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Morphological Study of The Skeletal Development in Duck Embryo (*Anas platyrhynchos domesticus*)

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Abstract

The present study is an experimental embryological research in the field of the avian anatomy which investigated natural skeletal development of the indigenous Iraqi duck embryos.Data on the normal stages of skeleton chondrogenesis and osteogenesis and growth of all skeletal parameters essential for interpretation clarifying data acquired on these studies.Fifty fertilized eggs of local breed ducks were collected from Kirkuk city villages, were incubated. As a mean of one egg daily at morning used to prepare well developed embryo from 7th day through 28th day of incubation. After separation of embryos from the egg yolk were skinned and evaserated before fixation in absolute ethyl alcoho l, and stained in a double staining of Alcian Blue and Alizarin Red stains for identification of both chondrofication of cartilage and ossification of bone elements respectively .The skeleton of macerated embryos were observed under stereoscopic microscope, giving spicefic attention to the time of onset of both chondrofication and ossification of bones created . Initial appearance of the primary ossification centre of each bone was chased serially. On 11thday of incubation the primary ossification centre observed firstly in the mid-diaphysis of the femur, tibia and fibula of the leg in addition to ossifying of the supraangular, quadratojugal of the skull followed by serial onset of ossification of other skeletal elements of the embryos.

The bones of the skull were determined to show the latest chondrofication and ossification. All elements of the hyoid apparatus were remained cartilaginous during hatching except the ceratobrancheal. Whereas the vertebral column showed chondrofication earlier than that of ribs and sternum, which ossified later. On the other hand chondrofication of all vertebral column elements were happened at the same time, and they ossified progressing from the cervical through the caudal region.

The bones of the leg showed chondrofication and ossification earlier than that of the wing. This phenomena seems to be normal because of the natural feature of the duck lets which attends to swimming after hatching.

Introduction

Natural events of pre-hatching bone formation and growth studied in different avian species. These studies gave attention to the economic importance by standing on of skeletal disorders. A review introduced by Sullivan, (1994), on some skeletal anomalies in various avian species leading to a considerable sever losses in the poultry production economy. On the other hand although, the avian is considered as a valuable model for human skeletal defects because of dissimilarities between human and avian skeletogenesis and developmental processes (Cook, 2001).

In the field of avian embryogenesis extensive investigations have been studied were mostly solicituded with the chicken as a representative subject. However,there are two different kinds of developmental models among birds, the nodifeugous type which leaves the nest immediatly after hatching, the young follow their parents but independently find their food),which gives rise in the precocial condition during hatching, which is described as capability to moving around on its own after hatching like duckling, chick and turkey young . But the nidicolous type (remain in the nest after hatching), which results to the altricial condition (Starek, and

Ricklets, 1998). Altricial species shows more postnatal growth rate than precocial species of birds (Ricklefs and Konarzewski, 1998; Lilja et al., 2001; Bloom and Lilja, 2005).Various gradation and degree of two types are simplified among the birds. A lot of studies and information were readily available on the chicken (Gallis domesticus), so it was the logical choice of the fundamental represented order.

Embryological researches uses avian embryos representing the model as experimental samples which reflects values including smaller size of the body and rapid growth and cost cheapness. Data on normal stages of skeletogenesis are essential indicators and indispensible parameters to clarify data on the developmental engineering,experimental embryology and developmental teratogenicity studies. Recent researches investigates embryonic skeletogenesis**(** Hashizume et al., 1993),development of parts of embryos cultured in vivo (Naito et al., 1990 ; 1994), in addition to reveal teratological tests for consequences of drugs or chemicals (Hashizume et al., 1992).Researchers have been accumulating evidences on stages of the bones development of the bones in various spices such as chicken (Hamburge and Hamilton,1951**;** Bellairs and Osmond 2005; Sawad et al., 2009).**),** quails **(**Nakane and Tsudzuki ,1999) **,** turkeys **(** Atalgin et al., 2008 **)** and Iraqi goose **(** Bayatli et al., 2012; Bayatli and Fadhil 2013). The whole mounts of double staining of embryos were prepared either partial or whole skeletal elements to stand on the fundamental values of basic embryonic knowledge.This study therefore,aimed to study the embryonic skeletogenesis and pursuit the serial morphological changes during the development of the various skeletal components of the embryo in the Iraqi local breed duck *(Anas platyrhynchos domesticus*) during the pre-hatching period. I have initiated in my mind the need to study the natural events occurs during osteogenesis of duck embryo skeleton as standard control.Ducks productions have an important role in the economy by large number of Asian countries. Continent alone is responsible for producing approximately 83% of the total duck meat produced in the world. Asia is famous in production duck meats , eggs , and enjoys their products and consumed by Asians.

