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Histological study for the development of Humerus and Femoral Bones in pre-hatched Embryos of Racing Pigeons and Japanese Quail

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Abstract

In developmental biology, studying the histological features of avian embryonic skeletal development might provide a reference standard. As a result, the purpose of this study was to determine the time of onset of ossification in femurs and humerous bones, as well as to characterize the most important histological changes found in these bones. This investigation used thirty fertilized eggs for each type of bird from various bird species, such as Japanese quail and racing pigeons. Embryos were collected at 8, 12, 16, and 18 days, based on the incubation time of each bird. Histological analysis of bone tissues was subsequently performed. The current study found that bones of racing pigeons and quails both underwent cartilage ossification on day 8 of incubation. During embryonic development, the ossification of the hindlimb persisted and even increased. The skeletogenesis ordering of ossification differed between the forelimb humerus bone and the hindlimb femour bone, as shown by comparing their calcification patterns. Results demonstrated that racing pigeons' humorous bones calcified more later than quail's embryonic bones. In conclusion, embryonic bone tissue staining and morphological variance analysis showed that the Japanese quail and pigeon embryos developed in different ways, which was linked to their bones growing at different rates during hatching.

Keywords: skeletogenesis, bone development, osteogensis, chondrification

Introduction

Limb development is an area of active research in both developmental and evolutionary biology (1). Birds primarily use their forelimbs as wings for aerial locomotion, while their legs serve as locomotor apparatuses for the necessary bipedal movement (2) ;(3). They continue to provide us with challenges and help develop our understanding of the path and appearance of the morphological characteristics that are

necessary for flight. Many cellular sources and differentiation mechanisms influence the formation of the vertebrate skeleton, reflecting the evolutionary history of the species (4); (5); (6). Bone is a composite material distinguished by a complex chemical makeup and a hierarchical structure. It has several functions, including offering structural support and supplying ions for the maintenance of homeostasis (7). Birds' bones are often characterized as lightweight because they evolved to reduce the energy expenditure required for flight. Moreover, they are essential in analyzing factors that may influence skeletal development and evaluating the consequences of these changes (8). In avians, intramembranous and endochondral ossification lead to bone development and proliferation. Intramembranous ossification directly

transforms mesenchymal cells into osseous tissue (9). Conversely, endochondral ossification enables their differentiation into cartilage templates followed by bone production (10). For bone development and full differentiation, there are three strategies. Each bone has an epiphysis at its terminal end. The term "diaphysis" denotes the shaft of a long bone. An epiphyseal plate constitutes the third kind of plate in the human body. Spongy bones, containing minute cavities, compose the epiphysis. The diaphysis is a compact bone characterized by few voids within its matrix (11). The epiphyseal plate is the principal site for substantial elongation following the cessation of bone development (12). Birds have several unique adaptations in their anatomical structure, resulting in their designation as a distinct class within the vertebrate phylum. Pigeons of the Columbia family exhibit highly advanced shoulder girdle and wing musculature, rendering them the most adept fliers among all bird species (13). Domestic fowl is a bird species that belongs to the pheasant family. (14) distinguish these avians by their terrestrial foraging habits and lack of flight capability. Scientists conducted several studies to establish a baseline for the gross anatomy of a flightless avian species and to compare its structural variances with those of other flight avian species. This experiment acquired and employed the sternum from four completely mature emus, in addition to that of a turkey and a duck. The sternum is a significant, unsegmented bony structure situated in the anterior ventral area of the body cavity. The egg possessed a bowl-shaped configuration, the turkey exhibited a triangular form, and the duck displayed a rectangle construction.(15) investigate the phenomenon of dynamic structures that experience ongoing alterations and remodeling in response to the ever-evolving environment (16) Numerous studies have routinely examined the prehatching development of the avian skeletal system. This study aims to document the normal staging for skeletal development in blue-breasted quail embryos. However, the histomorphological investigation of the quail skeleton's prehatching development is limited. Therefore, the purpose of this study was to determine when the wing bones chondrified and osseified, as well as to describe the

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histological stages of humerus formation in Japanese quail embryos.

