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Prenatal skull radiography and calvaria histogenesis in Uda and Yankasa breeds of sheep

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Abstract

The anterior fontanelle was opened and radiolucent in the second trimester; at the third trimester, the fontanelle began to shrink and finally closed at birth and became radiopaque. The borders of the orbit and the teeth were both radiolucent and undifferentiated at the second trimester and at the third trimester, and these regions became radiopaque at the second and third trimester, respectively. The histomorphology of the calvarium in the first-trimester foetus had three layers consisting of the mesenchymal and osteoblast cells; as the foetus ages within the second trimester, the mesenchymal cells were transformed into osteoblast, colonies, primitive bone spicules, matured bone spicules, and primitive trabeculae respective, in the third trimester, the primitive trabeculae developed into a matured trabeculae, in the day old, the matured trabeculae transforms into a primitive spongy bone. However, this process occurs earlier in Yankasa than in Uda. The histomorphology of the calvarium during foetal life is similar in the Uda and Yankasa. However, the stages of calvarium development occur earlier and faster in Yankasa than Uda of the same ages. This implies that foetal development and time of parturition could occur earlier in the Yankasa compared to the Uda breeds.

Keywords: Anterior fontanelle, Calvarium, Osteoblast, Radiopaque, Skull

Introduction

The bones of the skull are divided into the viscerocranium, which forms the face and the neurocranium, which forms the cranium that surrounds the brain. The neurocranium is subdivided into the base and the calvarium (Rice, 2008). The fontanelles are located in the calvaria, where two to three bones converge (Jane, 2000). In humans, at birth, fontanelles are large, but as the growth of the calvarial bones progresses after birth, their size rapidly reduces (Rice, 2008). Fontanelles are flexible structures that allow the temporary compression of the calvarium during parturition (Rice, 2008). Any bone may be classified in one of the following groups; long, short, flat, sesamoid, pneumatic or irregular bone (Dyce *et al.*, 2017). The flat bones of the skull develop by intramembranous ossification in sheets of well-vascularised mesenchyme. Some mesenchymal cells differentiate into osteoblasts which produce an osteoid matrix. Subsequently, this matrix becomes calcified forming bone spicules surrounded by a layer of osteoblasts. When more spicules form in the same location and increase in thickness by the process of appositional growth, they become interconnected forming a trabecular network of cancellous/flat bone, referred to as an ossification centre (Caetano-Lopes *et al*., 2007). The development of the skull has been extensively studied in humans. However, there are few pieces of literature on the development of the skulls in animals such as Camelidae skulls (Hena *et al*., 2012; Arencibia *et al*., 2005) and Bovine (Teja & Rajendranath, 2017). Evidence of study conducted by the authors on the histomorphology and radiography of the developing ovine skull revealed a dearth of information on the developmental anatomy of the ovine skull. Therefore, this study aims to bridge the gap by evaluating prenatal skull radiography and calvaria histogenesis in Uda and Yankasa breeds of sheep. The information in this study could be of significance in remedying the gap of information regarding the developmental anatomy of this animal species, as well as regarding its application in surgical interventions involving the brain.

Materials and Methods

Skull radiography

Sample collection: The ethical approval for this study was obtained from the Institutional Animal Care and Use Committee of Usmanu Danfodiyo University Sokoto with the reference number UDUS/FAREC/2019/AUP-RO-17. A total number of 40 wasted foetuses (20 each of Uda and Yankasa breeds of sheep) from slaughtered pregnant dams and 30 day-old postnatal samples (15 each of Uda and Yankasa breeds of sheep) were used for this study. The Uda and Yankasa foetal samples were sourced from Sokoto and Kaduna State abattoirs, respectively. The foetuses were aged and grouped into trimesters (Karen *et al*., 2001). The heads of the foetuses and day-old samples were decapitated and exposed to radiographs at the radiography laboratory of the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, using a portable X-ray machine (PLH Medical Ltd, Watford Herts, UK).

The foetal skulls were exposed to an X-ray. The skulls were placed on the radiographic film cassette loaded with an X-ray film (AGFA DT2B India and FUJI Japan Tokyo, size 24x30cm). A lateral position was adopted to obtain two standard radiographic views with appropriate exposure factors (Sirois & Anthony, 2009). A radiographic factor of 10mAs and 50KVp and 10mAs and 55KVp were used for second and third-trimester foetal heads, respectively, while a radiographic factor of 12 mAs and 48 KVp were used for day-old head samples. The processed X-ray films were illuminated and scrutinized using an X-ray film illuminator to determine the ossification process of the skull, and the radiographic images were captured using a Digital Camera and transferred to a Personal Computer.

