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A Morphological Study of Skeletal Development in Turkey during the Pre-Hatching Stage

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With 2 figures and 4 tables

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Summary

Skeletal chondrofication, ossification and growth of turkey embryos were investigated and analysed to enable assessment of the developmental status and evaluation of the experimental effects on skeletal development, skeletal mutations and development of cultured embryos. Ten embryos were prepared every 24 h from 8 to 28 days of incubation. The fixed embryos were cleared and stained *in toto* with Alcian blue & Alizarin red for cartilage and ossified components, respectively. Observation of the skeleton was performed under a stereoscopic microscopy, with special attention to the timing of chondrofication and ossification of the bones. The first occurrence of the primary ossification centres was observed in the femur, tibiotarsus, and the dentary and supra-angular of the mandible on the 12th day, followed immediately by the other long bones. Skeletal features of the skull were determined to show the latest appearance of cartilage and ossification. Hence, all elements of the hyolingual apparatus remained cartilaginous until hatching took place except for the ceratobranchial. Even though the vertebral column chondrified earlier as compared with the ribs and sternum, they ossified later. While chondrofication was present in all the regions of the vertebral column at the same time, ossification progressed from the cervical through caudal regions. The growth rate of the femur was eminently higher than that of the humerus with increase in time, particularly after the 20th day of incubation. This seems to be obviously natural because the eggs used in the study are from the broiler turkey, which gains giant muscle mass at a very short period; precocity is probably at the expense of the bones of the leg rather than those of the wing.

Introduction

Recently, studies in the field of experimental embryology of avian species have dealt frequently with natural skeletal development, teratological testing and developmental engineering. Hence, teratological experiments using artificial *in vivo* cultures are conducted on creating transgenic or chimeric birds from 1-cell or blastoderm stage (Perry, 1988; Etches et al., 1993; Naito et al., 1994; Ono et al., 1994, 1998). Furthermore, they are designed to investigate and analyse embryonic skeletogenesis (Hashizume et al., 1993), skeletal mutations (Nakane and Tsudzuki, 1998; Tsudzuki et al., 1998) and development of cultured embryos under artificial conditions (Naito et al., 1990). They also aim at revealing the teratogenic consequences of new drugs (Hashizume et al., 1992, 1993).

Using an avian embryo as an experimental model in research comprises several valuable possessions including smaller body size, more and fast prolificness, and precociousness of the species. A list of natural embryonic developmental stages needs to be acquired as the normal control in experimental embryology to design the experiments and to analyse the results. Thus, normal stages of skeletogenous development are essential indicators and indispensable parameters to evaluate the data acquired in the experimental embryology, and developmental and teratological experiments. They are also very important for the study of factors that might modify the skeletal development and for evaluation of their modifications (Baeriswyl, 1980).

Reports have been accumulating on the ossificatory developmental stages of the skeleton in various avian species including chicken and quail embryos (Hamburger and Hamilton, 1951; Padgett and Ivey, 1960; Zacchei, 1961; Nakane and Tsudzuki, 1999). They have documented ossification centres of either partial or whole fetal skeletal components to contribute significant basic knowledge to the studies in experimental embryology to acquire more precise and efficient data (Hamilton, 1952; Jollie, 1957; Nakane and Tsudzuki, 1999). This study, therefore, aimed at determining chondrofication and ossification centres, and growth of the bones of the skeleton in the pre-hatching period of the turkey. In this aspect, the time of gross appearance of the chondrofication and ossification centres was determined, pattern of the related bone growth was established and the data were compared with those of the literature. Overall, the findings of these studies may well contribute to the list of normal skeletogenetic stages in the development of avian species.

Materials and Methods

Fertilized eggs, weighing 85–89 g, of the Canadian white Hybrid Converter turkey were kindly obtained from the turkey production unit of Bolca of Bolu Feed AS/Turkey within 2 h after laying and stored at 15°C, and were put into an incubator (Brinsea Octagon 250, Sandford, UK) within 3 days of laying. The temperature and relative humidity of the incubator and the hatcher were adjusted to 37–37.82°C and 55–60%, and 36.3–37.0°C and 75–80%, respectively. Stages at 24-h intervals were taken for research to describe the external developmental occurrences of the embryos. Ten embryos were prepared every 24 h in the morning from 8 to 28 days of incubation; so a total of 210 embryos were used for the study. The external description was evaluated in accordance with the data of Hamburger and Hamilton, 1951.

