Nigerian Veterinary Journal
VOL:33 (1) 419-426
ARTICLE

Histological Studies of the Calvarial Development of One-Humped Camel (*Camelus Dromedarius*) Fetuses

HENA, S. A. *[1], SONFADA, M. L.[1], ONYEANUSI, B. I.[2], KENE, R. O. C.[3], UMAR, A. A.[1], SHEHU S. A.[1] and OYELOWO,F. O.[1]

[1] Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.[2] Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria.[3] Department of Medicine, Surgery and Theriogenology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.*Corresponding author:sundayhena@yahoo.co.uk, +2348060524623

SUMMARY

This study involved the use of thirty two camel fetuses, [23 (71.88%) males and 9 (28.12%) females]. Eleven fetuses were at the first trimester (34.4%), twelve at the second trimester (37.5%), and nine at the third trimester (28.1%). 1cm^2 of bone samples obtained from the different fetal calvaria at the first, second and third trimester stages were decalcified and processed for normal Hematoxylin and Eosin (H&E) staining. There was an initial evidence of loose mesenchymal cells condensed together with some blood vessels, osteogenic cells and ill-defined spicules. Ill-defined intertrabecular spaces were also seen at the first and second trimester levels. However, with advancement in age at the third trimester stage, prominent bone spicules or trabeculae were seen. Similarly, there were regularly arranged osteocytes within the trabecular matrix; and the intertrabecular spaces were more obvious. The findings of this research would help in understanding the microscopic anatomy of the developing calvaria in this animal species.

KEY WORDS: Histology, calvaria, one-humped, camel, fetuses.

INTRODUCTION

Camel is a well known desert animal and its climatic adaptations are mainly because of its peculiar physiological differences (Arnautovic, 1997). The activities of the camel are coordinated and controlled by the brain, and being so delicate, needs to be protected by the calvaria. The calvarium is an important bony apparatus as it encase and protects the delicate brain tissues lying within it. The calvaria comprised the frontal, parietal, occipital, temporal and interparietal bones (Evans, 1993; Smuts and Bezuidenhout, 1987). Each bone shows a central trabecular network that spreads to cover the brain (Evans, 1993). The flat bones of the cranial vault and sides are composed of an outer layer of ordinary compact substance, the lamina externa; an inner layer of very dense bone, the lamina interna or tabula vitrea, and between these a variable amount of spongy bone, here termed diploe (Getty, 1975).

Bone formation is an essential part of skull vault (calvaria) development in vertebrates. Calvarial bones, as well as most facial bones, form directly from mesenchyme by intramembranous ossification. Osteogenesis in these bones starts by mesenchymal cell condensation. These cells undergo differentiation into osteoprogenitor cells, which proliferate and ultimately differentiate into

osteoblasts that lay down the bone matrix (Hall and Miyake, 1992).

The mesenchymal condensations of the frontal and parietal bones form on the lateral aspects of the brain close to the cranial base. The calvarial bones grow as sheets between the brain and epidermis, and extend apically towards the top of the skull (Rice *etal.*, 2000). Osteoblast differentiation occurs at the bone margins or osteogenic fronts into which osteoblasts invade, and progenitor cells are recruited from the surrounding mesenchyme. Prior to birth, calvarial bones approximate each other with sutures forming between the bone margins and these sutures accommodate brain growth (Rice *etal.*, 2000). According to Zika and Klein (1975), the calvarium of rat was composed of fibrous tissues which became heavily calcified at birth. Calvarial bones form in a unique environment to other bones; they form in close contact with dura mater, which is the topmost layer of the meninges. Interactions between the dural cells and calvarial mesenchyme have been shown to be important in the regulation of calvarial bone development (Opperman *etal.*, 1995).

There are evidences of works conducted on: the study of the skull of one-humped camel by Ghaji *etal.*, (1987), anatomy of craniocephalic structures of camel by Arencibia, *et al.*, (2005), comparative gross anatomical studies of the skull of one-humped camel by Shahid and Kausar, (2005) and postnatal development in the linear and tric morphometrics of the camelidae skull by Al-Sagair and El-Mougy, (2002), but a histological study of the development of calvaria of one-humped camel (*Camelus dromedarius*) has not been elucidated.

This study is aimed at evaluating the histological developmental processes of the camel fetal calvaria at different levels of their gestational ages and such information could be a basis for understanding the anatomy of the fetal developing calvarium.

