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## Progressive Development of Appendicular and Axial Skeletons in Guinea Fowl (*Numida meleagris*) Embryo

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**Abstract:** From day one to day nine of embryonic development, no ossification centre was observed in the embryo. Small centres of ossification were seen on the 10<sup>th</sup> day at some locations like the cervical vertebrae, thoracic vertebrae, clavicle, coracoid, scapula, humerus, radius and ulna. Other areas with centres of ossification on this day included the ilium, pubis, femur and fibular. By day 12, these centres of ossification were very prominent at the points seen on day 10 and in addition, the Ischium had ossification centre. By day 13, ossification centres showed up in lumbosacral vertebrae, radius ulna, ulnecarpal and carpometacarpal joints, metatarsus, tibiotarsus, patella, first phalanx of digit ii and second phalanx of digit iii. Between days 15 and 16, additional ossification centres were observed at coccygeal vertebrae, digits ii, iii and iv of pectoral limb. By day 21, ossification centre appeared on the pygostyle and by days 23 and 24, the right and left clavicles had joined together although not properly fused.

**Key words:** Appendicular and axial, skeleton, guinea fowl, embryo

### Introduction

Bone is essentially a highly vascular, living and a constantly changing mineralized connective tissue. It is remarkable for its hardness, resilience, characteristic growth mechanisms and its regenerative capacity. It provides rigid support and protection to the soft parts and furnishes a lever system on which muscles are brought into play (Patten, 1948).

Bone does not form in a vacant space. It is always laid down in an area already occupied by some highly specialized member of the connective tissue family. Based on this, bone formation was broadly divided into two types. These are the membranous bone, which begins in areas already occupied by fibrous connective tissue and the endochondral bone which is laid down in areas already occupied by cartilage (Howlett, 1979). Therefore, it is the environment that determines the type of bone formation.

Three types of ossification were identified and described (Frandsen and Elmer, 1981). These include Intramembranous (mesenchymal), Endochondral and Heteroplastic types of ossification. Heteroplastic ossification occurs in tissues other than skeleton (Getty, 1975) and with the exception of the os penis in dogs, os cordis in bovine heart and os rostri in pigs, it is usually pathological (Frandsen and Elmer, 1981).

In long bones, which the appendicular skeleton belongs to, the first sign of an imminent endochondral bone formation appears in the middle of the diaphysis, giving

rise to a primary centre of ossification. From the primary ossification centre in the shaft or diaphysis of the long bone, endochondral ossification progresses gradually towards the end of the cartilaginous model.

At birth or during hatching, the diaphysis of the long bone is usually completely ossified but the two extremities known as epiphyses, still remain cartilaginous. Shortly after, however, ossification centres arise in the epiphyses and the endochondral ossification proceeds in a manner similar to that in the diaphysis. Finally the epiphyses are composed of spongy bone covered by a cartilaginous shell.

### Materials and Methods

80 guinea fowl eggs were procured from National Veterinary Research Institute, Vom, Nigeria and subsequently incubated with an electrically operated incubator at a temperature of 37.0°C.

Two eggs were utilized on daily basis. The embryos were removed from the shell and fixed in 10% formalin for a period of 16 days. After the 16<sup>th</sup> day in 10% formalin, the embryos were transferred into 95% alcohol for another 3 days. Rinsing in a fresh solution of 95% alcohol was followed by subsequent immersion in 2% potassium hydroxide for a period of 7-10 days depending on the size of the specimen.

The cleared and bleached embryos were again rinsed in 95% alcohol and transferred to 0.1% Alizarin Red

solution dissolved in 1% potassium hydroxide. After the fourth day in 0.1% Alizarin Red solution, embryos were transferred to another freshly prepared Alizarin Red solution for 3 days. Thereafter, the stained embryo skeletons were washed by passing them through a series of glycerol in 1% potassium hydroxide solution until no colour came out of the stained skeleton. Finally the stained skeletons were stored in glycerine solution until examined. This technique stained the bones deep red while the soft tissues were unstained.

It is pertinent to note that absolute care must be exercised during the following procedures:

- Removal of the embryo from their shell, this should be done with uttermost care to avoid breakage of the embryos when transferring them into 10% formalin.
- At the clearing phase, having appreciated the fact that potassium hydroxide is a corrosive agent which can readily destroy the bones, care must be exercised maximally to avoid undue maceration of the bones by the potassium hydroxide solution.
- Intact skeleton easily gets "dismantled" when transferring from one solution to another. This is an important stage as far as preservation of intact skeleton is concerned.
- The specimens should be allowed to remain in Alizarin Red solution for longer than 10 days, otherwise soft tissues tend to obscure the bony structures.