The embryos were fixed in 10% formaldehyde for 1 week and then 95% ethanol solutions for 10 days. This procedure was always started in the morning to acquire a timing fashion. Subsequently, they were cleared and the skeletons of the embryos were stained *in toto* with Alcian blue (C.I 74240; Merck, Germany) and alizarin red (C.I 58005, Merck, Munich, Germany) for cartilage and ossified components, respectively. The staining technique was modified from Peters, 1977 and Nakane and Tsudzuki, 1999, which dyes the cartilaginous tissue blue and the osseous tissue red. The technique displays the development of the cartilaginous components and localization of the early centres of the ossification areas in the bones.

Observation of the skeletons was performed under a stereoscopic microscopy (Olympus Optical Co Ltd, Tokyo, Japan; No: 20326), with special attention to the timing of chondrofication and ossification of the bones. Bone nomenclature was based on that described in Nomina Anatomica Avium (1993). The universal ethical rules were also strictly followed during the animal processing of the study.

Results

Developmental features of the bones of the 8–28 day embryos were described during the course of incubation. Transitions of the chondrofication and ossification of the bones are depicted in Tables 1–4 and Figs 1 and 2. On 8 days of incubation, the vertebral column and parachordal cartilage were stained blue around the notochord, along with the caudal region to the tip of the tail-bud even though no eminent chondrotic centres were observed. There was still no clear blue staining that reminded the skull and the fore- and hindlimbs (Fig. 2a).

At day 9 (Fig. 2b), the blue staining components including body and arch of the vertebrae became very clear. A slight blue staining was present in the orbitosphenoid, palatine, quadrate and trabeculae. Hence, small mandibular rudiments (dentary,

Table 1. Transition of ossification of the skull bones

	Days of incubation																				
Skull bones	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Occipital																					
Basioccipital	–	–	–	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
Exoccipital	–	–	–	–	B	B	B	R	R	R	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	R
Sphenoid																					
Parasphenoid	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Basisphenoid	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Laterosphenoid	–	–	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Temporal																					
Squamosal	–	–	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Prootic	–	–	–	–	–	–	–	B	B	B	B	B	B	B	B	B	R	R	R	R	R
Opisthotic	–	–	–	–	–	–	–	–	B	B	B	B	B	B	B	B	B	R	R	R	R
Epiotic	–	–	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Parietal	–	–	–	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
Frontal	–	–	–	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
Pre-frontal	–	–	–	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Mesethmoid	–	–	–	–	–	–	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Trabeculae	–	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Nasal	–	–	–	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pre-maxilla	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Maxilla	–	–	–	–	–	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Palatine	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Vomer	–	–	–	–	–	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pterygoid	–	–	–	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Jugal	–	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Quadratojugal	–	–	–	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Quadrate	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Mandible																					
Dentary	–	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Supraangular	–	–	–	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Angular	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Splenial	–	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Pre-articular	–	–	–	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Articular	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Ramus	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Hyolingual apparatus																					
Entoglossal	–	–	–	B	B	B	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	B	B	B	B
Urohyal	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Ceratobranchial	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Epibranchial	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

–, not stained with either Alcian blue or Alizarine red; B, stained blue with Alcian blue; R, stained with Alizarine red.

Table 2. Transition of ossification of the vertebral bones

Vertebral bones	Days of incubation																				
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Cervical vertebrae																					
Upper region																					
Centrum	B	B	B	B	B	B	B	B	B	R	R	R	R	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	R	R	R	R
Cervical rib	–	–	–	–	–	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Medial region																					
Centrum	B	B	B	B	B	B	B	B	B	R	R	R	R	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	R	R	R	R
Cervical rib	–	–	–	–	–	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Lower region																					
Centrum	B	B	B	B	B	B	B	B	B	R	R	R	R	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	R	R	R	R
Cervical rib	–	–	–	–	–	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Thoracic vertebrae																					
Upper region																					
Centrum	B	B	B	B	B	B	B	B	B	B	R	R	R	R	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	R	R	R	R
Medial region																					
Centrum	B	B	B	B	B	B	B	B	B	B	R	R	R	R	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	R	R	R	R
Lower region																					
Centrum	B	B	B	B	B	B	B	B	B	B	R	R	R	R	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	R	R	R	R
Synsacral vertebrae																					
Upper region																					
Centrum	B	B	B	B	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	B	B	B	B	R	R	R	R	R	R	R
Medial region																					
Centrum	B	B	B	B	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	B	B	B	B	B	R	R	R	R	R
Lower region																					
Centrum	B	B	B	B	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	B	B	B	B	R	R	R	R
Caudal vertebrae																					
Upper region																					
Centrum	B	B	B	B	B	B	B	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	B	B	B	B	B	B	B	R	R	R	R
Medial region																					
Centrum	B	B	B	B	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	B	B	B	B	B	R	R	R
Lower region																					
Centrum	B	B	B	B	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	B	B	B	B	B	R	R

