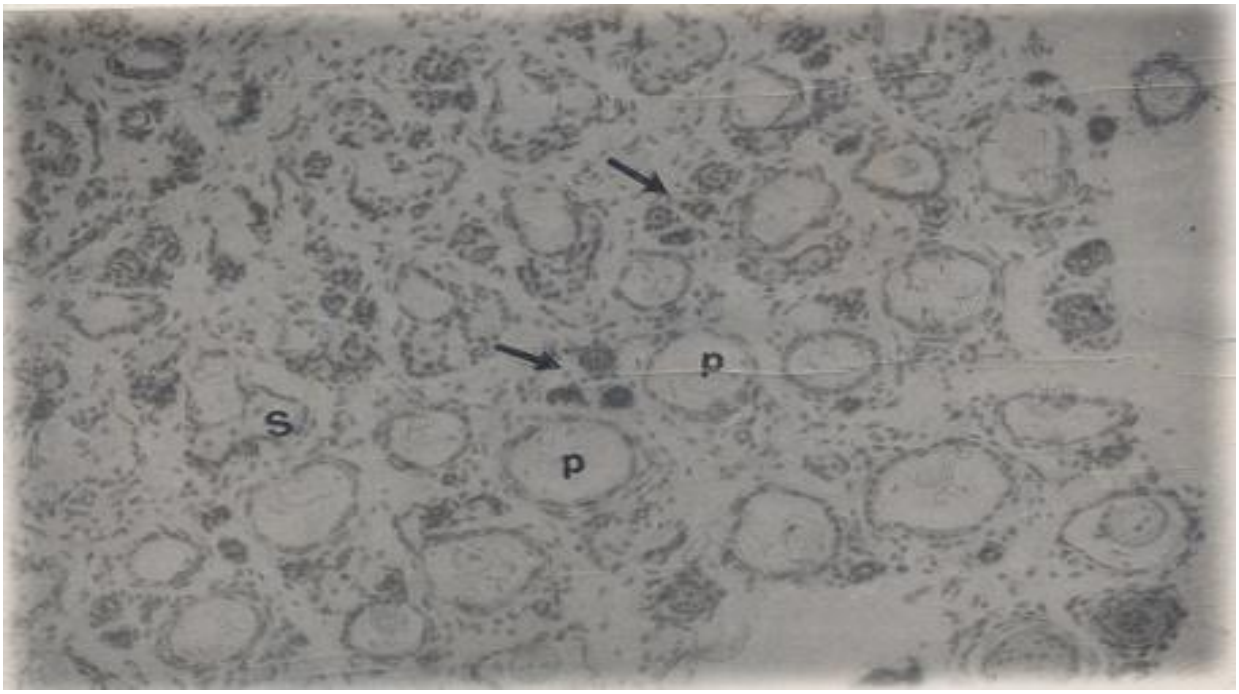
Group II (14.5 – 20.0cm C – RL; 9 – 13 weeks of gestation).
The epithelium became stratified. Basal layer consisted of columnar cells and the epithelium had 4-6 distinct cell layers. The cells of the stratum spinosum were large and oval in shape. The nuclei were irregularly arranged either centrically or eccentrically. Periderm, the topmost layer consisted of 1-2 layers of flattened nucleated cells. Hair follicular plugs were seen penetrating into the dermis from the basal epithelial layer, the prinordia of the tubular and sebaceous glands were seen associated with the follicular plug as solid buds.

Group I11 (23.0 – 30.5cm C –RL; 14 – 17 weeks of gestation).
The epithelium became stratified with many cell layers. Hair follicles opened into the surface. Basal layer became single layered and columnar. Topmost cell layers were pyknotic. The hair follicles were arranged in groups and well differentiated keratinized primary hair follicles were visible. In transverse sections, primary hair follicles were seen scattered in groups. Each follicle was seen associated with the rudimentary sebaceous gland, which consisted of large oval cells with clear cytoplasm and dark central rounded nuclei forming a group of about 3-7 cells. The hair follicle and the associated sebaceous glands were seen demarcated by a layer of cells separating them from the adjoining tubular gland which had a central lumen separated by a single layer of dark nucleated cells. Few solid groupings into 2-4 in number were seen in close association with primary follicles.

Group 1V (50.5 – 58.0cm C-RL; 19-20 weeks of gestation)

The epithelium along with hair follicles invaginated deeper into the dermis was observed. Sebaceous gland was seen adjoining the hair follicle in the papillary part of the dermis. The epithelium was corrugated but keratinisation was not evident. Smooth muscle cells were seen in small bundle adjoining sebaceous and hair follicles. Sweat glands were found deeper in reticular layer of the dermis in association with primary hair follicles. The sebaceous gland had simple columnar cells forming a wall surrounding a large lumen. In certain region, primary hair follicles were seen singly or in groups up to three. They were associated with sweat glands. Smaller hair follicles were seen in group in close proximity to the primary follicles but they were devoid of any sweat glands. These were the secondary hair follicles.