Materials and Methods

Thirty fertilized eggs of each type of bird including racing pigeons and Japanese quail were used in this study. Fertilized eggs were collected within 6 hours after laying and stored at 15°C for ~5 days. They were incubated in a locally manufactured incubator after adjustment of the temperature and humidity at 37.7 ± 0.2°C and a relative humidity of 70%, respectively. Embryos were collected and used for skeletal tissue staining on days 8, 12, 16, and 18 of incubation, depending on the incubation period of each bird. This study was carried out following the strictest standards for animal care and research. The experiment was conducted according to guidelines approved by the College of Veterinary Medicine, University of Diyala Animal Experimentation Ethics Committee, No. VM 202. October 2023. K&R, dated on 1/10/2023. The prepared embryos were preserved in 10% neutral buffered formalin for 24 hours. Tissues acquired for histological analysis comprised the humerus and femour bones from both birds embryos, which was subjected to standard histological processing techniques; slices of 5 µm thickness were prepared and stained with hematoxylin and eosin, thereafter inspected under a light microscope. The photographs displayed were obtained with a digital camera.

Results

The current study found that the histological development of the long bones (humerus) in the wings is different from that of the long bones (femour) in the legs. Endochondral ossification, or osteogenesis, is the process by which longitudinal growth becomes bone. This change happens gradually over time. This shift will last beyond the bone development phase.

Histological assessment of quail embryo

On the eighth day of the incubation, the cartilage template started to take on the shape of the potential humerus, complete with the middiaphysis and epiphyses at the end (Figure 1). The epiphyseal area was filled with tiny chondrocytes that were randomly dispersed throughout the extracellular matrix. These chondrocytes ranged in form from round to oval. According to the data presented here, the hypertrophy zone, which is composed of large chondrocytes, was seen to be present in the middle of the diaphysis, as well as on both of the hypertrophic zone's boundaries. Additionally, it was seen that the proliferative zone was made up of chondrocytes that had been crushed. On the twelfth day of incubation, the inner cellular layer of the perichondrium differentiated into osteoblasts on the eighth day. In the mid-diaphysis region, where the chondrocytes had grown too large, these osteoblasts deposited osteoid tissue in

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the form of a bone collar. This caused the perichondrium to gradually transform into the periosteum (Figure 1).

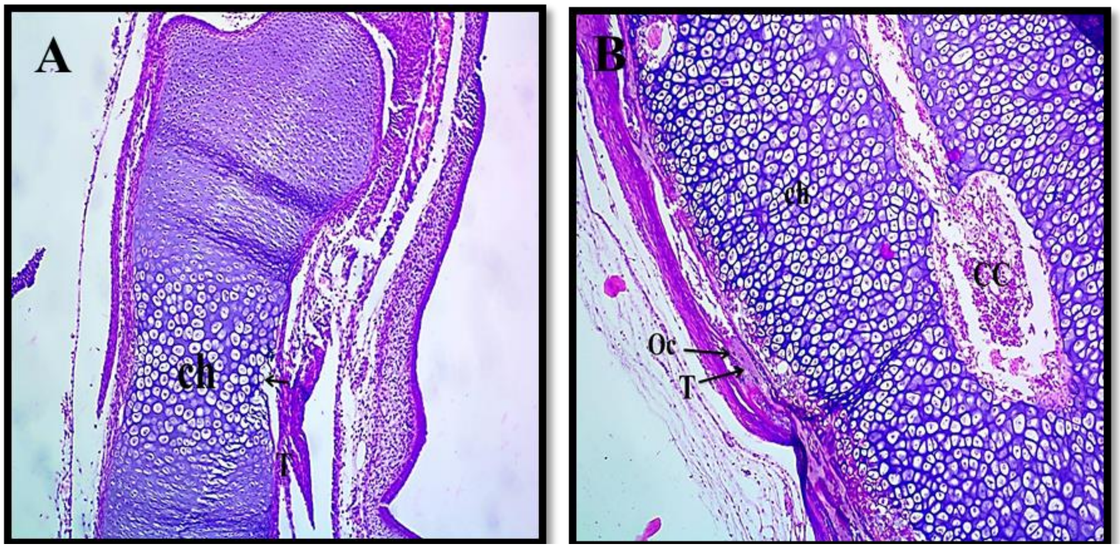

Figure 1: Photomicrographs show a bone tissue section from the humerus bone on 8-day-old (A) and 12-day-old (B) of the quail embryo, illustrating the initiation of osteogenesis (ch), trabecular bone (T); The hypertrophy zone, characterized by larger chondrocytes, is located in the center of the diaphysis, as indicated by the black arrows and the presences of chondroal canal (cc). (H&E stain, A &B 10X).