–, not stained with either Alcian blue or Alizarine red; B, stained blue with Alcian blue; R, stained with Alizarine red.

articular), very thin hyolingual apparatus (basihyal, urohyal, ceratobranchial, epibranchial) and periotic capsule were stained blue. The scapula, coracoid, humerus, radius, ulna, radial and ulnar carpals, and metacarpale majus and minus were also stained blue, reflecting the chondrotic drafts in general. Likewise, the femur, tibia, fibula and metatarsale II, III and IV were stained blue.

By the 10th day (Fig. 2c), no ribs and sternum existed. The chondrotic drafts of the vertebral arches and bodies of the cervical to synsacral components in the vertebral column bilaterally became prominent on the 10th day of incubation. Likewise, chondrotic drafts of the seven vertebral and four sternal ribs appeared at this stage. Blue staining was observed in the squamosal of the temporal bone. I chondrotic draft of the metacarpale alulare which was to be fused to that of the metacarpale majus, and the first phalanges of the alular and minus digits of the forelimb were stained blue while the tarsi tibiale and fibulare, first phalanges of the second, and third and fourth digits of the hindlimb were stained blue at this stage.

At day 11, the blue staining was seen in the pre-frontal, nasal, pre-maxilla, pterygoid and quadratojugal. All components of the mandible and hyoligual apparatus turned blue. The chondrotic drafts of the tibia, tarsi tibiale and fibulare fused to construct the tibiotarsus. At day 12, exoccipital of the occipital and jugal, and furcula stained partly blue. Chondrotic draft of the sternum was observed, attaching to the distal end of the sternal ribs and coracoid at this stage.

First ossification centres were determined in the dentary and supra-angular of the mandible on the 12th day. Diaphyseal ossified centres were also grossly visible in the femur and tibiotarsus at this stage. At day 13, parietal, frontal, maxilla and vomer stained partly blue. The uncinate processes of the third to sixth vertebral ribs were stained blue. Hence, squamosal part of the temporal bone, palatine, pterygoid, quadratojugal and pre-articular of the mandible began turning red at this stage. Ossified centres were also obvious in the clavicle, humerus, radius, ulna, femur, tibiotarsus, fibula and metatarsale II, III and IV. At day 14, basioccipital and

Table 3. Transition of ossification of the bones in the ribs and sternum

Ribs and sternum	Days of incubation																				
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Ribs																					
First vertebral rib	–	–	B	B	B	B	B	B	B	R	R	R	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	R	R	R	R
Uncinate process	–	–	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R
Third vertebral rib	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Uncinate process	–	–	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Fourth vertebral rib	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Uncinate process	–	–	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Fifth vertebral rib	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Uncinate process	–	–	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R
Sixth vertebral rib	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Uncinate process	–	–	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R
Seventh vertebral rib	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
First sternal rib	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R
Second sternal rib	–	–	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Third sternal rib	–	–	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Fourth sternal rib	–	–	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Fifth sternal rib	–	–	–	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Sternum																					
Body	–	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Laterocranial process	–	–	–	–	–	–	–	–	–	B	B	B	B	B	B	B	R	R	R	R	R
Inner laterocaudal process	–	–	–	–	–	–	–	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Outer laterocaudal process	–	–	–	–	–	–	–	–	–	–	B	B	B	B	R	R	R	R	R	R	R
Manubrium	–	–	–	–	–	–	–	–	B	B	B	B	B	B	B	B	B	B	R	R	R
Crest	–	–	–	–	–	–	–	–	B	B	B	B	B	B	B	B	B	R	R	R	R

–, Not stained with either Alcian blue or Alizarine red; B, stained blue with Alcian blue; R, stained with Alizarine red.

supraoccipital of the occipital and mesethmoid stained blue. Pre-frontal and ceratobranchial of the hyolingual apparatus joined the ossification. The coracoid and metacarpale majus and minus began ossifying at this stage.