Fetal and day-old histomorphology of the calvarium

Bone samples ($1cm^2$) were taken from frontal, parietal and occipital bone segments across all the prenatal and day-old age groups for histological technique. The samples were preserved in 10% formalin solution and decalcified in a solution consisting of 40mls of 10% nitric acid, 30mls of absolute alcohol, and 30mls of 0.5% chromic acid for three days and four days for foetal and day-old samples respectively before processing for routine Haematoxylin and Eosin technique according to a method described by Cardiff (2014).

Results

Fetal skull radiography

Second-trimester foetal skull radiography: The radiograph of the skull of the second-trimester foetuses of Uda and Yankasa showed the developing skull bones which appeared radiopaque. The skull of the early second trimester foetuses of the Yankasa appeared smaller in size than the Uda foetuses of corresponding age and crown vertebral rump length (CVRL) on the radiograph. The parietal and frontal bones were yet to fully ossify; as such, the neurocranium has a radiolucent area at the anterior fontanelle and the size of the radiolucent area varies depending on the foetal size, the radiolucent anterior fontanelle increases in size as the foetus grows bigger towards the late second trimester. The borders of the orbit and the teeth of the upper and lower jaws were yet to appear (Plate I).

(Inferior and superior alveoli) at the late third trimester. It was observed that the zygomatic process of the frontal bone and frontal process of zygomatic bone ossified and articulate to complete orbital margin at the caudal border of the orbit. A developing external auditory meatus was observed (Plate II).

Third-trimester foetal skull radiography: In the two breeds, the anterior fontanelle began to fuse (close) in the third trimester. The molar, premolar, and

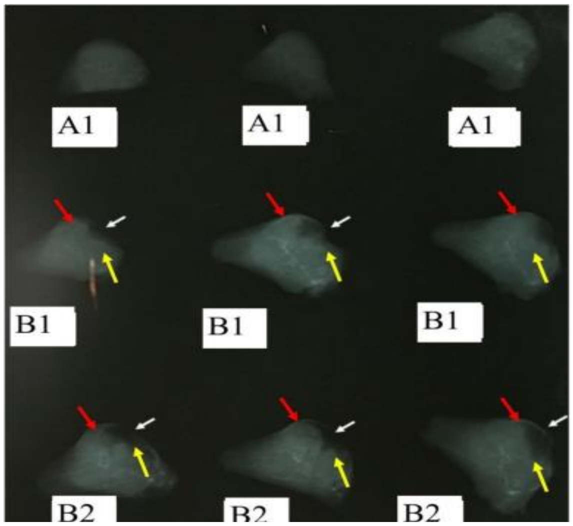

Plate I: A left lateral view of the radiograph of the skulls of the early second trimester foetuses in Yankasa (A1) and Uda (B1) and late second trimester foetuses in Yankasa (A2) and Uda (B2) showing the anterior fontanelle (white arrow), frontal bone (red arrow) and parietal bone (yellow arrow)

incisors teeth began to develop within the mandibular and maxilla alveolus.

Day old skull radiography

In the two breeds, the anterior fontanelle was closed, the external auditory meatus was fully ossified (radiopaque) and prominent, and the caudal margin of the orbit was complete. The upper premolar and molar of the maxilla and the incisors, premolar and molar of the mandible, began to erupt above their respective alveolus (Plate III).

Histomorphology of the foetal and day-old calvarium

In the early first trimester, foetuses (42-44 days of gestation) of the Yankasa and Uda, the histomorphology of the skull calvarium was free of osteocyte cells, osteoclast cells, bone spicules, trabeculae, and spongy bones. It has three (3) layers, one (1) middle layer containing osteoblast cells bounded by two (2) periosteal layers, largely dominated by connective tissues, collagen fibres, and spindle-shaped mesenchymal cells (osteogenic cells). However, the first trimester foetuses had numerous mesenchymal cells. The periosteal layers on both sides had two subdivisions, the periosteal and subperiosteal layers (Plate IV).