Materials and methods

Fifty fertilized eggs of indigenous Iraqi ducks were collected from villages surrounding Kirkuk city during three days, and stored at 15ºC , and incubated in the electric incubator in laboratories of Veterinary Medicine College in Kirkuk University. The incubator was adjusted on temperature of 37.5º C and humidity of 65 % , with continous turning of the eggs every six hours except first three days and last three days which needs no turning. Beginning from7thday until 28th day of incubation an embryo was prepared for fixation in absolute alcohol and double staining with alcian -blue and alizarin -red for detection of chondrofied and ossified parts of the skeleton of embryos, which stains the cartilages and bones with blue and red, respectively **(**Whitaker and Kathleen, 1979 **;** Erdodan et al., 1995). The whole mounts of embryos prepared in a modified procedure as following steps:

A. Complete skinning and removing of viscera and the eyes of the embryos of 12th day until the last day of incubation with careful separation of the embryo from the egg yolk to avoid damage and destruction of its skeletal structure

B. Fixation of skinned embryos in absolute ethyl alcohol for duration of(3-7)days according to the developmental stages of the embryo (late stages needs more time than earlly stages), as following:

a. Embryos of (7 - 10) days of incubation fixed for 3 days
b. Embryos of (11 - 16) days of incubation fixed for 4 days
c. Embryos of (17 - 22) days of incubation fixed for 5 days

d. Embryos of (23 - 28) days of incubation fixed for 7 days

C. Staining of embryos at (37 - 40ºC) for four days, prepared as following:

a. One volume of 0.3% (300mg alcian- blue dissolved in 100ml of 70% ethyl alcohol).
b. One volume of 0.1% (100mg alizarin- red dissolved in 100ml of 96% ethyl alcohol).
c. One volume of glacial acetic acid (100 ml).
d. 17 volume of 70% ethyl alcohol (1700 ml).

The solutions (a) mixed with (b), also (c) mixed with (d).Both mixtures added together forming one solution for double staining of embryos. 100 -500ml of this solution is sufficent for staining of each embryo according to the size of embryos which were stained.

D. Washing in tape water for 2 hours.

E. Maceration by using KOH(0.5 -2%) according to the age of embryos as following:

a. Embryos of (7 - 10) days of incubation macerated in 0.5% KOH for 12-18 hours
b. Embryos of (11- 16) days of incubation macerated in 1% KOH for 18- 24 hours
c. Embryos of (17 - 22) days of incubation macerated in 1.5% KOH for 2-4 days
d. Embryos of (23 - 28) days of incubation macerated in 2% KOH for 5-7 days

KOH is a destructive agent which can destroy the cartilages and bones of embryos when leaved for longer time. It needs chasing in intervals to avoid destruction of parts of the skeleton.

F. Clearing by using of glycerol. and storing the stained whole mounts in glycerol with addition of few granules of thymol to avoid fungi growth for a long time.

The skeletons observed under the stereoscopic microscope , with highlighting on the timing of each of chondrofication and ossification processes. The length of some selected long bones measured . Evaluated data were analyzed statistically to clarify the exact comparison during proceeding the ascending incubation times to evaluate the curve of the growth rate of these specific long bones .

