PRENATAL DEVELOPMENT OF RADIO-ULNAR AND TIBIO-FIBULAR BONES IN RED SOKOTO GOAT (*CAPRA HIRCUS*): A HISTOLOGICAL PERSPECTIVE

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ABSTRACT
Ability of ruminate neonates to run with the herd hours after birth necessitated this study. This study, therefore, was carried out to evaluate morphometry of radio-ulnar and tibio-fibular bones during prenatal period in Red Sokoto goat (RSG) and determined the specific gestation days of primary and secondary ossifications. A total of 45 abbatoir-sourced foetal samples obtained from slaughter houses were used for the study. The foetuses were categorized into eight age groups, based on established aging formulae. The radio-ulnar and tibio-fibular bones of foetuses were collected and prepared for histological examination. Results showed that primary ossification centre of the diaphysis was established by gestation day (GD) 54 in RSG fetuses with formation of tiny bone spicules in the diaphysis. This ossification spread towards the epiphysis by GD 57 while the epiphysis remained quiescent. Secondary ossification centre in the epiphysis was observed by GD 65 with numerous cartilage canals which coalesced into larger ones by GD 75, all associated with osteogenic tissues. Well advanced secondary ossification centre was observed by GD 90. Well-developed epiphyseal cartilage which separated well-formed and advanced diaphyseal and epiphyseal bones was observed at GD 105. Complete ossification of the diaphysis and epiphysis, leaving an epiphyseal plate, was observed at GD 124.
Key words: Red Sokoto goat; Foetal age; Radio-ulnar bone; Tibio-fibular bone; Ossification; Gestation

INTRODUCTION
The skeletal system stands out as one of the body structures that have been used for the characterization of different species of animals (Lochi *et al.*, 2014). Bone is the basic unit of the mammalian skeleton, providing the framework for weight bearing, protection of internal vital organs and hosts of other functions (Burr, 2019). The morphological development of mammalian bones is very important for diagnoses of skeletal diseases in young animals (Thompson et al., 2008; Ren et al., 2021). Mammalian appendicular bones are first seen as cartilage models and at precise periods during embryonic development, are replaced by complete bone through pre-existing cartilaginous model in a process of endochondral ossification (Carlson, 2014). To survive, ruminant neonates must have a fully developed and functional skeletal system to enable them run with the herd within hours of delivery. The goat, a ruminant, and the first herbivore to be domesticated have a worldwide distribution due to its remarkable adaptability to extreme climates and difficult terrain (Hirst, 2008). The ability of a kid to run with the herd within an hour of delivery shows a fully functional skeletal system that developed prenatally**.** Most studies on goats were on the prenatal and postnatal development of its internal organs (Kalita *et al.*, 2000; Nwaogu and Okolie, 2008; Abiaezute *et al*., 2021). Thus, the need to study prenatal development of the long bones of the indigenous Red Sokoto goat.

MATERIALS AND METHODS
A total of 45 goat fetuses collected as abattoir waste from slaughter houses in Nsukka Local Government Area of Enugu State Nigeria were categorized into eight groups starting from gestation day (GD) 50 – 130 using the Sivachelvan *et al.* (1996) aging formula for Red Sokoto goat fetuses. The radio-ulnar and tibio-fibular were dissected out and fixed by immersion in Bouin's fluid. Thereafter, the bones were decalcified in hydrochloric acid and prepared routinely for histology as described by Suvarna *et al.* (2018). Five µm thick sections obtained with the rotary microtome were mounted on clean glass slides, routinely stained with hematoxylin and eosin and studied under the light microscope. Photomicrographs were captured using Moticam Camera 1000 (Motic China group Ltd., Xiemen, China).

RESULTS

The radio-ulnar and tibio-fibular bones of the RSG fetuses consisted of a shaft and two extremities. The radio-ulna was a fused bone of the radius and ulnar bones while the tibio-fibular showed a stump of the fibular attached laterally to the proximal extremity of the larger tibia. Histological evaluation showed that at gestation day (GD) 51, both bones were only cartilaginous models surrounded by perichondrium and mesenchymal connective (Figure 2A). However, by GD 54, the primary center of ossification had already been established in their diaphysis which showed hypertrophied chondrocytes of the cartilage model on one side and tiny bone spicules on the opposite side (Figure 2B). At GD 57, the endochondrial ossification within the diaphysis had spread towards the two epiphyses, showing the chondrocytes close to the epiphysis were better organized with the different zones involved in ossification and ossified bone trabeculae (Figure 4C). At GD 60, the epiphysis was observed to still be in the cartilage forms (Figure 4D). By GD 65, numerous evenly spaced cartilage canals (blood vessels) were noticed within the epiphysis (Figure 5A) that increased in diameter and size. These canals, by GD 73, had coalesced into larger blood vessels surrounded by osteogenic tissues (Figure 5B) while between the diaphysis and epiphysis, the five zones were easily identifiable. At GD 90, the epiphysis had developed advanced secondary centre of ossification (Figure 6A) and at GD 105 the ossification of the epiphysis was in the advanced stage with numerous spongy bones and few areas of hypertrophied cartilage (Figure 8). At GD 129, the plane of ossification from the epiphysis was separated from that of the diaphysis by the epiphyseal cartilage (Figure 9).