In the late first trimester foetuses (48-50 days of gestation) of the Yankasa and early second trimester foetuses of the Uda (51-53 days of gestation), the

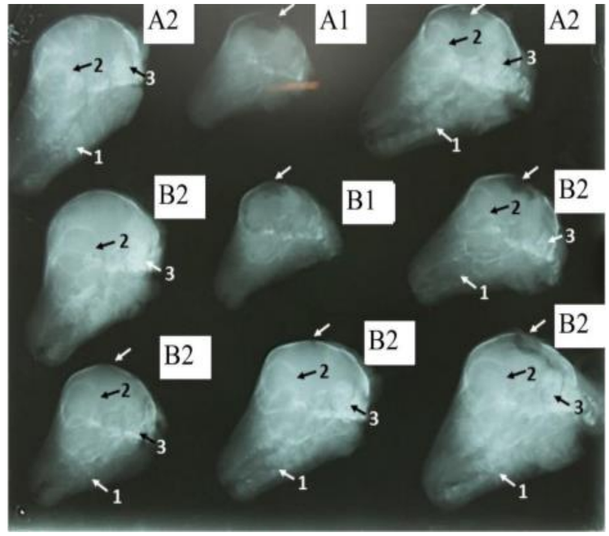

Plate II: A left lateral view of the radiograph of the skulls of the early third trimester foetuses in Yankasa (A1) and Uda (B1) and late third trimester foetuses in Yankasa (A2) and Uda (B2) showing the anterior fontanelle (arrow), teeth (1), complete orbital margin (2), and external auditory meatus (3)

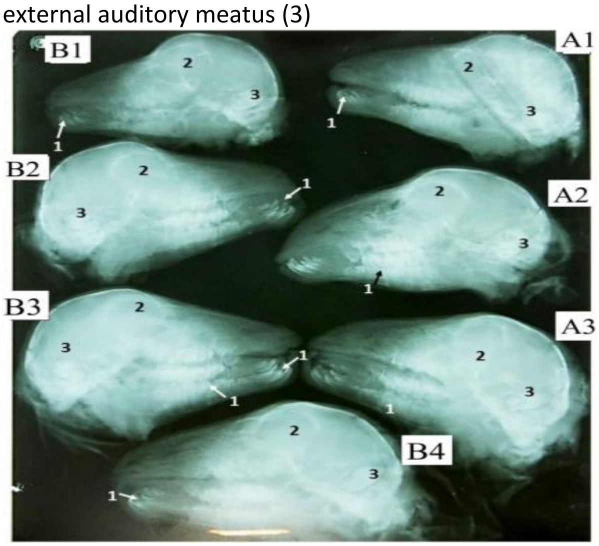

Plate III: A left lateral view (A1 – A3, B1 and B4) and right lateral (B2 and B3) of the radiograph of the skulls of the day-old Yankasa (A) and Uda (B) showing premolar, molar, and incisor teeth (1), orbit (2), and external auditory meatus (3)

mesenchymal cells in the periosteal layers develop into osteoblast cells; these osteoblast cells gather to form ovoid-shaped colonies at the periosteal ends of the middle layer, these colonies are referred to as the ossification centres. These colonies of osteoblast cells secrete collagen matrixes, which are seen to be surrounded by the osteoblast cells then form segments of primitive bone spicules, which developed into spicule bone spicules. The bone spicules were surrounded by the osteoblast cells and bounded on both sides by the periosteal layers (Plates V - VI). These processes continued into the second and third-trimester foetuses of the Yankasa and Uda. However, the first trimester foetuses had numerous mesenchymal cells. In the second trimester, the segments of the bone spicules began to appose to form primitive trabeculae (Plates VII-VIII). In the third trimester, the primitive trabeculae further appose with new bone spicules, increase in sizes, and further ossify to form matured trabeculae with connective tissue spaces (Plates IX - X). These trabeculae further develop/convert into a primitive spongy bone with interosseous spaces in a day-old Yankasa and Uda (Plates XI-XII).