MATERIALS AND METHODS

Included in the study were thirty two camel fetuses out of which twenty three were males representing 71.88% and nine were females representing 28.12%. Eleven fetuses were in the first trimester representing 34.4%, twelve were in the second trimester representing 37.5%, while nine were in the third trimester representing 28.1%.

This study was carried out in Sokoto metropolis which is located in Sokoto State, Nigeria. Wasted camel fetuses were collected from Sokoto main abattoir located in the metropolis through a daily visit for a period of six months. The fetuses were cleaned, put into polythene bag and transported to the Veterinary Anatomy laboratory of Usmanu Danfodiyo University, Sokoto where the samples were weighed, and the crown-vertebral rump lengths (CVRL) measured and recorded in centimetres. The gestational age of each fetus (in days) was calculated using the formula [X= (CVRL + 23.99)/0.366, where X is the gestational age in days and CVRL is the crown-vertebral rump length measured in centimeters] according to El-Wishy *et al.*, (1981) after which the fetuses were categorized into definite trimesters and then decapitated at the occipito-atlantal joint. The fetal heads were then dissected using a method as outlined by Mabbutt and Kokich (1979), removing the skin and muscle tissues as much as possible leaving only the skull. A piece of bone segment ($1cm^2$) each from the frontal, parietal, interparietal, occipital and temporal bones for the first, second and

third trimester fetuses was taken respectively. The samples were preserved in 10% formalin solution, decalcified in 5-7% nitric acid and processed for normal Hematoxylin and Eosin procedures as described by Gordon (1990), from which slides were eventually prepared. The slides were viewed using an Olympus Vanox microscope and representative photomicrographs were made using the Motic camera (Moticam 1000, 1.3M Pixel USB2.0).

RESULTS

Histological examination of the calvaria of camel fetuses in this study revealed that in the first and second trimesters, the membranous calvaria contained loose mesenchyme (Plates 1 and 2) with abundance of mesechymal cells which were light stained, ill-defined trabeculae and intertrabecular spaces were also seen (Plate 3).

At the second trimester, there was abundance of numerous osteoblasts scattered within the immature calvaria, with ill-defined trabeculae and intertrabecular spaces (Plates 4 and 5). With fetal advancement in age at the third trimester, there appeared to be an initial laid-down bony spicules, condensed mesenchymal cells, and ill-defined trabeculae. At this stage still, there were prominent bone spicules or trabeculae seen with fewer osteoblasts lining them (Plates 6 and 7). Regularly arranged osteocytes within the trabecular matrix and the inter-trabecular spaces were also obvious features observed at this trimester.