Fig 1: Transverse section of skin in the poll region of an ovine fetus of approximate 4-8 weeks (28.5 – 56.5 days) of age to show the large primary follicles (P) and smaller secondary follicles (arrows) appearing as solid cords in groups adjoining them, sebaceous glands (S) opening into the primary hair follicles could also be seen (H & E. X 200).



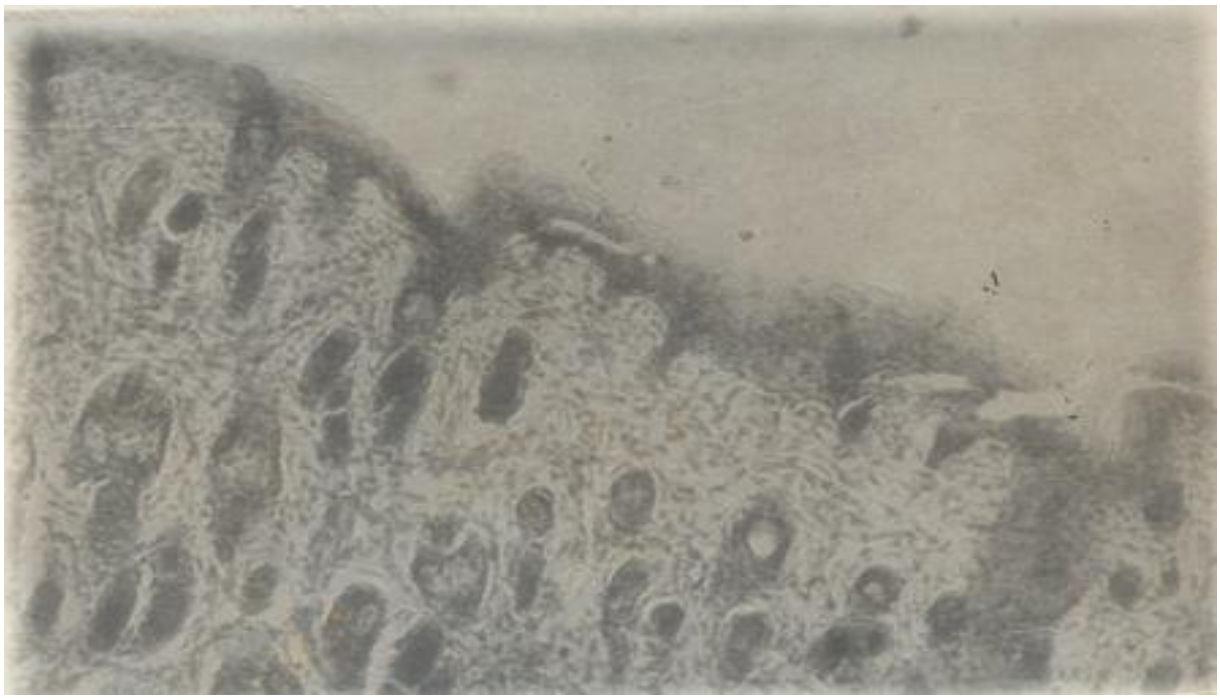


Fig 2: Vertical section of the skin of a fetal sheep of 9-13 weeks (63 - 91 days) of age. Note the corrugated epidermis. Primary hair follicles and sebaceous glands are seen (H & E. X 200).