At day 15, prootic of the temporal stained blue. Exoccipital of the occipital, parasphenoid and basisphenoid of the sphenoid, pre-maxilla, maxilla, jugal and ramus of the mandible began ossifying. Ossification began at the central portions of the six vertebral ribs. No ossification was observed in the sternal ribs and sternum yet. Ilium and pubis also ossified partly at this stage. At day 16, ophistotic of the temporal began turning blue while splenial of the mandible started ossifying. Three different ossification centres were seen in the cervical vertebrae of the one cadaver only. Ossification also appeared in the ischium. Epiotic of the temporal turned blue on the days 17 as laterosphenoid of the sphenoid began ossifying. A cylindrical ossification centre was also determined on the body of the each cervical vertebra. By this stage, all the skeletal components of the skull have already begun staining blue (Fig. 2e).

At day 18, no new blue staining was observed while basioccipital of the occipital, parietal and frontal were partly stained red. Ossification was determined on the body of the each thoracal vertebrae most of which constitute *Notarium*. Chondrotic drafts of the thoracal veretebrae ankylosed already to constitute the *Notarium*. Similarly, chondrotic drafts of the vertebrae from thoracal, lumbal, sacral and caudal regions fused to constitute *Synsacrum*. The metatarsale I and second phalanx of the first digit in the hindlimb also began ossifying while the metatarsal bones along with the tarsale I and II united at this stage to form the tarsometatarsus (Fig. 2f). At day 19, two additional ossification centres were observed on the vertebral arches of each cervical vertebra. The centres on each vertebra were attached through chondrotic tissues.

At day 20, supraoccipital of the occipital was partly stained red. Ossification centres on the cervical and thoracal vertebrae continued their extension, thus, those appeared on the vertebrae of the lumbal and sacral regions formed the *Synsacrum*. The first phalanx of the digit minus in the forelimb turned partly red. At day 21, mesethmoid and trabeculae turned red, latter fusing with the adjacent red staining on day 22. Transverse processes of the thoracal vertebrae began ossifying. Hence, double ossification centres were observed on the body of the caudal vertebra joining the formation of the *Synsacrum*. The central portions of the third and fourth sternal ribs turned red. The second phalanx of the fourth digit in the hindlimb also began ossifying at this stage.

At day 22, a very small ossification centre on the dens of the *Axis* was determined. Chondrotic drafts of the dorsal spines of the thoracal vertebrae appeared. Likewise, very small ossification centres were observed on the caudal vertebrae forming *Pygostyle*. The last four sternal ribs mostly finished their ossifications. The inner and outer processes of the laterocaudal processes were stained red. The tarsale I and II already united to tarsometatarsus also turned red. The fourth phalanx of the fourth digit in the hindlimb also began ossifying at this stage.

No new development was determined on the 23rd day while prootic of the temporal stained red on day 24. At day 24, the two ossification centres on the vertebral arches of each cervical vertebra except *Atlas* began uniting. No ossification was observed on the dorsal spines of the thoracal vertebrae yet. By this stage, all the vertebrae except the last three caudal ones had already begun ossifying. The laterocranial processes of the sternum were also stained red.

At day 25, ophistotic and ephiotic of the temporal and angular of the mandible began ossifying. Unification of the ossification centres on the vertebral arches of each cervical

