

PRENATAL DEVELOPMENT OF THE SPHENOID  
IN THE BUFFALO (*Bubalus bubalis*)

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**ABSTRACT**

The study on ‘Prenatal development of the sphenoid in the buffalo (*Bubalus bubalis*) was conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Tirupati. A total 75 buffalo embryos and foetii starting from the 26<sup>th</sup> day to the 310<sup>th</sup> day were collected from different slaughter houses. The specimens were fixed, processed for serial paraffin sectioning and the sections were subjected to different staining methods. The prechordal part formed the basisphenoid caudally and the presphenoid cranially. The anterior end of the prechordal part joined with the nasal capsule. The prechordal part was in precartilaginous form at 40 days and cartilaginous form at 45 days. The sphenoid was developed from six ossification centers. Ossification was recognized first in the orbitosphenoid, the body of basisphenoid and the alisphenoid at 62 days.

**Keywords:** prechordal part, sphenoid, buffalo

**INTRODUCTION**

The development of the skull was studied extensively in human and bovine by Arthur Keith (1949) and Matthews (1970), respectively.

The fate of the prechordal part of the skull was studied in detail only in human and has received very little attention among farm animals. The literature pertaining to developmental features of the sphenoid particularly in buffalo at different stages is not available. This study will provide a contribution to the existing anatomical knowledge and form the basis for further investigations.

**MATERIALS AND METHODS**

The study was made on 75 buffalo specimens at different embryonic and foetal stages starting from 26 days to 310 days at the Department of Veterinary Anatomy, College of Veterinary Science, Tirupati. The prenatal specimens of unknown age and irrespective of the sex were collected from slaughter houses in and around Hyderabad. The CVRL (Curved Crown Rump Length) of specimens was measured and ranged from 2.1 cm (38 days) to 105 cm (310 days). The age of the specimens was estimated by adopting Soliman's (1975) formula for buffalo, i.e  $Y=28.66 + 4.496x$  if CVRL is  $< 20$  cm and  $Y=73.544 + 2.256x$  if CVRL is  $\geq 20$  cms where Y is the age in d and X is the curved crown rump length in cms.

The embryos for serial microtomy were collected starting from the earliest possible age (26

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days) to study the development of the sphenoid during the prenatal period. The embryonic specimens from 26 to 101 days (16.1 cm CVRL) of age were fixed in 10% buffered neutral formaline and Bouin's fluids and processed for serial paraffin sections of 6-8  $\mu$  thickness (Singh and Sulochana, 1997).

The foetal heads of 70 days (9.2 cm CVRL) and above were subjected to decalcification by the Ffrmic acid - sodium citrate method after fixation (Singh and Sulochana, 1997) and the sections were subjected to Mayer's Haematoxylin and Eosin staining method for routine developmental study (Singh and Sulochana, 1997), Von Kossa staining method for calcium deposits (Humason, 1967), the Alcian blue staining method to differentiate cartilaginous tissue from bony tissue (Humason, 1967) and the Bismarck brown - methyl green method to differentiate cartilaginous tissue from bony tissue (Gurr, 1956).

The foetal heads from 70 days (9.2 cm CVRL) to 310 days (105 cm CVRL) were also subjected to the Alizarin Red S method for studying the extent of bone formation (Humason, 1967) and the Alizarin Red S and Toluidine Blue method for differentiating cartilage and bone (Burdi, 1965) in embryos. The slides were viewed under a microscope and the type of ossification, the time of ossification and number of ossification centers pertaining to the sphenoid bone were studied. The heads of the specimens from 90 days (13.7 cm CVRL) to 310 days (105 cm CVRL) of age were utilized to study gross morphological changes by taking various cross and longitudinal sectional profiles of the skulls after dissection.

## RESULTS AND DISCUSSION

The prechordal part was formed, as reported by Matthews (1972) in bovines, at 38 days from the dense mesenchyme ventral to the telencephalon and diencephalon, which lies cranial to the notochord. The anterior end of the prechordal part lying ventral and anterior to the tip of the telencephalon joined with the nasal capsule (Figure 1). The prechordal part was in precartilaginous form at 40 days (Figure 2) and cartilaginous form at 45 days (Figure 3). However Matthews (1972) reported the chondrification of the pterygoid process at 46 days in bovines. The prechordal part of the chondrocranium represented the sphenoid bone. The anterior end joined with the nasal capsules which concurs with the findings of Evans (1974) in dog. The transformation of mesenchyme into cartilage in the basisphenoid and pterygoid process was reported to occur during the second month in human (Arthur Keith, 1949).