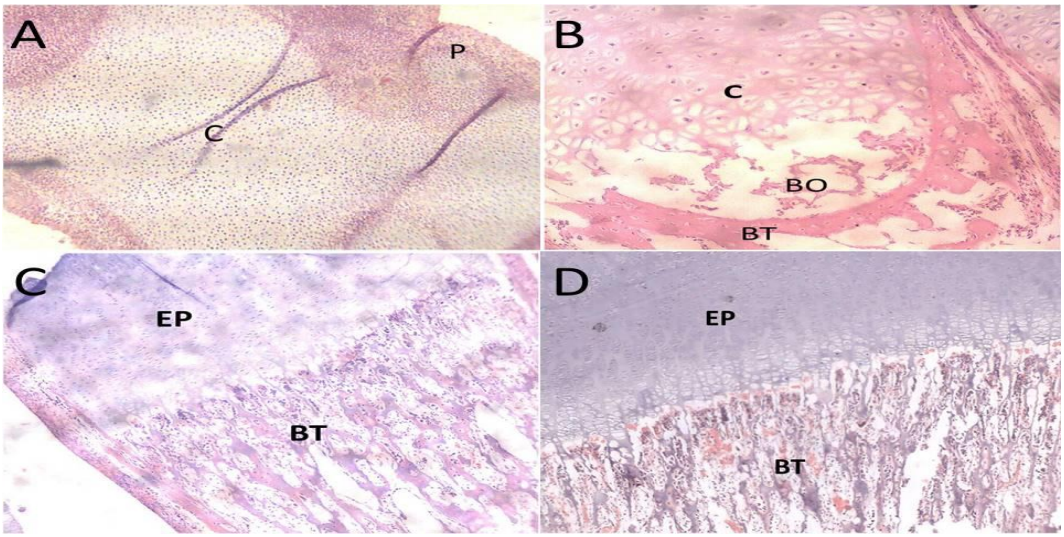

Figure 1. Photomicrograph of radio-ulna (A and B) and Tibia-fibula (C and D) showing stages of endochondrial ossification in Red Sokoto goat fetuses.

A – Gestation Day (GD) 51 showing the diaphysis that was all cartilaginous (C) with a covering pericondrium (P). B – GD 54 showing area of hypertrophied cartilage chondrocytes (C) and initial deposition of tiny bony spicules (BO) and trabeculae (BT). C and D – GD 57 and 60 respectively, showing active areas of diaphyseal ossification with well-defined zones and bony trabeculae (BT) and relatively quiescent cartilaginous epiphyseal areas (EP) respectively. H & E, × 40

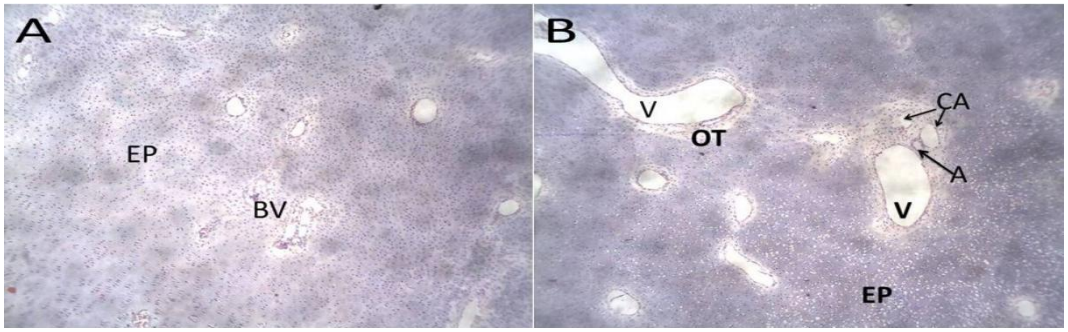

Figure 5. Photomicrograph of radio-ulna showing early stages of secondary epiphyseal ossification.

