

PRENATAL DEVELOPMENT OF THE SKULL DURING THE FIRST TRIMESTER
OF PREGNANCY IN THE BUFFALO (*Bubalus bubalis*)

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ABSTRACT

This study on the prenatal development of the skull during the first trimester of pregnancy in the buffalo (*Bubalus bubalis*) was conducted at Department of Veterinary Anatomy, College of Veterinary Science, Tirupati. A total of 100 buffalo embryos and foetii starting from the 26th to the 89 day were collected from different slaughter houses. The specimens were fixed and processed for serial paraffin sectioning, and the sections were subjected to different staining methods. The earliest indication of skull development was first recognized as a mass of dense mesenchyme enveloping the cranial end of the notochord at 27 days, followed by formation of the basal plate at 38 days. The ossification of the skull first appeared in the mandible, maxilla and malar bones at 45 days. Most of the chondrocranium was cartilaginous at 45 days. The chondrocranium began to ossify first at 62 days in the basioccipital and exoccipital, sphenoid and tympanic bulla. Most of the skull was ossified during the 45th to the 89th days of gestation in buffalo, during which period proper feeding of the mother by farmers is advised.

Keywords: prenatal development, skull, first trimester, buffalo

INTRODUCTION

The skull is most important, complex and specialized part of the axial skeleton, for it lodges the brain and sense organs. The development of the skull was studied in bovine by Matthews (1972). The prenatal development of buffalo skull had received very little attention among farm animals. Sequences in development of the skull will help in understanding the basis of developmental anomalies. The literature pertaining to developmental features of the skull during the first trimester of pregnancy is not available. Hence the present study was taken up.

MATERIALS AND METHODS

A total 100 apparently healthy buffalo embryos and foetii starting from the 26th day were collected from different slaughter houses. The study was conducted at Department of Veterinary

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Anatomy, College of Veterinary Science, Tirupati as a part of the ICAR ad hoc scheme "Pre and Post natal development of the Skull in the Buffalo (*Bubalus Bubalis*)". The CVRL (Curved Crown Rump Length) of specimens was measured and the age of the specimens was estimated by adopting Soliman (1975) formula coined for buffalo, i.e., $Y=28.66 + 4.496 X$ if CVRL is ≤ 20 cm and $Y=73.544 + 2.256 X$ if CVRL is ≥ 20 cm where Y is the age in days and X is the curved crown rump length in centimeters.

All the embryonic specimens and heads of foetii from 50 days were fixed in 10% buffered neutral formaline and Bouin's fluids and processed for serial paraffin sections of 6-8 μ thickness. The foetal heads of 70 days (9.2 cm CVRL) and above were subjected to decalcification by the formic acid - sodium citrate method after fixation (Singh and Sulochana, 1997). The sections were subjected to Mayer's haematoxylin and eosin method (Singh and Sulochana, 1997), the Alcian blue staining method to differentiate cartilaginous tissue from bony tissue and Von Kossa staining method for calcium deposits (Humason, 1967) to study the sequences in the development of skull. The foetal heads beyond the age of 70 days (9.2 cm CVRL) were stained by the Alizarin Red S method for studying extent of bone formation (Humason, 1967).

RESULTS AND DISCUSSION

Developmentally the bones of the buffalo skull were divisible into four groups i.e. Chondrocranium (cartilaginous neurocranium), Desmocranium (membranous neurocranium), Membranous viscerocranium and Cartilaginous viscerocranium based on developmental origin and nature of ossification. The skull was formed by

the fusion or joining of the above four originally separate components.

The mesenchymal, precartilaginous, cartilaginous and ossification stages were observed in the development of skull. The bones of skull were developed by differentiation of mesenchymal or cartilaginous cells into osteoblasts. The skull was completely mesenchymal at 26 days with otocysts and optic vesicle.

The earliest indication of skull formation was observed at 27 days (Figure 1) as a mass of dense mesenchyme around the cranial end of the notochord. It was followed by formation of the basal plate by the differentiation of occipital somites and paraxial mesoderm at 38 days. The basal plate cartilage formed the basioccipital, exoccipital and lower part of the squamous occipital. The basal plate formation was reported to be evident in bovines at 32 days of gestation by Matthews (1972). The occipital arches were evident as extensions of basal plate at 43 days. Most of the chondrocranium was in cartilaginous stage at 45 days (Figure 2).

The bones of the chondrocranium consisted of the lower part of the squamous occipital, exoccipital, basioccipital, petrous temporal, tympanic bulla, basisphenoid, presphenoid, ethmoid and turbinates. The frontal, parietal, interparietal and upper part of the squamous occipital formed the desmocranium. The premaxilla, maxilla, palatine, pterygoid except its hamulus, malar, squamous temporal, tympanic ring, lacrimal, nasal, vomer and mandible except its condyle constituted membranous viscerocranium. Meckel's cartilage and its derivatives malleus and incus, mandibular condyle, Reichert's cartilage and its derivatives including stapes, styloid process of temporal, tympanohyoid and hyoid formed the cartilaginous viscerocranium.