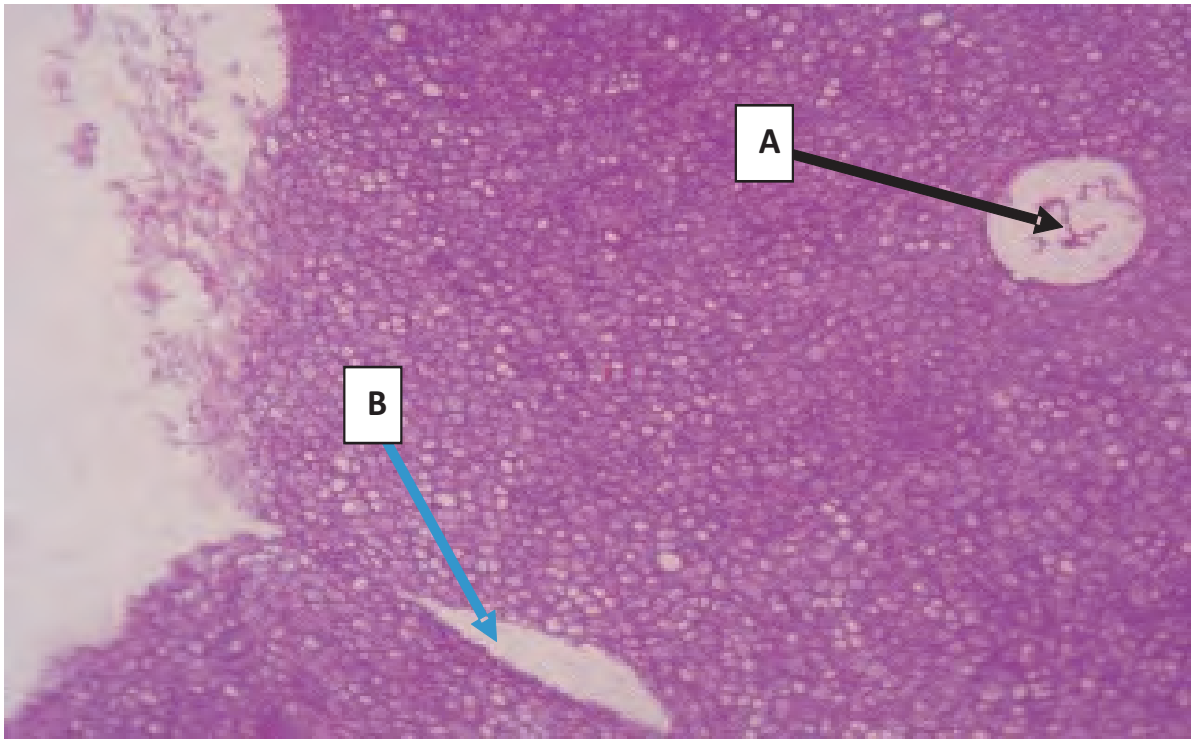

Plate 1: Photomicrograph of early-first trimester (35-43 days) **fetal calvarium:** Showing loose mesenchyme of developing calvarium with blood vessel (**A**- arrow) and intertrabecular spaces (**B**- arrow).(H&E ×400)

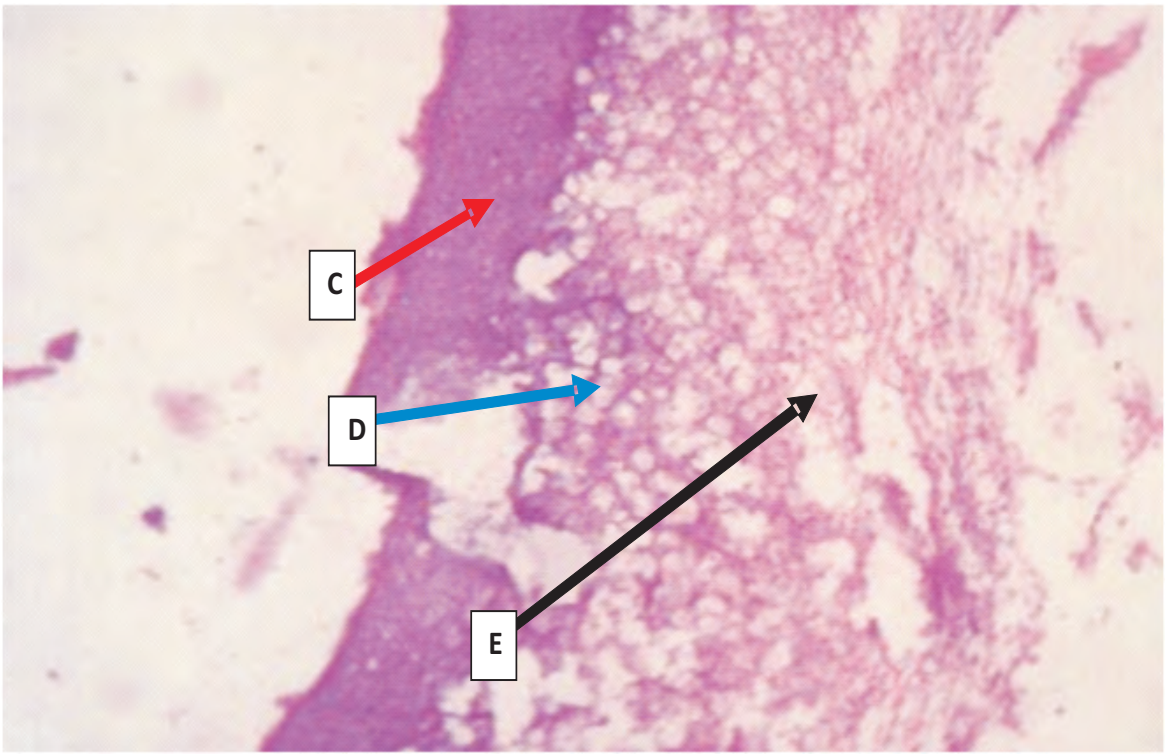

Plate 2:Photomicrograph of mid-first trimester (44-87 days) **fetal calvarium:** A typical micrograph of an immature calvarium undergoing intra-membranous ossification. Numerous osteoblasts (**D**-arrow), condensed mesenchymal cells (**C**- arrow) and ill-defined spicules (**E**- arrow). (H&E ×400)