The sphenoid consisted of two distinct parts, i.e the presphenoid and the basisphenoid developed in cartilage as described Sethi and Saigal (2002) in buffalo. The sphenoid was developed from six ossification centers, two for the orbitosphenoids, two for the alisphenoids and one each for the body of the presphenoid and the basisphenoid (Figure 4) as reported in human (Arey, 1965) and in bovines (Matthews, 1972). Contrary to this, the sphenoid was reported to be developed from five ossification centers by Hillmann (1975) in horse. However, four and eight ossification centers were reported by de Beer (1937) and Latshaw (1987) in man and domestic animals, respectively. Matthews (1972) observed a separate ossification center for the body of presphenoid making the total number of ossification centers six unlike in the present study wherein the body of the presphenoid was formed

by union of the orbitosphenoids and no separate ossification center was observed for the body. However, Hyman (1942) reported two ossification centers for the basisphenoid in mammals.

The presphenoid was developed from three ossification centers, one for the body of the presphenoid and one each for the wing of the presphenoid (Figure 4) as in rabbit (Bruce, 1941). Contrary to this, the presphenoid of pig was reported to arise as a paired endochondral center in the central stem by de Beer (1937). The presphenoid of human was reported to arise from not less than five centers by de Beer (1937), while Williams *et al.* (1989) recorded six ossification centers for the presphenoid of human.

The orbitosphenoid and alisphenoid were formed between otic and nasal capsules (Figure 3) as reported in domestic animals (Latshaw, 1987) and remained in the cartilaginous stage till 60 days (Figure 5). The orbitosphenoid cartilage was reported to be a derivative of Meckel's cartilage in domestic animals (Latshaw, 1987). In the present study, no evidence for a contribution of Meckel's cartilage in the formation of the orbitosphenoid could be seen. The root of the orbitosphenoids showed early ossification at 62 days, while Matthews (1972) reported the first ossification center of the presphenoid at 60 days. They were rectangular at 83 days (Figure 4) and fused with the body of the presphenoid at 89 days. The orbitosphenoid was the earliest component of the sphenoid complex to show complete ossification, first at 84 days (Figure 6).

The body of the basisphenoid, the alisphenoid and the pterygoid process showed early endochondral ossification at 62 days (Figure 7). Contrary to this, Arey (1965) reported the development of the alisphenoid and the pterygoid process (except the hamulus) by intramembranous

ossification in human, while Presley and Steel (1976) regarded the ossification of the alisphenoid in human as partly cartilaginous and partly membranous. The first ossification center of the basisphenoid was observed at 66 days in bovines (Matthews, 1972). Soana *et al.* (1996) observed the mineralization of the pterygoid process at 117 days in bovines. The body of the basisphenoid, the alisphenoid and the pterygoid process were completely ossified by 92 days (Figure 8). The sella turcica was distinct at 101 days and marked deepening of sella turcica was noticed at 238 days. The dorsum sellae was poorly developed throughout the prenatal period.

The consolidation of the body and the alisphenoids of the basisphenoid took place at 89 days. The basisphenoid was nearly joined with the basioccipital at 126 days while the presphenoid was nearly joined with the the basisphenoid at 132 days, the intervening cartilaginous tissue being minimal. Fusion between the presphenoid and the basisphenoid, between the basisphenoid and the basioccipital did not occur even at 310 days. However, Arthur Keith (1949) reported the union of the presphenoid with the basisphenoid during the eighth month in human. The dorsum sellae was reported to be greatly developed in the early foetal period of human by Arthur Keith (1949). However, in the present study, it was observed to be poorly developed throughout the prenatal period.

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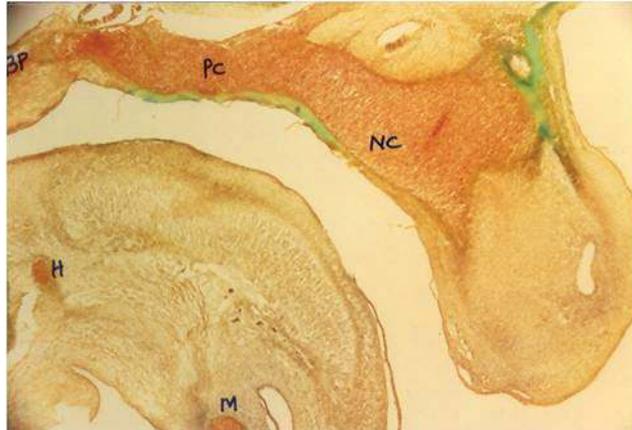


Figure 1. Photomicrograph of a sagittal section of the head of 47 day embryo showing the prechordal part (PC) located in front of the basal plate (BP) and contiguous cranially with the nasal capsule (NC) Bismarck brown -Methyl green method x 40 H. Hyoid M. Meckel's cartilage.