Most of the facial bones except the

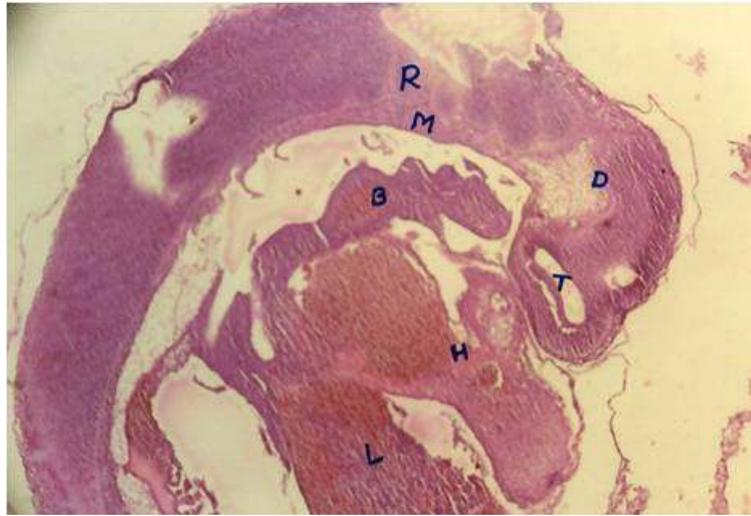


Figure 1. Photomicrograph of sagittal section of a 27 day buffalo embryo showing a mass of dense mesenchyme (M) enveloping the cranial end of the notochord. H & E x 40. Branchial arches (B), Telencephalon (T), Diencephalon (D), Heart (H), Liver (L), rhombencephalon (R) mesencephalon (ME).

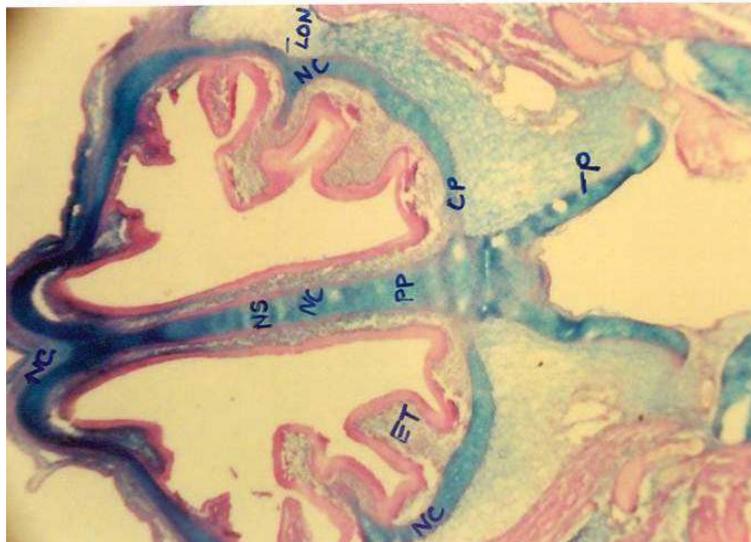


Figure 2. Photomicrograph of frontal section of the head of a 45 day buffalo embryo showing the cartilaginous form of the chondrocranium. Alcian Blue and Kernechtrot methodx 40. Lamina orbitonasalis (LON), Perpendicular plate of ethmoid (PP), Cribriform plate of ethmoid (CB), nasal capsule (NC), prechordal part (P), nasal septum (NS), ethmoturbinates (ET).

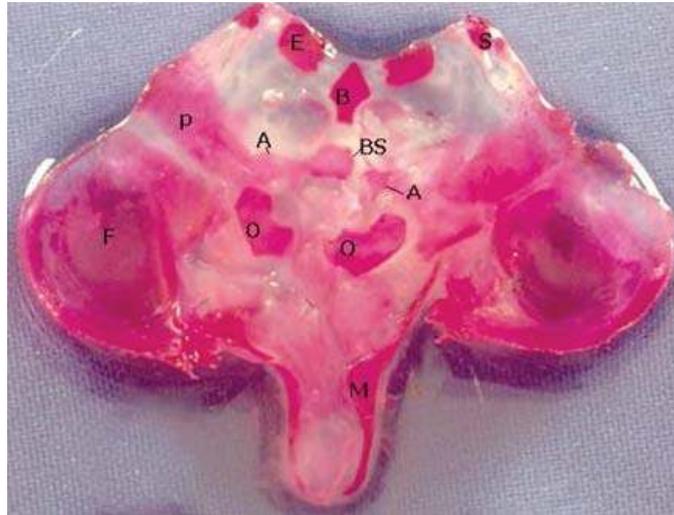


Figure 3. Photograph of an Alizarin Red S stained head of a 83 day buffalo foetus showing ossification centers. basioccipital (B), exoccipital (E), squamous occipital (S), orbitosphenoid (O), alisphenoid (A) and basisphenoid (BS), frontal (F), Parietal (P), Maxilla (M) and Tympanic bulla (T).

