Pre and postnatal morphological developments for the skull bones in guinea pig (Cavia cutleri)

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Abstract

The study designed to investigate the morphological changes from 25 day prenatal to the 30 day old postnatal in guinea pig, and found that, at the 25-35th day of gestation the result showed typical formation of the plates of all skull elements and show no ossification in any parts of cranium but, at the 35th days the blue color of plates were lighter than that of previous age. At the 45th day of gestation showed starting and extension of ossification centers in all plates of skull elements. At the 55-60th days of gestation the sutures line of articulation among the skull element in addition to the rami of mandible were not ossified. At the one -15 days postnatal showed ossification of the sutures line articulation and rami of mandible, while areas on the parietal, occipital, frontal and nasal bones were still not ossified. At the 30th day the nasal, temporal and occipital, baseoccipital, basespheniod, palatine and incisive bones have been ossified but, the ossification has not yet completed in frontal, parietal and maxilla.
Introduction

The laboratory guinea pig is a good experimental animal for developmental studies. It characterized by small size that provided easy maintenance and cure also it has gestation period of 65–72 days, with an average of 68 days (1). The vertebrate skull is consists of the neurocranium (skull vault and base) and the viscerocranium (jaws and other branchial arch derivatives). The skull is originated from cranial skeletogenic mesenchyme that derived from two distinct embryonic sources: mesoderm and neural crest. The neural crest cells origins of the viscerocranium and the anterior skull base are well established (2). During mammalian evolution an expansion of the frontal and parietal bones with incorporation of the squamosal and part of the alisphenoid to form neurocranium in addition to that the growth of skull is associated with articulation of skull bones by fibrous joints (sutures) all of which differentiate directly within the skeletogenic mesenchyme that lies between the brain and surface ectoderm (intramembranous ossification) (3). The present study has endeavored to demonstrate the type of ossification of skull bones, during the prenatal and postnatal development by using the bone dyes technique. Also for distinguish the sutures and fontanels between flat bones during prenatal and postnatal development of guinea pig skull.

Materials and methods

Fourty healthy guinea pigs included both sexes (10 males and 30 females) have been used in this study. Males and female were put together in mating cages for a half day and the pregnancy test was done by vaginal smear test. Five embryos have been collected at each age of pregnancy periods by cesarean section. Age detection, was done by dependent the crown-rump length (CRL) for corrections. CRL has measured from the vertex of the skull to the midpoint between the apices of the buttocks for prenatal only (4). The technique employed in this study has involved the double staining of bone and cartilage with alizarin red-s and Alcian blue. The double-staining technique has allowed the accurate localization of ossification centers within the cartilage primordia to be made (5).

Results and Discussion

At the 25th day of gestation

The result showed development of typical shape of skull with that organized by the formation of the plates of cheeks (mandibles), nose, mouth, frontal, parietal, temporal, occipital and the external ears get marked tips, also the basesphenoid (Fig.1). This stage, did not shows the ossification in any parts of cranium, these results were agreed with these of other studies in albino mice done by (6) at 15th day of gestation and in cat (7).

At the 35th days of gestation

At this period the plates of skull elements were did not displaying any visible center of ossification in cranium, but the blue color of plates were lighter than that of previous age (Fig.2). These results were parallel with the result of (8) in cat at the 21 day of gestation and (9) in domestic rabbit at the 18 day of gestation, who showed that the exoccipital part of occipital region was not ossified and appeared as bluish color and the tympanic part of temporal bone and base of skull was blue in color.
At the 45th days of gestation
This period has showed the development and extension of ossification centers in all plates of skull elements that represented by changed the blue coloration of skull into red (Fig.3). The pattern of ossification in this study was parallel with (10) in rat at the 17th day of gestation, (11) in house mouse at the 17th day of gestation, (8) in cat at the 38th day of gestation, (6) in mice at the 16th day of gestation and (12) in Japanese musk shrew who mentioned that, all remained occipital parts (except superior part) and tympanic bone were cartilaginous element through these stages of development. The result of this stage disagrees with result has been described in guinea pig (13) and mole by (14) who showed that the exoccipital, basioccipital and tympanic part of temporal which were not ossified are evident that most of cranium parts undergoes chondrogenesis pattern.

At the 55-60th days of gestation
The blue coloration was still in sutures articulation line between the nasal and frontal, frontal and parietal, parietal and occipital bones and inter nasal suture line (Fig.4), also the ossification has not yet completed in the rami of mandible (Fig.5). These results were compatible with (15) who observed that the sutures remain as non-ossified state, and with (9) in domestic rabbit at 28 day who found the frontal bones were still separated from the nasal bone cranially and from parietal bones caudally by the narrow sutures and in consistent with (6) in mice who mentioned that the sutures were very clear between the flat bones at 18 day of gestation.