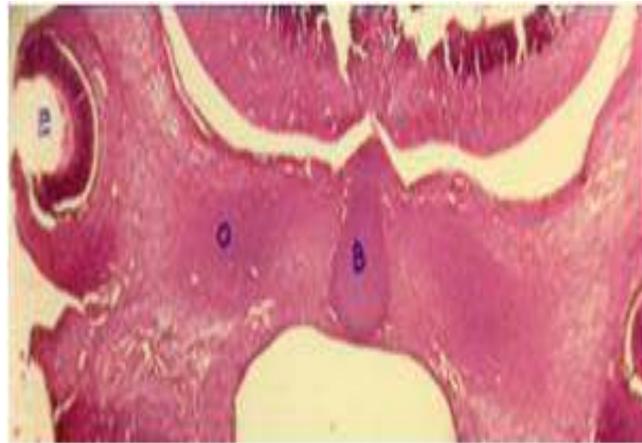


Figure 2. Photomicrograph of a cross section of the head of a 40 day embryo showing the precartilaginous form of the prechordal part i.e the orbitosphenoid (O), the body of the presphenoid (B). H & E x 40 E. Eye.

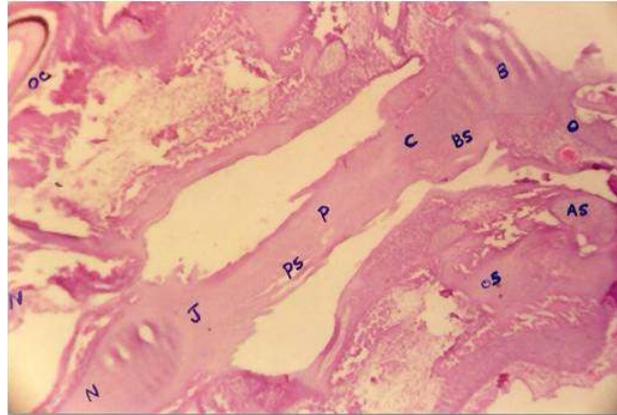


Figure 3. Photomicrograph of a frontal section of the head of a 45 day embryo showing the cartilaginous form of the prechordal part (P) in the floor of the cranial cavity. H & E x 40 O. Otic capsule B. Basal plate N. Nasal capsule C. Hyaline cartilage OS. Orbitosphenoid AS. Alisphenoid OC. Optic capsule J. Joining of nasal capsule with prechordal part BS. Basisphenoid PS. Presphenoid.

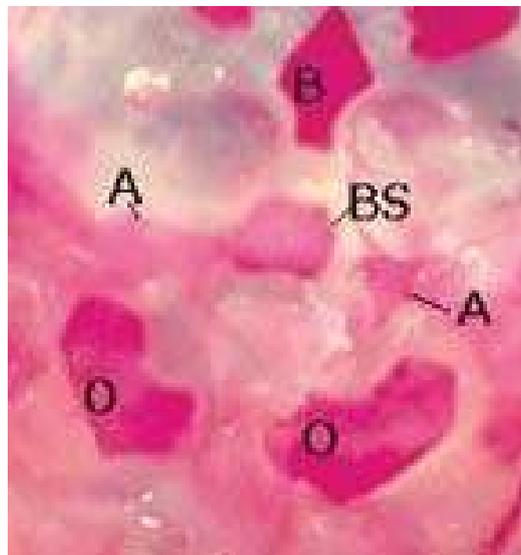


Figure 4. Photograph of an Alizarin Red S stained head of an 83 day foetus showing ossification centers in the floor of the cranial cavity i.e the orbitosphenoid (O), the alisphenoid (A) and the body of the basisphenoid (BS). B. Basioccipital.