Between the sixteenth and eighteenth days of incubation, developmental alterations in the long bones of the limbs resulted in noticeable longitudinal and radial expansion of the diaphysis. The radial growth was demonstrated to extend directly to the location of the cortical bone. Osteoblasts were detected originating from the inner layer of the periosteum (Figure 2). The bone trabeculae were heavily stained with hematoxylin and eosin. The osteoclasts resorbed the endochondral trabecular bone, and by the 18th day, the bone marrow cavity in the mid-diaphysis began to develop.

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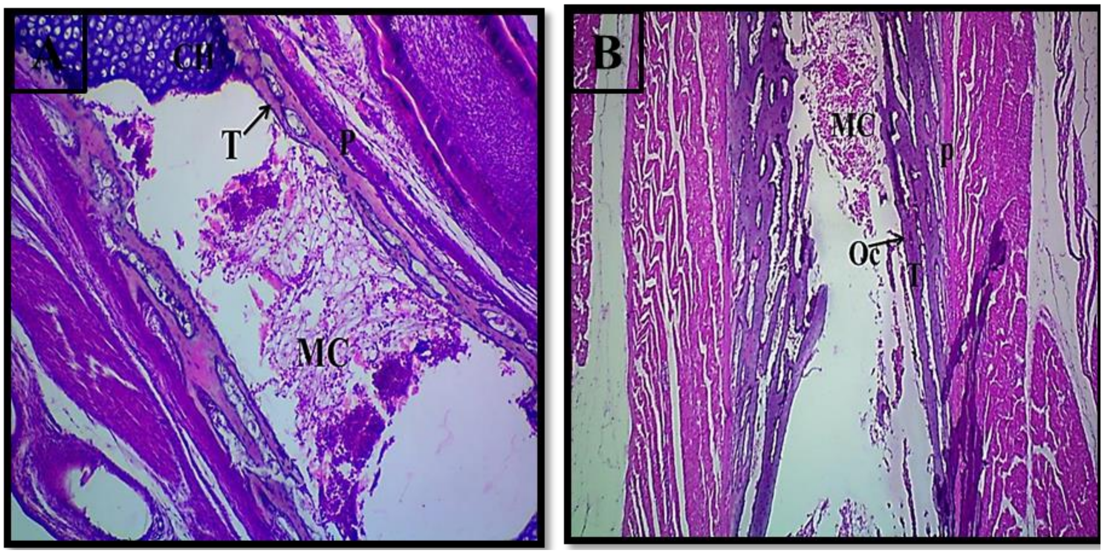

Figure 2: Photomicrographs show a bone tissue section from the humerus bone on 16-day-old (A) and 18-day-old (B) of the quail embryo, illustrating the formation of trabecular bone (T), medullary cavity (MC); periosteum (P); osteocytes (Oc). (H&E stain, A & B10X).

Histological assessment of racing pigeon embryo

By the eighth day, the cartilage template had developed into the prospective humerus, complete with a mid-diaphysis and epiphyses at the ends (Figure 3). The extracellular matrix in the epiphyseal site randomly distributes tiny, round- to oval-shaped chondrocytes. The result demonstrated the presence of a hypertrophy zone consisting of enlarged chondrocytes located in the center of the diaphysis, as well as on both sides of the hypertrophic zone. A region of increased chondrocyte growth and enlargement was observed (Figure 3).