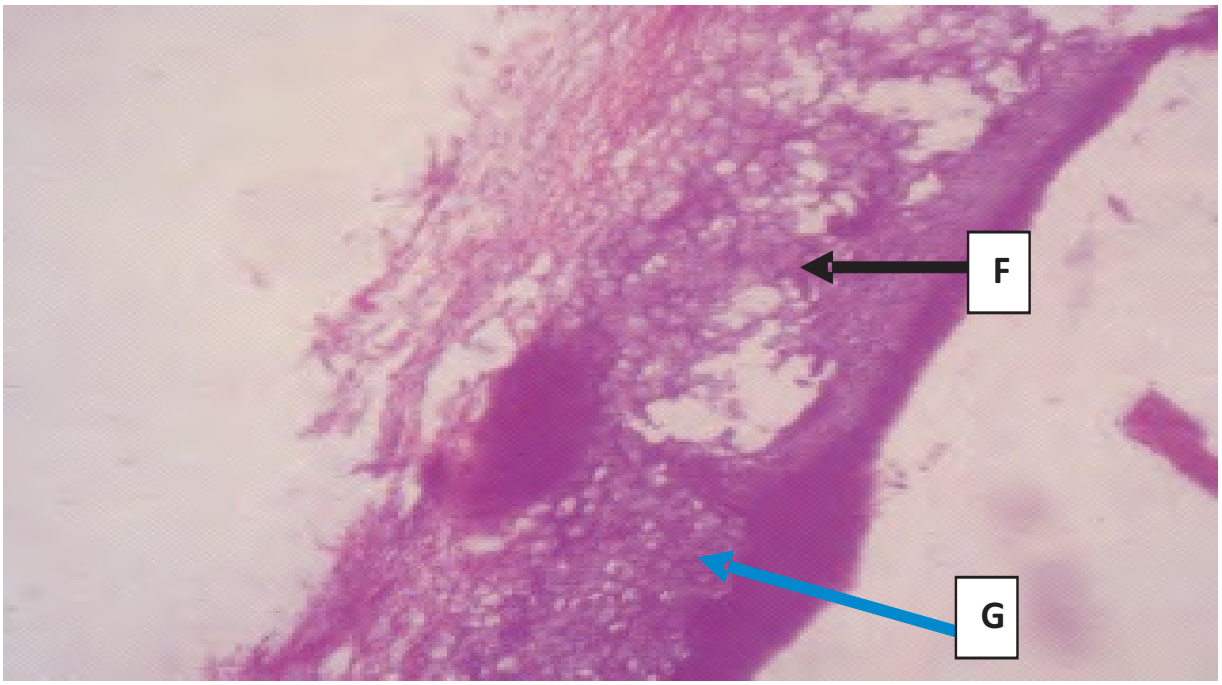

Plate 3: Photomicrograph of late-first trimester (88-130 days) **fetal calvarium:** Showing mesenchymal cells, light-stained (**G** - arrow), osteoblasts, dark-stained (**F**- arrow)(H&E ×400).

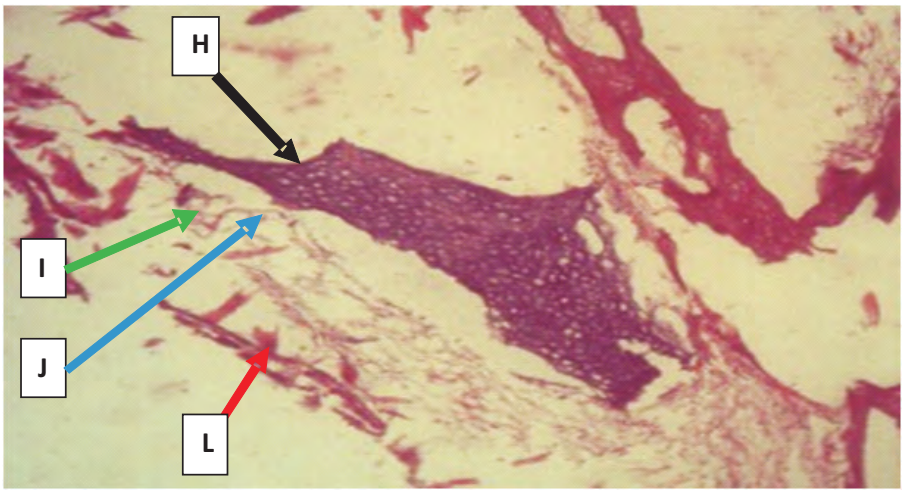

Plate 4: Photomicrograph of second trimester (131-260 days) **fetal calvarium:** Bone spicules (**L**-arrow), condensed mesenchymal cells (**H**- arrow), ill-defined trabeculae (**I**- arrow), and intertrabecular spaces (**J**- arrow). (H&E ×400)