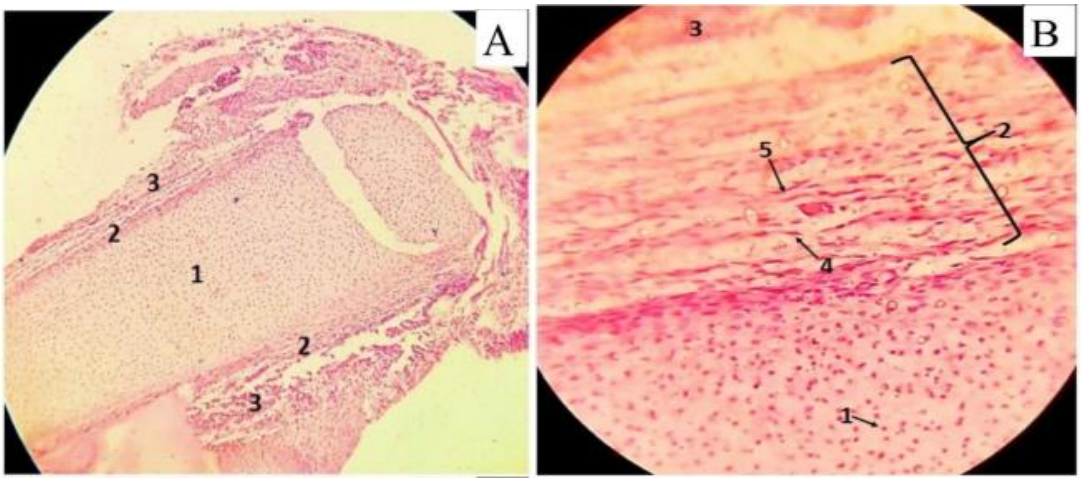

Plate IV: Photomicrographs of a transverse section of an early first trimester Yankasa frontal bone calvarium foetus showing the osteoblast cells **(1)**, subperiosteal bone layers **(2)**, periosteum **(3)**, mesenchymal/ostegenic cells **(4)**, collagen fibers **(5)**, X100 **(A)** and X400 **(B)**, H and E

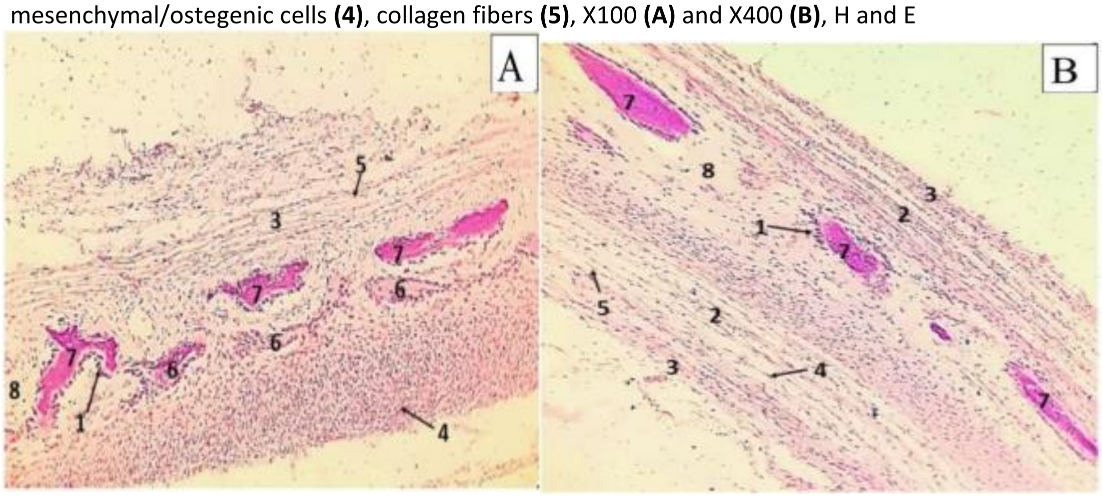

Plate V: Photomicrographs of a transverse section of a late first trimester Yankasa (A) and early second trimester Uda (B) frontal bone calvarium showing the osteoblast (1), subperiosteal bone layers (2), periosteum (3), mesenchyme cells (4), collagen fibers (5), developing/primitive bone spicules (6), developed bone spicules (7), and connective tissue (8) X100, H and E