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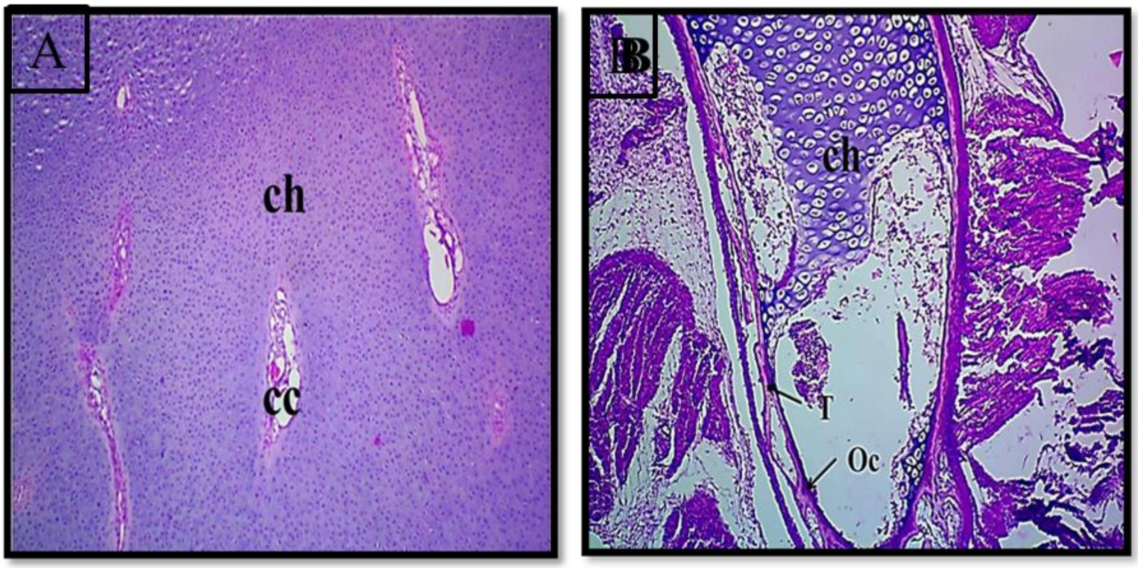

Figure 3: Photomicrographs show a bone tissue section from the humerus bone on 8-day-old (A) and 12-day-old (B) of the quail embryo, illustrating the initiation of osteogenesis, trabecular bone (T); The hypertrophy zone, characterized by larger chondrocytes (ch), is located in the center of the diaphysis, as indicated by the black arrows and the presences of chondroal canal (cc), osteocytes (Oc). (H&E stain, A & B 10X).

The diaphysis grew longitudinally and radially between the sixteenth and eighteenth days of incubation as a result of developmental alterations in the long bones of the limbs. The location of the cortical bone was demonstrated to be directly impacted by the radial growth. From the inner layer of the periosteum, osteoblasts were discovered (Figure 4). Hematoxyline and eosin stain were used extensively to stain the bone trabeculae. During resorption, osteoclasts broke down the endochondral trabecular bone, and by day 18, a bone marrow cavity had begun to develop within the mid-diaphysis.

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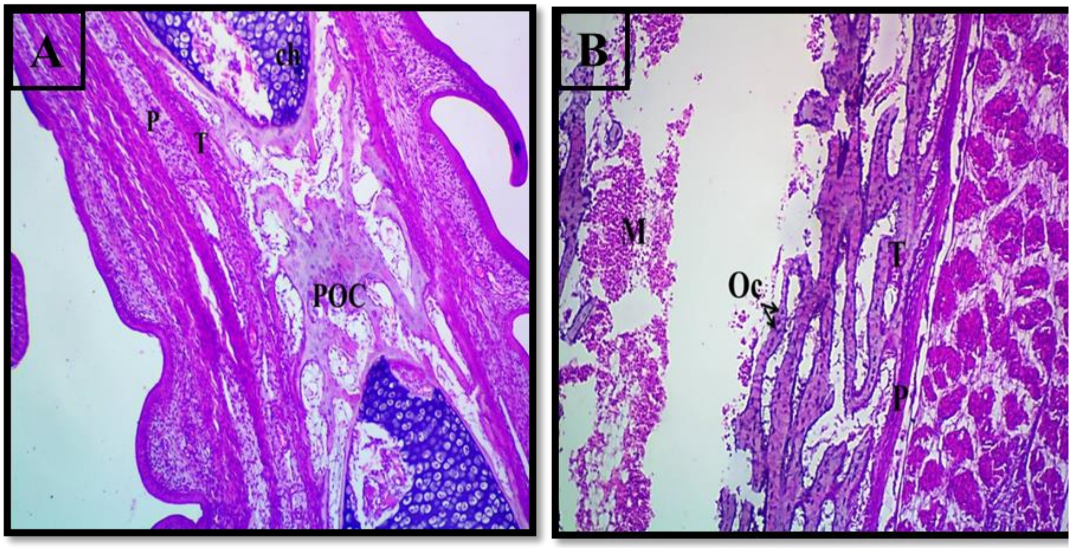