Results

The prepared whole mounts of duck embryos from the 7th day of incubation throughout the hatching showed a series of successive changes yelding skeletal elements represented by bones of different shapes and dimentions as flat,long,short and irregular bones.Similar to the skeleton of other birds and mammalian, the bones of the skeleton in the duck embryo were created through two different mechanisms depending upon the structural type of the bones. The flat bones including majority of the skull bones, scapula and Ilium of the pelvis were created by the intramembranous ossification, in which the mesenchymal precursor cells condense and convert directly to bone forming osteoblasts that secrete bone forming matrix in flat bones. Whereas the long, short and irregular bones were created by endochondral ossification, the processes by which the embryonic cartilaginous model of most bones contribute to longitudinal growth and is gradually replaced by the bone. Both chondrafication and ossification transitional developmental changes during processes of bones formation and their growth were illustrated in tables (1 - 4) and (fig.1 and 2). For convenience of description of the skeleton was divided into six parts; Skull, vertebrae, ribs, sternum, wing and leg.

Table 1: The developmental changes of bones of the skull :

Name of the bone	Age of the embryo(days)																
	7	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**22**	**24**	**26**	**28**
Occipital																		
Exoccipital	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
Supraoccipital	-	-	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Basioccipital	-	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Temporal																		
Squamasal	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R	R
Prootic	-	-	-	-	-	-	B	B	B	B	B	B	B	B	R	R	R	R
Opisthotic	-	-	-	-	-	-	-	B	B	B	B	B	B	B	B	R	R	R
Epiotic	-	-	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R
Parietal	-	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R
Trabeculae	-	-	-	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R
Prefrontal	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R
Frontal	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R
Premaxilla	-	-	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R
Maxilla	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R
Palatine	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R
Pterygoid	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R
Vomer	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R
Quadratojugal	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Jugal	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R
Sphenoid																		
Basisphenoid	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Orbitoshenoid	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Parasphenoid	-	-	-	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Mandible																		
Splenial	-	-	-	-	-	-	-	-	-	-	R	R	R	R	R	R	R	R
Dentary	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R	R
Angular	-	-	-	-	-	-	-	-	-	-	-	R	R	R	R	R	R	R
Supra-angular	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Articular	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Hyoid-apparatus																		
Ceratobrancheal	-	-	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Entoglossal	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Basihyal	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Epibranchial	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Urohyal	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

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Table 2: The developmental changes of bones of the vertebrae:

Name of the bone	Age of the embryo (days)																
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	24	26	28
Cervical vertebrae																		
Cranial region																		
Vertebral body	-	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Vertebral. arch	-	-	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Cervical rib	-	-	-	-	-	-	-	B	B	B	B	B	B	B	R	R	R	R
Middle region																		
Vertebral. body	-	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Vertebral arch	-	-	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Cervical rib	-	-	-	-	-	-	-	B	B	B	B	B	B	B	R	R	R	R
Caudal region																		
Vertebral. Body	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Vertebral. Arch	-	-	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Cervical rib	-	-	-	-	-	-	-	B	B	B	B	B	B	B	R	R	R	R
Thoracic vertebrae																		
Cranial region																		
Vertebral. Body	-	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Vertebral. Arch	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Middle region																		
Vertebral. body	-	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Vertebral .arch	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Caudal region																		
Vertebral. body	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Vertebral. arch	-	-	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Synsacrum																		
Cranial region																		
Vertebral. body	-	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Vertebral. arch	-	-	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R
Middle region																		
Vertebral. body	-	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Vertebral. arch	-	-	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Caudal region																		
Vertebral. body	-	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Vertebral. arch	-	-	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Caudal vertebrae																		
Cranial region																		
Vertebral. body	-	B	B	B	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	B	B	R	R	R	R
Middle region																		
Vertebral. body	-	B	B	B	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	B	B	B	B	R	R	R
Caudal region																		
Vertebral. body	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R
Vertebral. arch	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R

Table 3: The developmental changes of bones of the ribs and sternum:

Name of the bone	Age of the embryo (days)																	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	24	26	28
Vertebral ribs																		
First vertebral rib	-	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Second vertebral rib	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Uncinate Process	-	-	-	-	-	-	-	B	B	B	B	B	B	B	B	R	R	R
Third vertebral rib	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R
Fourth vertebral rib	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	B	R	R
Fifth vertebral rib	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	B	R	R
Sixth vertebral rib	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Uncinate process	-	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	R	R
Seventh vertebral rib	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Sternal ribs																		
First sternal rib	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Second sternal rib	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Third sternal rib	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Fourth sternal rib	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Fifth sternal rib	-	-	-	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R
Sternum																		
Body	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Crest	-	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	R	R
Laterocranial-process.	-	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	R	R
Inner latero-caudal prprocess	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R
Outer latero-caudal pr process	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R

Table 4: The developmental changes of the bones of the wing and leg:

Name of the bone	Age of the embryo (days)																	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22	24	26	28
Wings:																		
Scapula	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Clavicle	-	-	-	-	-	R	R	R	R	R	R	R	R	R	R	R	R	R
Coracoids	-	-	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Humerus	-	-	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Radius	-	-	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Ulna	-	-	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Carpi radiale	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Carpi ulnare	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Metacarpal II	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Metacarpal III	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
Metacarpal IV	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Second digit																		
First phalanx	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Second phala	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Third digit																		
First phalanx	-	-	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Second phal.	-	-	B	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R
Fourth digit																		
First phalanx	-	-	-	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R
Legs																		
Ilium	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
Ischium	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Pubis	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Femur	-	-	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Tibia	-	-	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Fibula	-	-	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Patella	-	-	-	-	-	-	B	B	B	B	B	B	B	B	B	B	B	B
Metatarsus I	-	-	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Metatarsus II	-	-	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Metatarsus III	-	-	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Metatarsus IV	-	-	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Digits																		
First digit																		
First phalanx	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Second phalanx	-	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Second digit																		
First phalanx	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Second phalanx	-	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Third phalanx	-	-	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R
Third digit																		
First phalanx	-	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Second phalanx	-	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Third phalanx	-	-	-	-	-	-	B	B	R	B	R	R	R	R	R	R	R	R
Fourth phalanx	-	-	-	-	-	B	B	B	B	B	B	R	R	R	R	R	R	R
Fourth digit					-													
First phalanx	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R
Second phalanx	-	-	-	-	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Third phalanx	-	-	-	-	-	-	-	B	B	B	R	R	R	R	R	R	R	R
Fourth phalanx	-	-	-	-	-	-	-	B	B	B	B	R	R	R	R	R	R	R
Fifth phalanx	-	-	-	-	-	-	-	B	B	B	B	B	R	R	R	R	R	R

(-) = Not stained with alcian blue or alizarin red-S, (B) = stained blue with alcian blue
(R) = Stained red with alizarin red-S

A : Chondrofication phase:

chondrafication indicated by blue staining of the skeleton of embryos as following:

At 7th day of incubation there was no any signs of chondrafication in any part of the skeleton. Whereas at 8th day of incubation slightly blue staining of the bodies of the vertebrae were appeared firstly.

At 9th day of incubation, some cranial bones including exoccipital, orbitosphenoid , hyoid apparatus except entoglossal, and upper parts of the wing and leg until the the distal end of the metacarpus and metatarsus except the carpals and patella were stained blue.

At 10th day of incubation, other skull bones including trabeculae basisphenoid , parasphenoid , articular, entoglossal of the hyoid apparatus were stained blue. Also the vertebral and sternal ribs, the body and inner latero-caudal process of the sternum, and 1st phalanges of the wing and leg first digits in addition to the 2nd phalanx of the 2nd digit of the wing showed blue colours staining of their chondrotic drafts.

At 11th day of incubation, the supraoccipital and basioccipital of the skull in addition to the 2nd phalanges of all digits of the wing and leg except the 2nd digit of the wing were stained blue.

At 12th day of incubation, the epiotic of the temporal bone , 2nd phalanx of the3rd digit of the wing , and 3rd phalanx of 2nd digit ,4th of 3rd digit of the leg were stained blue.

At 13th day of incubation**,** the prootic of the temporal bone , the uncinate processes of 3rd, 4th and 5th vertebral ribs were chondrofied.