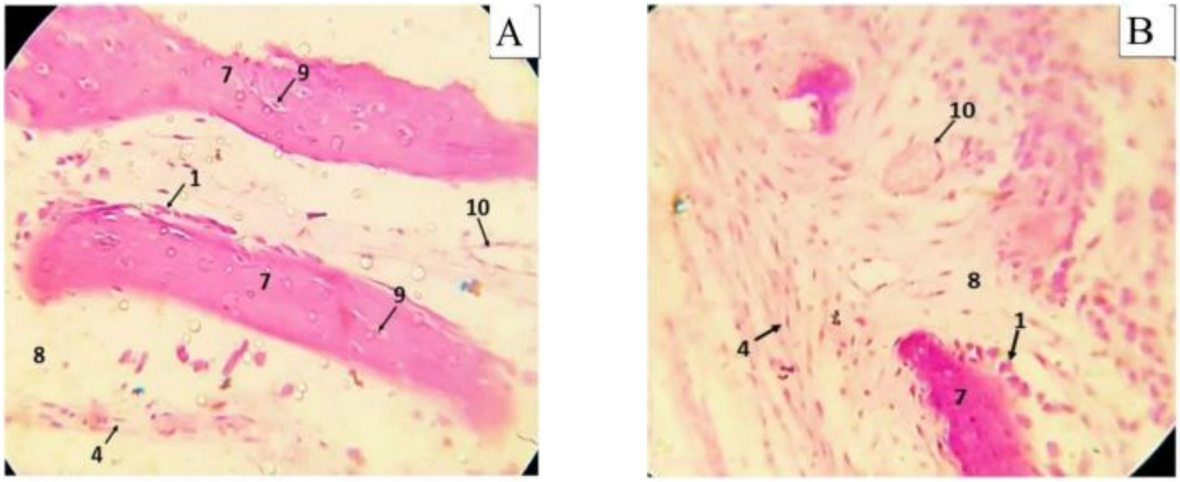

Plate VI: Photomicrographs of a transverse section of a late first trimester Yankasa (A) and early second trimester Uda (B) frontal bone calvarium showing osteoblast (1), mesenchyme cells (4), bone spicules (7), connective tissue (8) osteocytes (9), and emerging osteoblast cell colonies (10) X 400, H and E

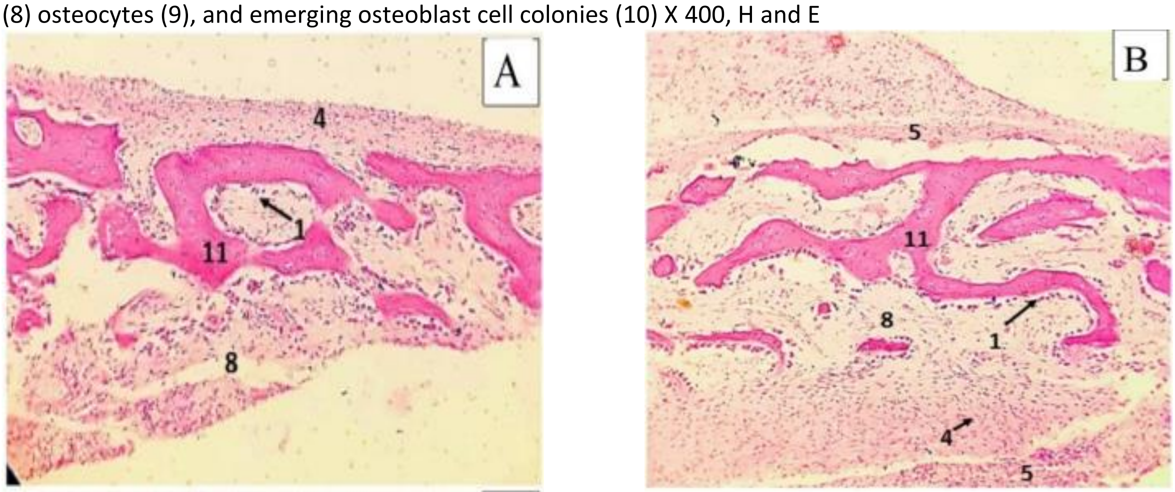

Plate VII: Photomicrographs of a transverse section of a second trimester Yankasa (A) and Uda (A) frontal bone calvarium showing the osteoblast cells (1), mesenchyme cells (4), periosteum (5), connective tissue (8), and primitive trabeculae (11), X400, H and E

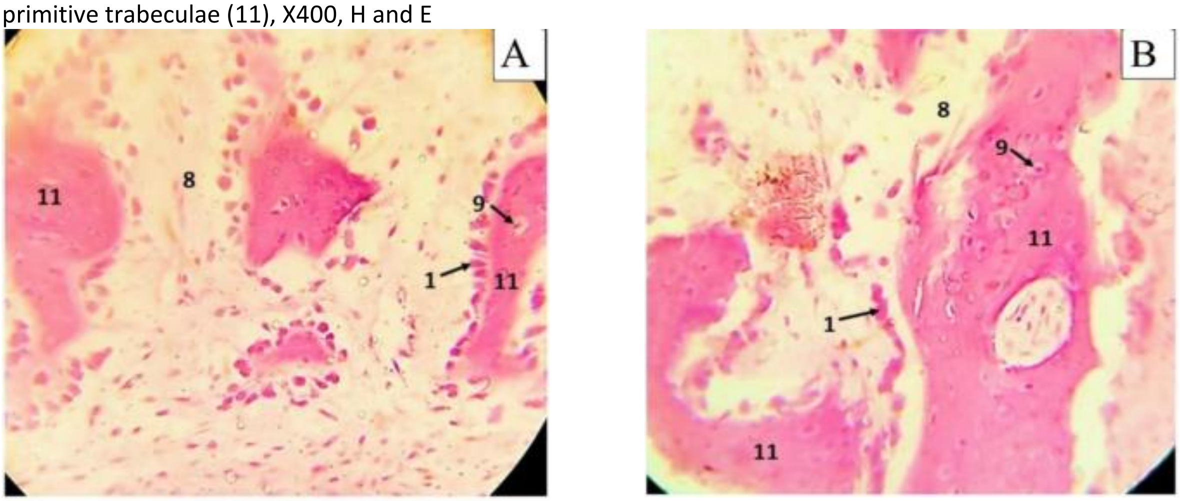

Plate VIII: Photomicrographs of a transverse section of a second trimester Yankasa (A) and Uda (B) frontal bone calvarium showing the osteoblast (1), osteocytes (9), connective tissue (8), and primitive trabeculae (11) X400, H and E