Figure 4: Photomicrographs show a bone tissue section from the humerus bone on 16-day-old (A) and 18-day-old (B) of the quail embryo, illustrating the formation of trabecular bone (T), medullary cavity (M); chondrocyte (ch); periosteum (P); osteocytes (Oc) and primary ossification center (POC). (H&E stain, A & B10X).

Discussion

In the field of comparative developmental biology, there has been an increase in interest regarding the scheduling of skeletogenic processes in a species-specific manner (17). During the embryonic stage, the degree of calcification of the hindlimbs has a significant influence on standing and locomotion (18). On the other hand, the forelimb is responsible for holding the body in order to facilitate flight. Skeletons are the fundamental mechanism responsible for carrying the body. The findings of the present study, which examines the histological features of bone tissues of two different bird's bones, suggest that the timing of early embryogenic skeletogenesis events may vary both within and across species (19). The present comparative study comprised Japanese quails which are

typically ground-dwelling species. The growth and development of hindlimbs in quials is essential in order to provide the necessary strength for walking, sprinting, and lifting off during flight. According to (20), the hindlimb is one of the parts of the quail and chicken bones that involvements ossification at an earlier stage. However, the quail embryos grew more quickly, and by the time they hatched, they had reached a more mature stage of development. (17) stated that the early growth rate of pigeons once they have hatched is substantially quicker than that of quails. The current study found that cartilage ossification occurred in the humorus bones of quails at day 8, and the same occurred of bones of racing pigeons. The ossification of the hindlimb continued, and it gradually increased during the embryonic development process. Furthermore, the majority of the hindlimb ossified prior to hatching, which is consistent with the findings of previous studies (18); (21). In contrast, racing pigeons birds are predominantly flight-dwelling species. As a result, their forelimbs must grow and develop in order to provide the necessary force for flight. When we compared the calcification patterns of the forelimb for example humorous bone and the hindlimb femour bone, we found that there were some differences in the skeletogenesis ordering of ossification. The findings showed that humorous of racing pigeons, in comparison to humorous of quail's embryonic bones calcified considerably later. Quail embryos, on the other hand, did in fact form these bones; nonetheless, they calcified at a somewhat later stage than other bones. This calcification delay occurs in the region between the terminals. When compared to quail embryos, racing pigeon embryos require days to grow their hindlimb and to be able to hold his body, but quail embryos require one to two days to be albe to stand and run. We can roughly compare the morphological features of chondrification of each bone in a bird's forelimb and hindlimb. The embryonic bone tissues staining and morphological variance analysis of different birds showed that the Japanese quail and pigeon had different patterns of embryonic development, which was linked to their different skeletal development speeds during hatching. These differences are related to the fact that precocial and altricial neonates have different skeletal development rates (17). This was the case in both species. Compared to changes in other developmental traits, the general ossification sequence pattern stays very stable. This is true even though there may be differences in how forelimb and hindlimb traits change (17). This is despite the fact that precocial and altricial species display heterochronies. According to these findings, the mechanical performance of limb bones appears to have a tight relationship with the locomotor capabilities of an animal. (22) provide a classic example of a ground-dwelling precocial bird, the Japanese quail, whose hatchlings have fully grown hind limbs. The femurs of hatching quails and pigeons differ in their geometrical and mechanical features (22, 23). According to (17), hatchling quails possessed bone tissues that were more rigid. In conclusion, morphological variance analysis and embryonic bone tissue staining indicated that Japanese quail and pigeon embryos exhibited distinct developmental patterns, correlated with their disparate bone growth rates during hatching.

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