At 14th day of incubation , the opisthotic of the temporal bone, the crest and latero-cranial and outer latero- caudal processes of the sternum, and 4th and 5th phalanges of 4th digit showed chodrotic signs.

At 15th day of incubation, All of the skeletal elements were chondrofied , no new element was stained blue in this stage. The chondrotic drafts were developed their chondrafication process until reaching the ossification phase.

B. Ossification phase

Red staining or Ossification of chondrotic drafts of different bones of the duck embryo was as following:

At 10th day of incubation there was no red colour staining of and skeletal parts of the duck embryo indicating absence of ossification centre yet. While on the day 11 the onset of ossification centre was appeared firstly in the chondrotic drafts of supraangular, quadratojugal of the skull. Primary ossification centres also observed in the mid-diaphysis of the femur, tibia and fibula of the leg.

At 12th day of incubation, ossified centres were obvious in the dentary of the mandible , clavicle ,mid-diaphysis of the humerus, radius and ulna of the wing.

At 13th day of incubation, the palatine and vomer elements of the skull, coracoids, metatarsal II, III and IV were began ossifying.

At 14th day of incubation, the exoccipital, prefrontal, frontal, maxilla, ptrygoid, jugal of the skull , in addition to the metacarpal III and ilium showed ossification.

At 15th day of incubation, the orbitosphenoid, basisphenoid , parietal of the skull began ossifying. Also ossification centres were seen in the vertebral ribs (2 nd through 5th) , metacarpal IV , schism, pubis, 1st phalanx of 2nd digit of the wing, and 1st phalanges of all of four digits of the leg.

At 16th day of incubation, the basioccipital , premaxilla and ceratobrancheal of the hyoid apparatus of the skull, bodies of the middle region of the cervical vertebrae, cranial and middle regions of the thoracic vertebrae, 1st vertebral rib, and 2nd phalanx of all digits of the leg were began ossifying. .

At 17th day of incubation, the parashpenoid, splenial of the skull, bodies of the caudal cervical and caudal regions of the thoracic vertebrae in addition to the arches of the cranial region of the cervical vertebrae. Metatarsal I, 3rd phalanx of 2nd , 3rd and 4th digits of the leg were started ossifying. In this stage the metatarsals united along with the tarsal I and tarsal II forming tarso-metatarsals.

At 18th day of incubation, angular of the skull, bodies of the cranial , middle and caudal regions of the synsacrum vertebrae , arches of the cranial and middle regions of the thoracic vertebrae, and 4th phalanx of 3rd and 4th digits of the leg were observed staining red indicating their ossification.

At 19thday of incubation,the articular element of the skull, arches of the caudal region of the thoracic vertebrae, first four sternal ribs, 1st phalanges of 4th digit of the wing and 5th phalanx of 4th digit of the leg were began ossifying by which all bonny skeletal elements of the limbs were ossified except the carpus , metacarpus II and 2nd phalanx of 2nd digit of the wing, tarsus and patella of the leg,

At 20th day of incubation, the trabeculae of the skull, bodies of the cranial and caudal vertebrae, arches of the cranial region of the of the synsacrum , 5th sternal rib were started ossifying. in this stage all bonny skeletal elements of the skull were ossified except the hyoid apparatus which remained cartilaginous until hatching. At 22th day of incubation, the bodies of the middle region of the caudal vertebrae and arches of the middle and caudal regions of the synsacrum were ossified. At 24th day of incubation, the bodies and arches of all parts of the vertebral column were ossified after red staining of the bodies of caudal region of caudal vertebrae, in addition to the arches of the middle and caudal regions of the caudal vertebrae. On the other hand the uncinate processes of 2nd and 3rd vertebral ribs and inner latero-caudal processes of the sternum were also began ossifying. At 26th day of incubation, in this stage the latest parts of the skeleton ossified were the uncinate processes of 4th, 5th and 6th vertebral ribs and remainder parts of the sternum including the crest and the latero-caudal process of the sternum by which all of bonny skeletal template of the body in the duck embryo were ossified with exception of some elements including, the hyoid apparatus, carpiradial, carpiulnar , metacarpal II and 2nd phalanx of the 2nd digit of the wing , patella, and the body of the sternum were remained cartilaginous without ossification during hatching.