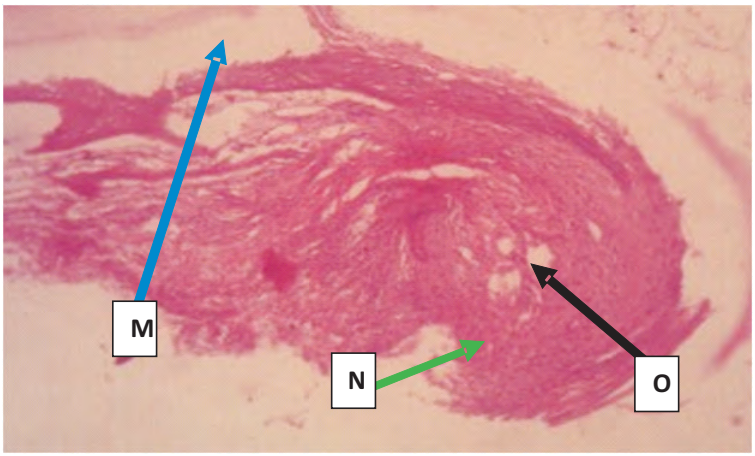

Plate 5:Photomicrograph of second trimester (131-260 days) **fetal calvarium:** Numerous osteoblats (**O** - arrow), ill-defined trabeculae (**N** -arrow), and intertrabecular spaces (**M** -arrow). (H&E ×100)

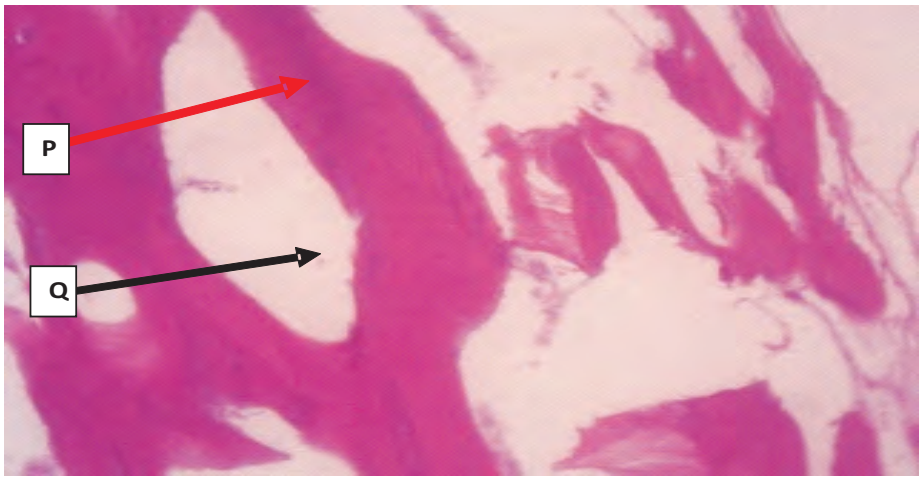

Plate 6: Photomicrograph of early-third trimester (261-303 days) **fetal calvarium:** Prominent bone spicule or trabecular (**P** - arrow), prominent intertrabecular spaces (**Q** – arrow). (H&E ×100)