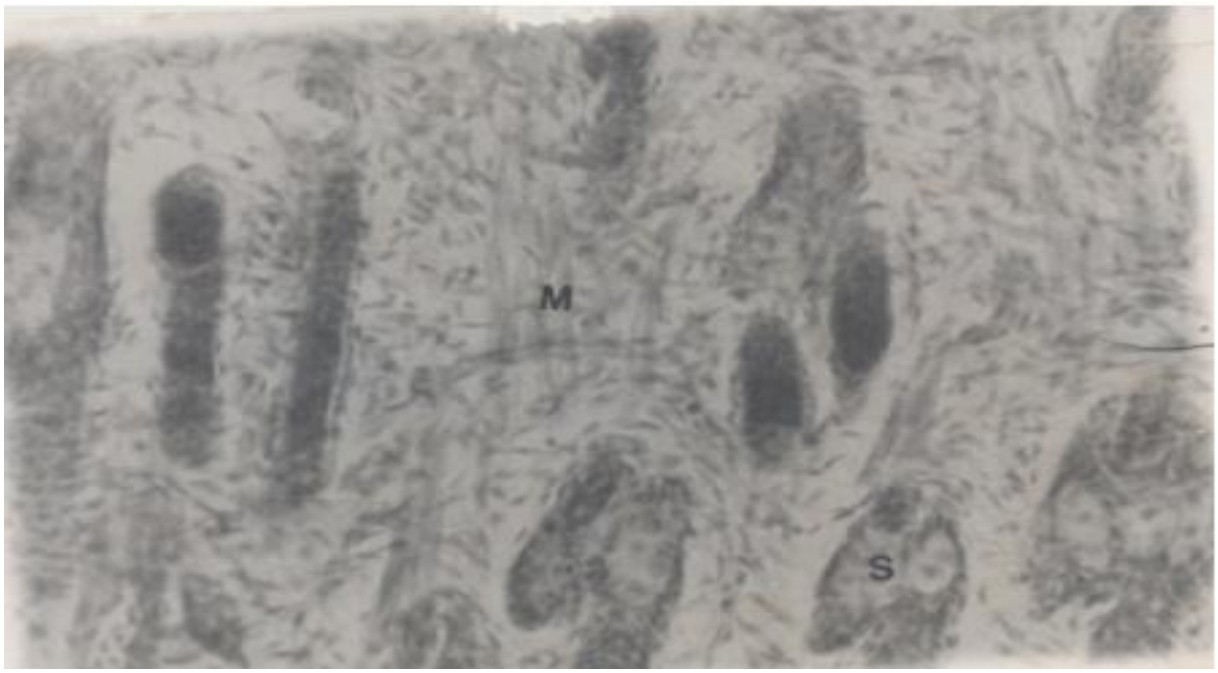


Fig 3: A higher magnification of the vertical section of the skin of a fetal sheep of 14-17 weeks (98-119days) of age to show the primary hair follicles, sebaceous glands (S) and the arrector pili muscle (M) (H &E. X 400).

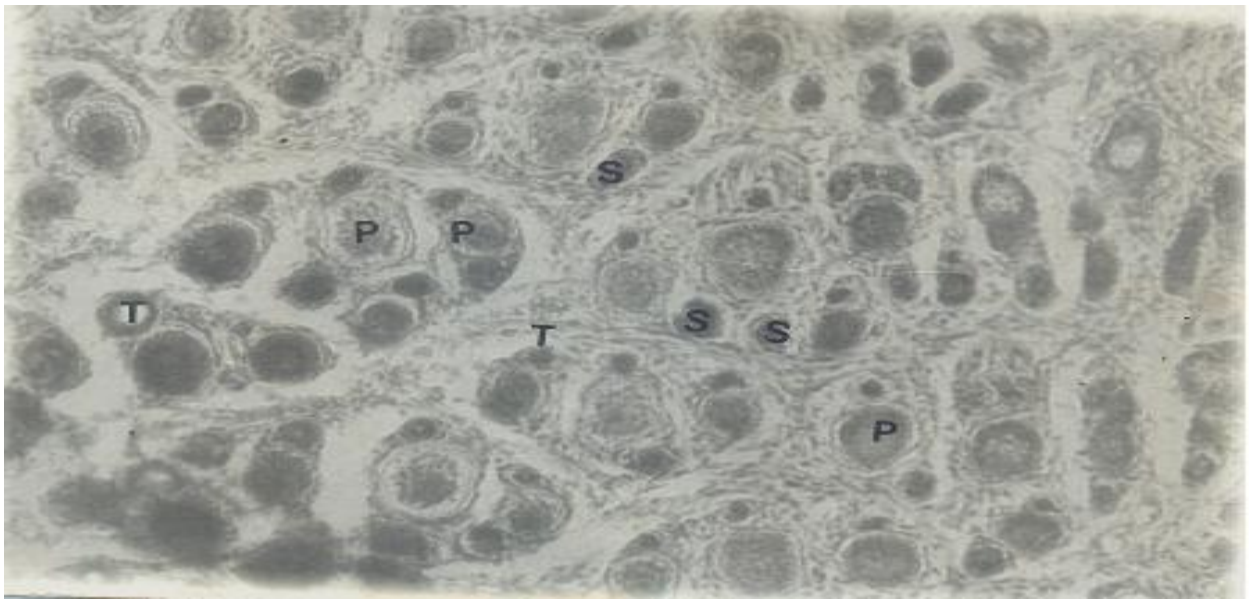


Fig 4: A vertical section of the skin of an ovine fetus of 19 - 20 weeks (133 – 140.5 days) of age. Primary follicles can be seen either singly or in groups (P) associated with tubular glands while the smaller secondary follicles (S) are seen in clusters close to the primary follicles. Note the presence of sweet glands (T) close to the primary follicles (H & E. X 200).

DISCUSSION

The micro morphological changes in the skin of Balami sheep as observed in these findings show that the epithelium developed as one or two cell layered structure at 4-5 weeks of gestation. The epithelium became more stratified by 7-9 weeks of gestation. Developing hair follicles associated with rudimentary sebaceous and sweat glands were observed at this stage. The stratified epithelium had stratum basale, stratum spinosum and the periderm at 10-12 weeks.