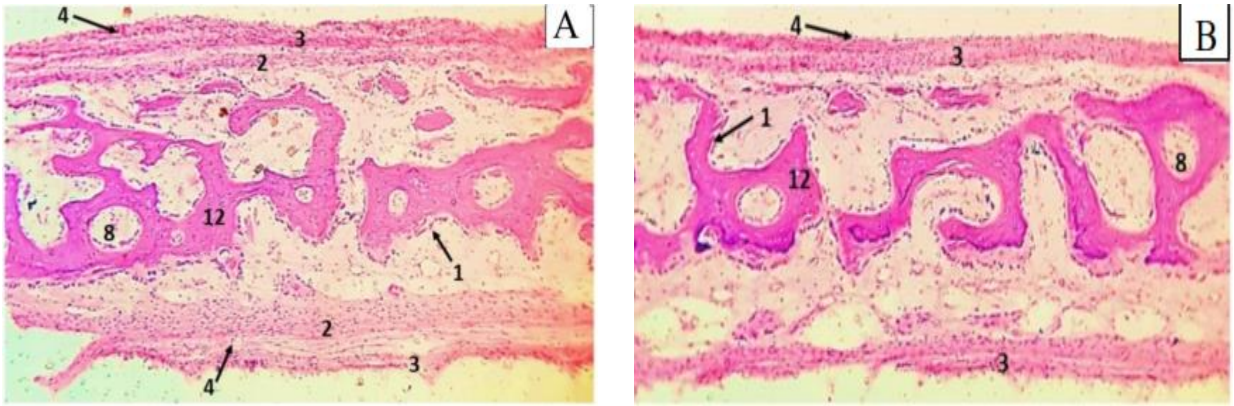

Plate XI: Photomicrographs of a transverse section of a day-old Yankasa (A) and Uda (B) frontal bone calvarium showing the osteoblast (1), periosteum (3), primitive spongy bone (13), and interosseous spaces (14) X100, H and E

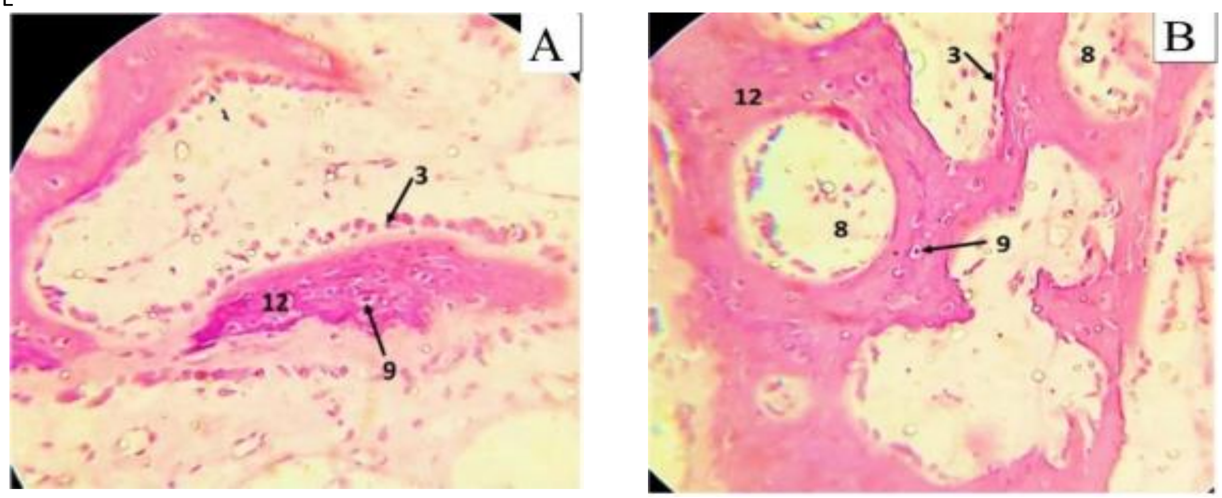

Plate X: Photomicrographs of a transverse section of a third trimester Yankasa (A) and Uda (B) frontal bone calvarium showing the osteoblast (3), connective tissue spaces (8), osteocytes (9), and matured trabeculae (12) X400 H and E