## Results

From days 1-9 of embryonic development, no ossification centre was observed in the embryos. The Alizarin Red stain did not detect any ossification densities at all (Tables 1 and 2).

On day 10 of embryonic development, small centres of ossification were seen in the cervical vertebrae, thoracic vertebrae, clavicle, coracoid, scapula, humerus, radius and ulna in the forelimb while they appeared in the ilium, pubis, femur and fibular in the hind limb (Tables 1 and 2).

By day 11, there were only slight increases in the size of the ossification centres. By day 12, the centre of ossification had also appeared in the ischium as well as those other points on day 10 (Table 2).

Day 13 of development showed new ossification centres at lumbosacral vertebrae, radius, ulna, ulnacarpal and carpometacarpal joints, metatarsus, tibiotarsus, patella, first phalanx of digit ii and second phalanx of digit iii.

At day 14, development of the bone was very similar to those features observed on day 13.

Between days 15 and 16, vestigial forms of coccygeal vertebrae, digit ii, digit iii and digit v of the pectoral limb and the more prominent 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> phalanges of digit iii and 4<sup>th</sup> phalanx of digit ii of pelvic limb were additional ossification centres detected.

By days 17-20 of development, there were prominent and clear increases in the ossification centres at

Table 1: Appearance of ossification centres in the pectoral limb of guinea fowl embryo as detected by Alizarin Red stain

DAY	Cl	Co	Sc	H	R	U	Uc	Cmc	Dg iii	Dg v	Dg ii	Dg i
1	No	No	No	No	No	No	No	No	No	No	No	No
2	No	No	No	No	No	No	No	No	No	No	No	No
3	No	No	No	No	No	No	No	No	No	No	No	No
4	No	No	No	No	No	No	No	No	No	No	No	No
5	No	No	No	No	No	No	No	No	No	No	No	No
6	No	No	No	No	No	No	No	No	No	No	No	No
7	No	No	No	No	No	No	No	No	No	No	No	No
8	No	No	No	No	No	No	No	No	No	No	No	No
9	No	No	No	No	No	No	No	No	No	No	No	No
10	X	X	X	X	X	X	No	No	No	No	No	No
11	X	X	X	X	X	X	No	No	No	No	No	No
12	X	X	X	X	X	X	No	No	No	No	No	No
13	X	X	X	X	X	X	X	X	No	No	No	No
14	X	X	X	X	X	X	X	X	No	No	No	No
15	X	X	X	X	X	X	X	X	No	No	No	No
16	X	X	X	X	X	X	X	X	X	X	X	X
17	X	X	X	X	X	X	X	X	X	X	X	X
18	X	X	X	X	X	X	X	X	X	X	X	X
19	X	X	X	X	X	X	X	X	X	X	X	X
20	X	X	X	X	X	X	X	X	X	X	X	X
21	X	X	X	X	X	X	X	X	X	X	X	X
22	X	X	X	X	X	X	X	X	X	X	X	X
23	X	X	X	X	X	X	X	X	X	X	X	X
24	X	X	X	X	X	X	X	X	X	X	X	X
25	X	X	X	X	X	X	X	X	X	X	X	X
26	X	X	X	X	X	X	X	X	X	X	X	X
27	X	X	X	X	X	X	X	X	X	X	X	X
28	X	X	X	X	X	X	X	X	X	X	X	X

Keys: Cl = Clavicle, Co = Coracoid, Sc = Scapula, H = Humerus, R = Radius, U = Ulna, Uc = Ulnacarpal, Cmc = Carpometacarpal, DgIII = Digit III, Dg V = Digit V, Dg II = Digit II, No = Absence of primary ossification centre, X = Presence of ossification centre.