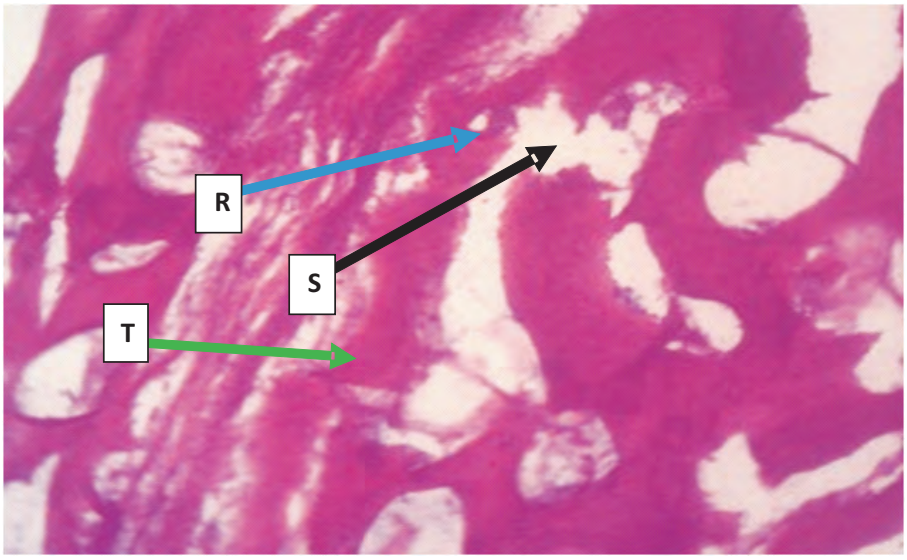

Plate 7:Photomicrograph of late-third trimester (349-390 days) **fetal calvarium:** A matured calvarium with prominent spicules showing homogenous matrix (**S** - arrow), osteoblasts lining the spicules (**T**- arrow) and intertrabecular spaces (R - arrow)(H&E ×400)

DISCUSSION

From the results obtained there was an initial evidence of loose mesenchymal cells condensed together with some blood vessels, osteogenic cells and ill-defined spicules and bone marrows. A characteristic feature of immature calvarial bone is the presence of greater abundance of cells than in a matured bone. However, with advancement in age, prominent bone spicules or trabeculae were seen with few osteoblasts lining the trabeculae; at this stage also there were regularly arranged osteocytes within the trabecular matrix and the inter-trabecular spaces were obvious. These findings were in agreement to those of Sivachelvan *et al.*, (1995) who worked on the calvaria of Sahel goat.

The first bone to arise, whether from mesenchyme or from cartilage (or in fracture repair post-natally), is in the form of spicules. These were also observed in this work where the initial bone development started from the condensation of mesenchymal cells thereby leading to bone spicule formation.

In immature bone, the collagenous lamellae are not arranged in parallel or concentric arrays as in mature spongy and compact bone, respectively, but are randomly oriented and loosely intertwined (Quarto *et al.*, 2009); this was also observed in this work. Immature bone also has more ground substance than mature bone and consequently, immature and mature bones showed different staining characteristics, immature bone stains more intense with hematoxylin and mature bone more intense with eosin. However, these staining variations had not been ascertained in this present work as mature calvarial bones were not used as a basis for comparison.

According to Junqueira and Carneiro (2005), flat bones of the skull have a middle layer of spongy bone sandwiched between two relatively thick layers of compact bone. Here the lamellae are of collagen and are not arranged concentrically round a central canal, but run parallel to one another. This work also observed that the bones of the calvarium were not arranged in concentric layers

around a central canal as seen in other bone types, but their trabeculae (lamellae) ran parallel to each other. Spongy bone is composed of bone spicules (trabeculae) and the spaces between the trabeculae are filled with bone marrows.

In intra-membranous ossification, bone is formed directly from mesenchymal tissue. Calvarial bones arise from two embryonic tissues, namely, the neural crest and the mesoderm (Quarto *et al.*, 2009). The flat bones of the skull and face, the mandible and the clavicle develop in this manner (Evans, 1993).The first step in intra-membranous ossification is the aggregation of mesenchymal cells in the area where bone is to be formed. The mesenchymal cells undergoing condensation processes were, however, noticed in this work. The tissue in this area became more vascularized and the mesenchymal cells began to differentiate into osteoblasts, which secreted the collagen and ground substance (proteoglycans) of bone matrix (collectively called osteoid). The osteoblasts maintained contact with one another via cell processes. The osteoid became calcified with time and the osteocytes became enclosed in canaliculi. Some of the mesenchymal cells surrounding the developing bone spicules proliferated and differentiated into osteo-progenitor cells. Osteo-progenitor cells in contact with the bone spicule became osteoblasts, and secreted matrix, resulting in appositional growth of the spicule (Quarto *et al.*, 2009).

