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RADIOGRAPHIC STUDY OF THE OSSIFICATION CENTERS OF PELVIC LIMBS AFTER HATCHING IN PARTRIDGE

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Abstract

The ossification centers and skeletal development of the pelvic limbs after hatching in partridge (Alectoris chukar) from day one to 98 days were studied by digital radiography. Alizarin Red S staining method was used to confirm the radiographic diagnosis in this study. The head of the femur was seen on day 28. The ossification of the patella was observed on day 49. In Alizarin-staining specimen, a ossification center was seen in the proximal epiphysis of tibia on day 28. The ossification centers of the tarsal region were seen from the first day. Fusion occurred between proximal tarsal bones and tibia on day 63. Distal tarsal bone fused with metatarsals on day 77. It seems that the appearance sequence of ossification centers in birds belonging to the Galliformes order is the same. And the difference between them is the time of the appearance of ossification centers. The use of digital radiography and Alizarin Red S staining simultaneously provided a set of benefical information for the further study on the skeletal development of partridge.

Keywords; hatching, ossification centers, partridge, pelvic limb, radiography.

Introduction

During the process of skeletal development in birds, cartilaginous templates are formed in the early days of emberyonic period, which then are replaced by bone.[1]

At the time of onset of ossification in cartilaginous templates of the pelvic limb bones, periosteal ossification occurs in the midregion of cartilaginous templates, and bony collar forms at this site. Then bony collar develops and vascularization takes place in it. In birds, like mammals, the primary ossification centers do not form. Finally, chondrocytes were removed and marrow cavity is created.[2]

There are many articles on ossification centers and skeletal development in birds, which have been related to pre-hatching period. A large number of these articles have been related to chicken (Gallus gallus domesticus).[3-5] Due to importance of the quail (Coturnix coturnix japonica), many studies were focused on this bird.[6-8] Also there are few articles on other species.[8-10]

The first centers of ossification were observed in chicken and turkey emberyos which were in the femur, tibia and fibula.[11,12]

The onset of perichondral ossification was observed in these bones in the 6th day of emberyonic period in the quail.[13]

Appearance time of ossification centers in the quail embryol leg skeleton was reported by Lansdown (1970). Also, he described that appearance sequence of these centers in the quail legs and chicken were very similar.[6]

Hogg (1980) studied the ossification centers in pelvic limb of chicken at three time including: hatching, post hatching and physical maturity.[14] similar study was performed by yahyaei et al. (2013) in quail.[15]

Many researches were interested in development of tarsal region, and some disagreement about the ossification of tarsal elements and distal tibial epiphysis were documented.

Latimer (1927) did not believe in the existence of real epiphyses in any of long bones of avian skeleton.[5] contary to his opinion, Franceschini (1967) considered that real epiphyses were found in the avian skeleton at the level of the tibia.[16]

Fusion time of tarsal ossification centers with tibia and metatarsals was identified as reliable and important criteria to assess the age of birds.[17]

The objective of present study is to investigate the ossification centers and ossification process of the pelvic limbs skeleton in partridge after hatching.

Materials and Methods

Fourteen partridge (Alectoris chukar) were kept under standard conditions related to temperature, nurishment, humidity and light. The temperature of the medium was adjusted to 35℃ in the first week, then at the end of each week the temperature decreased by 2℃. At the end of 7th week, the temperature was reduced to 21℃ and was kept constant until the end of study period.

Humidity was consistently 60% throughout the study period. Regarding the lighting program the 24 hours light was applied during the first week, subsequently it was decreased to 12 hours lighting and 12 hours darkness program.

The main diagnostic method in this study was digital radiography and Alizarin Red S staining was used to confirm the radiographic diagnosis. Digital radiographs were taken using portable X-ray machine Amadeo (P-90/20 VB) and 55 kVp, 0.6 mAs and 100cm FFD were adjusted. The digital imaging system consist of a flat–panel X-ray detector (Samsung: LLX240AB01).

Digital imaging and communication medicine (DICOM) software was used to process, display and save radiographic images.

Radiographs were taken of the birds on day one and then at the end of each week until the end of week 14.

At each stage of radiography two radiographs with ventrodorsal and lateral positions were taken from each bird. In the first day and then at the end of first, second, third and fourth weeks, each time a bird was randomly selected for Alizarin Red S staining and anesthetized with ether then fixed with 70% ethanol. Fixed specimens in ethanol were cleared with 1% KOH, then were stained with Alizarin Red S.[18] Finally stereomicroscope was used to observe and examine the leg skeleton.