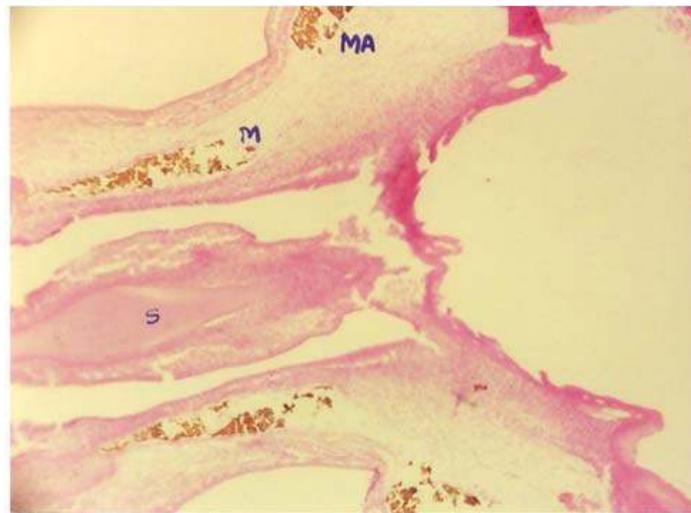


Figure 4. Photomicrograph of the frontal section of the head of a 45 day embryo showing early ossification in the maxilla (M) and malar (MA). Von Kossa method x 40. Septum nasi (S).

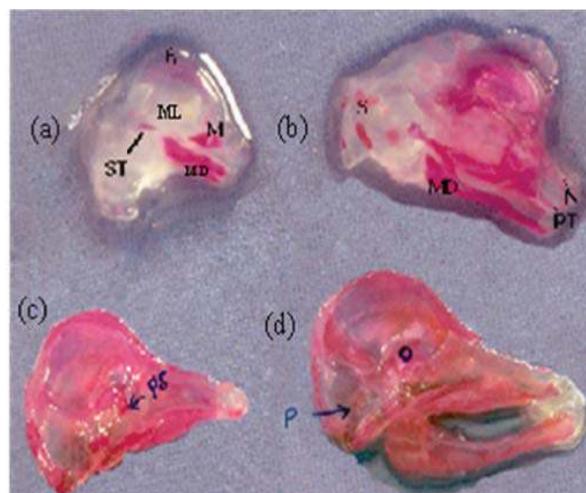


Figure 5. Photograph of Alizarin Red S stained heads (whole and sectioned) of foetuses showing a. 55 days - ossification in the maxilla (M), mandible (MA), squamous temporal (ST), frontal (F) and malar (ML) b. 64 days - early ossification in the lower part of the squamous occipital (S) and tympanic ring (TY) and complete ossification of the nasal (N) and palatine (PT) processes of premaxilla c. 89 days - formation of the body of the presphenoid (PS) by the fusion of the orbitosphenoids (O) and d. 111 days - early ossification with multiple centers in the petrous temporal (P).

mandible, vomer and hyoid as well as calvarial bones were ossified from single centers. The bones of floor of the cranium except the basioccipital were ossified from multiple centers. Most of the ossification centers of skull bones appeared before the end of the third month (Figure 3) except in the case of the petrous temporal, middle ear ossicles, ethmoturbinates and cribriform plate of ethmoid, turbinates and hyoid except stylohyoid.

The ossification of the skull first appeared in the mandible, maxilla and malar at 45 days (Figure 4). The ossification of the mandible was reported to begin at 52 days of gestation in bovines (Soana *et al.*, 1996). The chondrocranium began to ossify first at 62 days in the basioccipital and exoccipital, sphenoid and tympanic bulla. However the ossification of the petrous temporal was observed at 111 days of foetal life (Figure 5). The ossification of the desmocranium first appeared in

the frontal at 49 days while the ossification of the cartilaginous viscerocranium first appeared at 62 days in the stylohyoid.

The prechordal part formed the basisphenoid caudally and the presphenoid cranially while the nasal capsule formed the ethmoid, turbinates, nasal septum and nasal cartilages (Figure 2). The nasal capsule was precartilaginous at 40 days and cartilaginous at 45 days (Figure 2). The nasal capsule showed the beginning of ossification in the perpendicular plate of the ethmoid at 85 days. However, Vidic (1971) reported that chondrification and ossification of the nasal capsule appeared during the early 3rd and 5th month of prenatal life, respectively, and no ossification was noticed for the petrous temporal in the otic capsule till the 3rd month of age in man.

The bone wise appearance of ossification in the skull during the first trimester of pregnancy

in the buffalo was mandible, maxilla and malar (45 days), squamous temporal, frontal, lacrimal and tympanic ring (49 days), palatine (53 days), premaxilla and vomer at 56 days, parietal (60 days), nasal (61 days), stylohyoid, exoccipital, basioccipital, orbitosphenoid, body of basisphenoid, alisphenoid and tympanic bulla (62 days), lower part of squamous occipital and interparietal (64 days) (Figure 5), upper part of squamous occipital (65 days), pterygoid (83 days), perpendicular plate of ethmoid (85 days) and body of presphenoid (89 days) (Figure 5). Most of skull was ossified between the 45th and 89th days of gestation in buffalo, during which period proper feeding of the mother by farmers is advised.

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