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# RELATIONSHIP OF DEVELOPING SKULL WITH OTHER STRUCTURES AT DIFFERENT EMBRYONIC AND FOETAL STAGES OF BUFFALO *(Bubalus bubalis)*

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A**bstract:** The mouth was distinct between maxillary and mandibular prominences at 38 days. Early formation of mandibular and parotid salivary glands was evident at 45 days. The enamel organs of premolars of upper jaw and incisors and premolars of lower jaw were evident at 62 days. The canalisation of trachea and cartilaginous basis of larynx were evident at 26 days and 45 days respectively. The formation of choroid plexuses of ventricles was evident at 43 days while development of pituitary was observed at 41 days. The otic and optic vesicles were evident at 26 days. The vomeronasal organ was in the form of an epithelial tube at 43 days while cartilaginous support around it was evident at 49 days. A sensory nodule observed on skin at the cranial part of mandibular space from 40 days onwards histologically contained sinus hair follicles.
**Keywords:** Developing prenatal skull, related structures, Buffalo

## Introduction

The skull is a very complicated structure of the body and houses and supports the digestive, respiratory, nervous, sensory and endocrine structures (Nickel et. al., 1986). More literature is available pertaining to this work in cow (Evans and Sack, 1973) and bovines (Winters, 1942). But the same information in Buffalo (Singh et. al., 1963) is very scanty. Hence the present work was undertaken.

## Materials and Methods

The study was conducted on 509 embryos and foetuses of Buffalo at different embryonic and foetal stages starting from 26 days to 310 days. The prenatal specimens of unknown age and irrespective of the sex were collected from different slaughterhouses irrespective of age, nutritional status and breed of the mother. The CVRL (Curved Crown Rump Length) was measured in centimeters by using thread and scale. The CVRL measured ranged from 2.1 cm (38 days) to 105 cm (310 days). The age of the specimens was calculated by adopting Soliman's (1975) formula coined for buffalo i.e Y=28.66 + 4.496x if CVRL is <

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20cm and Y=73.544 + 2.256x if CVRL is ≥ 20 cms where Y is the age in days and X is the curved crown rump length in Centimeters.

The embryonic specimens less than 50 days (<4.8 cm CVRL) of age and heads of foetuses from 50 days (4.8 cm CVRL) to 101 days (16.1 cm CVRL) were fixed in 10% Buffered neutral formaline and Bouin's fluids and processed for serial paraffin sections of 6-8µ thickness (Singh and Sulochana, 1997). The foetal heads of 50 days (4.8 cm CVRL) and above were made into 2 to 3 pieces for proper fixation by adopting cross, frontal and sagittal planes. The foetal heads of 70 days (9.2 cm CVRL) and above were subjected to decalcification by Formic acid - Sodium citrate method after fixation (Singh and Sulochana, 1997) and the sections were subjected to Mayer's Haematoxylin and Eosin staining method (Singh and Sulochana, 1997).

**Results and Discussion**

Early formation of branchial arches was evident first at 26 days (Fig. 1) as reported in ox by Noden (2000). Distinct mouth was observed between maxillary and mandibular prominences at 38 days. However early formation of mouth was reported to be observed at 40 days (Singh et. al.,1963) in bovines. The fusion of medial and lateral nasal processes and maxillary processes was observed at 40 days. The lateral swellings of tongue were observed at 38 days and the distinct tongue formation was evident at 40 days.

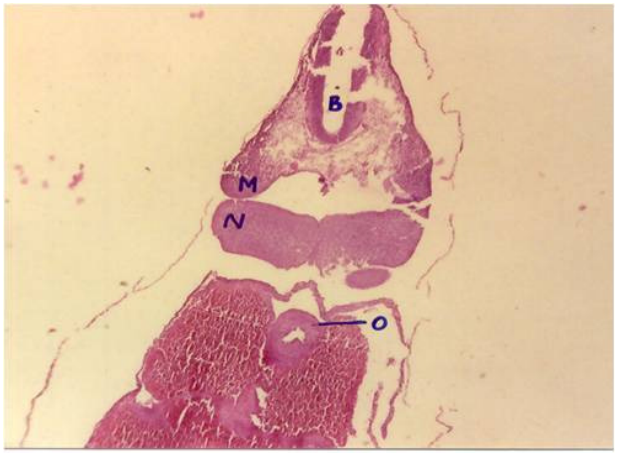


Fig.1. Photomicrograph of frontal section of 26 day embryo showing upper maxillary (M) and lower mandibular (N) prominences of Ist branchial arch
O. oesophagus, B. Brain H & E x 40

At 45 days early formation of parotid and mandibular salivary glands was evident while in human they were reported to arise during 6$^{th}$ week (Arey, 1965). The palatine tonsil formation was observed at 52 days. The primordia of sublingual glands were evident at 49

days. Distinct caruncula sublingualis was evident at 56 days. Early formation of buccal glands was evident at 59 days (Fig. 2). The separation of lips and gums was observed first at 90 days.