Results

Femur

The body of femur was seen on radiographs from the first day. The proximal and distal extremities of the femur were cartilaginous in the first day and were not seen on radiographs. In Alizarin-stained specimen on day 28 the femoral head observed for the first time and was also seen on radiographs in the same day. On day 14, ossification occurred in the middle part of the femoral neck (Fig. 1).

Femoral neck was completely ossified on day 28. The femoral neck was observable on radiographs from that day on.

Greater trochanter was cartilaginous in Alizarin-stained specimens before day 28. On day 28, it was ossified and was also seen on radiographs of that day. On day 21, condyles were formed at the distal extremity of the femur, while they were cartilaginous, then on day 28, the femoral condyles were completely ossified as seen on radiographs.

Fibula

In the first day, the fibula was seen on radiographs as a delicate bone on the lateral side of the proximal end of tibia. In Alizarin-stained specimen in the same day, the proximal end of fibula was ossified, while the distal end was cartilaginous.

In the following days, the distal end of fibula became gradually ossified and increased in length (Fig. 2).

On day 28 it was completely ossified and seen on radiographs as a rod-like bone, tapers distally, alongeside the tibia.

Fig. 1. Venteral view of pelvic girdle. Ossification of the middle part of the femoral neck on day 14. Alizarin Red S staining.

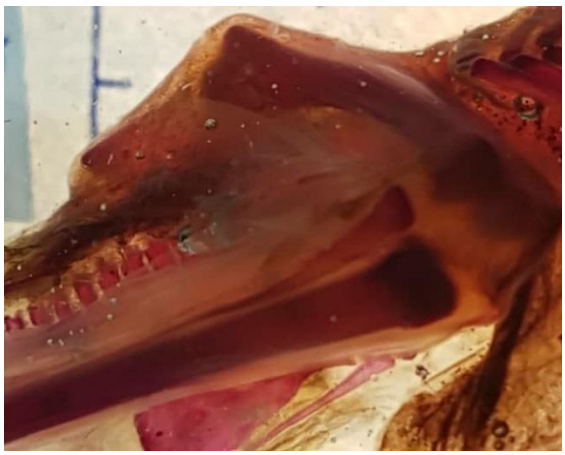

Fig. 2. Frontal view of the right tibia and fibula on day 7. Alizarin Red S staining.

Tibiotarsus (Tibia)

The body of the tibia was seen on radiographs in the first day and in Alizarin-stained specimen, the body of the tibia was ossified while proximal and distal ends were cartilaginous. At 8th day, tibial ossification was largely completed except at the proximal end. A ossification center was seen at the proximal end of the tibia on this day. It has been seen on radiographs since day 35 (Fig. 3). In the following days this center was expanded and on day 84, its fusion with the tibial epiphysis was seen on the radiographs. Tibiotarsus was formed by the fusion of the tibia with proximal tarsal bones.

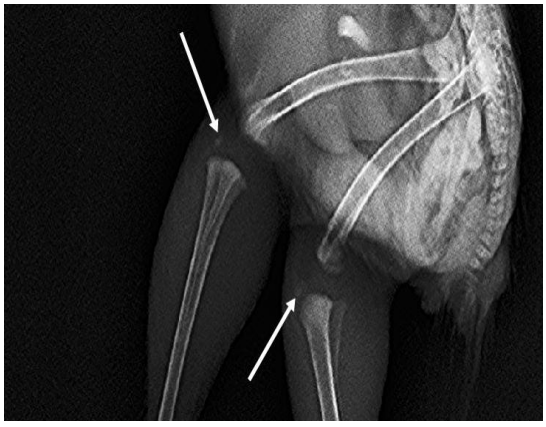

Fig. 3. Lateral view of stifle joint. Advent of the proximal tibial ossification center on day 35. Radiography.

Patella

The patella in birds is a small sesamoid bone in the anterior aspect of the stifle joint. On the 49th day, the patella was ossified and it has been seen on radiographs since that day (Fig. 4).

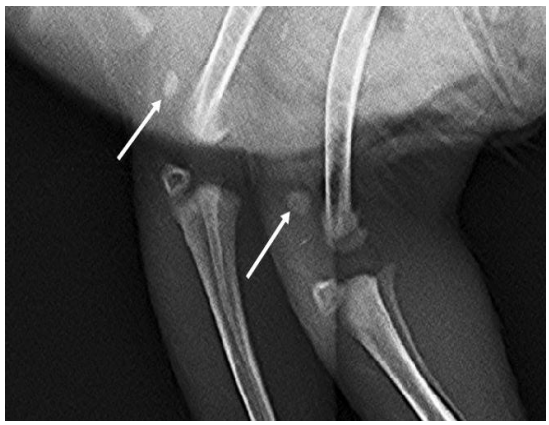

Fig. 4. Lateral view of stifle joint. Ossification of patella on day 49. Radiography.