Biometric measurements

After 26th day of incubation in which the latest bone elements were ossified, all of the ossified components of the skeleton continued their development until hatching at 28th day of incubation. Lengths of the humerus and femur as an examples were chased during the embryonic periods were displayed in (Fig.1) .

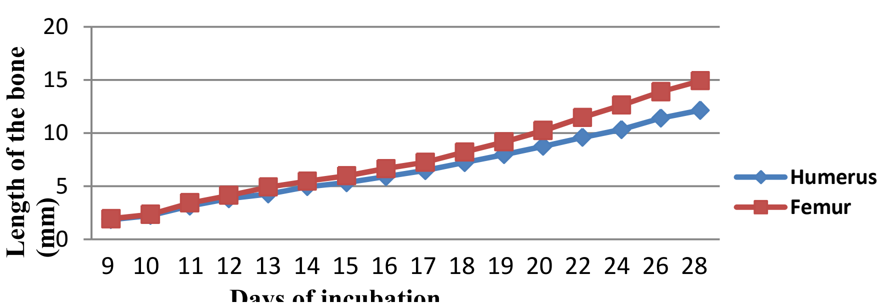

Fig.1: Growth curve of lengths of the Femur and humerus along the embyoembyonal stages of the duck skeleton.

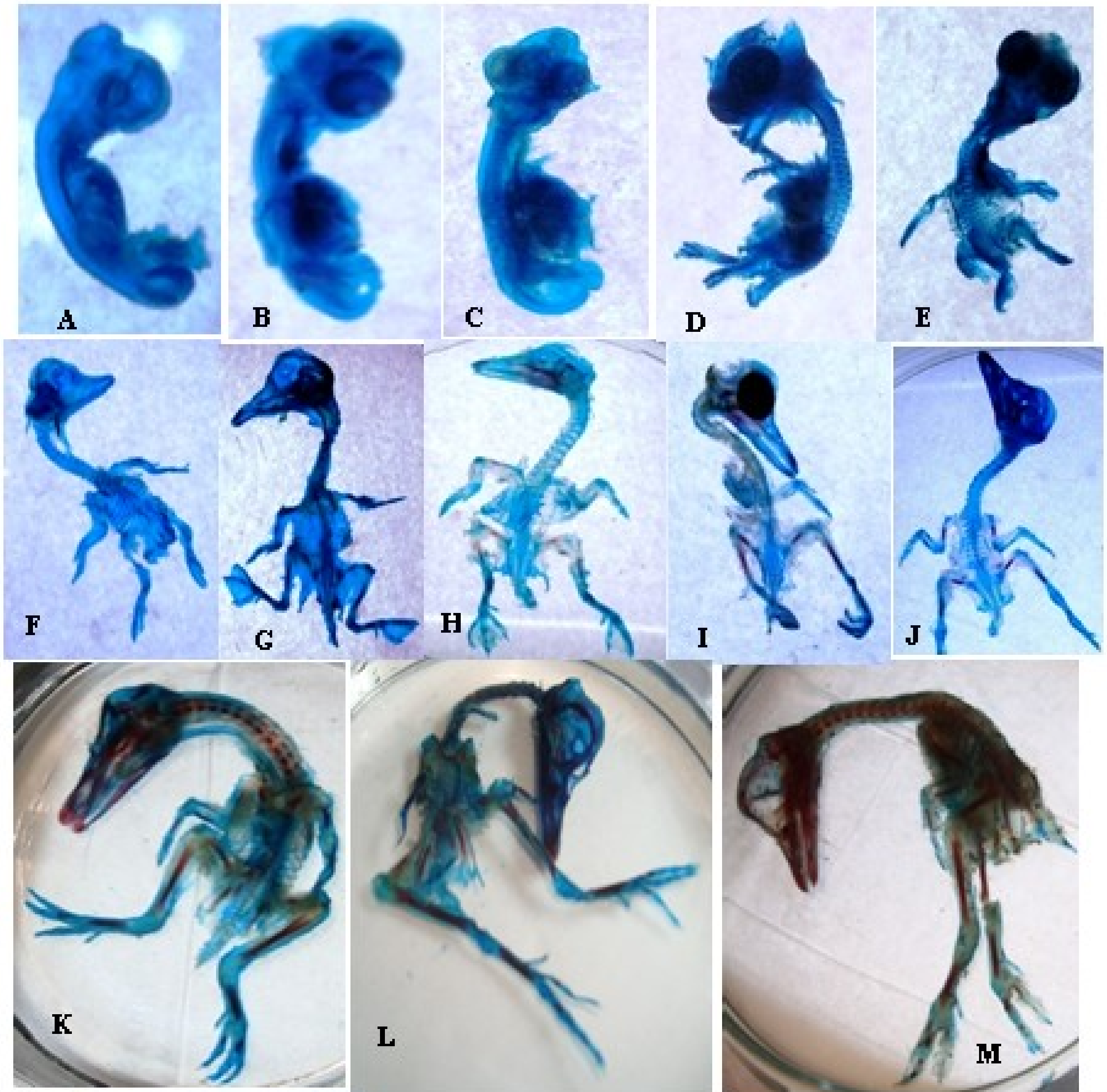

Fig. 2 : Skeletal features of duck embryos. A. 7th , B. 8th , C. 9th , D. 10th E. 11th , F. 12th , G.13th , H14th , I. 15th , J. 16th, K. 20th , L. 22th, M. 24th and N. 26th days of incubation.

Discussion

Tables(1-4) and Fig.2 illustrates as schemes or photographs of the processes of chondrofication and ossification of the skeleton in duck embryo, which are investigated and enable assessment of the normal developmental status during different stages of cartilages and bones formation and growth.

The accumulating data of the tables (1-4) represent the time of onset of chondrofication and ossification in almost bones of the skeleton of the duck embryo forming the template of the skeleton of the adult in the future including; the skull , vertebral column , ribs ,sternum and limbs. The developmental features of each part of skeletal system will briefly summarized as following:

Skull

The most bones of the skull were ossified intramembranously, and their ossification occurred in almost bones during the period between 11th and 24th day of incubation. By 11th day all parts of the skull bones had been created and appeared clearly. At 28th day of incubation (during hatching) , all parts of the hyoid apparatus were remained cartilaginous as chondrotic drafts except ossification of the ceratobrancheal element .The linage and pattern of chondrofication and ossification were similar to the chick (Jollie, 1957) , quail (Nakane and Tsudzuki, 1999) , turkey(Atalgin et al., 2008) and goose(Bayatli et al., 2012) embryos.