Fig.2. Photomicrograph of frontal section of head of 59 day foetus showing early formation of buccal glands (B) - C. Cheek T. Tongue H & E x 40

The dental lamina of upper and lower jaws were evident at 49 days while in human they were reported to appear during 7$^{th}$ week (Arey, 1965). The enamel organs of premolars of upper jaw and incisors and premolars of lower jaw were evident at 62 days (Fig. 3) while the enamel organs of molars appeared at 141 days. Enamel organs of cheek teeth were formed first at 60 days by Garlick (1954) in cow. Four bulgings covered by gum representing all 4 incisors were evident at 133 days.

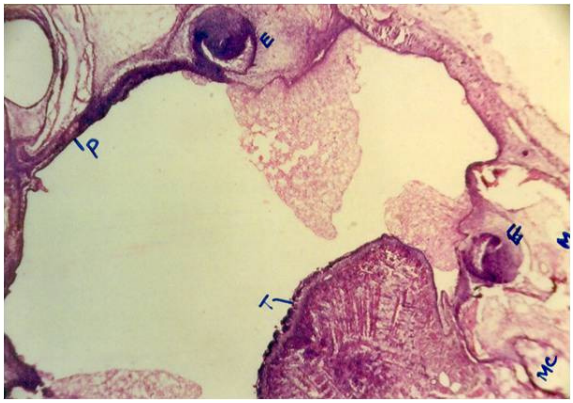


Fig.3. Photomicrograph of cross section of head of 62 day foetus showing enamel organs (E) of upper and lower jaws - T. Tongue P. Palate MC. Meckel's cartilage - M. Mandible H & E x 40

The horn buds were represented as rounded white spots at 72 days. However they were reported to appear at 60 days in cow by Evans and Sack (1973). The flexures of brain and midbrain prominence were distinct at 38 days. The basal ganglia were evident at 40 days.

The choroid plexuses of ventricles were evident at 43 days. Prominent cephalic dome which is supposed to be formed from the enlarged cerebral hemispheres was observed at 43 days while Singh et. al. (1963) reported the similar finding at 45 days in buffalo. The retemirabile cerebri was well established at 74 days. Rathke's pouch and the formation of pituitary was evident at 26 days and at 41 days (Fig. 4) respectively while cords of cells in pars distalis were evident at 57 days. However the primordium of pituitary was reported to appear at 20 days in domestic animals (Latshaw, 1987). Noden and de Lahunta (1985) observed the development of pituitary in calf embryo at 33 days.

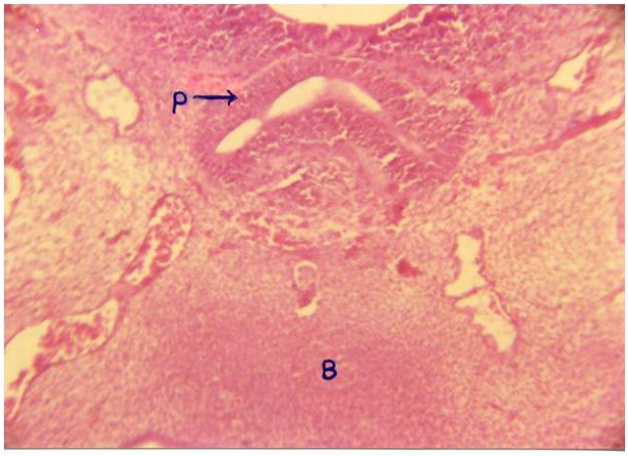


Fig. 4. Photomicrograph of cross section of head of 41 day embryo showing formation of pituitary (P) gland dorsal to basisphenoidal cartilage (B). H & E x 40

The otic vesicle was observed at 26 days. However it was reported to be prominent at 23 days (Evans and Sack, 1973) in cow and 28 days (Singh et. al., 1963) in buffalo. The growth of the pinna was evident at 46 days. The conchal cartilage was in precartilaginous form at 46 days while it was cartilaginous stage at 64 days. The nasal pit was evident at 40 days and it was distinct at 57 days. However nasal pit formation was reported at 30 days in bovines (Winters, 1942; Singh et. al., 1963; Evans and Sack, 1973). The appearance of nasal pit, otic vesicle and formation of eyelids were observed at a later stage compared to cow, which may be attributed to longer gestation period in buffaloes.

