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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Morphological Study of the Skeleton Development in Chick Embryo (*Gallus domesticus*)

A.A. Sawad, Balsam A. Hana[1] and Anwar N. Al-Silawi[1]
Department of Anatomy, College of Veterinary Medicine, University of Basra, Basra, Iraq
[1]Department of Biology, College of Science, University of Basra, Basra, Iraq

Abstract: The study comprised anatomical description of skeleton development in chick embryo *Gallud domesticud* which includes the appearance of ossification center during the embryological stages (5,10,14, 18 and 21) days. It was found that some of skull bones was formed by intramembranous ossification and that other by endochondral ossification. During the hatching, the skull was undergo complete ossification and there is a symmetry between the paired bones among their shape bone started with primary ossification center. The limbs were formed by endochondral ossification. The ossification begins centrally in the cartilage and proceed in all directions. The hind limbs ossified after fore limbs and there is an ossified signs in the tarsal and carpal bones before hatching. as well as, there is an obvious increases in length of primary ossification centers in both fore and hind limbs with further development. Histologically, three types of bone cells were studied, the osteoblasts, osteocytes and osteoclasts which covered by periosteium and their rules in intramembranous and endochondral ossification.

Key words: Chick embryo, skull bones, osteoblasts, osteocytes, osteoclasts

INTRODUCTION

Avian bones development and health are an important subject in avian research, especially because of its significant to the poultry industry. Every year, about 2-5% of the broilers raised are lost as a consequence of skeletal problems during growing and finishing fases due to mortality and condemnation (Sullivan, 1994). Many of the pathological skeletal deformities are still commonplace and do not appear to be linked to defined causes. Long bone distortions such as varus and valvus deformation and tibial dyschondroplasia are expressive examples for the mentioned pathologies. In addition although there are dissimilarities between human and avian bone development, the avian is considered a valuable model for human skeletal defects (Cook, 2001).

Birds mutual with other amniotes by that most of the skeleton laid down in cartilage which subsequently become ossified. Bones formed in this way are known as cartilaginous bones, as opposed to membranous \bones which are ossified directly from mesodermal tissue (Schepelmann, 1990).

Bone is remarkable for its hardness, resilience, characteristic growth mechanisms and its regenerative capacity. It provides rigid support and protection to the soft parts and furnishes a lever system on which muscles are brought into play (Potten *et al*., 1978).

The bone termed the woven bone on the first embryonic development which characterized by their immaturities and irregular collagen fibers and osteocytes contents and regards as a temporary bone, during the growth and developed stage transformed into the lamellar bone (Alder, 2000).

MATERIALS AND METHODS

Thirty chick embryo collected to investigates the skeleton development stages, they divides into five groups according to the chick embryo ages (1st : five days aged, 2nd; ten days aged, 3rd; 14 days aged, 4th; 18 days aged and 5th; one day after hatching).

The eggs were hatched in artificial incubators in 37-38°C temperature and 65 % humidity.

The following steps employed for the skeleton preparation and calcium identifications (Jenning, 1999):

- Opens the skin and removed the viscera carefully avoiding the damage in bony or cartilaginous skeleton or mesenchymal tissue.
- Fixed the embryos at 100% ethanol for 4 days without stirring and then three days with stirring.
- Imprisoned in the solution of Alizarine red and Alcian blue stain for five days with stirring, washed by tap water and imprisoned in solution of glycerol 20% with sodium hydroxide 1% in ratio 1;1 for 16 h in room temperature to removed tissue completely and stores in 15 glycerol for examined and photographed

For histological demonstration the samples were collected from (Skull, Vertebrates, Ribs, Fore and Hind limbs) then transfer into nitric acid 5% for 18 h- 3 days for decalcification.

Samples pass through a series of alcohol from 50%-100% for two hours for each concentration for dehydration. Then clearing in xylene for one and half hour .infiltration in paraffin and embedded with paraffin wax and cutting by using ratary microtome, the thickness

is about (5-8 micrometers) and mounting in slides (Luna, 1968) and stains with hematoxylene and eosine, Alizarine red and Alcian blue (Nori, 1980).