Table 4. Transition of ossification of the bones in the fore- and hindlimbs

	Days of incubation																				
Bones of the fore- and hindlimbs	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Forelimb																					
Scapula	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Coracoid	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Clavicle	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Humerus	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Radius	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ulna	–	B	B	B	B	R	R	R	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	B	B	B	B
Carpi ulnare	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Metacarpale alulare	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	–	–	–	–	–	–
Metacarpale majus	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Metacarpale minus	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
First phalanx of the digit alulae	–	–	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Second phalanx of the digit alulae	–	–	–	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
First phalanx of the digit majus	–	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Second phalanx of the digit majus	–	–	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
First phalanx of the digit minus	–	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R
Hindlimb																					
Ilium	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ischium	–	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Pubis	–	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Femur	–	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Tibiotarsus	–	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Fibula	–	B	B	B	B	R	R	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	B
Tarsale I	–	B	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Tarsale II	–	–	B	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Metatarsale I	–	–	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
Metatarsale II	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Metatarsale III	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Metatarsale VI	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
First phalanx of the first digit	–	–	–	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Second phalanx of the first digit	–	–	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R
First phalanx of the second digit	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Second phalanx of the second digit	–	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Third phalanx of the second digit	–	–	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
First phalanx of the third digit	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Second phalanx of the third digit	–	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Third phalanx of the third digit	–	–	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
Fourth phalanx of the third digit	–	–	–	–	–	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R
First phalanx of the fourth digit	–	–	B	B	B	B	B	B	R	R	R	R	R	R	R	R	R	R	R	R	R
Second phalanx of the fourth digit	–	–	–	B	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R	R
Third phalanx of the fourth digit	–	–	–	–	–	B	B	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Fourth phalanx of the fourth digit	–	–	–	–	–	–	–	B	B	B	B	B	B	B	R	R	R	R	R	R	R
Fifth phalanx of the fourth digit	–	–	–	–	–	–	–	–	B	R	R	R	R	R	R	R	R	R	R	R	R

–, not stained with either Alcian blue or Alizarine red; B, stained blue with Alcian blue; R, stained with Alizarine red.

vertebra completed even though the ossification centres of the each vertebral body were still separate. Arches of the sacral vertebrae began ossifying. The last three caudal vertebrae also turned red. The uncinate processes of the fourth to sixth vertebral ribs and dorsal aspect of the sternal body turned red.

At day 26, articular of the mandible turned red. After this stage, the ossified components of the observed bones continued to develop until the 28th day of incubation when the hatching happened (Fig. 2h,i,j). Transitions of the chondrofication and ossification of the bones observed are depicted in Tables 1–4. Serial development of the bones is also displayed in Figs 1 and 2.

Discussion

Nomenclature of the skull bones in avian species is still a very controversial issue and that indicated in the Nomina Anatomica Avium (1993) is the most commendable, and hence followed in this study. Tables and Figures schematically and photographically displayed the timing of ossification in the bones of turkey. The skeletal chondrofication, ossification and growth were investigated and analysed to enable assessment of the developmental status and evaluation of the experimental effects on skeletal development, skeletal mutations and development of cultured embryos under artificial conditions. The growth of the ossification of the bones was fundamentally equal bilaterally with time increase. The growth of the ossification of the measured bones was also determined in length to be fundamentally equal bilaterally with time increase. Because of that, very small numerical side variations were disregarded as they had no significant effects on the results.

The first primary ossification centres appeared in the diaphysis of the long bones of the hindlimb including the

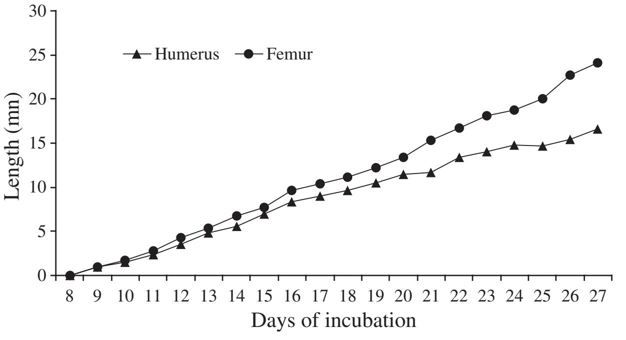

Fig. 1. Measurement of the lengths of the humerus and femur at all stages of the prenatal development.

femur and tibiotarsus, and in the dentary and supra-angular of the mandible on the 12th day, even though cartilaginous formation commenced earlier. Those of the forelimb were observed later on the 13th day. This slight delay indicates the precocity at the expense of the bones of the leg rather than those of the wing. This is in parallel with the accumulating data on domestic fowl (Baeriswyl, 1980; Pourlis et al., 1998) while controversial as compared with the situation where the long bones of the fore- and hindlimbs have showed similar patterns in Japanese quail (Nakane and Tsudzuki, 1999). It is also of interest to mention that skeletal features of the skull displayed the latest appearance of cartilage and ossification as compared with the other skeletal systems including extremities and vertebral column and thorax. Likewise, all components of the hyolingual apparatus remained cartilaginous except for the ceratobranchial. These results are similar to the accumulating data (Nakane and Tsudzuki, 1999).

