University of Al- Qadisiya
College of Science
Dept. of Biology

Developmental Study of Ossification Centers in Common Quail Embryo (*Coturnix japonica*)

A Thesis
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Supervisor's Certificate

I certify that this thesis was prepared by **Mohammed A- Ameer Haraj** entitled "**Study of Ossification Stages in Quail Embryo (*Coturnix japonica*)**" under my supervision at the University of Al-Qadissiya , College of Science , Biology department in partial fulfillment of the requirement for the degree of master of science – Biology – Zoology .

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Acknowledgment

First and fore most

Sincerely and greatly I feel urged to praise almightily **"ALLAH"** the most gracious and most merciful and his prophet **"MOHAMMED"** and his kinsfolk because this research has been completed under their benedictions .

My great thanks are due to my supervisor **Assist . prof. Dr. Hashim M. A-Kareem** who provided me with valuable assistance and scholarly instructions without which this work would not have been completed .

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Mohammed A- Ameer Haraj

AL-Husseiny

Dedication

To my parents ,

My father , for he is the one who established in me the love of knowledge .

My mother , for she is a pillar of strength .

To those who represent real love and brotherhood (My Brothers) .

To the strong and humble spirit (My Supervisor) .

MOHAMMED

Language Supervisor Certificate

This is to certify that I have read the thesis entitled (Study of Ossification Stages in Quail Embryo " *Coturnix japonica*") and have corrected every language mistakes ; therefore, this thesis is qualified for debate

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Abstract

A morphogenesis study has been made for the skeleton formation of the quail (*Coturnix japonica*) which included the analytical study to make the skeletal elements and the transitional changes that happens in the two processes of cartilage formation (Chondrofication) and bones (Ossification) during the growth of the different natural phases for the diverse bones of the body.

A quail was gathered of about (100) birds and brought up at home. Then the fertilized egg was gathered from those birds in about (7-9) days, then those egg were incubated by an Ukranian electronic incubator in a about (37,5-38 ^{o}C) and 70% of temperature and humidity respectively. The embryos were used

starting from day 3 of incubation to day 16 of incubation , which is the hatching day.

Egg were weighed individually before the incubation as well as at the time of it's taking out from the incubator in order to extract the embryo of it, the average of the weights of the egg for both periods was taken ,the results showed that the egg's weight decreased in about (0.5gm) after incubation in relation to it's weight before incubation, and this percentage increases gradually in relation to the increase of the incubation period. Also the measuring of the lengths of the humerus and femur successively started from day 6 of incubation till the hatching day.

The Embryos were extracted from the egg starting from day 3 of incubation, then they were stained with Alcian blue pigment and Alizarin Red – S stain.

The skeletal elements of the vertebral column showed earliest chondrofication in comparison with the other parts of the skeletal system, at day 3 of incubation only , but it showed delay in it's ossification which happened starting from day 11 of incubation, the ossification started in the cervical vertebral region and progressed toward the coccygeal region. Regarding ossification in the cervical and thoracic vertebral regions, it started from the middle region and then progressed toward the upper and lower regions, but in the lumbosacral and coccygeal vertebral regions , it started from the upper region and progressed toward the mid and lower regions. And within one vertebrae, the body of the vertebra which constitutes the central part showed early ossification and then extended to the vertebral arches on the both sides of the vertebra.

The thorax cage which is composed of the ribs and the sternum showed late chondrofication in comparison with the vertebral column and limbs, where chondrofied ribs appeared between days 6-8 of incubation. The vertebral ribs preceded sternal ribs in it's ossification , where the vertebral ribs ossified

starting from day 9 of incubation , while sternal ribs ossified starting from day 12 of incubation. In both vertebral and sternal ribs, ossification started at the middle region and then expanded toward proximal and distal ends of each bone. In the sternum, chondrofication appeared relatively at day 6 of incubation. While ossification occurred in the laterocranial process at day 14 of incubation as well as the laterocaudal process. In most cases, the sternal body remained cartilaginous at hatching.

Limbs, which most of it's parts consist of long bones, it succeeded the vertebral column in it's chondrofication starting from day 4 of incubation, yet it showed earliest ossification in comparison with the other skeletal parts of the bird, where the ossification started in these bones at day 8 of incubation. In long bones , ossification took place first at the middle region and then expanded toward the proximal and distal parts of each bone with the progress of the embryo's age.

Introduction

Embryology is defined as the study of morphogenesis of an individual life from the fertilization to the sexual maturation, including growth, differentiation and ways of controlling the activities of fetal configuration like regeneration, wound healing and hormonal control (Bazer*et al.*,1987) (AL Samarrai and Rabea, 1995).

Recent researchers in the field of experimental embryology of avian species have an extraordinarily advanced through focusing specially on natural skeletal development, teratological testing, and developmental engineering in avian species. This is important for the study of factors which could modify the skeletal development , and for evaluation of it's modifications in importance and time of onset of ossification (Baeriswyl, 1980).

The quail is a small migratory bird , domesticated for two purposes, to produce egg and meat, and it's called in our Arabian countries in different

names like (AL Samman and AL Salwa) ,but the most common name is (AL Salwa).

God almighty mentioned it in the great holy Quran more times

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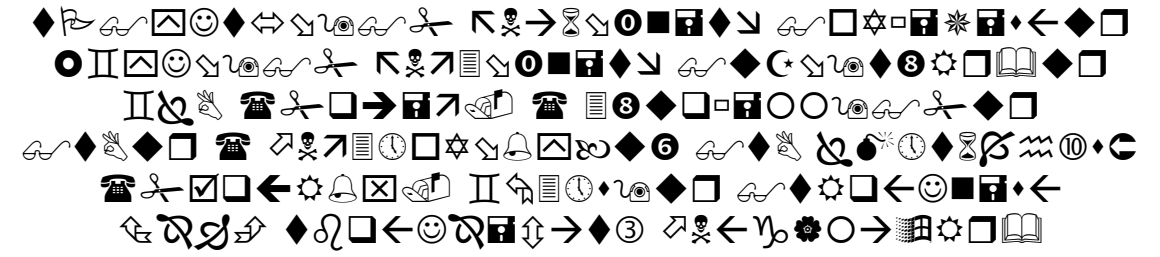

صدق الله العلي العظيم (سورة البقرة الاية ٥٧)

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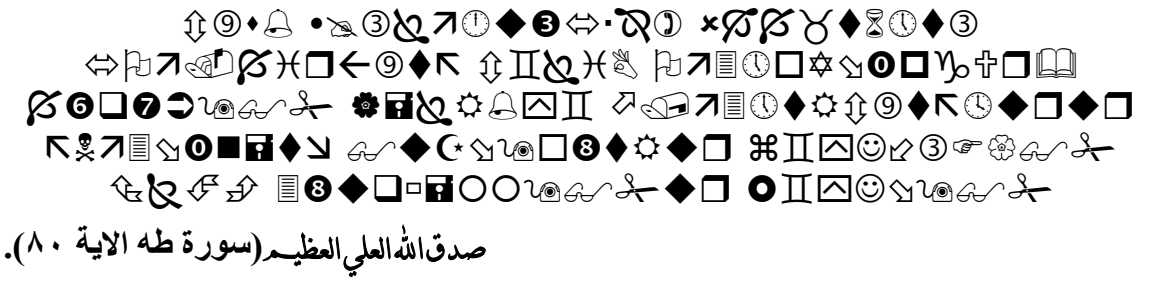

صدق الله العلي العظيم(سورة طه الاية ٨٠).

This bird is classified into two major species, The European species (*Coturnixcoturnix*) and the Asian species (*Coturnix japonica*).The source of all these species is the pharoah quail. More hieroglyphic graphics found in Egypt show the form of the bird and prestigious position that the bird gained in the era of the pharaohs, when it was called the holy bird (Ratnamohan, 1985; AL Kinany, 2002 ; AL Husseiny, 2003).

The Japanese quail is the most important species where it has all the characteristics of the other species. And it's used as a test animal in scientific

researches. (Davies and Follet, 1975; Ratnamohan, 1985 ; Wedty, 1988; AbidUda, 2007).

The domestication of the quail became one of the small projects that carried out by young and families in all over the world because it is characterized by many features: It's possible to live in cages, Inexpensive in domestication, It produces eggs in large quantities, characterized by rapidly growing because it has high speed metabolism, bears poor environmental conditions, relatively resistance to many diseases which became a real problem in the poultry industry and it is one of the fast and cheap ways to produce the animal protein. (ARaheem,2002; AL Husseiny,2003; Abid Uda, 2007; AL Sudani, 2009).

Aims of the study

A list of skeletal development is thought to be indispensable as a normal control in avian experiments, for example ; avian engineering , teratological testing and skeletal mutants. Also when using avian embryos in research, Japanese quail is thought to be superior to the chicken as an experimental animal, because it has smaller body size , is more prolific , and is more precocious than the chicken, the nature of Japanese quail will support efficient performance in experiments , thus the purpose of recent study is to establish a list of normal skeletogenetic stages in the development of Japanese quail embryos to contribute to the research fields. In addition on the previous aims , the study of the natural development of bones has an economic importance in the diagnosis of skeletal disorders that constitute a defect in the future , and it is significant in the poultry industry .

2-Review of Literature

2.1 The Classification of the Quail

Kingdom : Animalia

Phylum : Chordata

Class : Aves

Order : Galliforms

Family : Phasianidies

Genus : Coturnix

Species : *Coturnix japonica*

(General committee for agricultural researchs,2012)

Fig (1) The Quail (*Coturnix japonica*)

2.2 Information About the Quail Bird.

2.2.1 Periods of rearing up quail birds:-

1-Hatching period:

The length of the hatching period is about (16-17 days) and it is a short period in comparing with the length of hatching period of chickens which is (21 days), and turkeys (28 days). The temperature is almost (37.8ºC and humidity (84-86%) with continuous turning on the egg (3-5) times daily.).(General committee for agricultural researchs,2012).

2- Period of breeding or care:

It needs the same features and requirements of the chicken chicks.It demands taking care of the small size of the bird in the first days where it is about nearly (7 grams) for each chick, it may room inside it's small basins , so it needs to put small stones after clearing and putting them inside the basins. This process continues until the age of 3 weeks.The chicks need optimal heat (35^0C) because of it's small size which makes the temperature a very important factor, and in case of not , it expects deaths to be happened in high rates.

3- The productive period:

Producing egg starts in the age of 42 days which is the sexual maturing age for those birds mostly, the ratio of productivity increases weekly with ranges till it reaches to 95-100/year after the passage of 6 weeks if they find all the suitable environmental factors and this is through supplying the birds with number of lightening hours no less than 14 hours and temperature between (20-25ºC).(General committee for agricultural researchs,2012).

2.3 The Skeletal System.

The term skeleton is applied to the framework of hard structure which supports and protects the soft tissues of animals. The bones are divided into four classes according to their shape and function; Long bones, flat bones, short bones, and irregular bones. (Getty, 1975; Steele and Bramblet, 1988; Summerlee, 2013).

The skeleton serves two major functions in birds. First , mechanical levers and supporting arms operated by muscles form the locomotor system responsible for flight and other movements. Second, the bone which makes up most of the skeleton is a reservoir of calcium and phosphate which may be utilized in muscle contraction and nerve conduction. This reservoir is especially important during the reproductive cycle in female birds when the resorbtion of medullary bone provides an important part of the calcium necessary for egg shell formation (Chiasson, 1972).

2.3.1 Basic Anatomy of Bones

Descriptive anatomy divides bones into two major groups: long bones and flat bones. Initially, this classification was based solely on the gross appearance of the types of bone. The longbone category was extended to include two further types of bone that were neither flat nor long: short bones and irregular bones. (Summerlee, 2013).

Later, it was observed that bones of the skull (which comprise the majority of the flat bones of the body) and bones of the appendicular skeleton were derived from different embryonic tissues, which strengthened the emerging view that long and flat bones developed by different processes. During the 1980s, this classic view of bone development was challenged. Despite their

apparently different embryological origins, bones throughout the body develop by an identical process, and this has important implications for the organization and management of reparative processes (Summerlee, 2013).

2.4 Classical View of Ossification

2.4.1 Intramembranous Ossification.

Bone develops within stromal connective tissue that is characterized by mesenchymal stem cells, connected by thin cell processes, lying in a matrix of haphazardly arranged collagenous fibrils. Immediately before ossification commences two changes are observed; the mesenchymal stem cells proliferate and start to differentiate, finally forming osteoblasts, and the intercellular matrix becomes more dense and homogeneous (Summerlee,2013).

These changes alone are sufficient to induce a suitable environment for early calcification to commence, and the mineral content of the matrix increases rapidly. The osteoblasts augment the process by producing more matrix that is calcified, and some of these cells will become trapped in the tissue and will transform into osteocytes. Until the bone has reached the final size, a layer of osteoblasts remain on the periosteal surface. The same process occurs for flat bones and on the periosteal surface of the diaphysis of long bones (Summerlee,2013).

2.4.2 Endochondral Ossification.(Interacartilagenous).

Endochondral ossification occurs where bones elongate at a growth plate. This plate is arbitrarily divided into specific regions for descriptive purposes. At the epiphyseal front there is a layer of hyaline cartilage formed by cartilage cells, some of which may be embedded in matrix.

The older cartilage cells begin to multiply and form into columns separated by wide parallel bands of interstitial substance. The cells are separated from each other by a thin capsule of matrix. These cells hypertrophy and incorporate stores of glycogen. Providing there are adequate concentrations of minerals available, the intercellular matrix then starts to calcify, particularly between adjacent columns of cells. This zone forms a provisional structural framework between the growth plate and the cancellous bone of the metaphysis. Loops of blood vessels then invade the connective tissue and penetrate into the vertical columns. The interstitial tissue is removed, leaving calcified vertical columns of matrix known as the primary spongiosa. This primary spongiosa is considered to be the necessary scaffolding upon which the bone matrix can be deposited (Summerlee,2013).

In this way the newly formed endochondral bone mirrors the cartilage model which it has replaced. The key feature of this hypothesis is that the cartilage model forms first and the bone is laid down onto that model. As bone matrix is laid down upon the primary spongiosa they are transformed into

secondary spongiosa, a more permanent set of trabeculae. These will be modified by the joint action of osteoblasts and osteoclasts to form the thickened adult trabeculae, which are clearly visible upon gross examination of the cut surface of bone. The pattern of mineralization at the growth plate can be clearly demonstrated by autoradiography and is of some interest. Comar *et al*., (1952) showed that soon after calcium 45 (^{45}Ca) administration heavy deposits of radioactive ion are seen in the growth plate and adjacent trabecular bone of the metaphysis. Thirty days after (^{45}Ca) administration, the radioactive content of the plate is relatively low and concentration in the trabecular bone is less than on day one. By 60 days, osteoclastic activity has removed and remodeled almost all the newly formed bone and the level of radioactivity observed is low in all areas.Once an animal achieves skeletal maturity, bone stops growing in length and there is no further new formation of bone. The skeleton continues to be modeled and remodeled but the rate of change is considerably less than during the growth phase. Radioactive calcium introduced into bone at this stage may take years to be resorbed and removed. This underlines concerns about the hazards from certain radionuclides, for example strontium 90 (^{90}Sr) or strontium 89 (^{89}Sr), which have been shown to accumulate selectively in the skeleton (McLean and Budy, 1964).

2.5 Formation and Differentiation of Avian Somite Derivatives.

During somite maturation, the ventral half of the epithelial somite disintegrates into the mesenchymal sclerotome, whereas the dorsal half formsa transitory epithelial sheet, the dermomyotome , lying in between the sclerotome and the surface ectoderm. The dermomyotome is the source of the majority of the mesodermal tissue in the body, giving rise to cell types as different as muscle, connective tissue, endothelium and cartilage. Thus, the dermomyotome is the most important turntable of mesodermal cell fate choice in the vertebrate embryo. Sclerotome development is characterized by cranio-caudal polarization, resegmentation and axial identity. It's formation is controlled by signals from the notochord, the neural tube , the lateral plate mesoderm and the myotome. These signals and cross-talk between somite cells lead to the separation of various subdomains, like the central , ventral , dorsal and lateral sclerotome. (Christ and Scaal, 2008).

2.6 Studies on the Ossification in Common Quail.

2.6.1 Early Bone and Cartilage Histogenesis in Embryonic Japanese Quail in the Conditions of Microgravity.

Total preparations of 4-day old embryos from both groups (spaceflight microgravity " space group" and laboratory " control group") demonstrated clearly that the cartilaginous anlage of the femoral bone had dyaphisial , 2 epiphysial and 2 proliferation zones. By day 7 of embryogenesis , cartilaginous

anlage had grown in size in both groups due to intensive chondrocyte multiplication and gain in the intercellular substance mass. Tibial cuff in space embryos measured half and femoral cuff was 2.3 times smaller in comparison with these parameters in the control group. In addition , intensity of chondrocyte multiplication was reduced histological profiles of femur and tibia in 10-day old embryos in the control pointed to enhancement of osteogenesis. The metaphysis zone contained distinct mitosis figures of different stages of division. Bone deposition could be seen below the peristoma. The osteogenesis cuff spread up to the femoral anlage metaphysis; cartilage was calcined . Space embryos display retard osteogenesis. There were ingrown blood vessels in the region of cartilage destruction; however, vessels grown in the periosteum were less in number as compared with the laboratory control. Also, the perichondral ossification layer was considerably thinner , whereas the osseous cuff was 1.3 and 1.45 shorter in the femur and tibia , respectively . (Komissarova*et al*., 2012).

2.6.2 Histogenesis of Japanese Quail Bone and Cartilage Tissues at the Final Stages of Embryonic Development in Microgravity.

The results of comparative histological studies of skeleton development in 14 and 16 day Japanese quail embryos grown in space flight and in the control groups revealed retardation of cartilage replacement by bone in the femur and tibia in space embryos as compared with their controls. Perichondral ossification metaphysis was reached by day 14 both in the space and control embryos. Destruction of cartilaginous diaphysis advanced onto the proximal and distal parts including the periphery. Tibia and femur cartilaginous cores in space embryos were destructed worse than in the controls in consequence of insufficient minerals supply. Perichondral ossification in the 16 day space and control embryos was alike close to completion spreading as far as the epiphysis.