RESULTS AND DISCUSSION

Anatomically: The roof of the skull before ossification composed of transparent mesenchymal tissue (Fig. 1) and their base from adherent cartilages, the ossification centers are small and becomes gradually clear at tenth day and have special dispensation (Fig. 2). The skull bones completed their ossification at the fourteenth day in the (Frontal bone, Parietal bone, Occipital bone, Supraoccipital bone, Nasal bone, mandibular bone, maxillary bone) (Fig. 3).

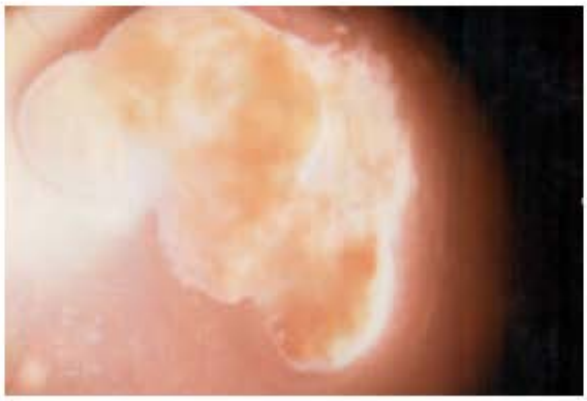

Fig. 1: Five day age chick embryo shows mesenchyme tissue Alizarine red stain, 3.3X

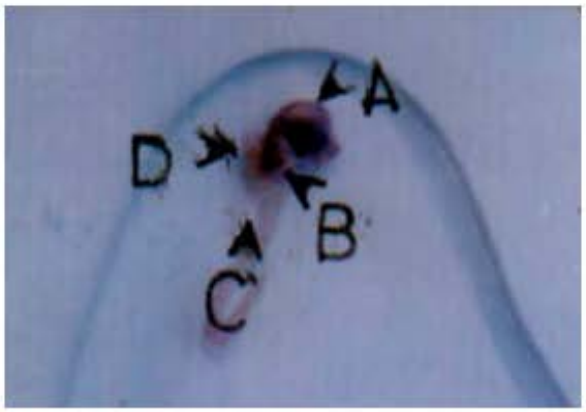

Fig. 2: Five day aged chick embryo, A-Skull, B-Left wing bud C-Left foot bud, D-Prevertebra. Alizarine red stain, 2.5X

The special allocation of the ossification centers in the skull of chicks in agreement with the El-Sayad and Mohammed (1985) in the ossification centers in mice and Hall (1981) of the first stage of skull ossification center in poultry at the ninth day of embryo age.

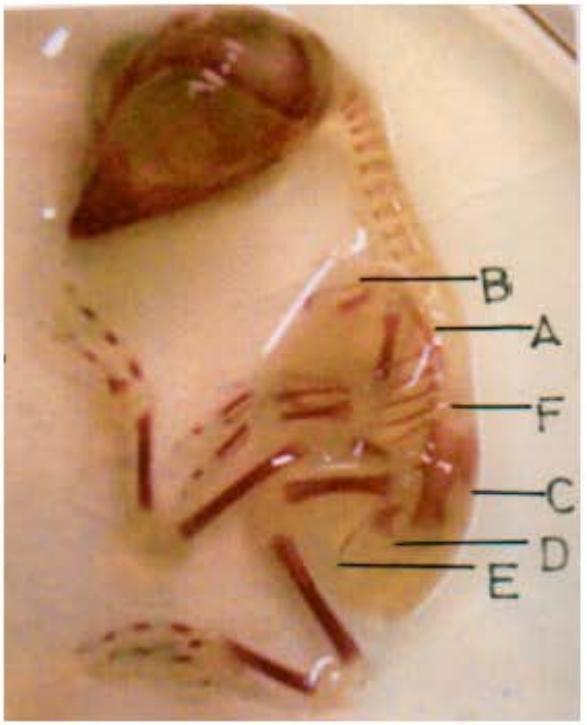

Fig. 3: 14^{h} day chick embryo, A-scapula, b-clavicle c-Pubis, D-Ischium, E-ilium, F-Ribs Alizarine red stain, 2X

The vertebrates ossified by endochondral ossification, the vertebral arch appears different ossification times due to vertebral difference.