Vertebral Column

The vertebrae showed the earliest process of chondrofication in all parts of the vertebral column at the same time. Most vertebrae stained blue at 8thday, whereas the vertebral arches chondrofied at 9th day of incubation. However, they ossified during the period between 16th and 24th day of incubation progressing from the cervical toward the caudal (coccygeal) regions of the vertebral column. A tendency was observed for ossification to progress from the bodies to the arches of the vertebrae. The same pattern had been seen in chick (Jollie,1957 ; Sawad et al., 2009), quail (Nakane and Tsudzuki,1999) , Turkey(Atalgin et al.,2008) and goose (Bayatli and Fadhil, 2013) embryos .

The ribs and sternum

The ribs and sternum showed the latest occurrence of chondrofication in comparison to the four parts of the skeleton development, during the period between 10-14th day of incubation. On the other hand primary ossification centres appeared at 15th day in the middle area of the vertebral ribs and progressed proximally and distally . This means even though the vertebrae chondrofied earlier as compared to the ribs and sternum, they ossified later. ossification in the sternal parts later at 19th day of incubation at which the ossification of limbs and skull elements were terminated, was is similar pattern with that occurred in quail (Nakane and Tsudzuki, 1999). turkey (Atalgin et al.,2008) **,** and goose (Bayatli, 2011) embryos**.**

The ossification of the uncinate processes progress from the caudal border of the vertebral ribs is in agreement with (Tickle and Codd 2009) described these processes in most species of existent birds. Although the processes were lastly thought to be assist for flight **(**Welty, 1988 **)** or strengthening to the thoracic cage (Kardong, 1988). These processes have recently been employed to be fundamental components of the ventilator apparatus of birds.