Tarsal bones

In the first day, in the specimen stained with Alizarin, three ossification centers were observed in the tarsal region including two centers in the proximal row and one in the distal row (Fig. 5). These centers had grown in size on the 7th day and were seen on radiographs in the same day. On the 14th day, the two proximal tarsal bones were formed like tibial condyles (Fig. 6). On the 28th day, the two centers were the shape of the distal tibial epiphysis. In the same day the distal center was seen as a large bone in the vicinity of the proximal and of metatarsals (II, III, IV). Fusion between two proximal bones was seen on day 49 by radiography (Fig. 7). Finally proximal tarsal bones were fused to the tibia on day 63 and tibiotarsus was formed.

Fig. 5. Lateral view of intertarsal joint. Tarsal ossification centers on the first day. Alizarin Red S staining.

Fig. 6. Lateral view of intertarsal joint. Tarsal bones on day 14. Alizarin Red S staining.

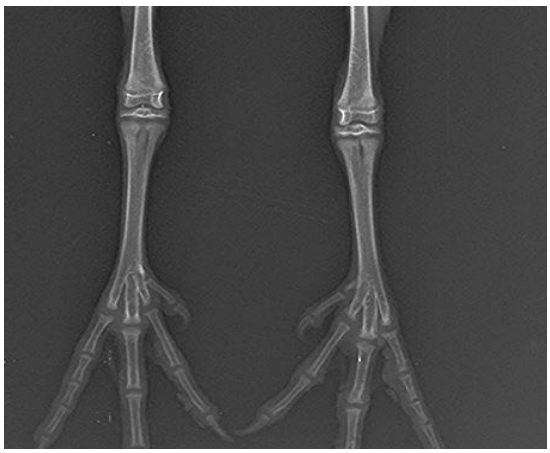

Fig. 7. Dorsoplantar view of intertarsal joint. Fusion between two proximal tarsal bones on day 49. Radiography.

Metatarsal bones (Tarsometatarsus)

The metatarsus bone was formed by the connection between the main metatarsals (II, III, IV), and was seen on radiographs from the first day. In the Alizarin-stained specimen of the first day, three main metatarsals (II, III, IV) were seen while they were fused together in the diaphysis, and at the distal extremity they were completely separated from each other. Proximal extremity of the main metatarsals were connected, which in this region the three bones were separable.

Tarsometatarsus was formed by fusion between the metatarsus and distal tarsal bone on day 77. The proximal extremity fusion of metatarsals was seen on radiographs in the same day (Fig. 8).

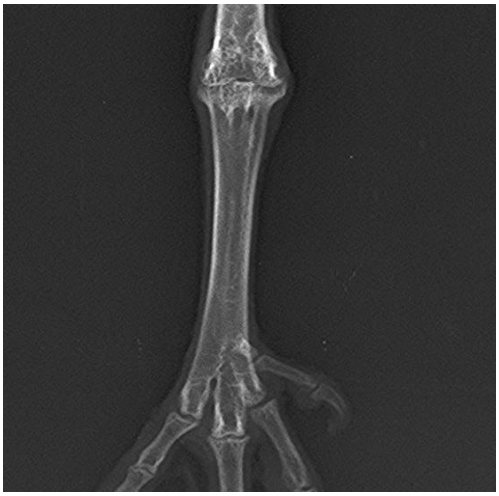

Fig. 8. Dorsoplantar view of intertarsal joint. Complete fusion between metatarsals in the body and proximal extremity on day 77. Radiography.

Metatarsal I

Metatarsal I was seen on radiographs from 7th day.

Digits

Phalanges of digits were seen on radiographs from 7th day. The phalanges were ossified from the first day as seen in the Alizarin-stained specimens.

In the first day, the apex of all the terminal phalanges was ossified, and their base was cartilaginous. In the rest of the phalanges of all digits, the body was ossified and their extremities were cartilaginous.

On day 14, the ossification of the phalanges was complete and the distance between phalanges was reduced.

Discussion

In present study, the ossification centers and skeletal development of the pelvic limbs of partridge were investigated at weekly intervals after hatching. There are not many studies related to the ossification centers after hatching in birds and most of these studiese have been done on chicken and quail. The information about the ossification centers of the pelvic limbs in chicken and quail after hatching is more complete than other birds. Therefore results of this study comared with obtained results of studies performed on these two birds.