A – Gestation day (GD) 65 section showing numerous cartilage canals containing blood vessel and nerves (BV) that invaded the epiphysis (EP). B – GD 73 section, showing the cartilage canals that coalesced into larger canals containing venules (V), arterioles (A) and capillaries (CP) surrounded by osteogenic tissues (OT). H & E. × 40

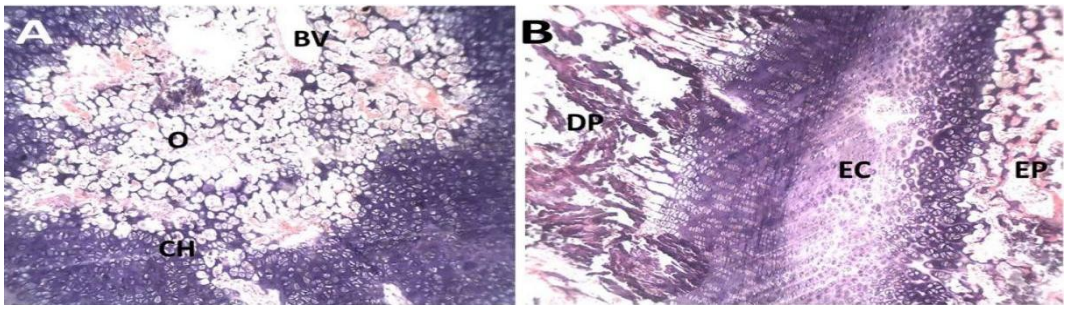

Figure 6. Photomicrograph of Tibio-fibula, showing advanced stage of epiphyseal and diaphyseal ossification. A – Gestation day (GD) 90 section, showing well-defined secondary centre of ossification (O) with hypertrophied chondrocytes (CH) and blood vessel (BV). B – GD 105 section, showing well developed epiphyseal cartilage (EC) demarcating the ossifying diaphyseal (DP) and epiphyseal (EP) areas.

Discussion

The observation of only cartilaginous models in the foetuses below GD 51 suggests that bone formation had not been initiated. Similarly, long bone formation was reported to commence in GD 55 in ewe fetuses (Cerny and Brandstatter, 1990) indicating near similarity in ages of bone formation in goats and sheep. Wenham (1981), further clarified that in sheep fetuses, the bones of the body ossify at different gestation days with first observance of primary ossification centre being in the mandibular bones at GD 41, followed by the ribs, humerus, femur, tibia, radius, ulna and metacarpals at GD 55. Furthermore, differences in the number and age of observation of ossification centres occurring both within and between litters of the same gestational age has been noted (Wenham, 1981). Moreover, Shapiro (2008) and Danmaigoro *et al*. (2020) suggested that long bone formation occurs in the second term of gestation in goat fetuses. This observation may account for the absence of ossification centres before GD 51 in the RSG fetuses in this study. Similarly, the epiphyseal area showed lack of bone formation during this period which was comparable to the observations reported by Danmaigoro et al. (2020).

Observation of bone trabeculae in the diaphysis after GD 51 indicated initiation of bone ossification in RSG fetuses. The gestation period of goat doe is 145-50 days, showing that osteogenesis of long bones was initiated during the second term of gestation, similar to the observations of other authors (Ahmed, 2008; Shapiro, 2008; Danmaigoro *et al*., 2020). The mode of the spread of the ossification in this study from the diaphysis to the proximal and distal epiphysis has been reported in other mammals (Ahmed, 2008; Bacha and Bacha, 2012). Furthermore, the present result showed continued deposition of trabeculae in the diaphysis at GD 60 with no noticeable blood vessels in the epiphysis. However, the appearance of blood vessels in the epiphysis at GD 65 indicated initiation of ossification which culminated in the visualization of the secondary centres of ossification in the epiphysis. The structural changes the foetal diaphysis and epiphysis underwent in this study which included increase in deposition and mineralization of the bone tissues with the ultimate formation of long bones that can carry weight of the neonates hours after birth is an indication that fetal bone formation were completed before birth.

CONCLUSION

Study showed that lengths and weights of the radio-ulnar and tibio-fibular bones of RSG increase with advancing age of the foetuses. It was also found that primary ossification of the diaphysis in the RSG foetuses sampled started at gestation day 54, while secondary ossification in the epiphysis was observed by GD 65. Complete ossification of the diaphysis and epiphysis was observed at GD 124 suggesting that the bones of the RSG foetuses were completely formed before parturition.

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