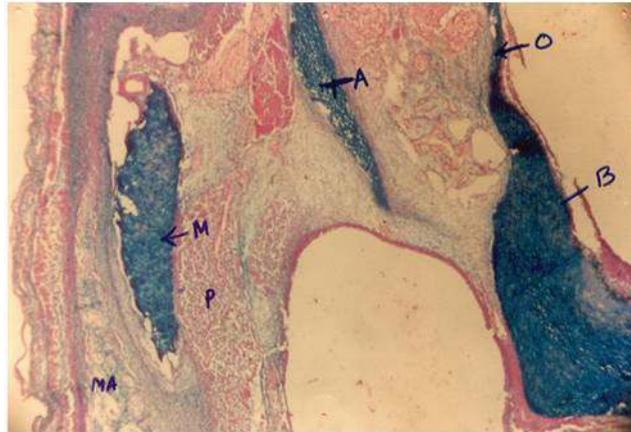


Figure 5. Photomicrograph of a cross section of the head of a 60 day foetus showing the cartilaginous form of body of the presphenoid (B), orbitosphenoid (O), alisphenoid (A). Alcian Blue and Kernechtrot method x 40 M. Meckel's cartilage, P. Pterygoideus medialis muscle, MA. Mandible.

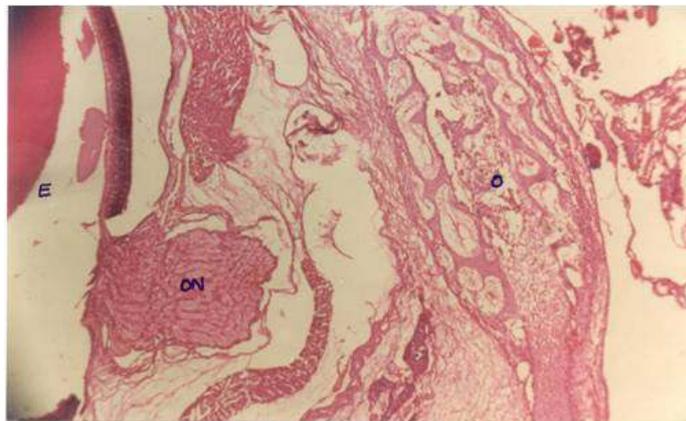


Figure 6. Photomicrograph of a cross section of the head of an 84 day foetus showing complete ossification of the orbitosphenoid (O) H & E x 40 ON. Optic Nerve E. Eye.

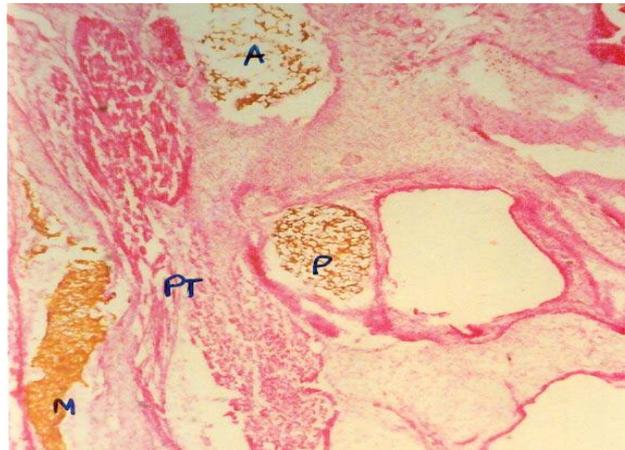


Figure 7. Photomicrograph of a cross section of the head of 62 day foetus showing early ossification in the prechordal part i.e. the alisphenoid (A) and the pterygoid process (P) Von Kossa method x 40 M. Ossification in the mandible PT. Pterygoideus medialis muscle.

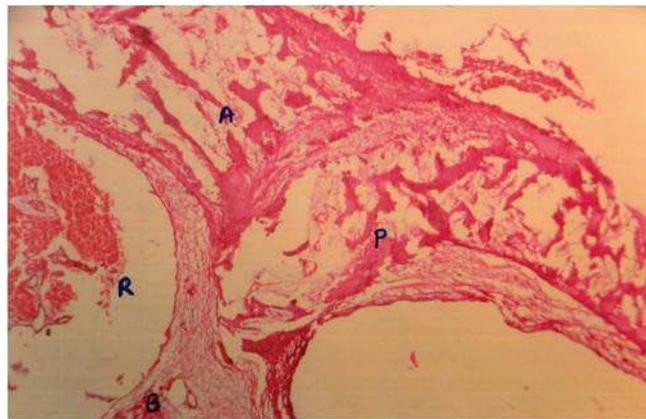


Figure 8. Photomicrograph of a cross section of the head of 92 day foetus showing complete ossification of the alisphenoid (A) and the pterygoid process (P) of the basisphenoid (B). H & E x 100 R. Retemirabile cerebri.

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