The vertebral arches of the cervical, thoracic, lumbar and sacral vertebrates starts at the fourteenth day (Fig. 4), While the ossification at the coccygeal vertebrates starts at the eighteenth day, the cervical vertebrates remained uncompleted after the hatching (Fig. 5). This is in contrast to Andreo *et al.* (1998) as they found that the vertebrates in mice ossified at the 16^{h} day and Fernando *et al.* (2000) at the 15^{h} day, While Shapiro (1992) noticed that the ossification starts at the cervical vertebrate body in chick embryo at 12^{h}-13^{h} day and at the sacral vertebrae at 19^{h} day.

The primary calcification of the rib body at the proximal extremity at the 10^{h} day which extends remained cartilaginous area towards the vertebrae until the hatching, while the distal extremity forms the cartilaginous ribs (Fig. 6). That in agreements with Drew and Alexander (1985) in farm animals ribs.

The hind and front limbs formed by endochondral calcification and starts at the bones center and extends towards the extremities remained cartilaginous area at bone ends termed growth plates (Fig. 7).

The fore hind bones shows similar appearance of ossification centers that starts at the 10^{h} day at the scapulae, clavicle, humerus, radius and ulna.

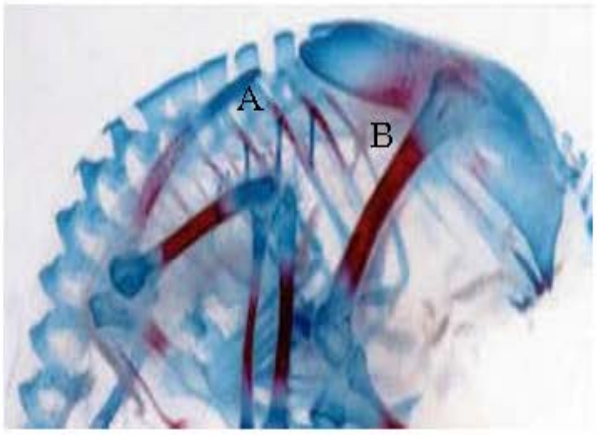

Fig. 4: 14th day chick embryo, A-Vertebrae, B-Ribs Alizarine red stain, 2x

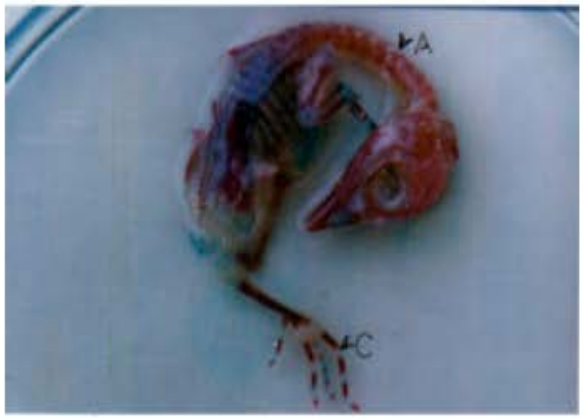

Fig. 6: 18th day chick embryo consists A- Vertebrae B-Sternal ribs PhalangesAlizarine red stain 2X