Hair follicular plugs were seen penetrating into the dermis from the basal epithelial layer. Primordia of tubular and sebaceous glands were seen associated with the follicular plug as solid buds at 10-12 weeks of fetal life.

The topmost cells of the stratified epithelium became pyknotic and keratinized primary hair follicle opened into the surface by 13-15 weeks of gestation. Primary hair follicles were seen scattered in group and each follicle was seen associated with the rudimentary sebaceous gland demarcated by a layer of cells separating them from adjoining tubular gland at this stage.

The sebaceous glands and the arrestor pili muscles were observed in the superficial part of the hair follicles whereas the sweat glands were seen deeper at 16-18 weeks of gestation. Primary hair follicles were seen either singly or in groups while the secondary hair follicles were observed in groups close to the primary follicles at this stage of fetal life. Lyne and Hollis, (1972) described the skin of prenatal sheep and reported that the initiation of both primary and secondary follicles from epidermis starts at 105-115 days gestation, they also reported that secondary follicles were formed from existing primary follicles.

Hammond *et al.* (1971) reported that in sheep fetuses, the primary follicles develop first followed by the secondary follicles. Generally two types of hairs exist and are pigmented. Hickmann *et al.*

(1962) studied the histology of fetal skin and reported that in sheep hair follicles are arranged in groups and well differentiated keratinized hair follicles were visible at a time corresponding to fetal ages of 106-108 days. Ahmed *et al.* (1985) described the histological changes in the skin of black face sheep that a single layered epithelium appeared by 26th day of fetal life, and a two layered epithelium appeared by 52nd day of intrauterine. In 78 days old fetus, primitive stages of hair follicles appeared with primordial of tubular glands and sebaceous glands. Keratinized primary hairs and arrestor pili muscle were observable by 104th day. The epithelium had four layers and was corrugated at 130 day. The primary hair follicles were arranged single or in groups neighboring the primary hair follicles. These observations compare favourably with the findings of the present study in the tropical Balami breed and are also comparable to New Zealand Romney sheep (Andrews, 1998), Madras red sheep (Purnshotmen *et al.*, 2010) German black head (Kurtdede and Asti, 1999) and Swedish pelt sheep (Bulteler *et al.*, 2004).

Mobini, (2012) described primary hair follicle as having porcine sweat glands, arrector pili muscles and sebaceous glands, these observations are in agreement with the report of the present investigation. In this work, all the hairs (Primary and secondary) of the various regions of the prenatal Balami sheep, medulla was seen; the sweat glands, sebaceous gland and arrector pili muscles were observed around all the primary hair follicles. The arrector pili muscle associated with sebaceous glands were seen around all the hair follicle located in the dermis. Similar observations were reported by Mobini, (2012). Findings presented for Merino sheep (Warren *et al.*, 2008), Tsigai sheep (Genko Vski and Gerchev, 2007) and for Balami sheep in the present study, suggest that initiation of primary hair follicle stops after the first half or two third of gestation at the time when the fetus has achieved highest density of primaries hairs.
In man, however, primary hair follicles are initiated until birth while secondary hair follicles do not exist (Serri and Ceremele, 1979).

Developmental features of sweat glands, sebaceous glands and their distribution pattern have also been described by (Amakiri, 1974; Ahmed *et al.*, 1985; Mobini, 2012). The observations are in agreement with the reports of the present findings and in Balami sheep, the sequence of development and time followed a pattern similar to that described by these workers. In determining age of fetuses, physical parameters as well as the developmental changes were used in the present study. These change, followed a pattern similar to that reported by Boyd, (1979) and compare, favourably with that of Ojo, (1982). In these findings, no significant difference in various structures of the skin between male and female was observed. Similar results were also reported by Mobini, (2012) in Bakhtiari Iranian native breed, Yeruham *et al.* (1979) in Assad, Merino and Awassi breeds.

CONCLUSION:

The findings reported in this work are a preliminary step to further pre and post histomorphological investigations of the epidermis, hair follicles and skin glands of other breeds of sheep in Nigeria. At the present time, there is widespread interest in various aspects of the adaptation of sheep to tropical environments. It was also observed from the present study that changes seen in the epithelium, hair follicles sweat and sebaceous glands of fetal skin of Balami sheep indigenous to Nigeria were similar to those reported for the temperate breeds. The study also shows that both primary and secondary hair follicles were seen in the skin of tropical breeds of sheep, thus the potential of these sheep producing wool require further investigation. It was

concluded from this study that the morphological changes which occurred in the fetal skin of Nigerian Balami tropical breed are similar to those of the temperate breeds and the pattern of development followed the same sequence and time suggesting that these structural changes are influenced genetically and not by environmental factors.

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