As expected, the chondrofication was completed by the 13th day in the vertebral column while it was by the 18th day in the thorax. On the other hand, the first ossification areas appeared on the 15th day of incubation in the medial region of the vertebral ribs and progressed to the proximal and distal regions studied while the occurrence of first ossification in the cervical elements of the vertebral column was on the 17th day. In other words, even though they chondrified earlier as compared with the ribs and sternum, they ossified later. This coincides mostly with the accumulating data (Baeriswyl, 1980; Pourlis et al., 1998; Nakane and Tsudzuki, 1999). It is also interesting hereby to mention that, in our study, while chondrofication was present in all the regions of the vertebral

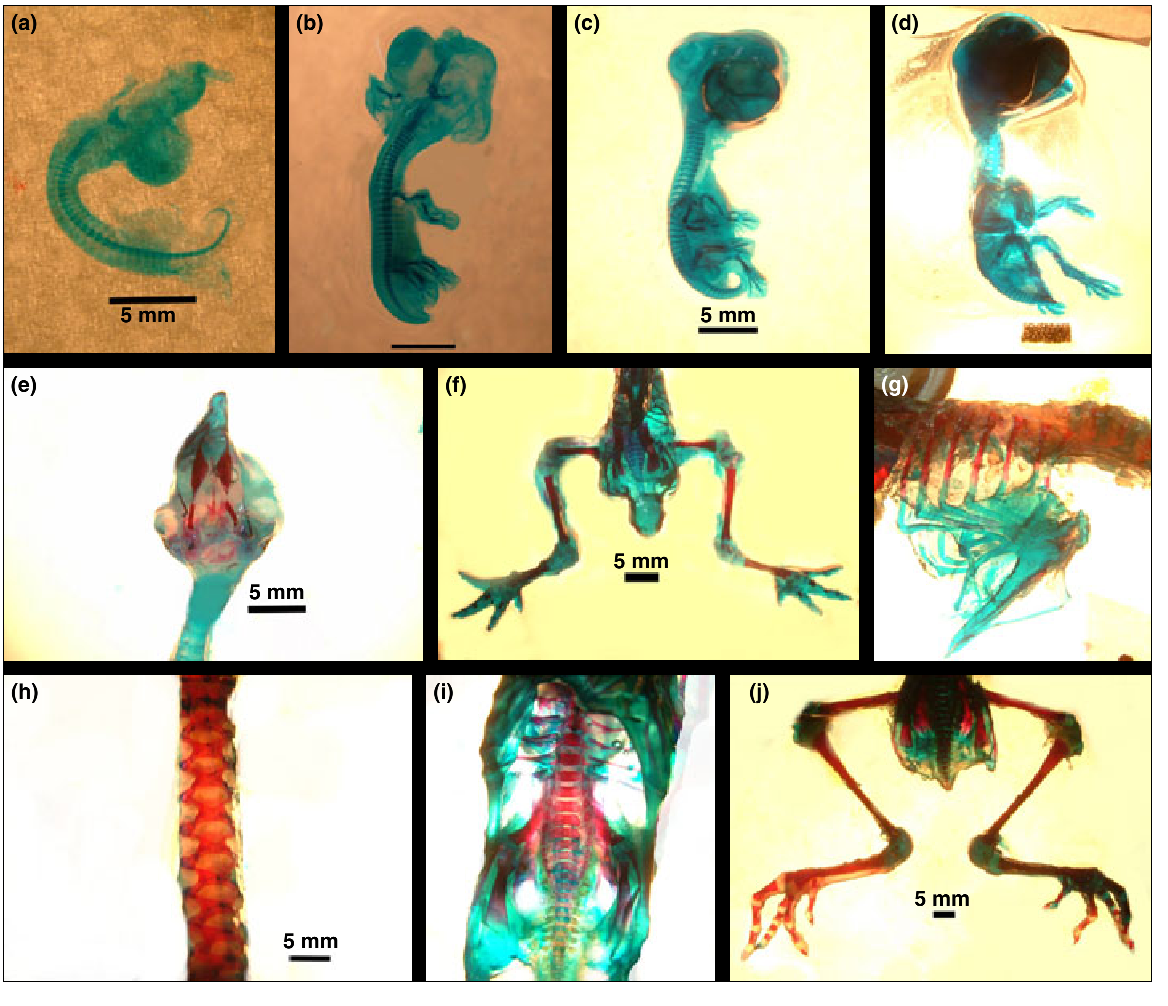

Fig. 2. Skeletal features of the turkey in the pre-hatching period on various days. Detail explanations are in the text. (a) 8th day, (b) 9th day, (c) 10th day, (d) 12th day, (e) skull, ventral, on 17th day, (f) hindlimbs on 18th day, (g) sternum, lateral, on 19th day, (h) cervical vertebrae, dorsal, on 26th day, (i) sacrum, ventral, on 27th day, (j) hindlimbs on 27th day of incubation.

column at the same time, ossification progressed from the cervical through caudal regions. Thus, ossification started at the medial region in the cervical and thoracic levels while it began at the upper region in the synsacral and caudal regions and extended downwards.