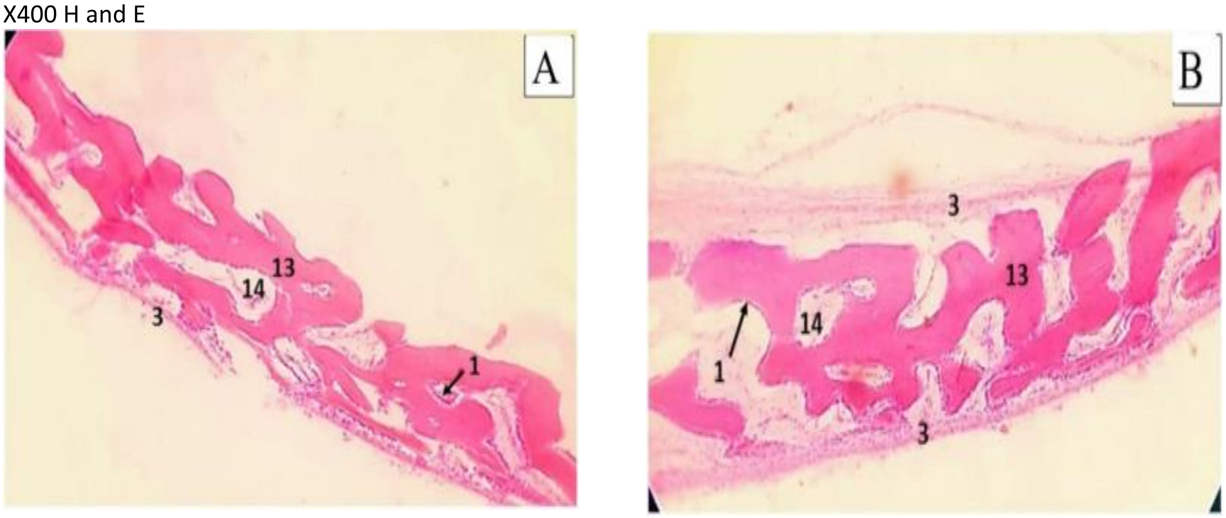

Plate XI: Photomicrographs of a transverse section of a day-old Yankasa (A) and Uda (B) frontal bone calvarium showing the osteoblast (1), periosteum (3), primitive spongy bone (13), and interosseous spaces (14) X100, H and E

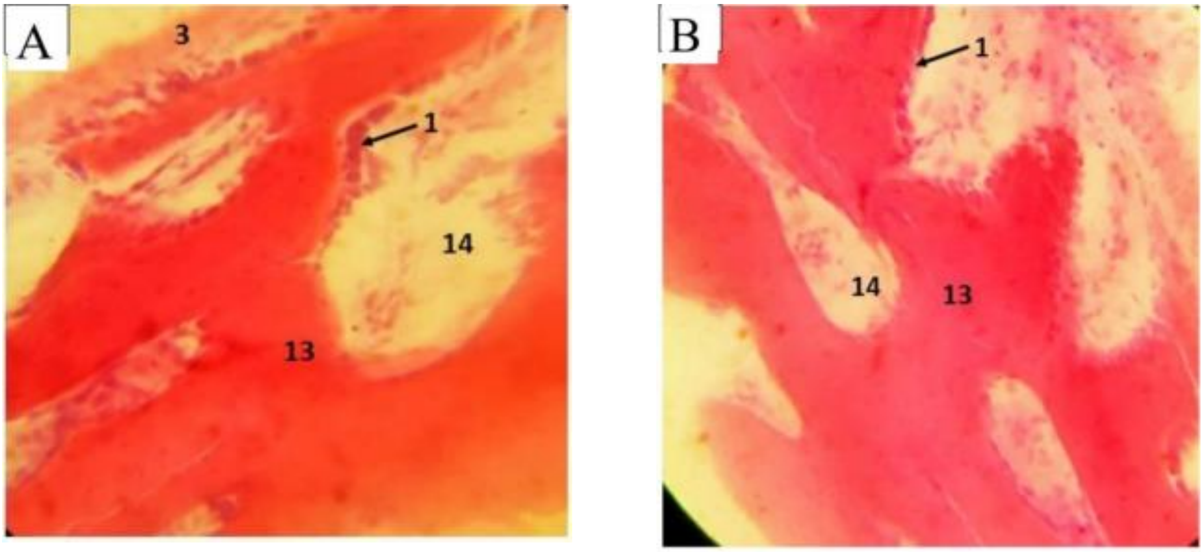

Plate XII: Photomicrograph of a transverse section of a day-old Yankasa (A) and Uda (B) frontal bone calvarium showing the osteoblast cells (1), periosteum (3), primitive spongy bone (13), and interosseous spaces (14), X400, H and E