The recent study revealed that the ossification of the sternum appears at later stage in avian embryo development , beginning from latero-caudal and latero-cranial process , whereas the body of the sternum remained cartilaginous during hatching. This observation is in agreement with that in quail (Nakane and Tsudzuki ,1999) , chick (Bloom and Lilja, 2005) ; Kurtul et al., 2009**,** turkey (Atalgin et al.,2008)**;** Tickle and Codd, 2009) , and Iraqi local breed goose (Bayatli, 2011).. They noted that the body of the sternum had been still mainly a chodrotic in nature at hatching, and it had ossified after hatching. While Atalgin et al., (2008) mentioned that sternal body showed area of ossification shortly before hatching on day 20 of incubation in the Hubert strain chick embryo. This may be due to the heriditory factor of the strain of the bird.

The Limbs (wings and legs)

The limbs showed chondrofication late(9thday) after the vertebrae(8thday) and early before the ribs and sternum(10thday). While the transitional ossification by onset of appearance of primary ossification centre in some bones of the leg was earliest among all parts of the skeleton at 11th day of incubation appeared in the mid-diaphysis of the femur, tibia and fibula of the leg, in addition to ossification of the clavicle of the wing. The consequences of both chondrofication and ossification of limbs were parallel to that of the goose embryo **(** Bayatli and Fadhil, 2013**).**

The earliest appearance of the primary ossification of the femur and tibia of the hind limb rather than that of the fore limb in the domestic duck embryo in this study is in a similar parallel pattern with that of the waterfowl goose embryo which is revealed by Bayatli, (2011) who interpret that the newly hatched goslet has ability for swimming soon after hatching. This needs gaining giant muscle and more developed skeletal elements, responsible for provide an energy requirement for specific movement of the legs essential for swimming. While in other species of birds the onset of ossification of long bones of both wing and leg including the humerus , radius and ulna of the wing and the femur , tibia and fibula of the leg took place at the same time (Nakane and Tsudzuki, 1999) in quail (Atalgin et al.,2008) and (Holder, 1978) in chick embryos. The early hatched duckling is considered as precocial bird, which has ability to moving around on its own after hatching **(** Starek and Ricklets,1998**).** Altricial species shows higher posnatal growth rate than precocial species of birds **(**Hamburger and Hamilton, 1951; Ricklefs et al.,1998**;** Lilja et al., 2001; Bloom and Lilja, 2005**).** Various gradation and degree of two types are simplified among the birds.

Ossification of all of the skeletal elements of the fore limb except the non-fused carpal elements including carpiradial and carpiulnari, and 2nd phalange of the alular (2nd) digit. On the other hand the union of metatarsals along with the tarsal I and tarsal II to tarso-metatarsals was similar to that observed by Atalgin et al.,(2008**)** in turkey**,** **(** Bayatli and Fadhil, 2013 **)** in goose embryos.

The ossification of all the skeletal elements of the leg were ossified except the patella, which remained cartilaginous during hatching. These observations were compatible with Nakane and Tsudzuki (1999). ; Pourlis et al.,(1998**)** in quail , Kurtul et al.,(2009) **;** Holder, (1978). in chick and Atalgin et al., (2008**)** in turkey embryos.

The growth curve of long bones of both wings and legs in the recent study were followed the similar pattern with increase the time of incubation as it drawn clearly in Fig.1. This was contradictory observation with (Nakane and Tsudzuki , 1999) in the quail, and it has been explained the fact that rate of growth of the organs in bird species differ according to form and function(Lilja et al., 2001 ; Pourlis et al.,1998).

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