Literature reports indicate that ossification in the sternum of the avian species occurs at the later stage of development (Nakane and Tsudzuki, 1999; Blom and Lilja, 2004, 2005). Interestingly, ossification begins in the laterocaudal and laterocranial processes of the sternum, and completion of the ossification of the body of the sternum occurs at and after hatching.

It has been well known that birds possess different postnatal growth rates, i.e. altricial species show higher postnatal growth rates than precocial birds (Ricklefs et al., 1998; Lilja et al., 2001; Blom and Lilja, 2005). This is directly related to the degree of precocity of the new born chick. Altricial species with higher postnatal growth rates allocate such rates particularly to the rapid development of supply organs including those in the digestive system. The early development of these organs is probably at the expense of growth directed to demand organs such as muscles and skeletal features, particularly those in the extremities. This might be another reason why bones of the skull showed later appearance of cartilage and ossification. The growth pattern of the components of the vertebral column, ribs and sternum in avian species also varies significantly (Naito et al., 1990; Nakane and Tsudzuki, 1999; Lilja et al., 2001), even though they show in general later appearance of ossification as compared with those of the forelimb and hindlimb. It is again very interesting to remind that even though the vertebral elements chondrify earlier as compared with the ribs and sternum, they ossify later, which was also clearly documented in our study. Hence, the body of the sternum has been still mainly a chondrotic in nature at hatching. Calcium mobilization and mineralization began at first in the components of the hindlimb in turkey embryos of our study.

The growth rate has been reported to vary significantly among different bones of the same species also (Hamburger and Hamilton, 1951; Holder, 1978; Blom and Lilja, 2004, 2005). There is a significant increase during the first few days of the development in the cartilaginous and ossified components of all the bones. The development of the ossification continues throughout the hatching while that of the cartilaginous parts displays a plateau, followed by a steady decrease (Hamburger and Hamilton, 1951; Holder, 1978). In this study, despite the later appearance, there was an increase in the ossification rate of the bones of the skull except almost all components of the hyolingual apparatus. The ossification in the ribs and sternum began later and seemed to display a steady increase through the hatching.

Likewise, the growth pattern of the long bones of the wing and leg in Japanese quail was indicated to differ significantly (Pourlis et al., 1998). It has been highlighted in this report that the long bones of the wing reach their mature length more quickly than those of the leg. On the other hand, as can be clearly drawn from the Fig. 1, the results have shown in our study that the growth rate of the femur is eminently higher than that of the humerus with increase in time, particularly after the 20th day of incubation. This seems to be obviously natural because the eggs used in the study are from the broiler turkey, which gains giant muscle mass at a very short period; precocity is probably at the expense of the bones of the leg rather than those of the wing.

Chondrofication and calcification usually begin from different regions of the skeletal system. They usually occur in the medial region and progress to the proximal and distal regions in the ribs (Hamilton, 1952; Nakane and Tsudzuki, 1999). These reports also indicated that the uncinate processes of the ribs in the Japanese quail appeared as cartilage tissue and then ossified while those of the chicken were directly calcified without any chondrofication. We also observed in this study that the uncinate processes of the ribs in the turkey showed early chondrofication followed by an eminent ossification. In the metatarsal and digital bones, calcification begins either at the proximal or at the distal region. Hence, ossification began at the mid-region and progressed to the proximal and distal regions in the long bones and spread outwardly in the flat bones.

Finally, as indicated by the literature (Nomina Anatomica Avium, 1993), the carpal bones first appeared as chondrotic drafts, and then distal ones fused with the carpometacarpus while the carpi radiale and ulnare remained as separate chondrotic elements. Likewise, the proximal tarsal bones united to the tibia while the distal ones joined the formation of the tarsometatarsus with the metatarsal bones.

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