Discussion

The radiographic observations of the foetal skulls made in this study revealed the decalcification of the skull bones of the second-trimester foetuses of the Uda and Yankasa through the radio-opaque appearance of the neurocranial and viscerocranial bones. This is similar to studies on foetal camel skulls (Hena *et al*., 2012). The difference in the skull of the early second trimester foetuses of the Yankasa and Uda foetuses could be associated with the small-sized nature of the adult Yankasa breed. The radiolucent area is seen at the anterior fontanelle of the second trimester, suggesting the presence of soft tissue or poor ossification, and the chronological increase in the size noted in this anterior fontanelle can be associated to the subsequent increase in the size of the calvarium. The presence of the fontanelles is to allow further increase or growth of the skull (Rice, 2008). In the third trimester, the radiolucent areas of the anterior fontanelle began to reduce, signifying progress in its calcification or closure. In the day-old, the anterior fontanelle was said to be closed because of the absence of the radiolucent areas noted in the second and third trimesters. The anterior fontanelle was said to persist after birth and appear as a soft spot, which closes after 2 years of birth (Rice, 2008).

No evidence of the development of the teeth of the upper and lower jaws was seen at the second trimester, at the third trimester (52-102 days), the calcification of the teeth of the upper and lower jaws (molars, premolars and incisors) became evident as shown by the presence of radiopaque dental germs within the maxilla and mandibular alveolus, this suggests the onset of development or growth of these teeth. This is similar to the work of Soanag *et al*. (1997), who reported that the time of appearance of the first maxilla dental germ in the Italian breed of cow was 97 days of gestation; however, in this cow, only the third premolar was seen (Soanag *et al*., 1997). In the day-old, the emergence of the upper premolar and molar of the maxilla and the incisors, premolar, and molar of the mandible from their alveolus into the buccal cavity could indicate progress in teeth ossification and lengthen. In the second trimester, the incomplete radiopaque circle that forms the margin of the orbit at the caudal border suggest that the calcification of the zygomatic process of the frontal bone and the frontal process of the zygomatic bone which form was yet to occur and in the third and day-old trimester, the complete radiopaque orbital margins noted suggest complete calcification and articulation of the zygomatic process of the frontal bone and frontal process of the zygomatic bone. In the third trimester, the spiral-shaped opaque region observed at the caudal parts of the skull indicates the ossification of the external auditory meatus became well established at day-old due to the increased calcification and size noted. In the day-old, the external auditory meatus was fully ossified.

The histological findings in this study, whereby the mesenchymal cells transformed into osteoblast, bone spicules, trabeculae and spongy bone, respectively revealed that the skull calvarium developed via intramembranous ossification (Moore *et al*., 2015). It has been documented in humans that flat bones of the skull develop by intramembranous ossification in sheets of well-vascularized mesenchyme (Moore *et al*., 2015). The stages and processes of bone tissue development of the skull calvarium observed in this study are similar to that of humans, where it was reported that some mesenchymal cells differentiate into osteoblasts which produce an osteoid matrix (Gilbert, 2010). Subsequently, this matrix becomes calcified forming bone spicules surrounded by a layer of osteoblasts (Schoenwolf *et al*., 2015). Appositional growth is the increase in the diameter of bones by the addition of bony tissue at the surface of bones (Gilbert, 2010). When more spicules form in the same location and increase in thickness by the process of appositional growth, they become interconnected forming a trabecular network of cancellous bone referred to as an ossification centre (Cochard *et al*., 2012). These stages and processes of bone tissue development occur faster and earlier in the Nigerian breeds of sheep compared to a human, probably because they have shorter trimester and gestation periods. The radiographic and histomorphologic development of the calvarium in this study showed that the development occurs faster in Yankasa than in Uda; this could imply that foetal development and parturition could occur earlier in the Yankasa compared to the Uda breeds.

In conclusion, radiographically, the anterior fontanelle appeared and remained open throughout the foetal life and closed at day-old. The histogenesis of the calvarium during foetal life is similar in the Uda and Yankasa; however, the stages of development occur earlier and faster in Yankasa than Uda of the same ages. This means that foetal development and time of parturition could occur earlier in the Yankasa compared to the Uda breeds.

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Conflict of interest

The authors declare that there is no conflict of interest.

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