At the one -15 days postnatal
These periods were showed disappearing of blue coloration in the articulation line between nasal frontal, frontal and parietal, parietal and occipital bones, while various sized areas on the parietal, occipital, frontal and nasal bones have not ossified (Fig.6), the rami of mandible revealed completed ossification (Fig.7). This results have paralleled with the results of (9) in domestic rabbit at 1 day old who observed the metopic suture (between the frontal bones), lambdoid suture (between the parietal and interparietal bone) and sagittal suture between the parietal bones can be observed as very narrow sutures and have smooth borders, while coronal suture (between the parietal and frontal bones) has irregular edges and also seen by (15) in other species such as mice and rats who mentioned in human, the lambdoid suture was between the supraoccipital and parietal bones not between interparietal and parietal bones. At age of the 15th day the results have showed the presence of those non ossified areas on the dorsal aspect of skull, also on the ventral aspect of skull showed incomplete ossified of incisive bone (Fig.8). These results incompatible with (9) in domestic rabbit at 4 day old which presented the primary ossification center in ventral part of tympanic and with (16) in Marsupials (Macropodidae) who mentioned the first sign of ossification in the tympanic was during the third day old postnatal.

At the 30th day of gestation
The skull elements on the dorsal aspect (nasal, frontal, parietal, temporal and occipital bones) and ventral aspects (baseoccipital, basesperichondrium, palatine and incisive bones) were appearing red color of alizarin red that indicates the ossification of these bone have been started as early as before the 30th day, but the ossification has not yet completed that represented by blue coloration which seen in the skull elements; frontal, parietal, maxillary, sutures line in between bones and nasal turbinates' (Fig.9,10).This can be determined by histological trails, because the whole mount staining was focusing the gross appearance of the ossification only. The parietal bone has showed its ossification center just caudo-laterally of frontal bone. The first ossification center single rose at each side of the frontal and parietal. These results of
ossification state had been observed in 10; 11; 16; 12; 8; 9 and 6). Many authors but in different degree of variation by (17; 10; 11; 16; 12; 8; 9 and 6).

Fig. 1: Skull of 25 days old guinea pig embryo. A: lateral view shows: frontal plate (1), parietal plate (2), occipital plate (3), temporal plate (4), nasal plate (5), mandibular plate (6), ear (7), and nasopharyngeal plate (8). B: caudal view.

Fig. 2: Lateral view of Skull (35 days old guinea pig embryo) shows: nasal plate (1), frontal plate (2), parietal plate (3), occipital plate (4), temporal plate (5), orbit (6), mandibular plate (7) and palatine plate (8).
Fig. 3: Lateral view of whole-mount skull (45th days old guinea pig embryo) shows: nasal bones (1), frontal bones (2), parietal bones (3), temporal bone (4), incisive bone (5) and non-osified suture lines between bones (arrows). Alcian blue & alizarin red stains.

Fig. 4: Dorsal view of whole-mount skull (At the 55-60th days old guinea pig embryo) shows: nasal bones (1), frontal bones (2&3), parietal bones (4), occipital bone (5), and non-osified suture lines between bones (arrows). Alcian blue & alizarin red stains.

Fig. 5: Ventral view of whole-mount skull (At the 55-20th days old guinea pig embryo) shows: incisive bone (1), mandible (2) and non-osified region in mandible (arrows). Alcian blue & alizarin red stains.
Fig. 6: Dorsal view of whole-mount skull (At the one day postnatal guinea pig) shows: inter nasal suture line (black arrow), frontal-parietal suture line (yellow arrows), inter parietal suture line (white arrow), occipital bone (green arrow) not-completed ossified areas (two heads arrows). Alcian blue & alizarin red stains.

Fig. 7: Ventral view of whole-mount mandible (At the one day postnatal guinea pig) shows completed ossified mandible (black arrows). Alcian blue & alizarin red stains.

Fig. 8: Ventral view of whole-mount of maxilla (At the 15th day postnatal guinea pig) shows incomplete ossified of incisive bones (black arrows). Alcian blue & alizarin red stains.
Fig.9: Dorsal view of whole-mount skull (30 days postnatal guinea pig) shows: frontal bones (1), parietal bones (2), occipital bone (3), temporal bones (4), nasal bones (5), incisive bones (6) and ear plate (7), non-ossified parts (arrows). Alcian blue & alizarin red stains.

Fig.10: Ventral view of whole-mount skull (At the 30th days old guinea pig) shows: foramen magnum (1), baseoccipital (2), basephenoid (3), bulla (4), occipital condyle (5), sphenoid (6) palatine (7) and incisive bones. Alcian blue & alizarin red stains.
References