Long bones metaphysis was abundant in monomorphic cells as a growth bank existing during and 7 days post hatching. However, absence, in contrast to the controls, of osteogensis sites in long bones epiphysis, suggested retardation of chondrocytes calcification in these areas, as well as of ossification in space embryos. (Komissarova*et al*., 2013).

2.6.3 The Ossification of the Pectoral Girdle and Wing Skeleton of the Quail.

The onset of ossification centres of the pectoral girdle and wing skeleton of the quail has been studied in embryos and juvenile birds, which were sampled daily from the 4^{th} day of incubation up to the 12^{th} day after hatching. Specimens, which were cleared and were stained with Alcian Blue and Alizarin Red S, were examined at the stereomicroscope. The furcula ossified with intramembranous ossification at the 7^{th} day of incubation. The first centres of perichondral ossification in the scapula and the coracoid bones were observed at the 7^{th} day of incubation. In the humerus, radius and ulna, perichondral ossification was observed at the 6^{th} day of incubation. The carpal bones were ossified between the 8^{th} and 10^{th} day of the post-hatching period. The 2^{nd} metacarpal bone began to ossify at the 6^{th} day of the post-hatching period, whereas the 3^{rd} and 4^{th} metacarpal bones were ossified at the 7^{th} day of incubation. At the 9^{th} day of incubation, ossification was observed in the 1^{st} phalanx of the alular digit and in the phalanges of the major digit. The 2^{nd} phalanx of the alular digit began to ossify at the 12^{th}day of incubation , whereas the phalanx of the minor digit at the 14^{th} day of incubation. (Pourlis and Antonopoulus, 2011).

2.6.4 The Ossification of the Pelvic Girdle and Leg Skeleton of the Quail.

The onset of ossification centers of the pelvic girdle and leg skeleton of the quail in embryos and juvenile birds were studied. Specimens, which were cleared and were stained with Alcian Blue and Alizarin Red S, were examined at the stereomicroscope. The ilium and the pubis began to ossify at the 8th day, whereas the ischium at 9th day . Perichondral ossification was observed at the 6th day in the femur, tibia and fibula. A secondary ossification center was detected in the proximal epiphysis of the tibiotarsus at the 15th post-hatching day. The patella began to ossify at the 30 post hatching day. Regarding the tarsal bones tibiale, pre-tibiale and fibulare, ossification was observed at the 15th , 12th and 16th days , respectively. The metatarsals II, III, IV ossified at the 7th day , whereas the metatarsal I at the 11th day. The centers of ossification of the 1st phalanges of all digits were observed at the 9th day. At the same day, the ossification centers of the 2nd phalanx of digits II and III as well as the 3rd phalanx of digit III appeared. At the 10th day , ossification was observed in the 2nd phalanx of digit I, in the 3rd phalanx of digit II and in the 2nd and 3rd phalanges of digit IV. In the 4th phalanx of digit III and in the terminal phalanges of digit IV, ossification was observed at the day 11th .

2.6.5 Long Bone Development in the Japanese Quail Embryos.

Ahmed and Soliman described the main histological development stages of long bones (tibia and femur) from Japanese quail embryos. Limb bud was established at day 5 of incubation. Mesenchymal cells differentiated into chondrocytes forming a cartilage template in the position of the future tibia and femur at day 6 of incubation. At day 7 of incubation, the cartilage template enlarged and had the shape and position of the future tibia and femur. At day 8, central chondrocytes underwent hypertrophy and were surrounded by a periosteal bone collar. Cellular and vascular invasion from the bone collar into the central zone of the cartilage template, cartilage resorption and formation of marrow tunnel and finally peripheral calcification was seen. Vascular cartilage

canals penetrating the epiphysis were observed at day 9 and the canals gradually increased in thickness and number toward the hatching day. Articular epiphyseal growth cartilage with resting, proliferative and hypertrophic zones was clearly established by day 10 of incubation. After 17 days of incubation, the zonation of the articular epiphyseal cartilage were much clear, many cartilage canals were present within the epiphyses. In epiphyses of tibia but not femur, foci of chondrocytes hypertrophy appeared close to the cartilage canal.(Ahmed and Soliman,2013).

The first appearance of the primary ossification centres in the diaphysis of the studied six long bones (humerus , radius , ulna, femur, tibia and fibula) was found to occur between the sixth and the seventh day of incubation, humerus and tibia showed the greatest growth rate while the radius and fibula showed the lowest. (Pourlis*et al*., 1998).

The ossification began at the same time in the long bones of the wing and leg. At the embryonic day 6, all the cartilaginous rudiments consisted of three zones. The central zone composed of hypertrophic chondrocytes, a second zone on either side of the central zone, which consisted of flattened cells and a third zone, which represented the epiphyseal region. A thin sheath of osteoid and a bi-layered perichondrium-periosteum surrounded the central zone of the cartilaginous rudiments of the long bones. The perichondrium consisted of a layer of osteoblasts, in contact with the cartilage, and a layer of fibroblasts. At the embryonic day 7, the thickness of the calcified osteoid ring increased and a vasculature appeared between the layer of osteoblasts and the layer of fibroblasts. At the embryonic day 8, a second sheath of periosteal bone began to be formed. Concurrently, vascular and perivascular elements began to invade the cartilage. The ossification spread towards the distal ends of both the diaphysis. At the electron microscopic level, the osteoblasts of the perichondium showed cytoplasmatic characteristics of cells involved in protein

synthesis. The perichondral ossification is the first hallmark of the osteogenesis in the long bones.(Pourlis*et al*., 2006)

2.7 **Characteristic anatomical features of avian skeleton.**

The skeletal system provides the strong framework for the support and protection of the remainder of the systems, organs and tissues making up the body of the fowl. There have been a number of modifications of the bones founds in mammalians that enhances the ability of the bird to fly. While fowls that are not able to fly well, they still remains the ability to some extent. These modifications are:-

1- A hollow bone filled with air connected to the respiratory system. Many of bird's bones are pneumatic, with struts across their hollow interior to provide combination of lightweight and strength as an adaptation to flying ,these bones include ; skull , clavicle , sternum (keel) , humerus , pelvic girdle , and synsachram vertebrate (Peter , 1981; Ritchison , 2007). But (Schepelmann, 1990) noted that pneumatization of bones in birds does not take place until after hatching.

2- The medullary bones are important sources of calcium for the lying hens. These bones are , femur , tibia , pubis , ribs , ulna , scapula , and toes, (Chiasson , 1972).

3- The size of the head had been reduced significantly when compared to other species . A large head would be a hindrance when flying , bones of the skull are flat and fused together . It is difficult to distinguish many of bones of skull due to obliteration of the sutures . The orbit of the eye is relatively large (Chiasson , 1972).

4- The skull consists of five major bones: the frontal (top of head), parietal (back of head), premaxillary and nasal (top beak), and the mandible (bottom beak). The weight of the skull of the normal bird usually weighs about 1% of their total body weight (Chiasson , 1972).

5- Birds lack teeth and even a true jaws, instead having evolved a beak, which is far more lightweight. The beaks of developed chick embryo have a projection called an egg tooth, which facilitates their exit from amniotic egg during hatching (Proctor and Lynch, 1993; Ritchison, 2007).

6- The neck is quite long in most species , that consists of 13 – 25 cervical vertebratae.(Chiasson , 1972).

7- Vertebral formula is C_{14} , T5 , L_6 , S_2 , Ca_{15} , in the pigeon (Chiasson , 1972) ; C_{14} , T_7 , $L+S_{12}$, Ca_8 in the quail (Nakane and Tsudzuki, 1999) ; C_{14} , T_7 , L_5 , S_2 , Ca_{10} , in the chick (Bellairs and Osmand, 2005), with some considerable variations due to different life styles , involve:

- Number of cervical vertebrae ranged between 9 in parrot and 25 in swan.
- Last thoracic , lumbar, sacral, and some first caudal vertebrae fused together making up one mass known as (*Synsacram*).
- The long tail of the many other animals has been reduced to a very short section of fused caudal vertebrae called the (*Pygostyle*).
- The rigidity has been achieved by fusing these groups of vertebraein birds, important in both flight and bipedal locomotion.(Chiasson, 1972).

8- There are seven pairs of ribs in quails, chicken and turkys (Nakane and Tsudzuki, 1999; Bellairs and Osmand, 2005; Kurtul*et al* ., 2008; Atalgin and Kurtul, 2009), and five pairs in pigeons (Chiasson, 1972). The ribs have been modified by the inclusion of uncinate process (UP), extends of the posterior edge of the vertebral ribs . Although UP was previously thought to be adaptations for flight (Welty, 1988) or to strenghthen the ribs and ribs gage (Kardong , 1988), these processes have recently been demonstrated to be integral components of the ventelatory mechanisms of birds (Codd*et al* ., 2005).

9- The sternum modified into structure called (*Keel*) , which provides a large surface area for the strong attachment of muscles of flight . It is noted that swimming birds have a wide sternum, while walking birds had a long sternum, and flying birds have the width and height equal (Ayhan*et al* ., 2006).

10- Carpal bones have two free infused bones , radial and ulnae articulate with the radial and ulna bones respectively , other carpal bones fused with the metacarpus forming carpometacarpal bone. Whereas tarsal bones are entirely fused , forming tibiotarsal and tarsometatarsal bones (Nakane and Tsudzuki , 1999; Kurtul*et al* 2008).

11- There is well developed pectoral girdle which consists of scapula , clavicle (furcula or wish bone), and coracoids (collar bone)bones. Scapula is narrow and thin, Two rod-shaped clavicle combined forming (*Furcula*) (Chiasson, 1972). The shoulder consists of scapula , coracoides. Humerus forms the upper arm which joins the radius and ulna (fore-arm) to form the elbow. The carpus and metacarpus form the wrist and hand respectively , and digits form fingers. Bones in the wing are

extremely light so that the bird can fly more easily (Proctor and Lynch, 1993).

12- Innominate correspondes the oscoxae of mammals, consists of ilium, ischium and pubis, the later is long slender bone (Chiasson, 1972).The upper leg consist of the femur . At the knee joint, the femur connects to the tibiotarsus and fibula (lower leg).The tarsometatarsus forms the upper part of the foot , digits makes up the toes. The leg bones of the birds are the heaviest , contributing to a low center of gravity (Proctor and Lynch, 1993).

13- Digits of the wing are three in number (1^{st} and the 5^{th}) are lacking . But pelvic limb in most of birds has four digits (fifth is lacking). The first digit (hallus)has two phalanges, the second has three , the third has four and the fourth has five.The distal phalanx of each digit bears a claw (Chiasson, 1972). Many birds have three toes forward and one back, others have two forward and two back. The fifth toe is lost completely except in some birds where it has become a defensive (Spur, such as in the chicken). Ducks, geese and swans all have medium length toes joined together by a web of skin called (palmate foot) to make excellent paddle for rowing themselves through the water during swimming.(Proctor and Lynch, 1993).

3. Materials and Methods

3.1 Materials

3.1.1 Equipments and Apparatus

The following equipments and apparatus were used in this study:

No.	Equipment	Company

1	Automatic hatching incubator	Ukraine
2	Curved Scissor	Pakistan
3	Digital camera	U S A
4	Dissecting microscope	Zeiss binocular microscope
5	Electric sensitive balance	Mettle PC 2000
6	Glass bottles of wide mouths	Germany
7	Glass petri dishes	Germany
8	Glass flasks	Germany
9	Graduated cylinder	Germany
10	Light microscope	China
11	Oven	China
12	Scalpel and plats	Pakistan
13	Stopped glass vials for storing subjects	Germany
14	Thermometer	Ukraine
15	Thump forceps	Pakistan

3.1.2 Chemicals

The following chemical materials were used in this study:

No.	Materials	Company
1	Absolute alcohol	BDH England
2	Alcian blue	($C_{56}H_{58}Cl_{14}CuN_{16}S_4$) Germany
3	Alizarin Red-s	($C_{14}H_7NaO_7S$) Germany
6	Formalin	England
7	Glacial acetic acid	BDH England

8	Hydroxide potassium	BDH England
9	Glycerol	BDH England
11	Parrafin wax	Merk, Germany
12	Picric acid	BDH England
13	Thymole	BDH England
14	Bouin's solution	BDH England
15	Methyl salicylate	BDH England
16	Benzyl benzoate	BDH England

3.2 Methods

3.2.1 Experimental design

Fertilized egg (90 egg) ,weighing (8-14) grams (Fig.52). Obtained from 100 quail birds (50 males and 50 femalesreared at home),were stored at 15 ºC. They were incubated in an Ukrainian manufactured incubator ,after adjustment of the temperature and humidity at 37.5 - 38 ºC. and 70% respectively .With continous turning of egg three times 0.Skeletons of the embryos were stained with alcian blue and alizarin red-s pigments for cartilage and ossified bones , respectively.(Nakane and Tsudzuki, 1999).

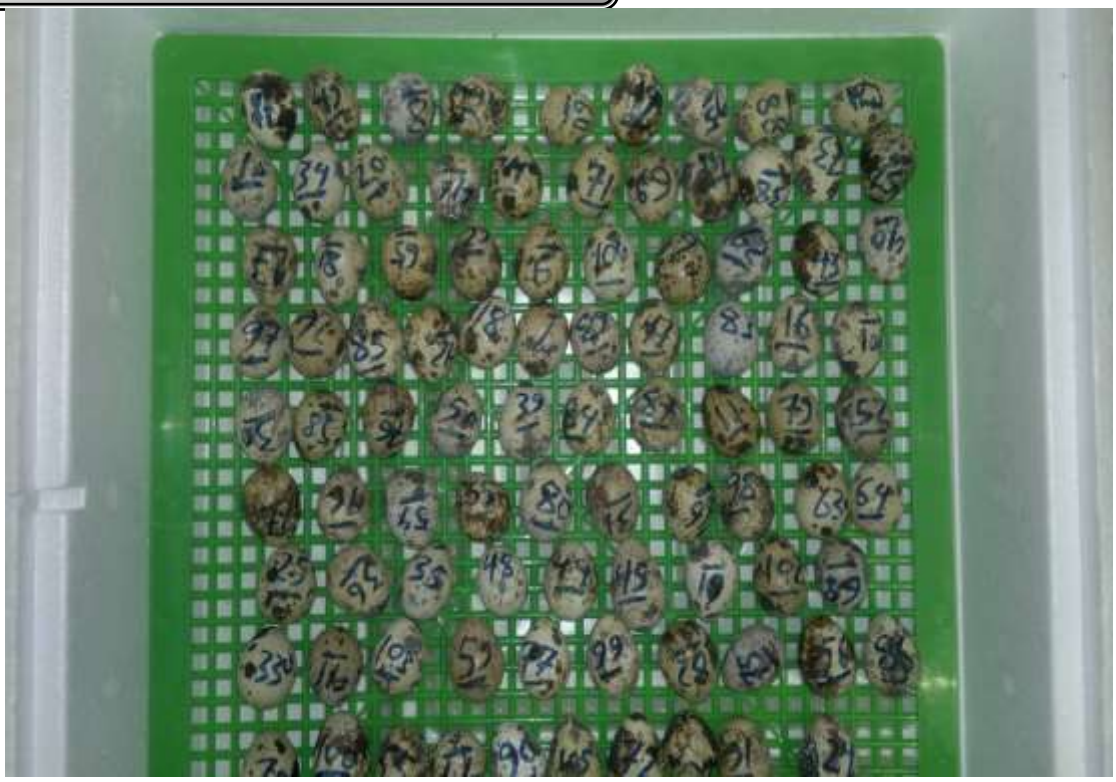

Fig (2). All the incubated egg at the first time of incubation

3.2.2 Preparation of whole mounts of embryos

The general procedure of staining of the bones in whole mounts was developed to examine bones deposition during development . As an independent project , one could prepare a series of fetuses (embryos) of different ages such that the rate of bone growth and development can be compared. The technique that they described is for use with frozen or fresh organisms and thus avoids having to fixed the specimens in formalin first. However other procedure are available that use formalin-preserved material (Wassersug, 1976).

3.3 Visualization of skeletal system by double staining of Alizarin Red-S and Alcian Blue.

Studies of the visualization of the skeletal system of fetuses or embryos go back to the beginning of the 19th century. Alden carried out the first successful skeletal staining in 1962 (Alden, 1962). Double staining method of skeleton developed consequently by (Ojeda *et al*., 1970) in chick embryo, (Whitaker and Kathleen,1979; Selby, 1987) in rat and mice, (Nakane and Tsudzuki, 1999) in Japanese quail, (Atalgin and Kurtul, 2009) in turkey embryos.In previous studies , the whole skeletal system of the different embryos were stained with Alcian blue and Alizarin red-S for both cartilage and ossified bone components, respectively. The staining technique and steps of it's procedure were modified from the literatures above , which dyes the cartilage tissue blue and the osseous tissue red. The technique displays the chondrofied components and location of the early appearance of the centers of the ossification area of the bones and their developmental sequences.

3.3.1 Alcian Blue.

It isa family of polyvalent basic pigment,and it has been historically the most common and the most reliable member. It is used to stain acidic polysaccharides such as glycosaminoglycans in cartilages and other body structures, some types ofmucopolysaccharides, sialylated glycocalyx of cells etc. Use of Alcian blue has historically been a popular staining method in histology especially for light microscopy in paraffin embedded sections and in semithin resin sections. The tissue parts that specifically stain by this pigment become blue to bluish-green after staining and are called **"Alcianophilic"**.(http://en.wikipedia.org/wiki/Alcian_blue_stain).

3.3.2 Alizarine Red-S.

It is an organic compound that is important as a prominent dye; it is extracted from root of a plant called Modder, which grew in sandy soils of Netherlands. Alizarine red-S is used in biochemical assay to determine

quantitavely , by colormetry the presence of calcific deposition by cells of an osteogenic lineage, during matrix mineralization associated with true bone . (http://dictionary.reference.com/rowse/alizarine(January02/2007).

3.3.3 Principle steps of double staining technique:

The modified procedure of Ojeda *et al*. (1970) , Watson (1977) , and Tsudzuki and Wakasugi (1988) were used as follows.

A- For 3-6 day embryos.

1- Fix in Bouin's solution over night.

2- Drain, wash off the adhering fixing fluid with distilled water and place in 70% ethanol containing 1% ammonia.