In conclusion, the findings in this research generally showed that the calvarial bones of the fetuses at the first and second trimester stages contained abundance of cells chiefly mesenchymal cells as well as osteoblasts and irregularly ill-defined trabeculae as fetal age advanced. At the third trimester, the cells tended to decrease in number and the bone trabeculae became well organized.The information obtained in this study mayserve as a baseline data in understanding the microscopic anatomy of the developing calvaria in this animal species.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support and efforts of Mr. I.M. Wiam who greatly helped in the histological procedures.

REFERENCES

AL-SAGAIR, O. and EL-MOUGY, S. A. (2002): Postnatal development in the linear and tric Morphometrics of the camelidae skull. *Anat. Histol. Embryol. 31:232-236.*

ARENCIBIA, A., RIVERO, M. A., GIL, F., RAMIREZ, J. A., CORBERA, J. A., RAMIREZ, G. and VAZQUEZ, J. M. (2005): Anatomy of the craniocephalic structures of the camel (*Camelus dromedarius*) by imaging techniques: A magnetic resonance imaging study. *Anat. Histol. Embryol.* 34: 52-55.

ARNAUTOVIC, I. (1997):A contribution to the study of some structures and organs of camel (*Camelusdromedarius*). *J. Cam. Prac.and Res. Vol.4. 2:287-293.*

EL-WISHY, A. B., HEMEIDA, N. A., OMAR, M. A., MOBARAK, A. M. and EL-SAYED, M. A. I. (1981): Functional changes in the pregnant camel with special reference to fetal growth. *Br. Vet. J., 137:527-537.*

EVANS, H. E (1993): Miller's Anatomy of the Dog. Pub.Saunder's Company.3rd Edition. Pp 59-71.

GHAJI, A. and SHIRIKANT, S. T. (1987):The skullof one-humped camel (*Camelusdromedarius*). *Zariya Veterinarian, Vol. 2 No. 2 Pp 36-44.*

GETTY, R. (1975):The Anatomy of the Domestic Animals. W.B. Saunders Company, Philadelphia, 5th Edition, vol. 2. Pp 20-22.

GORDON, K. C. (1990): Tissue Processing. *A Theory and Practices of Histological Techniques*. Bancroft, J.D. and Stevens Eds. Churchill Livingstone, Edinburg. Pp 45-46.

HALL, B. K and MIYAKE, T. (1992):The membranous skeleton; the role of cell condensation in vertebrate skeletogenesis. *Anat. Histol. Embryol.186:107-124.*

JUNQUEIRA, L. C. and CARNEIRO, J. (2005):Basic Histology. Text and Atlas.11th Edition.Published by: The McGraw-Hill Companies, Inc. Pp 135-148.

MABBUTT, L. W. and KOKICH, V. G. (1979):Calvarial and sutural re-development following craniectomy in the neonatal rabbit. *J. Anat. (1979), 129, (2):413-422.*

OPPERMAN, L. A., PASSARELI, R. W., MORGAN, E. P., REINTIES, M. and OGLE, R. C. (1995): Cranial sutures require tissue interactions with dura mater to resist osseous obliteration in vitro. *J. Bone Miner. Res. 10:1978-1987.*

QUARTO, N., WAN, D. C., KWAN, M. D., PANETTA, N. J., LI, S. and LONGAKER, M. T. (2009): Origin matters: Differences in embryonic tissue origin and Wnt signaling determine the osteogenic potential and healing capacity of frontal and parietal calvarial bones. *J. Bone Miner. Res. 25:1680–1694.*

RICE, R., DAVID P. C., BJORN, R. O. and IRMA, T. (2003):Progression of calvarial bone development in mice. *Develop. Biol. 26: 75-87.*

SHALID, R. U. and KAUSAR, R. (2005): Comparative gross anatomical studies of the skull of one-humped camel (*Camelus dromedarius*). *Pakistan Vet. J., 25 (4):2005-2006.*

SIVACHELVAN, M. N., HAJARA, B. U. and CHIBUZOR, G. A. (1995):Fetal development of the calvarium in Sahel goat: its post-natal significance. *Tropical Vet. 13, (3&4) 109-118.*

SMUTS, M. S. and BEZUIDENHOUT, A. J. (1987): Anatomy of the Dromedary. Oxford University Press, Wanton Street, Oxford OX2 6DP. Pp 1-10.

ZIKA, J. M. and KLEIN, L. (1975): Comparison of whole calvarial bones and long bones during early growth in rats. Histology and collagen composition.Pub. Pringer-verlag, New York. *J. Calc. Tiss. Res. Int'l. 18:101-110.*