Table 2: Appearance of ossification centres in the pelvic limb of guinea fowl embryo as detected by Alizarin Red stain

Day	I	Is	P	F	Fb	Pt	Mt	Tt	Tmt	1Px DgII	2Px DgII	1Px DgIII	2Px DgIII	3Px DgIII	4Px DgII
1	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
2	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
3	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
4	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
5	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
6	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
7	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
8	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
9	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
10	X	No	X	X	X	No	No	No	No	No	No	No	No	No	No
11	X	No	X	X	X	No	No	No	No	No	No	No	No	No	No
12	X	X	X	X	X	No	No	No	No	No	No	No	No	No	No
13	X	X	X	X	X	X	X	X	X	X	X	No	No	No	No
14	X	X	X	X	X	X	X	X	X	X	X	No	No	No	No
15	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
16	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
17	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
18	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
19	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
20	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
21	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
22	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
23	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
24	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
25	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
26	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
27	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
28	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Keys: I = Ilium, Is = Ischium, P = Pubis, F = Femur, Fb = Fibular, Pt = Patellar, Mt = Metatarsus, Tt = Tibiotarsus, Tmt = Tarsometatarsus, 1 P x Dg II = 1<sup>st</sup> phalanx digit II, 2 P x Dg II = 2<sup>nd</sup> phalanx digit II, 1 P x Dg III = 1<sup>st</sup> phalanx digit III, 2 P x Dg III = 2<sup>nd</sup> phalanx digit III, 3 P x Dg III = 3<sup>rd</sup> phalanx digit III, 4 P x Dg II = 4<sup>th</sup> phalanx digit II, No = Absence of ossification centre, X = Presence of ossification centre.

cervical, thoracic, lumbosacral and coccygeal regions (Table 3).

On day 21, ossification centre was first detected on the pygostyle. Between days 23 and 24, the right and left clavicles had joined together but were not properly fused.

Day 27 of development showed that the epiphyseal growth plate on the large and long bones were still open.

## Discussion

The primitive axial support of vertebrates is the notochord that appears during the first week of embryonic life and it serves as the primitive back bone and is later replaced by vertebrae (Zobriskey, 1969).

Gimba (1990) observed that ossification centres first appeared in the chicken embryos (with incubation period of 21 days) on the 8<sup>th</sup> day but in the guinea fowl with an incubation period of 28 days, the centres were seen on day 9.

Development of bones in some other animal species like the *Rattus rattus*, commenced on the 10<sup>th</sup> day (gestation period of 21-23 days). The zebu cattle with a

gestation period of about 288 days had its appendicular skeleton begin to appear from 42<sup>nd</sup> day (Ojo, 1977).

From the foregoing, it is apparent that there is a relationship between the time of appearance of centre of ossification and length of incubation period as in the avian species and length of gestation period in mammals.

One obvious fact from the information above is that bone development starts at a very early stage in all animals. It is also apparent that ossification centres may start at both limbs at the same time but become completed earlier in the fore limb than the hind limb.

The clavicles were reported to appear on day 8 in chicken and become fused on day 16 (Gimba, 1990). It was observed on day 10 in the guinea fowl but the fusion that occurred on 24<sup>th</sup> day was only a few days before hatching. Considering the incubation periods of the two species, it could be said that fusion occurred at the same time lag.

It is pertinent however to note that as the days of embryonic development progress, it becomes difficult to "sight" new centres of ossification, because the existing ones tend to increase in size and length thereby partially

Table 3: Appearance of ossification centre in the axial skeleton of guinea fowl as detected by Alizarin Red stain

DAY	Cv	Tv	Ls	Cc
1	No	No	No	No
2	No	No	No	No
3	No	No	No	No
4	No	No	No	No
5	No	No	No	No
6	No	No	No	No
7	No	No	No	No
8	No	No	No	No
9	No	No	No	No
10	X	X	No	No
11	X	X	No	No
12	X	X	No	No
13	X	X	X	X
14	X	X	X	X
15	X	X	X	X
16	X	X	X	X
17	X	X	X	X
18	X	X	X	X
19	X	X	X	X
20	X	X	X	X
21	X	X	X	X
22	X	X	X	X
23	X	X	X	X
24	X	X	X	X
25	X	X	X	X
26	X	X	X	X
27	X	X	X	X
28	X	X	X	X

Keys: Cv = Cervical vertebrae, Tv = Thoracic vertebrae, Ls = Lumbosacral vertebrae, Cc = Coccygeal, No = Absence of ossification centre, X = Presence of ossification centre.

obliterating the new centres of ossification as they link up with the new centres of ossification.

It is worthwhile to note that there is a definite peculiarity of the bones of birds in that the epiphysis is absent in embryonic life until the chick is a day old or more (Cameron, 1972).

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