3- Change the ammoniated alcohol at 2 or 3 hours intervals with frequent agitation until the specimens loses most of the picric acid. Three changes may suffice. Then, place in 95% ethanol for 1 night.

4- Stain embryos for 1 day at 37 ^{o}C in a freshly prepared solution of 95% ethanol (80 ml), 20 ml of glacial acetic acid, and 30 mg of alcian blue.

5- Dehydrate in 95% ethanol for 2 days and 99% ethanol for 3 days.

6- Macerate and store in a 3:1 mixture of methyl salicylate and benzyl benzoate.

B-For 7- day embryos.

1- Fix and stain for 2 days at 37^{o}C in a freshly prepared solution of 95% ethanol (80 ml) , 20 ml of glacial acetic acid , and 15 mg of alcian blue.

2- Dehydrate in 95% ethanol for 3 days , three changes of the ethanol may suffice .

3- Remove viscera.

4- Stain and macerate for 1 day in 0.002% alizarin red-s / 0.2% KOH .

5- Clear in glycerine / H_2O solutions of increasing concentrations (25 , 50 , and 75) of glycerins for 7 days each, to 100% glycerine for storage.

C-For 8-10 day embryos.

1- Fixation , staining , and dehydration as in step (1) and (2) for 7-day embryos.

2- Remove viscera , skin , and adipose tissue.

3- Stain and macerate for 1 day in 0.002% alizarin red-s / 0.5% KOH.

4- Clearance and storage as earlier .

D- For 11-12 day embryos.

1- Fixation , staining , dehydration , and removing of tissues as earlier.

2- Stain and macerate for 1 day in 0.002% alizarin red-s / 1% KOH.

3- Clearance and storage as earlier.

E- For 13-17 day embryos.

1- Remove skin, viscera , and adipose tissue.

2- Fixation , staining , and dehydration as in step (1) and (2) for 11-12 day embryos.

3- Macerate for 1 day in 1% KOH.

4- Transfer to 0.002% alizarin red-s / 1% KOH for 2 days.

5- Clearance and storage as earlier.

Observation of the skeletons of (3-7 day embryos) was performed under a dissecting microscope paying attention to the timing of chondrofication and calcification. Observation of the skeletons of (8-16 day embryos) was performed under a digital camera , since the size of the embryos became bigger than the eye pieces and the objective lenses of the dissecting microscope , and can't display the whole size of the embryo. Chondrofication was confirmed by blue color stained with Alcian blue pigment and calcification by red color stained with alizarin red-s pigment.

4-Results and Discussion

The developmental features of the whole skeleton of 3-16 day embryos were described during the course of incubation . For convenience of description , the skeleton was divided into five parts , which are vertebrae, ribs, sternum, forelimb , and hind limb, on and after 5 days of incubation . The forelimb and hindlimb include the pectoral girdle and pelvic girdle , respectively. For (3 – 7) day embryos were observed and examined through dissecting microscope , but for (8 – 16) day embryos were observed and examined through digital camera, because of it's large size which was bigger than the eye piece of the dissecting microscope, that prevented taking pictures for the whole mount of the skeleton.

At day 3 of incubation.

Average (AVG) egg weight before incubation was 12.9 gms , and at time of extraction was 12.32 gms. (Diagram. 5).

Only the upper parts of the vertebral column were stained blue around the notochord. But the coccygeal region of the vertebral column was not

stained(Fig.4). Nakane and Tsudzuki (1999) observed in quail also that the parachordal cartilage and the vertebral column were stained blue.

At day 4 of incubation.

AVG egg weight before incubation was 11.87 gms, and at time of extraction was 11.72 gms (Diagram. 5).

Vertebrae:-

the coccygeal region was stained blue from the upper region to the tip of the tail-bud (Fig.6). The base of the vertebral (neural) arches in the cervical to lumbosacral regions was bilaterally stained blue on each centrum (vertebral body)(Table. 1). In addition to the above observations, Nakane and Tsudzuki (1999), also observed in quail that the axis of the parachordal cartilage met at approximately right angles to the axis of the upper region of the vertebral column.

Forelimb:-

the humerus , radius and ulna were stained blue.

Hind limb:-

the femur , tibia and fibula were stained blue.That was identical to what Nakane and Tsudzuki (1999) observed.

At day 5 of incubation.

AVG egg weight before incubation was 12.57 gms, and at time of extraction was 12.08 gms (Diagram.5).

Vertebrae:-

The vertebral column now is completely stained blue (Table.1). Nakane and Tsudzuki (1999) mentioned that the base of the vertebral arches in the coccygeal region was bilaterally stained blue on each centrum.

Ribs, and Sternum:-

No ribs and sternum were recognized till now(Fig.8).

Forelimb:-

The scapula, coracoid and the metacarpus were slightly stained blue (Table.3).

Hindlimb:- The metatarsus and the ilium were stained blue (Table.3).

At day 6 of incubation.

AVG egg weight before incubation was 13.27 gms, and at time of extraction was 12.92 gms (Diagram.5).

Vertebrae:-

The vertebral column now is more clear and well recognizable.The vertebral foramen that resulted from the fusion of the vertebral arches exist bilaterally on the centra from the cervical to lumbosacral regions with each other at the midline stained blue (Table.1).

Ribs:-

About four vertebral ribs and two sternal ribs were observed stained in blue (Table.2).While,Nakane and Tsudzuki (1999) said that seven vertebral ribs and four sternal ribs (the first to fourth), were stained blue. And also observed that the first to fourth sternal ribs were jointed to the third to sixth vertebral ribs , respectively.

Sternum:-

A pair of the sternal rudiments was stained blue in the dorsolateral wall of the thorax(Table.2). Nakane and Tsudzuki (1999) added (this rudiments jointed to the distal end of the sternal ribs and coracoid).

Forelimb:-

The length of the humerus (LH)= (3.7 ± 0.25 mm) (Diagram.1) . Only the humerus , radius, ulna and the metacarpus are now blue (Table 3), but the phalanges of the digits are still not stained in this day. Nakane and Tsudzuki, (1999) observed blue staining in the first phalanges of the third and fourth digits in quail at this day of incubation.

Hindlimb:-

The length of the femur (LF)= (4.3 ± 0.25 mm) (Diagram. 3). The femur, tibia, fibula , ilium and metatarsus are still blue in this day of incubation and no blue colored phalanges were observed till now (Table.3). Nakane and Tsudzuki(1999) said that the pubis , ischium , first metatarsus , first phalanx of the second digit , first and second phalanges of the third digit , and the first and second phalanges of the fourth digit were stained blue. They also said that the proximal ends of the second to fifth metatarsi fused with the nodule of the fused distal tarsi.

At day 7 of incubation.

AVG egg weight before was incubation is 12.53 gms, and at time of extraction was 12.15 gms (Diagram.5).

Vertebrae:-

The dorsal spines were recognized from the cervical to lumbosacral regions. Also the cervical ribs were appeared bilaterally at the ventral side of the cervical vertebrae. Bayatli(2011) (in goose), observed at this day the parachordal cartilage and the vertebral column around the notochord were stained blue. The vertebral column extends caudally to the parachordal cartilage along the notochord beginning from the cervical until the tip of the tail bud . Including cervical , thoracic, lumbosacral , and coccygeal regions. The above observations of Bayatli are identical to the observations that happened at day 3 of incubation for the quail in the current study.The same process occurred at day 5 of incubation in chick embryo. (Bellairs and Osmond, 2005).

Ribs:-

The fifth, sixth and seventh vertebral ribs were stained blue, as well as the third, fourth and fifth sternal ribs were stained blue also (Table.2).

Sternum:-

The sternal rudiments that appeared bilaterally in an earlier stage expanded ventrally and still stained blue. Also the inner processes of the laterocaudal processand mediocaudal process were stained blue.

Forelimb:-

LH=(4.2 ± 0.3 mm) (Diagram.1) . The humerus , radius and ulna are still stained blue, and no phalanges were stained blue till now (Table.3). That is not agreed with Nakane and Tsudzuki(1999), since they said that the humerus , radius , and ulna turned red at the central portion of each bone, also they said that the first phalanx of the second digit and the second phalanx of the third digit were stained blue.

Hindlimb:-

LF=(5.45 ± 0.3 mm) (Diagram. 3). The femur , tibia, and fibula are still stained blue at this day of incubation. No blue stained phalanges were observed at this day, only the metatarsus was stained blue (Table.3). While Nakane and Tsudzuki (1999) watched red regions at the center portion of the femur , tibia and fibula. Also they observed blue staining in the first phalanx of the first digit, second phalanx of the second digit, third phalanx of the third digit and the third and fourth phalanges of the fourth digit.

At day 8 of incubation.

AVG egg weight before incubation was 12.32 gms, and at time of extraction was 11.89 gms (Diagram.5).

At day 8 of incubation , ossified red regions first appeared along the whole skeleton system.

Vertebrae:-

The transverse processes of the 6^{th} to 10^{th} lumbosacral vertebrae were stained blue(Fig.10) , and that is identical to what Nakane and Tsudzuki(1999) mentioned. In this stage there was observable blue staining of the vertebral element for the first time which became more obvious cartilaginous (Table.1).

Ribs:-

All uncinated processes of the vertebral ribs were stained blue(Fig.10).While,Nakane and Tsudzuki, (2011), observed that the uncinated processes of the third to sixth vertebral ribs were stained blue only. Bayatli(2011), didn't mentioned the ribs at this day of incubation in goose yet.

Sternum:-

The sternal rudiments were closely adjacent to each other at the midline, and they are still blue stained (Table.2).

Forelimb:-

LH= (6.4 ± 0.2 mm) (Diagram.1).The percentage of the red (ossified) region in the humerus (PH)=(25± 5%) (Diagram.2).

The humerus , radius , and ulna turned red at the central portion of each bone (Table. 3). The scapula is still blue, as well as the clavicle. The first phalanx of the second digit and the second phalanx of the third digit were partly turned red at the mid region of each bone(Fig.10) .Nakane and Tsudzuki (1999), observed the second phalanx of the second digit was stained blue. The scapula and the third and fourth metacarpi turned red at the central portion , also they observed that the clavicle was stained red . At this day , Bayatli(2011), observed blue staining of the scapula and coracoid of the pectoral girdle in goose. Time of appearance of these events disagreed with Chevallier(1977) , while he noted that chondrofication at about the end of the sixth day in both of the wing and leg buds of the chick embryo.

The onset of ossification of forearm parts of the wing including humerus, radius, and ulna in quail was at day 8 of incubation, in comparison to goose occurred at day 12 of incubation (Bayatli, 2011), in chicks at day 8 of incubation (Hamburger and Hamilton, 1951), (7.5 – 8) days (Holder, 1978), 7 days (Bellairs and Osmond, 2005), 10 days (Sawad*et al*., 2008) in domestic chick, 9 days (Kurtul*et al* ., 2009) in hubbert strain chick embryo. While in

turkey the osteogenesis of long bones of forelimb observed at day 13 of incubation (Atalgin and Kurtul, 2009). It has been well known that birds posses different postnatal growth rate, i.ealtricial species show higher postnatal growth rate than precocial birds (Ricklefs*et al*., 1998 ; Bloom and Lilja, 2005).

Hindlimb:-

LF=(7.85 ± 0.3 mm) (Diagram.3). The percentage of the red (ossified) region in the femur (PF)= (12± 5%) (Diagram.4).The femur , tibia , and fibula turned red at the central portion of each bone (Table.3). The first phalanx of the second digit , the second phalanx of the third digit and the fourth phalanx of the third digit were stained blue(Fig.10). Nakane and Tsudzuki (1999) also observed blue staining of the phalanges but in different sequences, also they observed red color turning at the central portion of the third and fourth tarsometatarsi. At this day of incubation , Bayatli(2011), in goose observed the precursor of the femur , tibia , and fibula has developed from the mesenchyme of the leg bud. These observations were in the similar pattern of establishment of the wing and leg buds of the precartilaginous elements of chick embryo. With different time of occurrence of these events (Bellairs and Osmand , 2005) noted that it occurs at the 6^{th} day in the wing and fifth day in the hind limb of the check embryo.

At day 9 of incubation.

AVG egg weight before incubation was 12.52 gms, and at time of extraction was 12.08 gms(Diagram.5).

Vertebrae:-

(Hereafter, in the description , each of the cervical , thoracic, lumbosacral , and coccygeal vertebral regions will be divided into three regions, that is upper, medial , and lower regions).

The cervical , thoracic , lumbosacral , and coccygeal vertebrae were well recognized and stained blue all(Fig.11) (Table.1). Nakane and Tsudzuki (1999),

said that only the transverse processes of the 11th lumbosacral vertebra and the fourth to sixth coccygeal vertebrae were stained blue at this day of incubation. Bayatli(2011), observed very clear blue staining of the vertebral components in goose, including the bodies and arches of all vertebrae, the vertebral developmental features atthis day of incubation are similar to that of turkey embryos that observed by (Atalgin and Kurtul, 2009) .

The individual vertebra of birds posses the typical structure found in all vertebrae consist of vertebral body or centrum and bilateral arches (Bellairs and Osmond , 2005).

Ribs:-

The uncinate processes of the vertebral ribs are still blue, and the central portion of the first to fifth vertebral ribs turned red (Table.2). This is almost near to Nakane and Tsudzuki (1999),observations at this day of incubation. Bayatli(2011), observed only the pre-cartilaginous drafts of the 6th and 7th vertebral ribs at this day in goose.

Sternum:-

The crest and the outer process of the laterocaudal process, and the laterocranial process were stained blue. The upper regions of the bilateral sternal rudiments were fused to each other at the ventral midline (table.2).

Forelimb:-

LH= (8.2 ± 0.15 mm) (Diagram.1). PH= (35 ± 5%) (Diagram.2).The red regions of the humerus, radius , and ulna expanded from the center to toward the proximal and distal ends(Fig.11). The first phalanx of the first and second digits as well as the coracoid turned red at the central portion of each bone (Table.3). Bayatli , (2011), said that the developmental features of the shoulder girdle in early day 9 of incubation in goose revealed blue staining of the scapula and coracoids, with no appearance of the clavicle chondrofication is in consistence with Chivallier (1977) who found that at 6th day of chick embryo

there was no sign of clavicular mesenchymal condensation. Whereas (Hall, 1986) found out that at (7.5 days) there was cartilaginous scapula, coracoid, and humerus with mesenchymal condensation for the future clavicle arises.AlsoBayatli said that at day 9 of incubation in goose there was observable blue staining of the humerus , radius , and ulna of the forelimb, also there was elongation of the wing through addition of the new elements including carpal and metacarpal elements.

Hindlimb:-

LF= (9.5 ± 0.2 mm) (Diagram.3). PF= (40 ± 5%) (Diagram.4).The patella stained blue. Ilium and ischium turned red at the centeral portion of each bone (Table.3). The first phalanx of the second digit, first phalanx of the third digit, and the first and second phalanges of the fourth digit turned red at the central portion of each bone(Fig.11). That is typical to what Nakane and Tsudzuki (1999), observed in quail embryos also.Bayatli(2011), said that there was blue staining of femur, tibia, and fibula of the hind limb elements in addition to chondral drafts of the proximal and distal tarsal elements and blue staining of the second, third , and fourth metatarsal. While carpals , tarsals , and metacarpals of turkey embryo chondrified at 10^{th} day.(Atalgin and Kurtul, 2009).Bellairs and Osmond, (2005) observed that carpals and metacarpals appears between the 6^{th}-8^{th} days of incubation, whereas tibiotarsls and metatarsals appear during the early part of day 6 of incubation in check embryo. While,Hosseini and Hogg, (1991) observed recognizable perichondrium surrounding cartilage core in the diaphyseal portion of the shaft of tibia in check embryo at 7^{th} day of incubation.

At day 10 of incubation.

AVG egg weight before incubation was 12.54 gms, and at time of extraction was 12.24 gms) (Diagram.5).

Vertebrae:- The centrapartly turned red in the medial cervical , thoracic , and lumbosacral regions(Fig.13). Nakane and Tsudzuki(1999) observed the fusion of the transverse processes of the 7^{th} to 10^{th} lumbosacral vertebrae to each other at the tips. The dorsal spines of the first to third lumbosacral vertebrae fused with each other, and partly turning red of the centra in the cervical , thoracic and lumbosacral regions also.

Ribs:-

The sixth and seventh vertebral ribs turned red at the central portion(Fig.13) (Table.2). While, in goose seven vertebral ribs (1^{st}-7^{th}) and the first four sternal ribs (1^{st}-4^{th}) were stained blue (Bayatli, 2011). That observations of Bayatli was parallel to that noted by (Atalgin and Kurtul, 2009) in turkey.

Sternum:-

Still stained blue and the fusion of the bilateral sternal rudiments reached to the tip of the mediocaudal process (Table.2). That was identical to what (Nakane and Tsudzuki , 1999) observed.

Forelimb:-

LH= (8.4 ± 0.15mm) (Diagram.1). PH= (50 ± 3%) (Diagram.2).The first and the second phalanges of the third digit turned red at the central portion(Fig.13). Bayatli(2011) said that the chondrotic drafts of carpal elements including carpiradiale and carpiulnare, and 1^{st} phalanx at 3^{rd} digit of the forelimb were stained blue. Likewise the pelvic girdle elements including the ilium , ischium, and pubis, in addition to chondrotic drafts of the first metatarsal and first phalanx of the second ,third , and fourth digits were stained blue. That is approximately parallel to that of turkey except pelvic girdle and digits occurred at day 11 of incubation (Atalgin and Kurtul, 2009).

Hindlimb:-

LF= (9.7 ± 0.15mm) (Diagram.3). PF= (58 ± 2.5%) (Diagram.4).The pubis , first phalanx of the first digit, second phalanx of the second digit, and the third phalanx of the third digit were stained red at the central portion of each bone(Fig.15) (Table.3). That is near to Nakane and Tsudzuki (1999) observations , but they added that a cartilaginous process extended toward the distal direction from the back portion of the proximal region of the tarsometatarsus.

At day 11 of incubation.

AVG egg weight before incubation was 13.15 gms, and at time of extraction was 12.53 gms (Diagram.5).