The vomeronasal organ was in the form of an epithelial tube at 43 days while cartilaginous support around it was evident at 49 days (Fig. 5). However primordium of Vomeronasal organ was reported to be appeared during 37-43 days in human (Smith and Bhatnagar, 2000). The canalisation of nasolacrimal duct was evident at 64 days in concurring with the findings of Arthur Keith (1949) in human. The precartilaginous and cartilaginous support around nasopalatine duct was evident at 56 days and 74 days respectively. The

canalisation of trachea was evident at 26 days. The precartilaginous and cartilaginous basis of larynx was evident at 43 days and 45 days respectively.

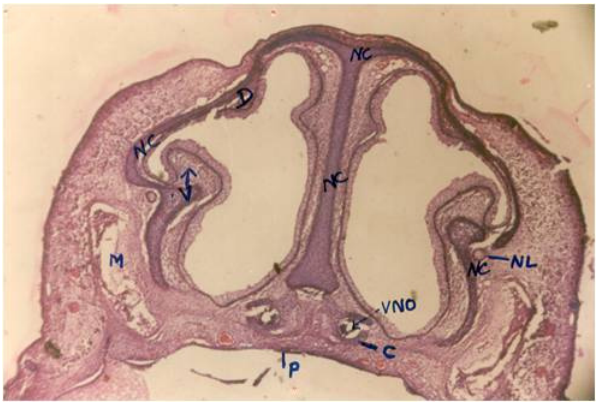


Fig.5. Photomicrograph of cross section of head of 49 day foetus showing formation of cartilaginous support (C) around the vomeronasal organ (VNO) – Dorsal (D) and Ventral (V) turbinates, NC. Nasal capsule NL. Nasolacrimal duct P. Palate M. Ossification in maxilla. H & E x 40

The optic vesicle was first seen at 26 days (Fig. 6) while it was reported to appear during 4$^{th}$ week in human embryo (Sadler, 1985). The establishment of eye was distinct at 38 days. Early formation of eyelids was evident at 43 days (Fig. 7) while initial eye lid formation was reported by Evans and Sack (1973) at 38 days in cow. The fusion of eyelids was observed at 55 days while the same was reported in cow during 50-60 days (Noden and de Lahunta, 1985). Black pigmentation of free margins of eyelids was evident at 120 days. The eyelids were separated at 200 days. Early primordium of lacrimal gland was evident at 55 days while the branching of parenchyma of lacrimal gland was evident at 69 days. The canalization of lacrimal duct was evident at 64 days. Nasolacrimal duct was solid at 49 days and its canalization was distinct at 101 days.


Fig.6. Photomicrograph of frontal section of head of 26 day embryo showing optic vesicles (O) - D. Diencephalon T. Telencephalon H. Heart H & E x 40

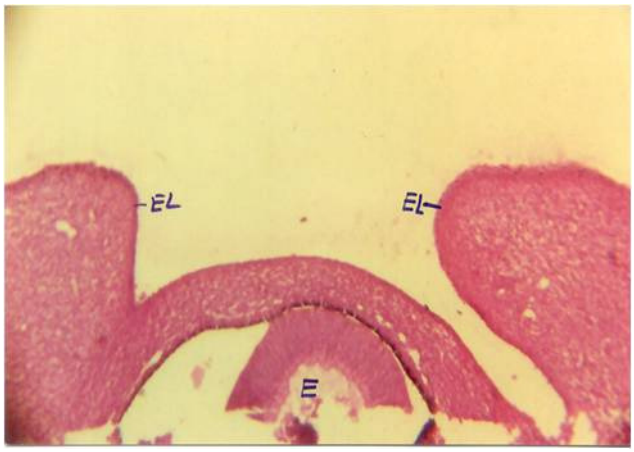


Fig. 7. Photomicrograph of sagittal section of head of 43 day embryo showing early formation of eyelids (EL) - E. Eye H & E x 100

A sensory nodule was observed on skin at cranial part of mandibular space of specimens from 40 days onwards and histologically it consisted of sinus or tactile hair follicles (Fig. 8). The skin over parietal and frontal regions of the skull, eyelids, upper and lower lips and pinna attained black pigmentation at 169 days. The skin over entire head showed black pigmentation at 193 days.


Fig. 8. Photomicrograph of sensory nodule showing sinus hair follicles (SH) - S. Sinus H. Hair F. Hair follicle H & E x 40

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