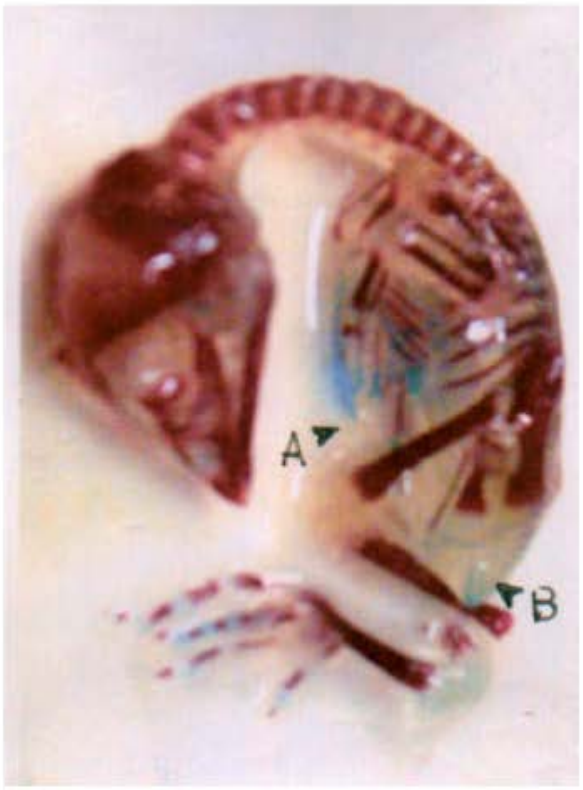

Fig. 5: 18th day complete chick embryo A- Sternum B-Coccygeal Alizarine red stain, 2X

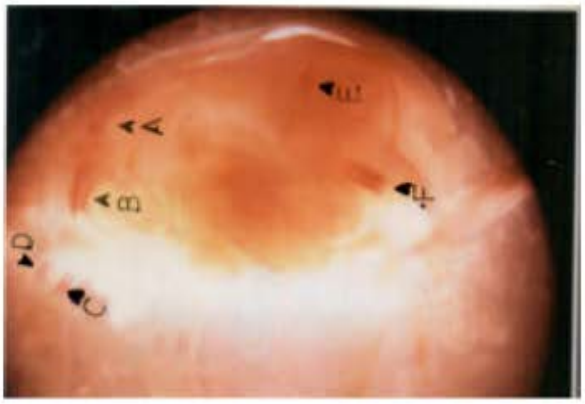

Fig. 7: 10th day chick embryo A-Scapula B-humors C-Radius D-Ulna E-Femur F-Tibia and Fibula. Alizarine red stain1.6X

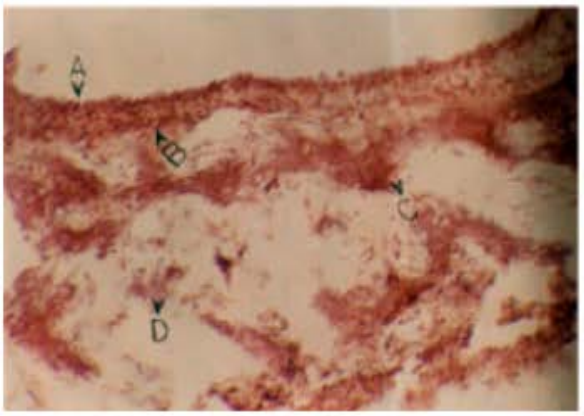

Fig. 8: Chick embryo skull 10th day ages A-Mesenchymal Plate B-Osteocyte C-Interstitial substance D-Medullary cavities, Alizarine red stain 280X

At the fore limbs the calcification start at the 14th day in Ilium, Ischium and pubis and at the 5th day in femur, while start at 10th day in tibia and fibula.

The study of Daniel *et al.* (2003) are the same in the hind limb of chicks and disagreement with Janina *et al.*, (2003) in mice and coincided by Hill (2000) in fore limbs of pigs and Saunder (1998) in chicks.