Vertebrae:-

The red ossified regions in the upper, medial and lower regions of the cervical, thoracic and lumbosacral vertebral regions expanded toward the proximal and distal ends of each vertebra (Table.1).While, the coccygeal vertebral region still not ossified yet (Fig.15).Nakane and Tsudzuki (1999) saw partly turning red in the centra of the upper and lower cervical regions, in the vertebral arches of the medial cervical region and in the centra of the medial thoracic region. Bayatli(2011), didn't observe ossification centers appearance yet in goose at this day of incubation as well as Atalgin and Kurtul, (2009) in turkey.

Ribs:-

The red ossified regions of the first to seventh vertebral ribs still expanding toward the proximal and distal ends(Fig.15). While, in goose at this day of incubation , the same expanding observed also by Nakane and Tsudzuki (1999) in quail also at this day of incubation. Bayatli(2011) said that the last sternal rib (5^{th}) showed blue staining , attached to the sternal ribs and coracoids elements at this stage. Blue staining of the body and inner laterocaudalprocessof the sternum in goose was in agreement with Atalgin and Kurtul(2009) in turkey.

Sternum:-

The manubrium was staind blue, but didn't connected to the sternal body (Table.2). That is parallel to Nakane and Tsudzuki (1999) observations at this day of incubation in this bone.

Forelimb:-

LH= (8.9 ± 0.15mm) (Diagram.1). PH= (55 ±3%) (Diagram.2).The first and the second phalanges of the second digit turned red at the distal portion(Fig.15) (Table.3). In goose there was blue staining of the 1st phalanx of the 1st and 4th digits. And 2nd Phalanx of the 2nd digit. Bayatli(2011).

Hindlimb:-

LF= (10.3 ± 0.2 mm) (Diagram.3). PF= (67 ± 3%) (Diagram.4).The distal end of the tibia fused with the nodule of the fused proximal carpi.The second, third, and fourth phalanges of the fourth digit turned red at the central portion, the second phalanx of the first digit, the third phalanx of the second digit, and the fourth phalanx of the third digit partly turned red at the distal portion of each bone(Fig.15). While, Nakane and Tsudzuki (1999) observed partly turning red in the first metatarsus at the proximal portion, but their other observation were agreed with the current observations , since they also observed partly red turning in the third and fourth phalanges of the fourth digit at the central portion, also the second phalanx of the first digit, the third phalanx of the second digit, the fourth phalanx of the third digit and the fifth phalanx of the fourth digit at the central portion of each bone. Bayatli(2011), said that at this day of incubation in goose there was blue staining in the 1st phalanx of the 1st digit, and 2nd phalanx of the 2nd, 3rd, and 4th digits of the hind limb, and that was inconsistent with what had taken place in the turkey, in which the phalanges of the fore and hindlimbs and sternal ribs except the last rib took place at 10th day of incubation (Atalgin and Kurtul, 2009).

The time of appearance of the ossification centers in long bones of the fore and hind limbs showed different patterns of the sequence of ossification process. The observations that were accumulated on the quail at this day of incubation differed from the accumulated data that were observed on goose , which were agreed with the accumulated data on turkey embryos, whose hind limb long bones showed ossification centers on the 12th day of incubation, those of the fore limb including humerus , radius and ulna showed slightly later ossification at the 13th day of incubation. This slight delay indicates the precocity at the expence of the bones of the leg, rather than those of the wing (Atalgin and Kurtul, 2009) in turkey and (Baerswyl, 1980; Pourlis*et al* ., 1998) in domestic fowl. While the current study showed similar pattern of ossification in both fore and hind limbs, as well as Holder, (1978) in chick embryo.

At day 12 of incubation.

AVG egg weight before incubation was 12.44 gms, and at time of extraction was 11.84 gms) (Diagram.5).

Vertebrae:-

The coccygeal vertebral region still not ossified yet (Table.1). The vertebral arches of the cervical , thoracic and lumbosacral vertebral regions started to be ossified (Fig.17).The above observations agreed with Nakane and Tsudzuki(1999), in such sides and disagreed with their observations in other sides, while they observed that the upper and lower cervical regions , the centra of the upper and lower thoracic regions, and the centra of the upper lumbosacral region partly turned red. The second to sixth thoracic vertebrae had a ventral spine and the first two were fused with each other at the tips.

Ribs:-

The central portion of the first to fourth sternal ribs turned red(Fig.17).

Sternum:-

The manubrium was fused into the sternal body(Fig.17).

Forelimb:-

LH= (9.4 ± 0.2mm) (Diagram.1). PH= (70 ± 2.5%) (Diagram.2).The humerus , radius and ulna reached late stages in it's process of ossification.The first and the second phalanges of the second digit partly turned red (Fig.17). While Nakane and Tsudzuki (1999) observed only elongation in the fore limbs at this day of incubation. Bayatli(2011),observed the first red turning of the humerus at this stage, which occurred later in relation to the femur was typically parallel to accumulating data in turkey embryo (Atalgin and Kurtul, 2009). But he disagreed with them in the timing, which occurred in the fore and hind limb at 12 and 13 days of incubation , respectively. This delay can be considered as an indicator for the difference of the precocity degree.The lifestyle and feeding may plays an important role in the sequences of the skeletal developmental differentiation between species (Daniel, 1957).

Hindlimb:-

LF= (10.8 ± 0.2 mm) (Diagram.3). PF= (80 ± 3%) (Diagram.4).The red (ossified) regions of the ossified phalanges still expanding. The red ossified regions of the femur , tibia and fibula are now well recognized and reached late stages at each bone. Also they were elongated in high percentage(Fig.17) (Table.3).

At day 13 of incubation.

AVG egg weight before incubation was 11.76 gms, and at time of extraction was 11.2 gms (Diagram.5).

Vertebrae:-

The Vertebral arches of the upper, medial , and lower cervical, thoracic and lumbosacral vertebral regions partly turned red (Table.1). In this case the cervical , thoracic and lumbosacral vertebral regions are completely turned red .The centrum of the vertebrae of the coccygeal vertebral region partly turned

red(Fig.19). That was almost agreed with Nakane and Tsudzuki, (1999) observations.

Ribs:-

The central portion of the fifth sternal rib partly turned red(Fig.19), and the previously ossified sternal ribs now completely turned red and elongated also (Table.2).

Sternum:-

Larger than that of the previous day (Fig.19).

Forelimb:-

LH= (10 ±0.2 mm) (Diagram.1). PH= (77 ± 3%) (Diagram.2). Elongated, and all the phalanges of the fore limb digits are now ossified (red colored)(Fig.19).

Hindlimb:-

LF = (11.6 ± 0.4 mm) (Diagram.3). PF=(85 ± 2.5%) (Diagram.4). Elongated, also all the phalanges of the hind limb digits are now ossified (red colored)(Fig.19).The above observations are almost near to what Nakane and Tsudzuki(1999) observed in quail at this day of incubation. But they didn't observe completely turning red in the metacarpus and metatarsus, only observed that in the digits.

At day 14 of incubation.

AVG egg weight before incubation was 11.79 gms, and at time of extraction was 11.21 gms (Diagram.5).

Vertebrae:-

The vertebral arches existing bilaterally on the centrum of the coccygeal vertebrae fused with each other at the midline resulting in formation of the vertebral foramen, also they were partly turned red(Fig.21). The cervical ribs of the lower cervical region , the vertebral arches of the lower thoracic region, the centra of the medial and lower lumbosacral regions, the vertebral arches of the

upper lumbosacral region and the ventral side of the transverse processes of the eighth lumbosacral vertebrae partly turned red, the centra of the third to eighth lumbosacral vertebrae were fused. (Nakane and Tsudzuki, 1999).

Ribs:-

Elongated only (Fig.21) (Table.2). While Bayatli(2011) observed (in goose) that the uncinated processes of the 2^{nd} , 3^{rd} and 4^{th} vertebral ribs , connected to the caudal edges of ribs and directed caudo-dorsally, were stained blue.

Sternum:-

The inner and the outer processes of the laterocaudal process turned red at the central portion (Table.2).

Forelimb:-

LH= (10.8 ± 0.2 mm) (Diagram.1). PH = (85 ± 2%) (Diagram.2). Elongated and the metacarpus partly turned red(Fig.21) (Table.3). Nakane and Tsudzuki(1999) said that the forelimb only elongated at this day of incubation without mentioning any other changes.Bayatli(2011) observed at this day of incubation in goose , the coracoid elements of the pectoral girdle at it's mid – diaphyseal portion turned red. For comparison , this element turned red in chick at stage (7.5-8) days (Hall, 1986) , and 12 days (Bellairs and Osmond, 2005), and in the turkey at 14^{th} day of incubation (Atalgin and Kurtul, 2009).These variations of time of onset of ossification of this bone may be related directly to the degree of precocity (Kurtul*et al*., 2009).

Hindlimb:-

LF= (12.9 ± 0.25 mm) (diagram.3). PF= (88 ± 1.5%) (Diagram.4).The femur , tibia, and fibula almost completely turned red. Ilium turned red, but ischium and pubis still partly ossified(Fig.21).

At day 15 of incubation.

AVG egg weight before incubation was 12.04 gms, and at time of extraction was 11.19 gms (Diagram.5).

Vertebrae:-

The cervical vertebral region (14 bones), thoracic vertebral region (7 bones) , lumbosacral vertebral region (12 bones) , and coccygeal vertebral region (8 bones) were completely turned red at this day of incubation(Fig.23) (Table.1).

The above truth almost agreed with Nakane and Tsudzuki(1999) since their accumulated observations showed almost completely ossification in the vertebral regions, they said that the vertebral arches of the medial and lower lumbosacral regions , the transverse processes of the 7^{th} , 9^{th} , and 10^{th} lumbosacral vertebrae, and the centra and vertebral arches of the upper coccygeal region partly turned red. The centra of the 7^{th} thoracic vertebrae to 11^{th} lumbosacral vertebrae were fused.

Ribs:-

All the vertebral and sternal ribs are now completely ossified (Fig.23). That is identical to Nakane and Tsudzuki (1999) , since they observed at this day red turning of the uncinated processes of the second to fifth vertebral ribs and of the central portion of the fifth sternal rib. This complement of their observations on ribs when joined with their previous observations will supports the observations of the current study .But in goose at day 15 of incubation, the vertebral ribs from 2^{nd} to 5^{th} began ossifying at the mid-longitudinal plane, but there were no signs of ossification in the uncinated processes of these ribs, (Bayatli, 2011).While, Tickle and Codd(2009), observed that all vertebral ribs began ossifying at day 18 of incubation in the turkey embryo, whereas observed at day 15 of incubation by Atalgin and Kurtul(2009) in the same bird. On the other hand (Kurtul*et al* ., 2009) observed the first occurrence of the ossification in the ribs at day 11 of incubation in chick embryo. But (Sawad*et al* ., 2008)

noted the primary ossification of the rib body at the proximal extremity at day 10 of incubation in chick embryo also.

Sternum:-

Completely turned red (Fig.23).

Forelimb:-

LH = (12.5 ± 0.3 mm) (Diagram.1). PH = (90 ±3%) (Diagram.2). Elongated only (Fig.23)..

Nakane and Tsudzuki (1999) said that the proximal end of the third metacarpus fused with the nodule of the fused distal carpi.Bayatli(2011) observed the 3rd and 4th metacarpals of the fore limb were slightly turned red at their mid-diaphyseal as a beginning of ossification at this day of incubation.

Hindlimb:-

LF = (14 ± 0.4 mm) (Diagram.3). PF= (92 ± 2%) (Diagram.4).Elongated. In goose and at this day of incubation , the 1st phalanx of the 3rd digit of the hind limb began ossifying , the ilium and ischium of the innominate of the hind limb were turned red in this stage (Bayatli, 2011). Atalgin and Kurtul(2009), noted occurrence of that at 15th and 16th day of incubation in the ilium and ischium in turkey. And at 14th day of same time with the pubis in chick embryo (Sawad*et al* ., 2008).

At day 16 of incubation.

AVG egg weight before incubation was 12.39 gms, and at time of extraction was 11.73 gms) (Diagram.5).

At the end of this day all the rest of the incubated egg were hatched (Fig.26). While Nakane and Tsudzuki (1999), needed extra one day for hatching.

Vertebrae:-

All the vertebral bones that were ossified in the previous stage enlarged significantly (Fig.25).The centrum of the cervical vertebrae at the cranial and middle regions began ossifying by turning red appeared like spot at the central portion of the body of each ossified vertebrae (Bayatli, 2011).Bayatli also said that the onset of ossification appeared in some of the cervical vertebrae in 16th day of incubation in his study on goose. Whereas in chick embryo the onset of bone formation of the vertebral bodies was occurred at 13th day of incubation and extended dorsally within the neural arches at 13.5th days and vertebrae developed entirely through 16th days of incubation (Shapiro, 1992). While,Bellairs and Osmond (2005), noted the appearance of the primary ossification centers in the chick embryo at 11th-13th days of incubation.

Ribs:-

Elongated and enlarged significantly (Fig.25).While, in goose , the 6th and 7th vertebral ribs began ossifying at the mid-longitudinal plane, and there was distinguished progression of the ossification to the proximal and distal region in the vertebral ribs (2nd – 5th), while there was no appearance of ossification in their uncinated processes (Bayatli, 2011).

Sternum:-

Elongated and enlarged significantly (Fig.25).

Fore limb:-

LH = (14.8 ± 0.4 mm) (Diagram.1). PH = (95 ± 2%) (Diagram.2). Elongated and enlarged significantly (Fig.25). That was typically similar to Nakane and Tsudzuki(1999).

Hindlimb:-

LF = (17.3 ± 0.5 mm) (Diagram.3). PF = (95 ± 0.25%) (Diagram.4). Elongated and enlarged significantly (Fig.25).In goose and at this day of incubation the phalangeal elements including the 1st phalanx of the 2nd digit of the forelimb , in addition to the 1st phalanx of the 1st, 2nd , and 4th digits and 2nd

phalanx of the 3rd digit in hind limb showed appearance of ossification centers (Bayatli, 2011).

Table1. Transition of chondrofication and ossification of the vertebral ones

Vertebral bones	Day of incubation													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cervical vertebrae														
Upper region														
Centrum	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	R	R	R	R	R
Middl region														
Centrum	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	R	R	R	R	R
Lower region														
Centrum	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	R	R	R	R	R
Thoracic vertebrae														
Upper region														
Centrum	B	B	B	B	B	B	B	B	B	R	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	B	R	R	R	R
Middl region														
Centrum	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	B	R	R	R	R
Lower region														
Centrum	B	B	B	B	B	B	B	B	B	R	R	R	R	R

Vertebral arch	-	B	B	B	B	B	B	B	B	B	B	R	R	R
Lumbosacral vertebrae														
Upper region														
Centrum	B	B	B	B	B	B	B	B	B	R	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	B	B	R	R	R
Middl Medial region														
Centrum	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	B	R	R	R	R
Lower region														
Centrum	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Vertebral arch	-	B	B	B	B	B	B	B	B	B	R	R	R	R
Coccygeal vertebrae														
Upper region														
Centrum	-	B	B	B	B	B	B	B	B	B	R	R	R	R
Vertebral arch	-	-	B	B	B	B	B	B	B	B	B	R	R	R
Middl Medial region														
Centrum	-	B	B	B	B	B	B	B	B	B	R	R	R	R
Vertebral arch	-	-	B	B	B	B	B	B	B	B	B	R	R	R
Lower region														
Centrum	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Vertebral arch	-	-	B	B	B	B	B	B	B	B	B	R	R	R

-, Not stained with either Alcian blue or Alizarin red S ; B, Stained blue with Alcian blue; R, partly stained red with Alizarin red S

Table 2. Transition of chondrofication and ossification of the bones in the ribs and sternum

Bones of ribs and sternum	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ribs														
First vertebral rib	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Second vertebral rib	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	B	B	B	B	B	B	B	R	R
Third vertebral process	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	B	B	B	B	B	B	B	R	R
Fourth vertebral rib	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	B	B	B	B	B	B	B	R	R
Fifth vertebral rib	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	B	B	B	B	B	B	B	R	R
Sixth vertebral rib	-	-	-	-	B	B	B	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	B	B	B	B	B	B	B	B	R
Seventh vertebral rib	-	-	-	-	B	B	B	R	R	R	R	R	R	R

Firs sternal rib	-	-	-	B	B	B	B	B	B	R	R	R	R	R
Second sternal rib	-	-	-	B	B	B	B	B	B	R	R	R	R	R
Third sternal rib	-	-	-	B	B	B	B	B	B	R	R	R	R	R
Fourth sternal rib	-	-	-	B	B	B	B	B	B	R	R	R	R	R
Fifth sternal rib	-	-	-	-	B	B	B	B	B	B	R	R	R	R
Sternum														
Body	-	-	-	B	B	B	B	B	B	B	B	B	B	B
Laterocranial process	-	-	-	-	-	-	B	B	B	B	B	R	R	R
Laterocaudal process	-	-	-	-	-	B	B	B	B	B	B	R	R	R
Manubrium	-	-	-	-	-	-	-	-	B	B	B	B	B	B
Crest	-	-	-	-	-	-	B	B	B	B	B	B	B	B

Table 3.Transition of chondrofication and ossification of the bones in the fore – and hind limb

Bones in the fore- and hind limbs.	Day of incubation													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Forelimb														
Scapula	-	-	B	B	B	R	R	R	R	R	R	R	R	R
Coracoid	-	-	B	B	B	B	R	R	R	R	R	R	R	R
Clavicle	-	-	-	-	-	B	B	B	R	R	R	R	R	R
Humerus	-	B	B	B	B	R	R	R	R	R	R	R	R	R
Radius	-	B	B	B	B	R	R	R	R	R	R	R	R	R
Ulna	-	B	B	B	B	R	R	R	R	R	R	R	R	R
Second metacarpus	-	-	B	B	B	B	-	-	-	-	-	-	-	-
Third metacarpus	-	-	B	B	B	R	R	R	R	R	R	R	R	R
Fourth metacarpus	-	-	B	B	B	R	R	R	R	R	R	R	R	R
First phalanx of the second digit	-	-	-	-	B	B	R	R	R	R	R	R	R	R
Second phalanx of the second digit	-	-	-	-	-	B	B	B	R	R	R	R	R	R
First phalanx of the third digit	-	-	-	B	B	B	B	R	R	R	R	R	R	R
Second phalanx of the third digit	-	-	-	-	B	B	B	R	R	R	R	R	R	R
First phalanx of the	-	-	-	-	B	B	B	B	B	B	B	R	R	R

fourth digit														
Hind limb														
Ilium	-	-	-	B	B	B	B	B	B	B	B	B	R	R
Ischium	-	-	-	-	B	B	B	R	R	R	R	R	R	R
Pubis	-	-	-	-	B	B	B	R	R	R	R	R	R	R
Femur	-	B	B	B	B	R	R	R	R	R	R	R	R	R
Tibia	-	B	B	B	B	R	R	R	R	R	R	R	R	R
Fibula	-	B	B	B	B	R	R	R	R	R	R	R	R	R
Patella	-	-	-	-	-	-	B	B	B	B	B	B	B	B
First metatarsus	-	-	-	B	B	B	B	B	R	R	R	R	R	R
Second metatarsus	-	-	-	B	B	R	R	R	R	R	R	R	R	R
Third metatarsus	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Fourth metatarsus	-	-	-	B	B	B	R	R	R	R	R	R	R	R
First phalanx of the first digit	-	-	-	-	B	B	B	R	R	R	R	R	R	R
Second phalanx of the first digit	-	-	-	-	-	B	B	B	R	R	R	R	R	R
First phalanx of the second digit	-	-	-	-	B	B	B	R	R	R	R	R	R	R
Second phalanx of the second digit	-	-	-	-	B	B	B	R	R	R	R	R	R	R
Third phalanx of the second digit	-	-	-	-	-	B	B	B	R	R	R	R	R	R

First phalanx of the third digit	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Second phalanx of the third digit	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Third phalanx of the third digit	-	-	-	-	B	B	B	R	R	R	R	R	R	R
Fourth phalanx of the third digit	-	-	-	-	-	B	B	B	R	R	R	R	R	R
First phalanx of the fourth digit	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Second phalanx of the fourth digit	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Third phalanx of the fourth digit	-	-	-	-	B	B	B	B	R	R	R	R	R	R
Fourth phalanx of the fourth digit	-	-	-	-	B	B	B	B	R	R	R	R	R	R
Fifth phalanx of the fourth digit	-	-	-	-	-	B	B	B	R	R	R	R	R	R

-, Not stained with either Alcian blue or Alizarin red S ; B, Stained blue with Alcian blue; R, partly stained red with Alizarin red S.