The first day after hatching is the end of skeletal development during the emberyonic period. In fact, on this day, we saw the ossified parts of leg skeleton during emberyonic period. In this regard, Hogg (1980) observed an ossification center in the femur, tibia, fibula and main metatarsals in chicken at the time of hatching.[14]

Latimer (1927) reports the ossification time of the proximal and distal extremities of the femur simultaneously in a day which is consistent with our findings.[5]

In this study, we observed the head, greater trochanter and condyles of the femur on day 28, while yahyaei et al.[15] (2013) observed condyles and greater trochanter of the femur in quail from the 21st day after hatching. Then, on the 56th day after hatching, they observed the femoral head.

In general, ossification of the head, greater trochanter and condyles, as well as the thickness of articular cartilage, indicate the development extent of the femur.[4]

During the avian emberyonic period, the distal end of fibula becomes part of the distal end of tibia, then the fibula losses all of its distal part.[19,20] Lack of distal end of fibula is a unique property of avian leg skeleton development.[21] According to previous studies, the onset of patella ossification in chicken was from day 63,[14] and in the case of quail from day 30.[31]

In present study, as seen in previous studies, patella ossification did not occur until several weeks after hatching (day 49).

In many birds, the patella is a attached to the tibiotarsus by patellar ligament. While in some birds, the tibial crest extends proximally from the front of the stifle joint and in these birds there is no patella, and in others, there is a small patella with the tibial crest.[22] Ostrich is the only bird that has two proximal and distal patella.[23]

Few authors pointed to the presence of sesamoid bones in intertarsal joint of birds. Cause of these bones formation and leg tendons calcification is considered to stress forces on the leg tendons, especially at the landing time.[14,23] The appearance of an ossification center in the proximal tibial epiphysis has been reported in most bird species.[24, 25]

Pourlis et al. (2014) observed this center in quail from the 15th day after hatching.[13] Church reported that the onset of ossification proximal tibial center in chicken is at 35 days. He also announced that fusion time of this center with tibial diaphysis is in week 18.[4]

Some authors believed that this ossification center is a secondary ossification center.[4,13,16,26] Hanies (1940) claimed that secondary ossification centers in avian are found only in the proximal tibial epiphysis. He stated that the lack of secondary ossification centers in avian is due to the extensive pnumatization of long bones.[26] Hogg (1980) refuted this hypothesis, arguing that he did not accept extensive pnumatization of avian long bones.[14]

Carter et al. (1987) suggested that the reason for the low abundance of secondary ossification centers formation in birds is the lower density of epiphyseal cancellous bone in birds compare to mammalian cancellous bone.[27] regarding the ossification centers in the tarsal region at the time of hatching, in present study three ossification centers were observed including two in proximal row and one in the distal row.

Tarsal region has always been considered by many authors and there were different opinions about the development of this region.[3,16,17,29] The existence of these centers at the time of hatching in chicken has already been confirmed.[28-30] A number of authors have reported the onset of ossification of these centers in quail on the 16th day of the emberyonic period,[6,7,13] Which confirms our observation in the first day after hatching.

In current study the fusion occurred between two proximal tarsal bones at the 49th day after hatching. The time of occurrence of this fusion in chicken was reported on the 72nd day after hatching.[14,16]

According to various reports regarding the time of disappearance of the distal growth plate of the tibia in chicken,[3,4,16,17] it seems that sex and breed are two influential factors on the disappearance of these growth plates.[17]

The findings of this study regarding tarsometatarsus development is consistent with information provided by Namba et al.[31](2010)
By comparing the observations of the present study with findings of Pourlis et al.[13](2014) and Holder[19] (1983) it can be inferred that the phalangeal formula as well as the pattern of ossification of phalanges in partridge is the same as chicken and quail.
According to the available data, it seems that the completion time of ossification process of the pelvic limbs skeleton is 84th day after hatching (Fig. 9). On this day all the elements of the pelvic limbs were totally formed. From day 84 until the end of study (day 98) no changes were seen in the pelvic limbs skeleton. Therefore, it is recommended that this day be considered in future studies on the skeletal development of the pelvic limbs in partridge.

Fig. 9. Lateral view of leg skeleton. Completion of the pelvic limbs skeleton ossification on day 84. Radiography.

Comparison of the skeletal development of the pelvic limbs of three birds, including chicken, partridge and quail showed that the ossification centers appearance sequence in the pelvic limbs of all three birds is very similar.
In addition to, it was found that the pattern of ossification centers appearance in the pelvic limbs of all three birds does not follow a obvious proximal- distal sequence.
The time of ossification centers appearance was different among these birds, so that was earlier in quail and later in chicken. The another different is the time of growth plates closure and physical maturity, which are earlier in quail and later in chicken.

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