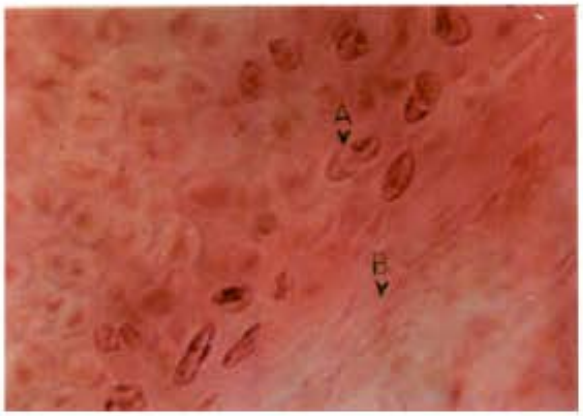

Fig. 9: Humors in 10^{h} day chick embryo A-swallowing chondrocytes B-Collagenous fiber H and E Stain 600X

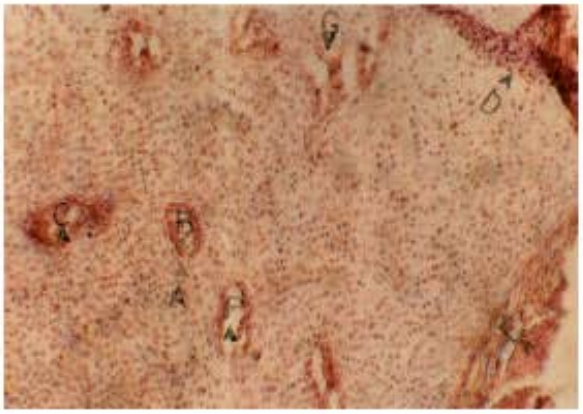

Fig. 10: Tibia and Fibula of 10^{h} chick embryo A-Young Haversian canal B-Bony plate C,D- perosteum bud E- periosteum F-Medullary cavity, Alizarine red stain 600X

Histologically: The skull roof forms from mesenchymal tissue contains a star shaped processed cells with amorphous substance, contains of trabeculae filled by bone marrow, the trabeculae lined by osteoblasts, the mesenchyme cells differentiates into osteoblast which transform into osteocytes (Fig. 8), that the same founds of Couly *et al.* (1993) in chicks and Theiler (1972) in mice.

The differentiation of growing and maturation area of humerus at 10^{h} day by chondrocytes that appears large and flattened at maturation area and small and scattered in grow area due to the recurring chondrocytes mitosis, these results have been the same observation by Pool (1991).

At 14^{h} day chick embryo the chondral grow shows abutment of maturation and calcification areas and that with agreement by Change *et al.* (1994), the mature

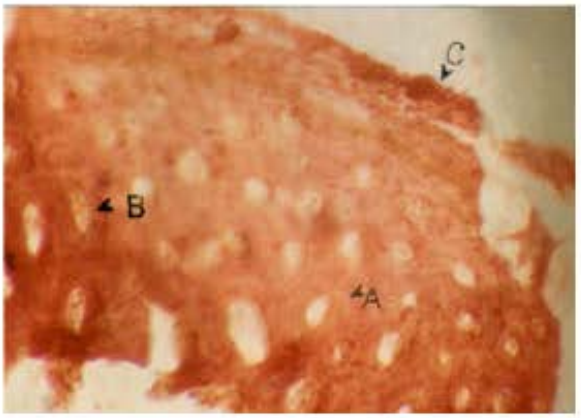

Fig. 11: Tarso-metatarsal bone in one day ages chick embryo A-Haversian canal, B-Medullary cavity, C-Periosteum, Alizarine red stain 1000X

chondrocytes appears swallowing nucleus while the calcified cells appear clear due to the calcium participative, the changes in the structure and character the cartilage amorphous substance on the swallowing and maturation areas followed by the invasion of blood vessels and the programmed dead of the peripheral chondrocytesand replaced by a trabeculated osseous substance secreted from osteocytes (Fig. 9) this finding similar to Al-Barawi and Suliman (1987).

At the late embryonic stages the haversian canals manifestation (tibia and fibula) at 10^{h} day age on the hard bones while the carpo-metacarpal bones have a young haversian canal as unitive axis cylinders form a complete haversian apparatus fills by bone marrow (Fig. 10,11), this in agreement with Leslie and Robert (1974) and Dellman and Brown (1976).

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