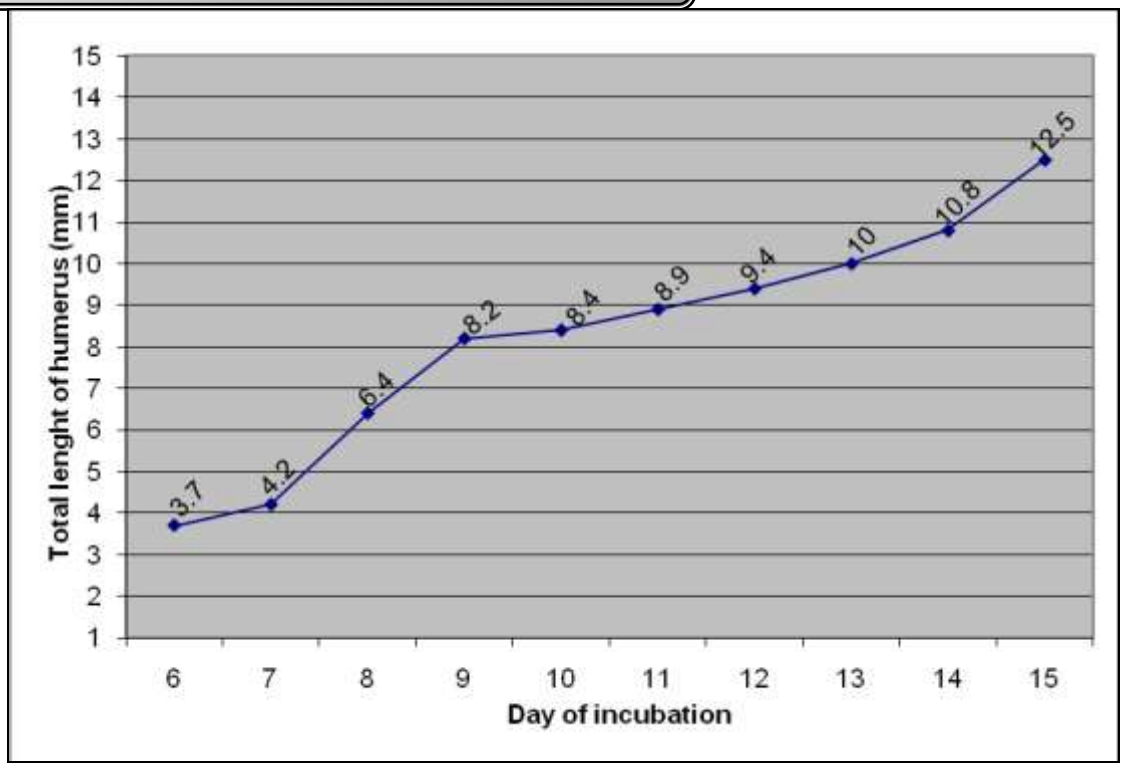

Diagram (1). Growth curve of the total length of the humerus

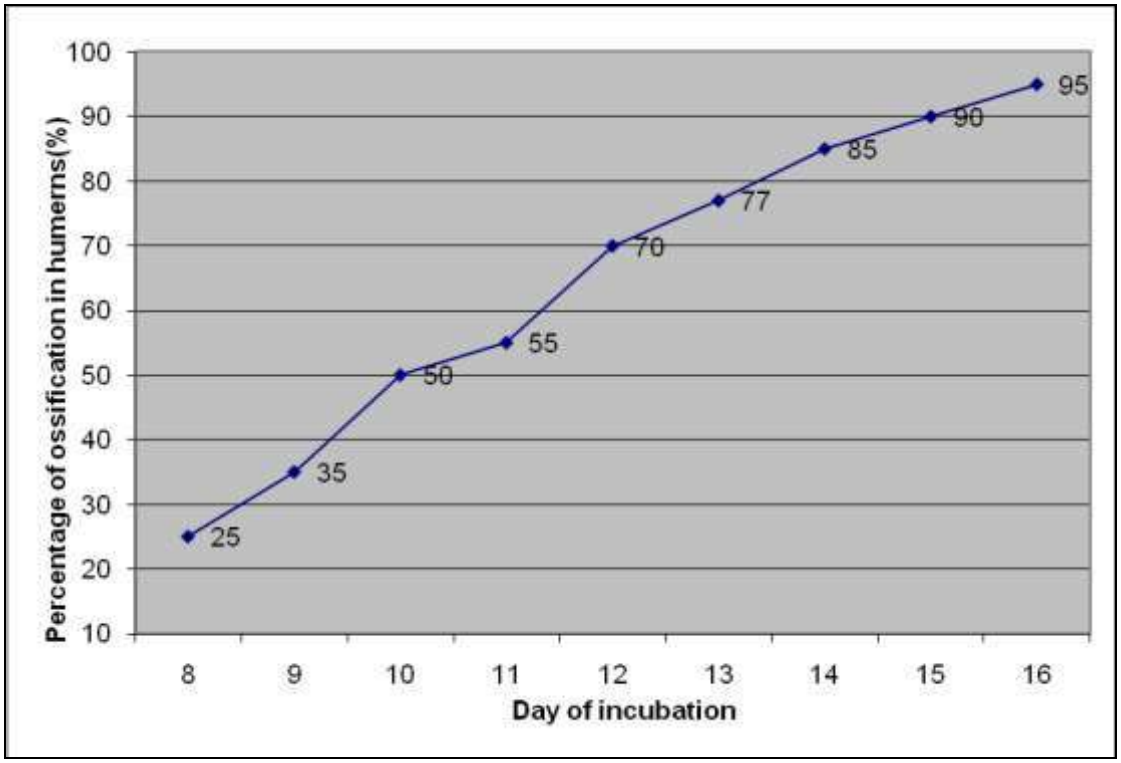

Diagram (2). Percentage of ossification in humerus

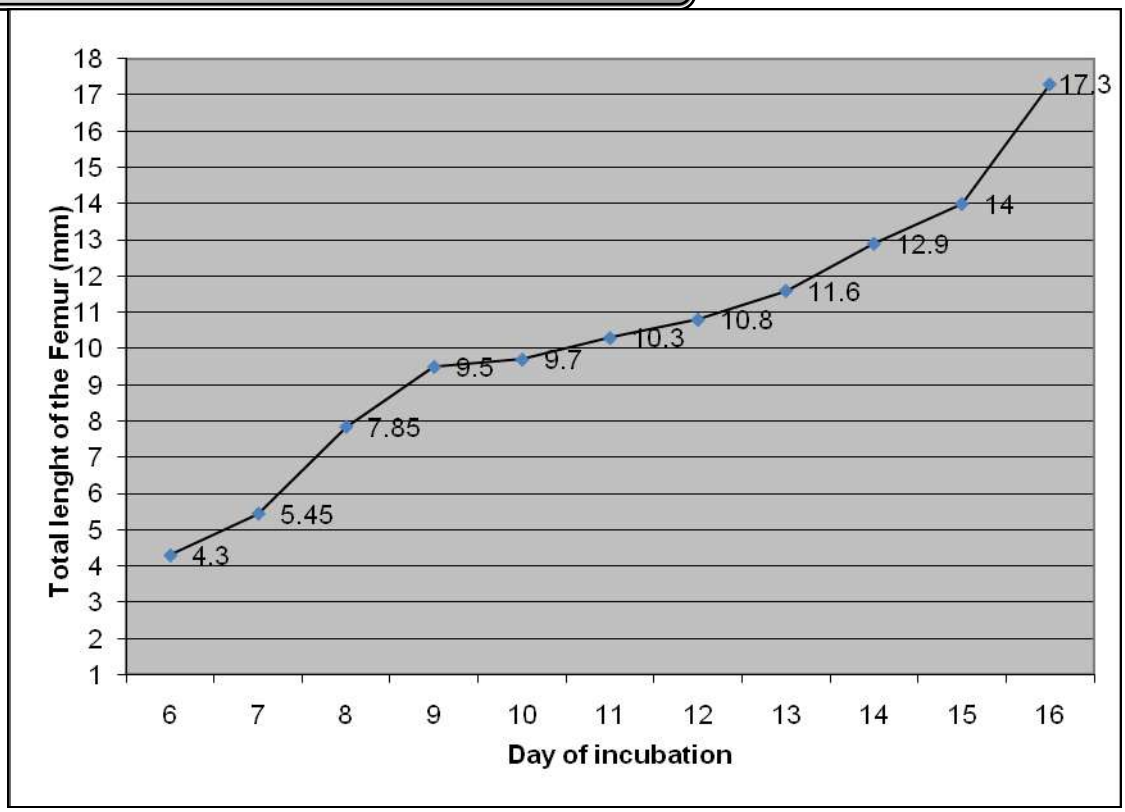

Diagram (3) Growth curve of the total length of femur

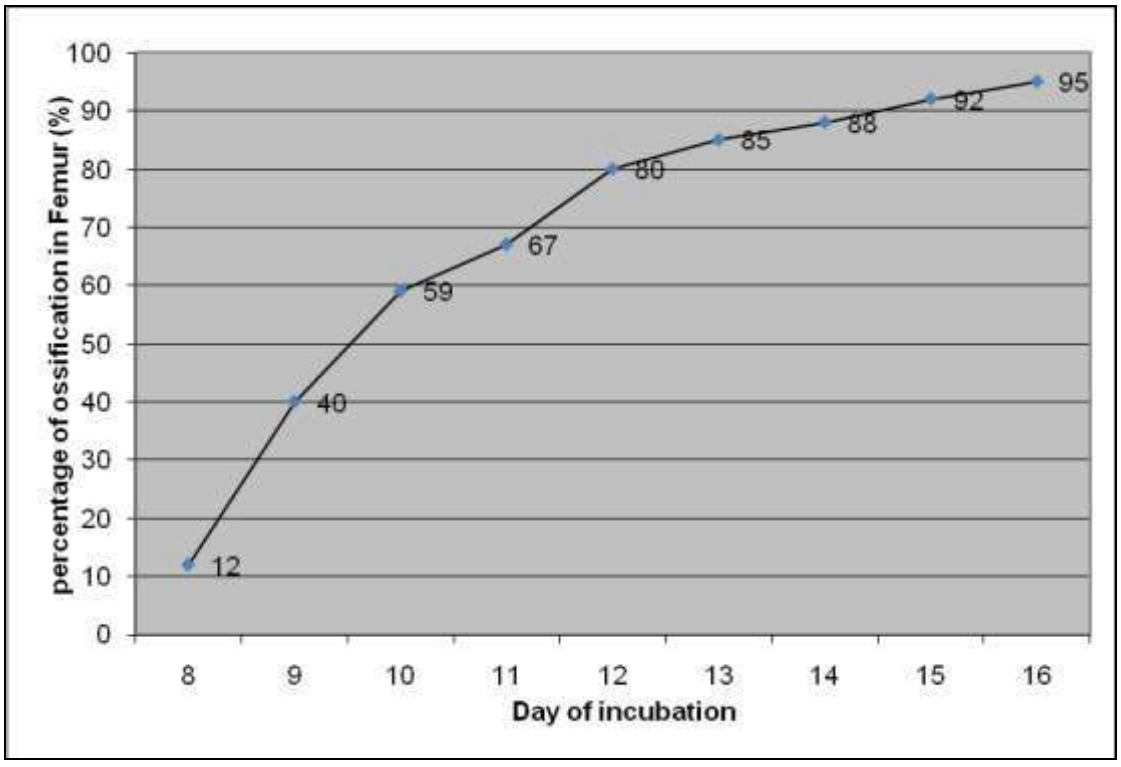

Diagram (4) Percentage of ossification in femur

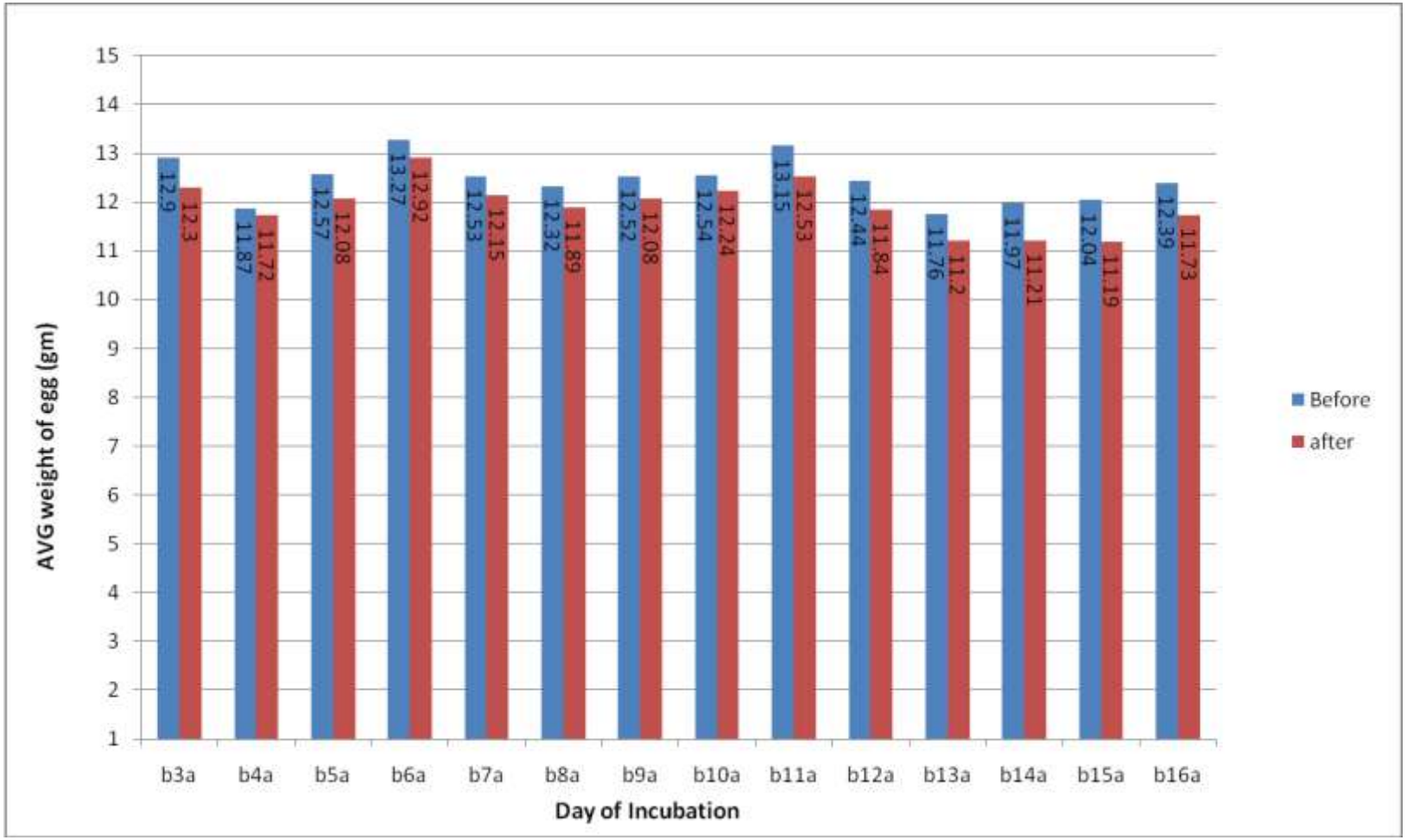

Diagram (5) Average weight of the egg before and after incubation

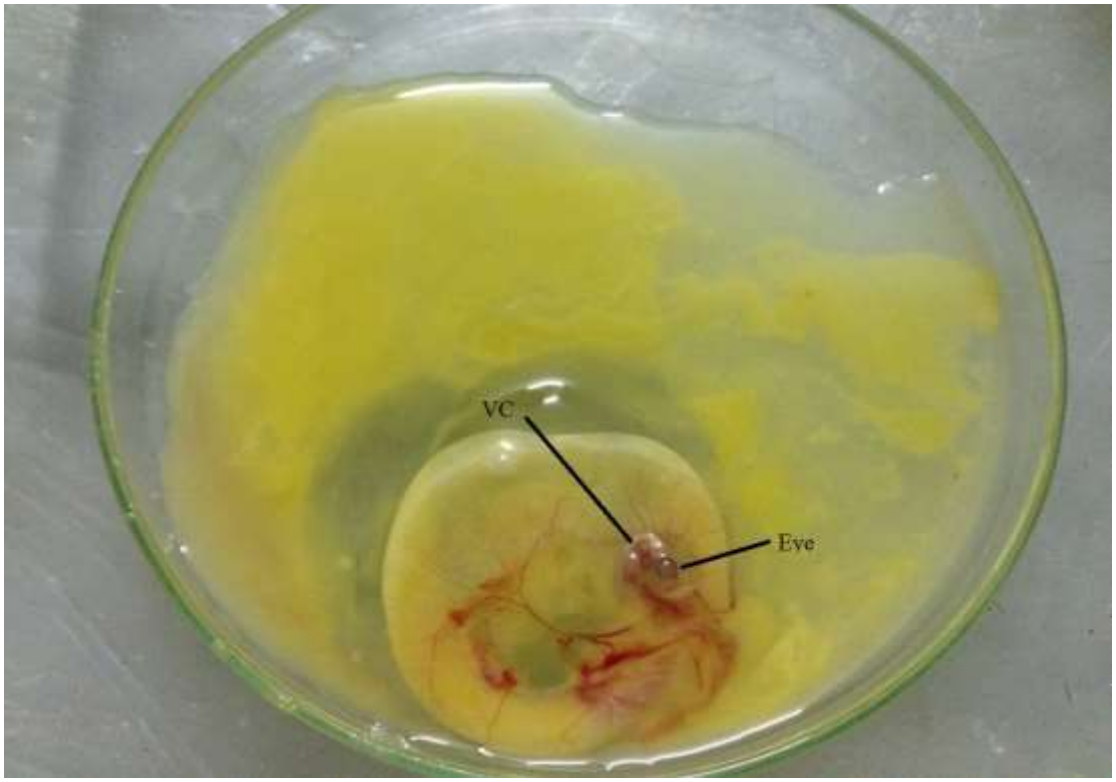

Fig (3). Embryo of 3 days of incubation, Vertebral column (VC).

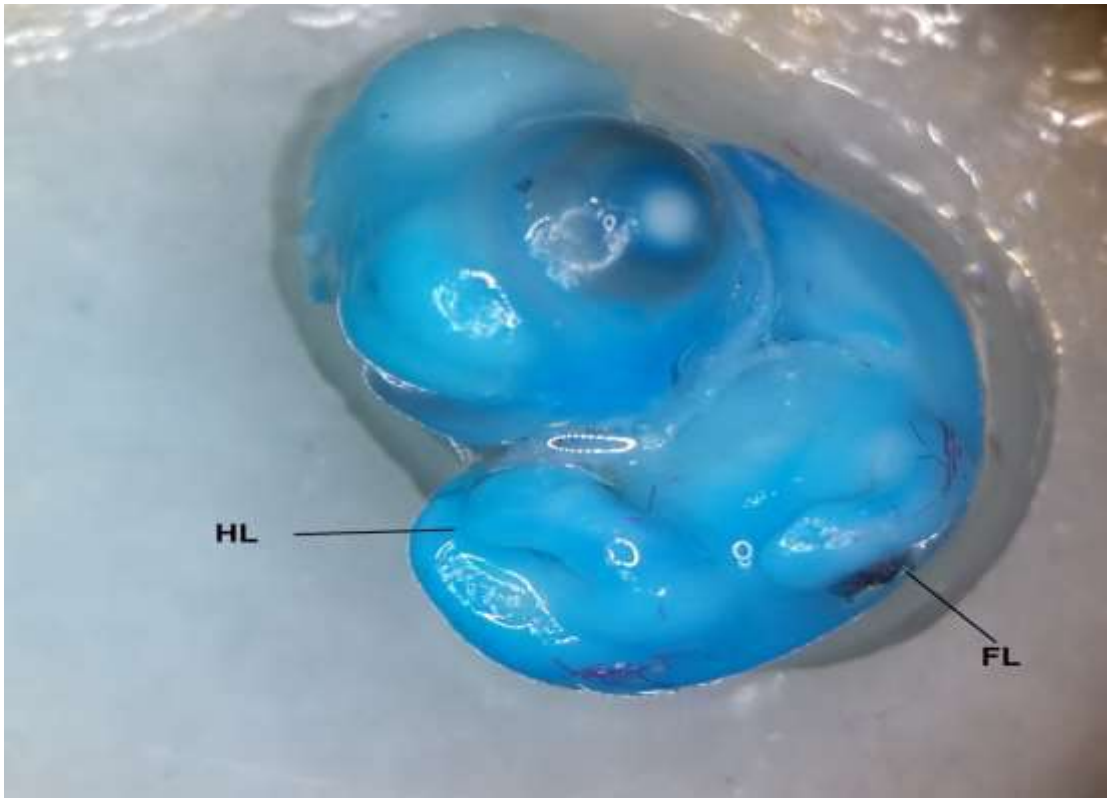

Fig (4). Whole mount of 3 days of incubation embryo stained with Alcian blue, hindlimb (HL), forelimb (FL).

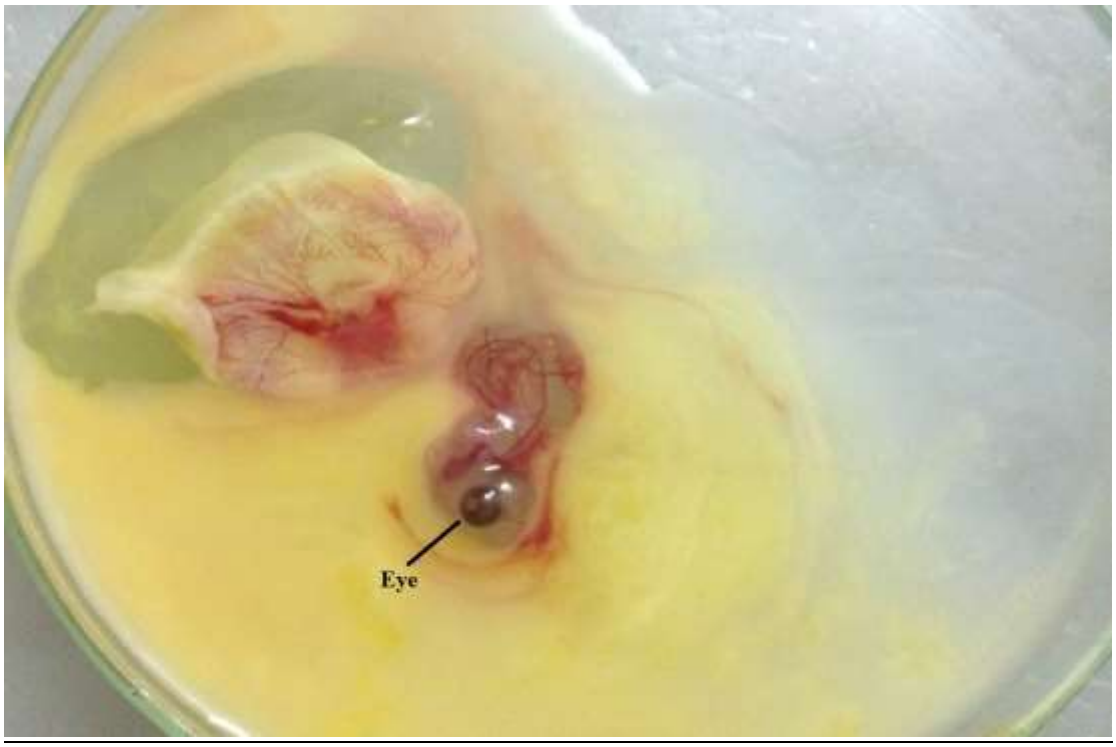

Fig (5). Embryo of 4 days of incubation

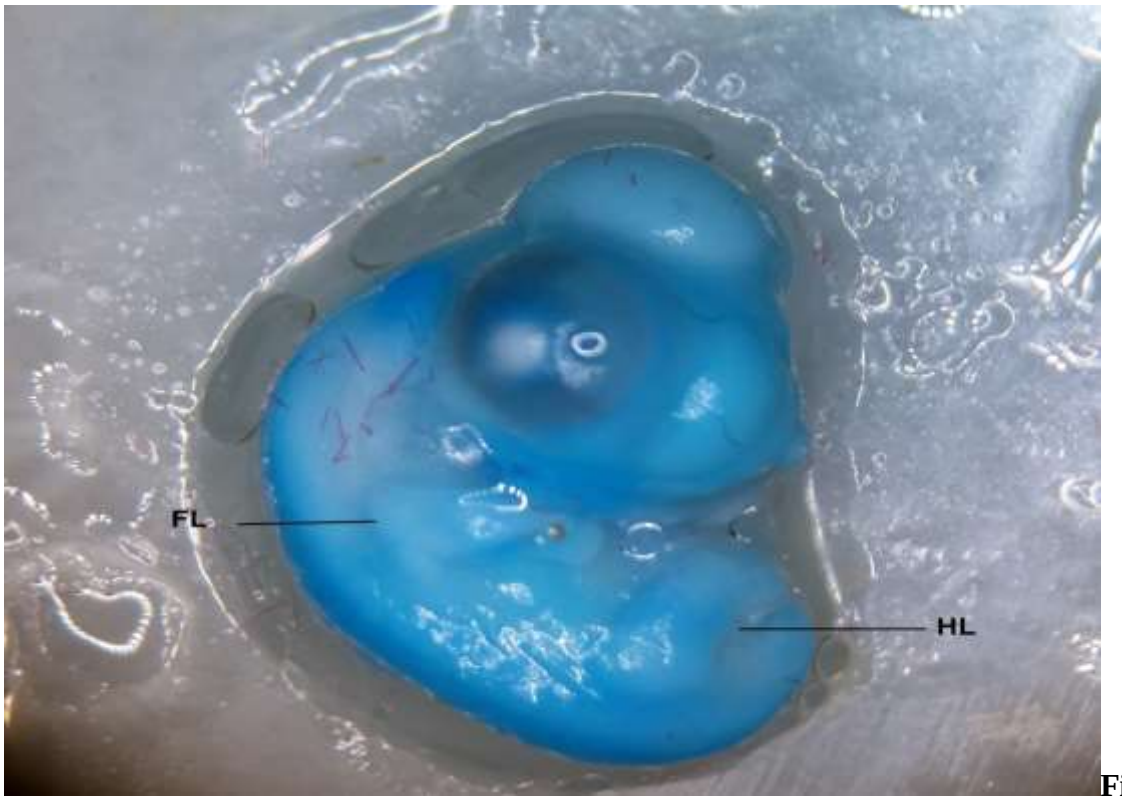

Fig (6). Whole mount of 4 days of incubation embryo stained with Alcian blue, hindlimb (HL), forelimb (FL).

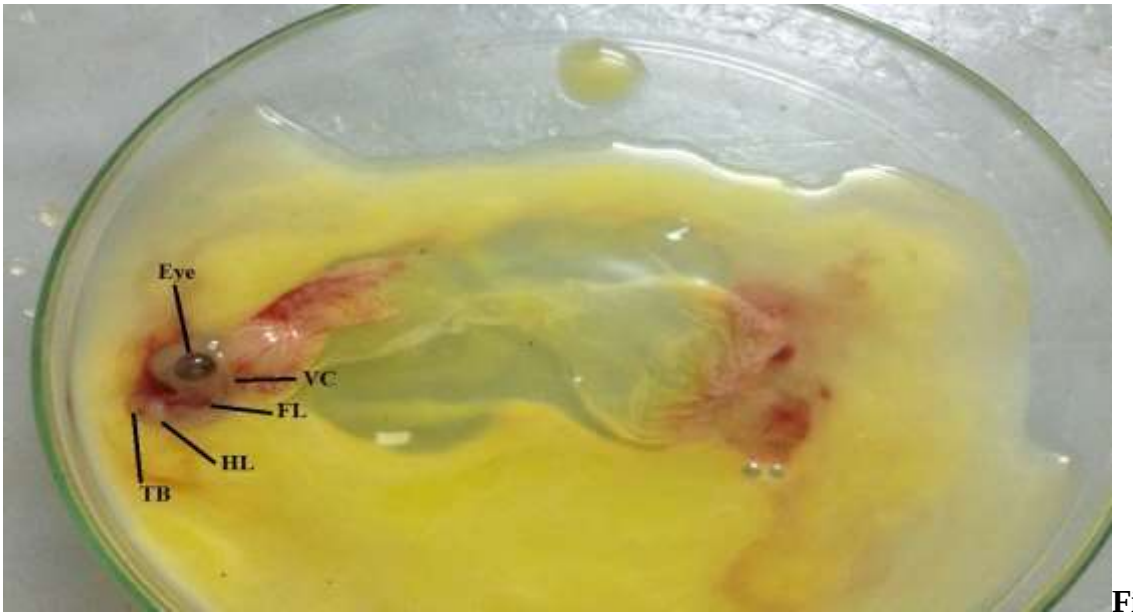

Fig (7). Embryo of 5 days of incubation, Vertebral column (VC); Fore limb (FL); Hind Limb (HL); Tail Bud (TB).

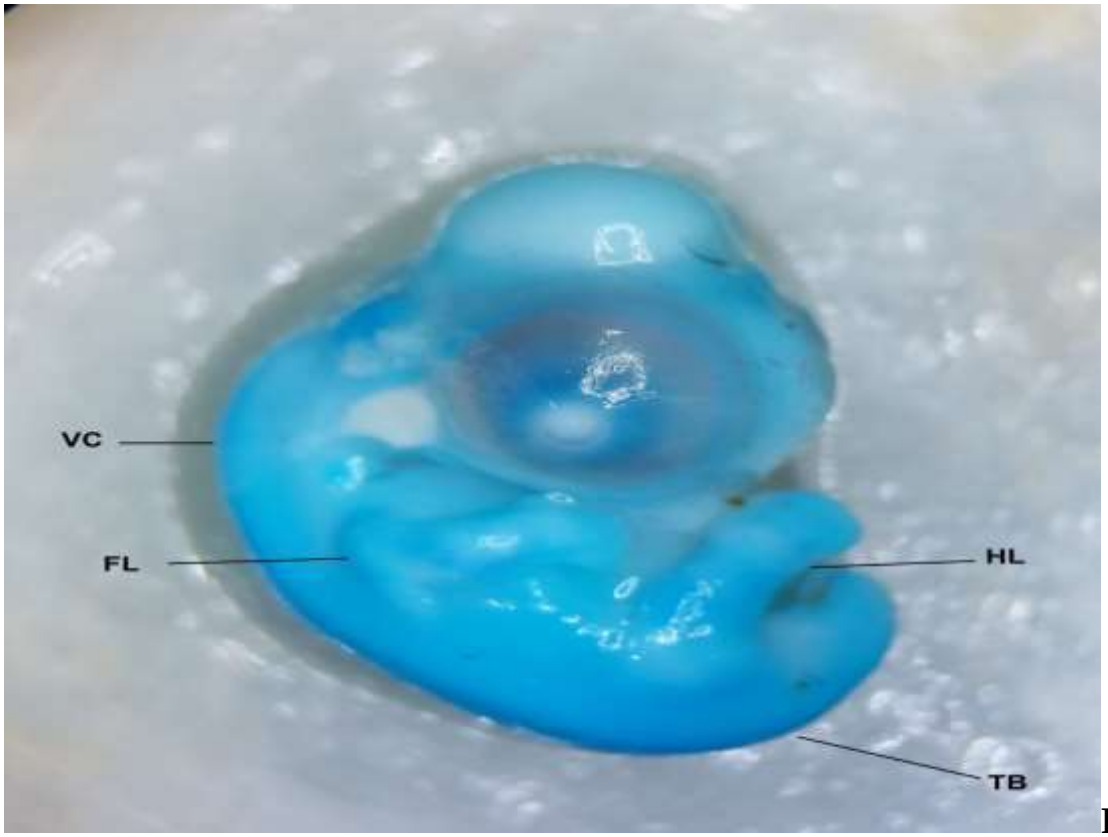

Fig (8).Whole mount of 5 days of incubation embryo stained with Alcian blue, hindlimb (HL);forelimb (FL); Chondrofied vertebral column (VC); Tail bud (TB).

Fig (9). Embryo of 8 days of incubation

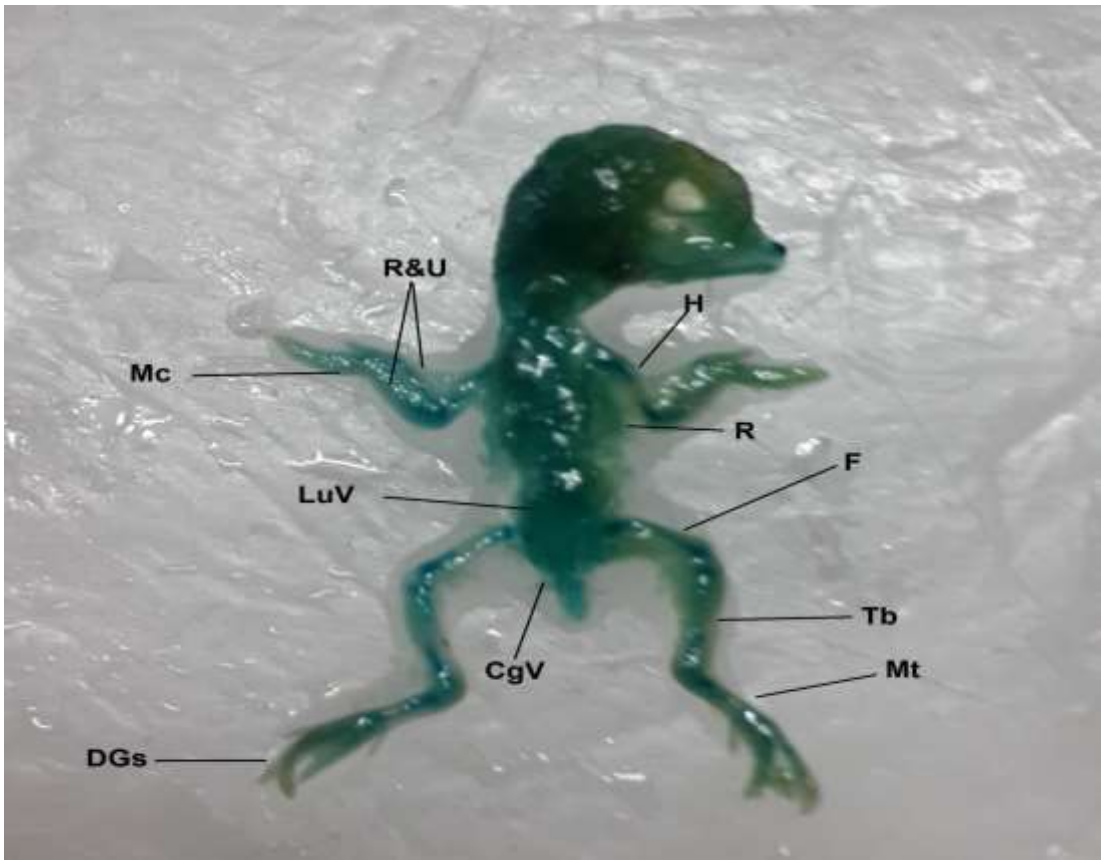

Fig (10).Whole mount of 8 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, Humerus (H), Radius and Ulna (R&U); Metacarpus (Mc); Ribs (R); Femur (F); Tibia (Tb); Metatarsus (Mt); Digits (DGs); Lumbosacral vertebral region (LuV); Coccygeal vertebral region (CgV)

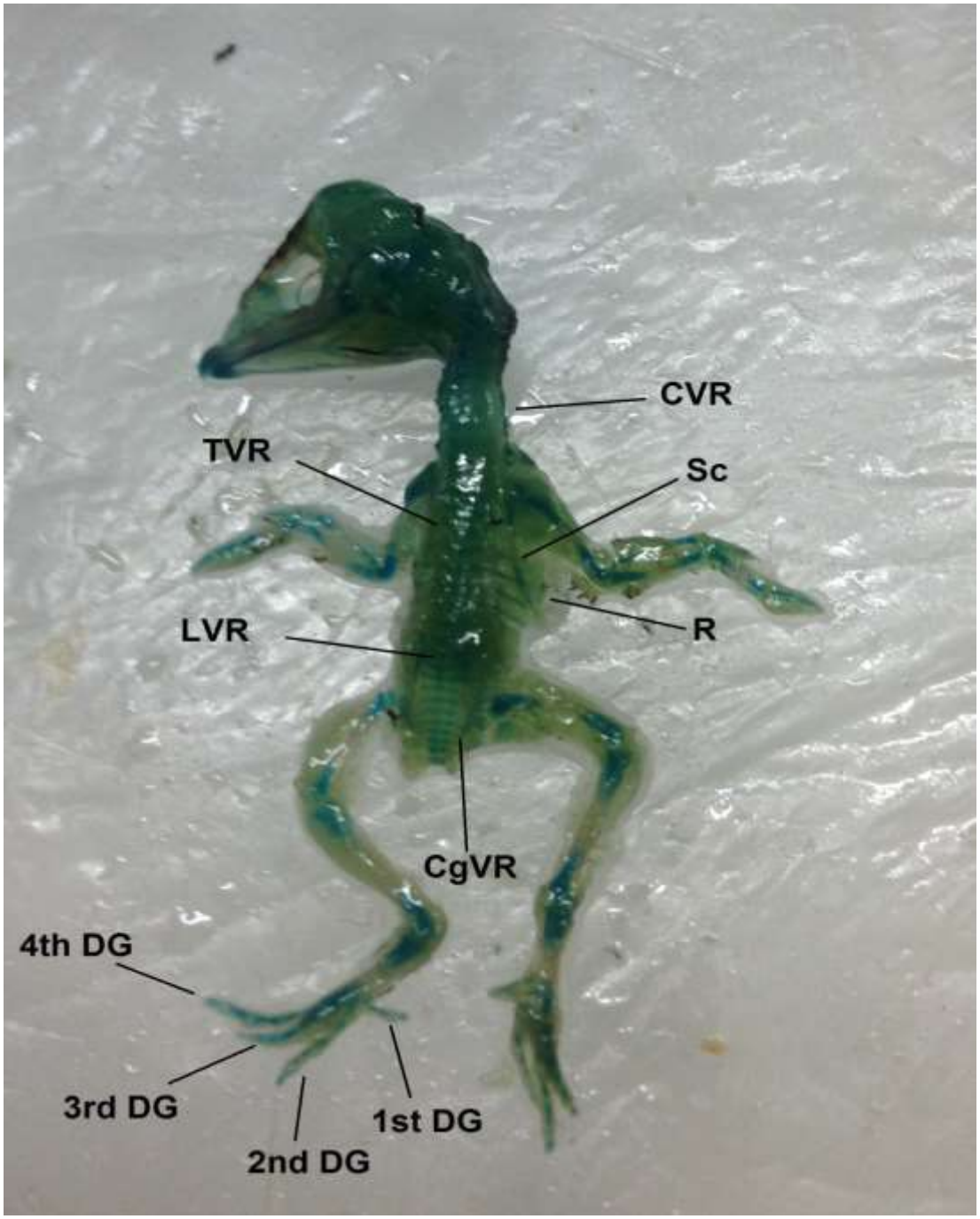

Fig (11). Whole mount of 9 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, Cervical vertebral region (CVR), Thoracic vertebral region (TVR); Lumbosacral vertebral region (LuVR); Coccygeal vertebral region (CgVR); Scapula (Sc); Ribs (R); Digits (1st, 2nd, 3rd, and 4th DGs) .

Fig (12). Embryo of 10 days of incubation

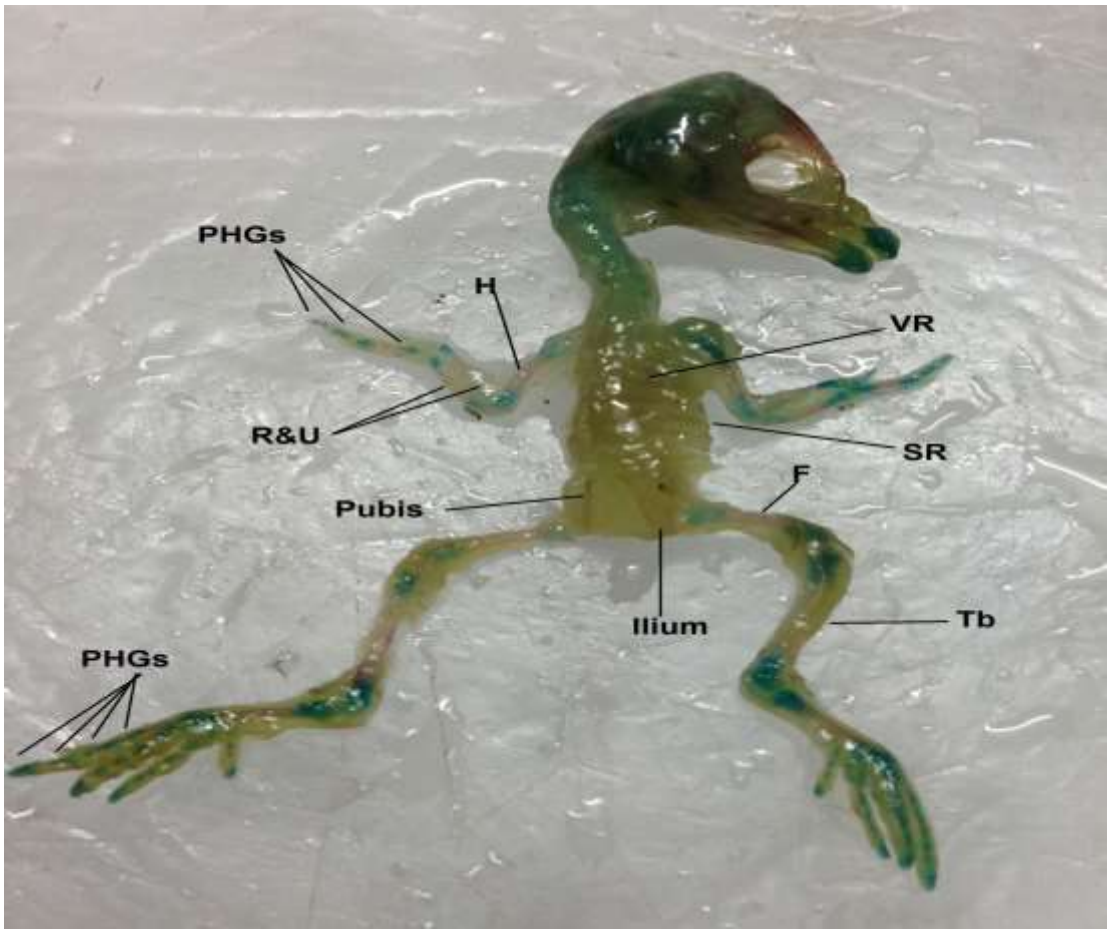

Fig (13). Whole mount of 10 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, Humerus (H), Phalanges (PHGs); Vertebral ribs (VR); Sternal ribs (SR); Radius and Ulna (R&U); Femur (F); Tibia (Tb).

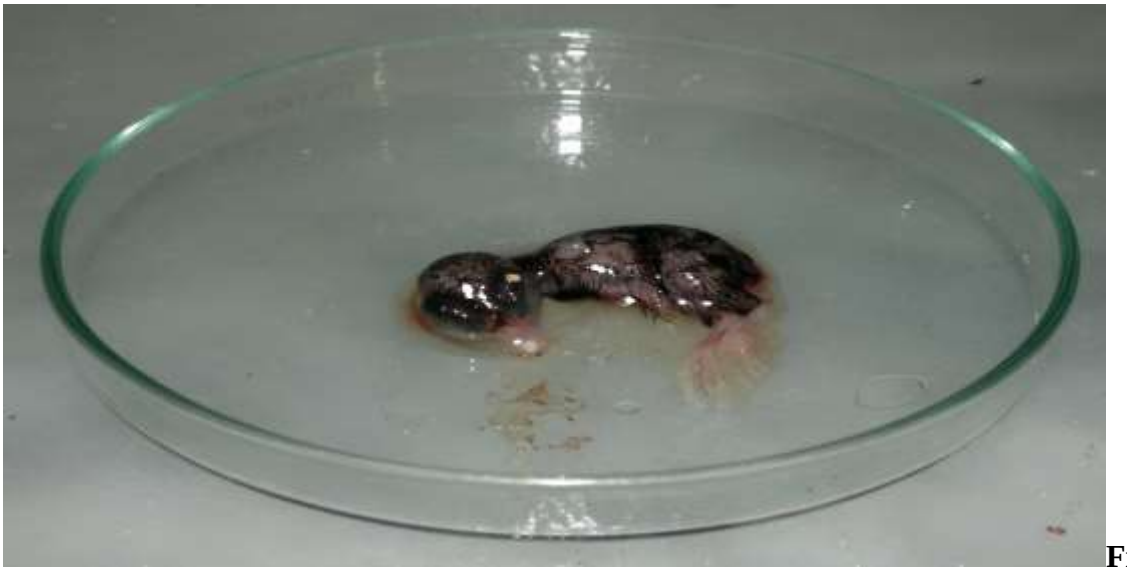

Fig (14). Embryo of 11 days of incubation

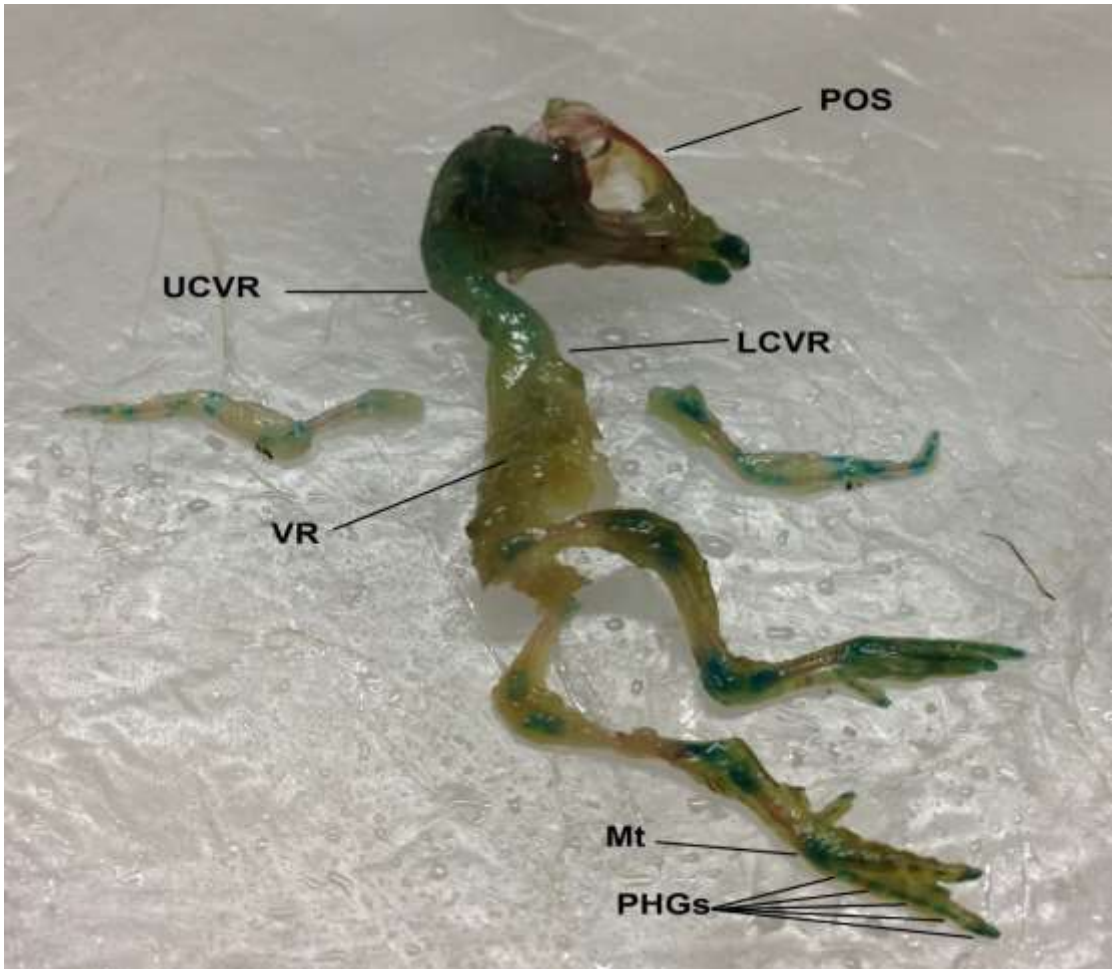

Fig (15). Whole mount of 11 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, Partly ossified skull (POS), Upper cervical vertebral region (UCVR); Lower cervical vertebral region (LCVR); Vertebral ribs (VR); Metatarsus (Mt); Phalanges (PHGs).

Fig (16). Embryo of 12 days of incubation

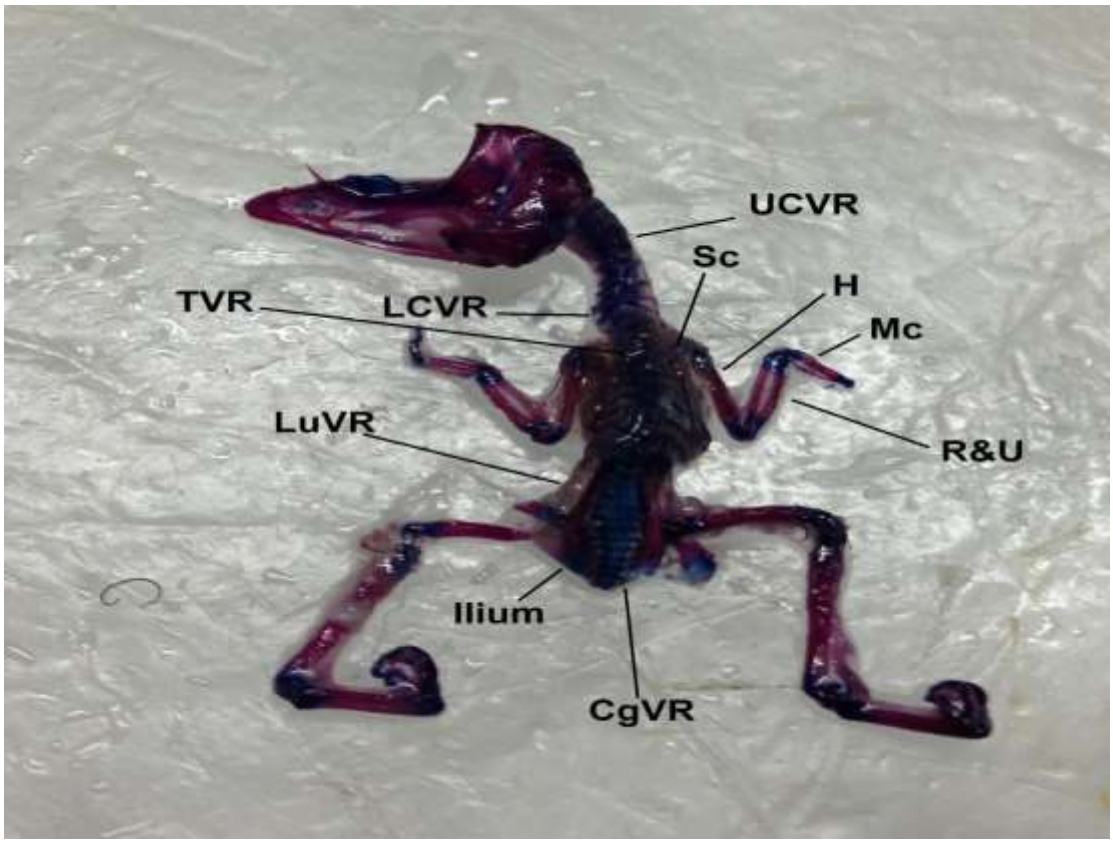

Fig (17). Whole mount of 12 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, Upper cervical vertebral region (UCVR); Lower cervical vertebral region (LCVR); Thoracic vertebral region (TVR); Lumbosacral vertebral region (LuVR); Coccygeal vertebral region (CgVR); Scapula (Sc); Humerus (H); Radius and Ulna (R&U); Metacarpus (Mc).

Fig (18). Embryo of 13 days of incubation

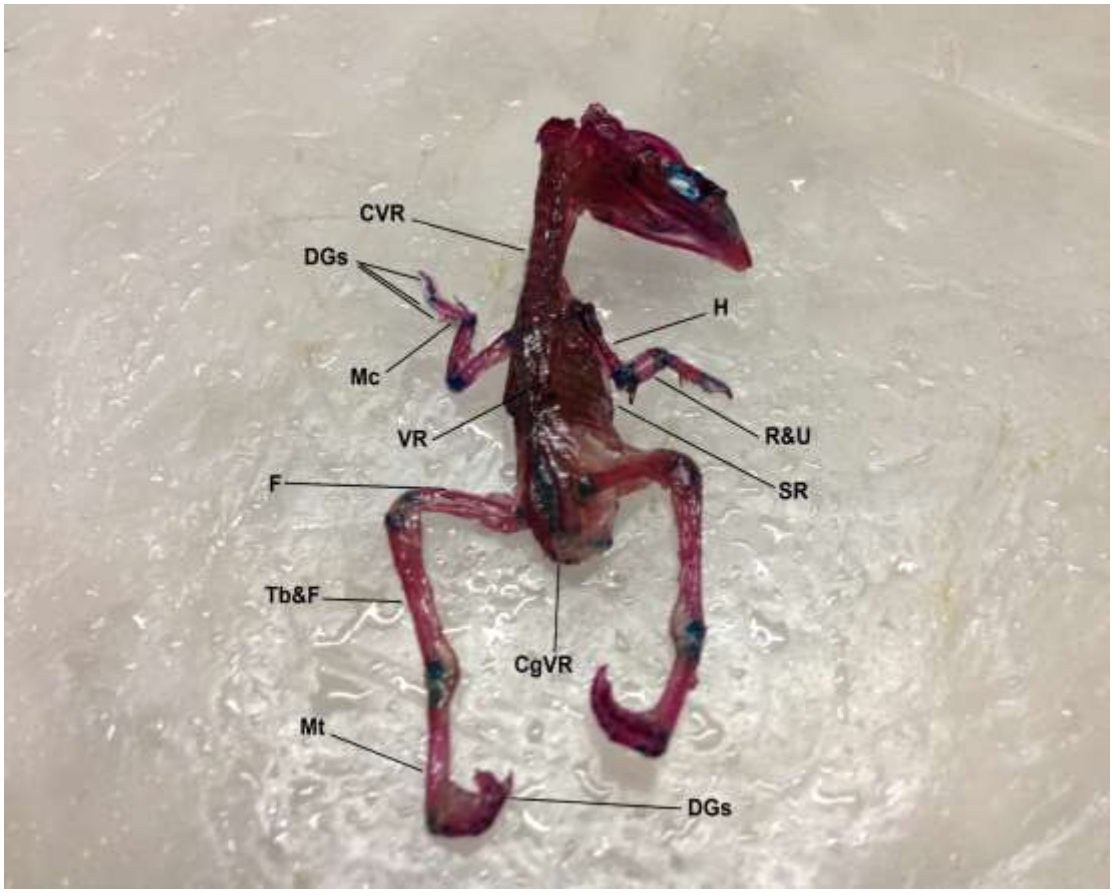

Fig (19). Whole mount of 13 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, cervical vertebral region (CVR); Humerus (H); Radius and Ulna (R&U); Metacarpus (Mc); Digits (DGs); Vertebral ribs (VR); Sternal ribs (SR); Femur (F); Tibia and Fibula (Tb&F); Coccygeal vertebral region (CgVR); Metatarsus (Mt) .

Fig (20). Embryo of 14 days of incubation

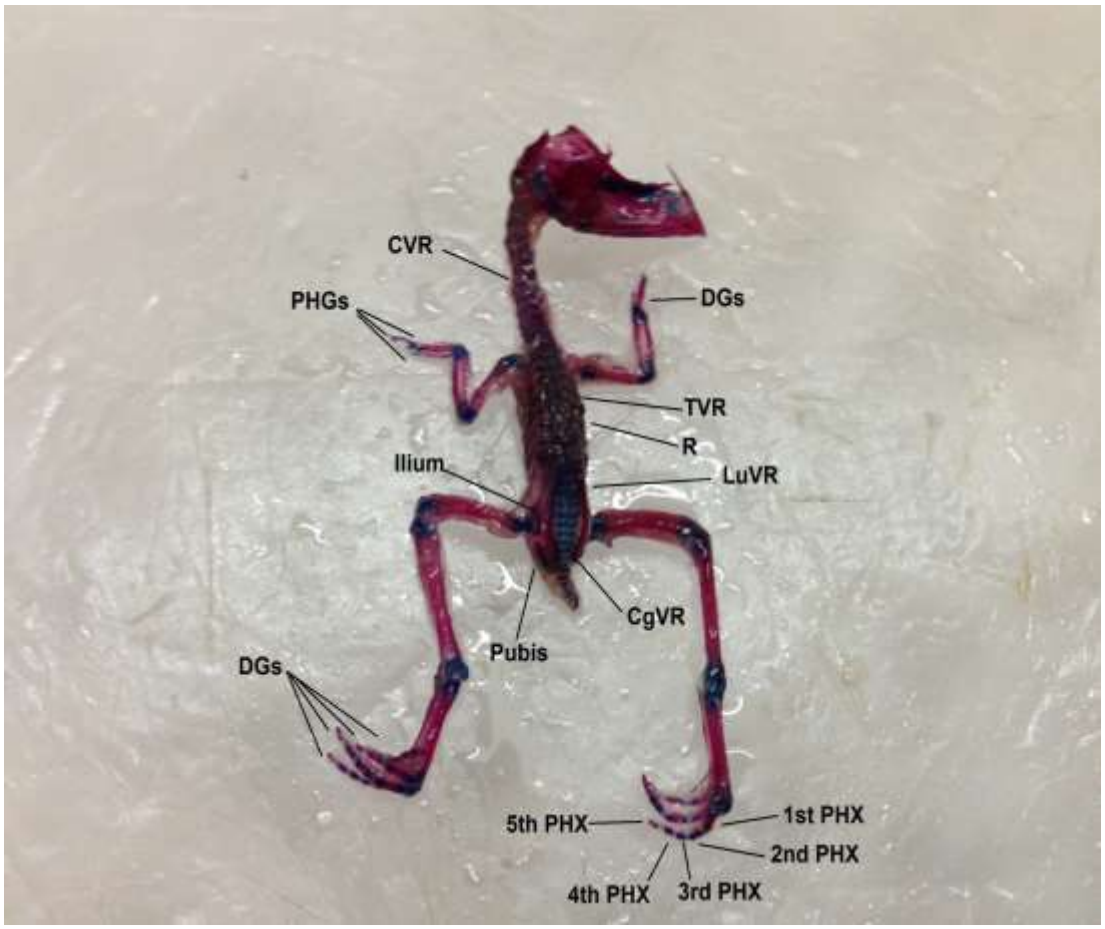

Fig (21). Whole mount of 14 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, cervical vertebral region (CVR); Thoracic vertebral region (TVR); Lumbosacral vertebral region (LuVR); Coccygeal vertebral region (CgVR); Ribs (R); Digits (DGs); Phalanges (PHGs); 1st, 2nd, 3rd, 4th, and 5th Phalanges (1st,2nd,3rd,4th,and 5th PHX).

Fig (22). Embryo of 15 days of incubation

Fig (23). Whole mount of 15 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, Ribs (R) ; Sternum (St) ; Scapula (Sc).

Fig (24). Embryo of 16 days of incubation

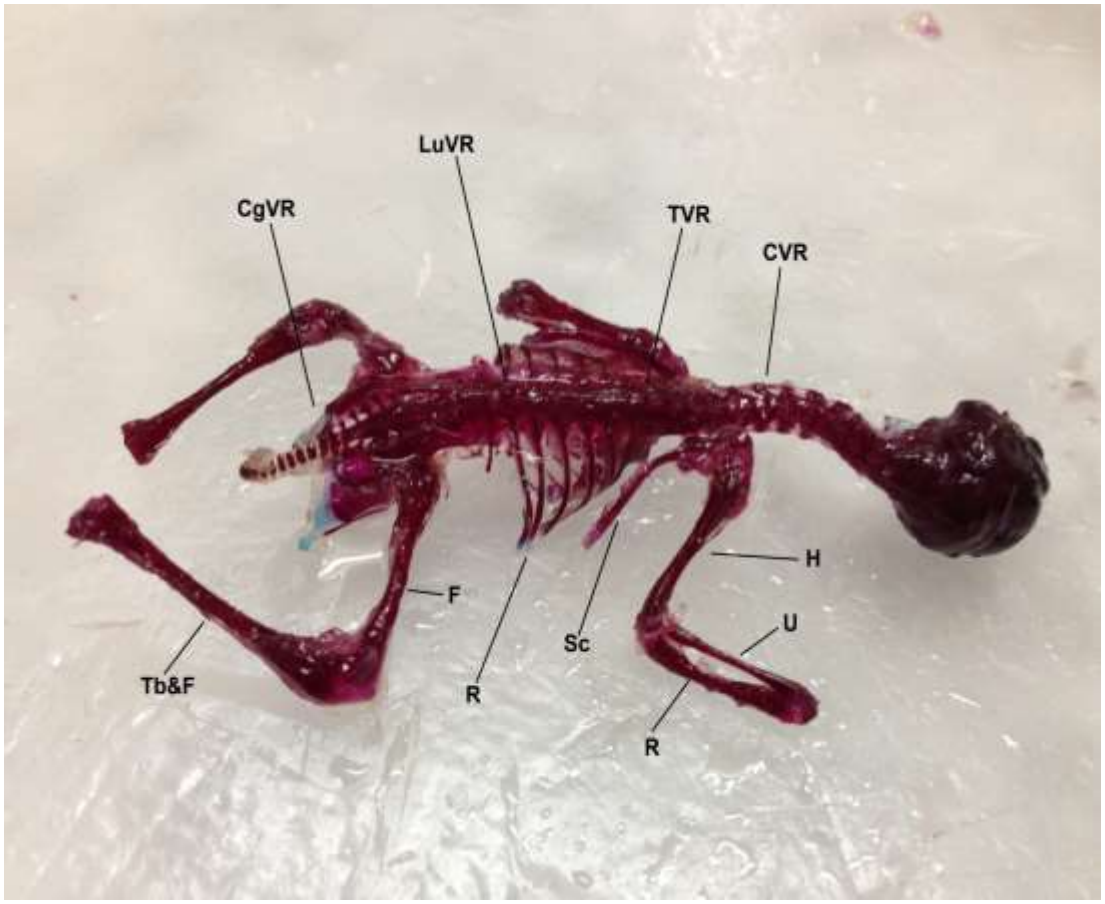

Fig (25). Whole mount of 16 days of incubation embryo stained with Alcian blue and Alizarin red-S double staining method, cervical vertebral region (CVR); Thoracic vertebral region (TVR); Lumbosacral vertebral region (LuVR); Coccygeal vertebral region (CgVR); Humerus (H); Radius and Ulna (R&U); Scapula (Sc); R (Ribs); Femur (F); Tibia and Fibula (Tb&F).

Fig (26). Hatching of the incubated egg at day 16 of incubation

Conclusion

1- The vertebral system showed the earliest appearance of cartilage in the three systems, While , it's ossification showed delay.Within one vertebrae, progression observed for calcification to progress from the centrum to the vertebral arch.

2- Ribs showed the latest appearance of cartilage as compared with the other bones of the skeleton. Vertebral ribs showed earlier ossification as compared with the sternal ribs. In both vertebral and sternal ribs, ossification first occurred at the middle region and then progressed to the proximal and distal regions.In the sternum, chondrofication appeare relatively at 6-9 days of incubation, while ossification occurred at day 14 of incubation . In most cases, the sternal body remained cartilaginous at hatching.

3- The appearance of cartilage was late in the fore and hind limbs skeletal system, next after the vertebral skeletal system and before the ribs and sternal system . But the onset of ossification was earliest among the other systems. Chondrofication and calcification progressed from the proximal to distal bones.In all long bones, Calcification first occurred at the middle region and then progressed to the proximal and distal ends of each bone. The patella appeared at day 9 of incubation and remained cartilaginous at hatching.

4- Scapula appeared at day 5 of incubation, also the coracoid at the same day and then calcified at day 8 and 9 respectively.While the clavicle appeared at day 8 of incubation as cartilage , and calcified at day 11 of incubation.

Recommendations

1- It is important to complete a post hatching study on the non-ossified cartilaginous elements , in order to determine the time of calcification of these elements.

2- Studying the fine structure of the beginning of cartilaginous cell by using electron microscope .

3- Study the hormonal interference , particularly parathyroid hormones accompanied with the skeletal development of this bird.

4- Studying the genetic material that affect the normal developmental morphogenesis of the skeletal system of this bird (gene expression).

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الخلاصة

اجريت دراسة النشأة المظهرية لتكوين الهيكل العظمي في طائر السلوى ،(*Coturnix japonica*) والتي تضمنت الدراسة التشريحية لتخلق العناصر العظمية والتغيرات الانتقالية التي تطرأ في عمليتي تكوين الغضاريف (التغضرف) والعظام (التعظم) خلال نمو الانماط الطبيعية المتنوعة لعظام الجسم المختلفة.

تم جمع طائر السلوى بعدد (١٠٠) طائر وتمت تربيتها منزليا . ثم تم جمع البيض المخصب من هذه الطيور بفترة ٧-٩ ايام. تمت عملية حضن هذا البيض المخصب بواسطة حاضنة كهربائية اوكرانية الصنع بدرجة حرارة ٣٧،٥ – ٣٨ درجة مئوية ونسبة رطوبة بلغت ٧٠%. استخدمت الاجنة ابتداءا من عمر ٣ ايام ولغاية ١٦ يوم حضانة (يوم الفقس).تم وزن البيض بشكل مفرد لكل بيضة قبل عملية الحضن وكذلك اثناء استخراجها من الحاضنة بغرض استخراج الجنين منها . ثم تم اخذ

معدل اوزان البيض للفترتين والنتائج اظهرت ان وزن البيضة يقل بنسبة نصف غرام بعد الحضن عن وزنه ما قبل الحضن ، وان هذه النسبة تزداد بشكل طفيف تدريجيا كلما زادت فترة الحضن .كذلك تمت عملية قياس اطوال عظمتي الزند والفخذ بالنسبة للطرف الامامي والخلفي للجسم على التوالي ابتداءا من اليوم السادس من فترة الحضن وحتى يوم الفقس.

أستخرجت الاجنة من البيض ابتداءا من عمر ٣ ايام ، ثم تمت معاملتها بصبغتي (Alcian Blue) و صبغة (Alizarin Red – S).

بالرغم من ان عناصر العمود الفقري اظهرت نشوء مبكر للغضروف مقارنة مع الاجزاء الاخرى من الهيكل العظمي ، في اليوم الثالث من الحضن ، الا انها اظهرت تأخرا في تعظمها الذي حصل ابتداءا من اليوم الحادي عشر من فترة الحضانة . ألتعظم بدأ بالفقرات العنقية وسار باتجاه الفقرات الذيلية .بالنسبة للتعظم في مناطق الفقرات العنقية والصدرية ،فقد ابتدأ من المنطقة الوسطى ثم توسع باتجاه المنطقة العليا والسفلى، لكن في المنطقتين الفقريتين القطنية العجزية والذيلية ابتدأ التعظم من المنطقة العليا او الجزء العلوي لهذه المناطق ثم توسع باتجاه الجزء الوسطي والسفلي.اما على نطاق الفقرة الواحدة، ابدى جسم الفقرة الذي يشغل الجزء المركزي منها تعظما مبكرا أمتد منه الى قوسي الفقرة على جانبي جسمها .

أظهر القفص الصدري المتكون من الاضلاع وعظم القص تغضرفا متأخرا مقارنة بالعمود الفقري والاطراف. حيث ظهرت الاضلاع الغضروفية بين الايام ٦–٨ من فترة الحضانة، الاضلاع الفقارية سبقت الاضلاع القصية في تعظمها . حيث تعظمت الاضلاع الفقارية ابتداءا من يوم ٩ من فترة الحضانة فيما تعظمت الاضلاع القصية ابتداءا من اليوم ١٢ من فترة الحضانة.في كلتا الاضلاع الفقارية والقصية ، التعظم ابتدأ من المنطقة الوسطى للعظم وتوسع نحو المناطق القاصية والدانية عن المركز. أظهر عظم القص تغضرفا واضحا ابتداءا من اليوم السادس للحضن، لكن مراكز التعظم ظهرت في اطرافه الرأسية والذيلية ابتداءا من اليوم ١٤ من فترة الحضن، في اغلب الحالات بقي جسم العظم القصي غضروفا الى يوم الفقس.

الاطراف التي تتكون معظم اجزاؤها من العظام الطويلة فأنها تلت العمود الفقري في تغضرفها ابتداءا من اليوم الرابع من فترة الحضانة، ألا انها اظهرت تعظما مبكرا بالمقارنة مع الاجزاء الاخرى من الهيكل العظمي للطير .حيث بدأ التعظم في هذه العظام في اليوم الثامن من فترة الحضانة.حصل التعظم في العظام الطويلة في منتصف جسم العظم وبدأ يمتد دانيا وقاصيا بتقدم عمر الجنين .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة القادسية
كلية العلوم

دراسة تطورية لمراكز التعظم في جنين طائر السمّان (ألسلوى)

رسالة مقدمة الى

مجلس كلية العلوم/ جامعة القادسية وهي جزء

من متطلبات نيل درجة ماجستير علوم في علم الحيوان

من قبل

محمد عبد الامير حرج

بكالوريوس علوم/ علوم حياة/ كلية العلوم جامعة القادسية ٢٠٠٧

إشراف

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١٤٣٥ هـ ٢٠١٤م