# Radiological and histological assessment of the ossification centers of pectoral limb in quail

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**Key words:**
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## Abstract:

**BACKGROUND:** The growth and differentiation of skeletal pectoral limb girdle, wing and the ossification centers in these regions after hatching were investigated in some groups of quails. **OBJECTIVES:** The aim of this study was to determine the age of physical maturity and radiological and histological assessment of the ossification centers of pectoral limb in quail. **METHODS:** 14 quails after hatching were reared in similar and standard conditions and sampled once every 7 to 90 days. **RESULTS:** According to radiological and histological results, differentiation of the wing in quail commences with the appearance of centers of undeveloped cartilages in diaphyseal humerus, radius, and ulna at the end of 7 days, and also carpal regions at the beginning of the 14 days. The growth sequence in humerus, radius, ulna, carpus, metacarpus, and digits are observed in various stages that the high growth is related to the maximum cartilaginous activity and their ossification stages and humerus keeps its growth connection constant with the length of the whole wing skeletal, although its growth scale lessens after the 21st day. The histological results were evaluated based on prepared tissue sample from the proximal humeral portion. Lack of bone marrow was observed in the all 1st day`s tissue samples and bone marrow conformation was commenced after 7th day. The growth plate was not observed in all the samples and this issue is complementary to the information obtained from radiographic examination. **CONCLUSIONS:** According to this study, time, which could be as the completion of the ossification process and the formation of all parts of the pectoral limb girdle and wing is 70 days after hatching.

## Introduction

Numerous studies have been done about the forming of the ossification centers in birds before and after hatching. There are studies about quail before hatching and embryonic period but there is no fundamental investigation on the time and place of the ossification centers after hatching while there is little information about the wing skeletal on quail (*Coturnix japonica*).

The anatomy of bird wings and the pat-

terns related to it is widely studied (Alexander 1983, Rubin and Lanyon 1984). There have been many attempts for the analysis of the factors involved in controlling the differentiation of it (Hamilton 1961, Rayner 1979, Livezey and Zusi 2007). Most of these studies are about domestic poultry (*Gallus domesticus*) (Sullivan 1962, Koch 1973). As there is a great deal of information about the development of skeleton in *Gallus domesticus*, we can consider them to compare with the results of this study on quail. Such a comparison between the two species which have been classified under various branches of Galliformes is important (Blom and Lilja 2004).

The development of long bones in poultry from the histogenesis process of all the wing skeletal parts to the differentiation of mesenchymal cells to chondroblasts and osteoblasts are described in considerable detail (Guedes, de Abreu Manso et al. 2014).

Lansdowne's study has surveyed the differentiation of mesenchymal cells to chondroblasts and osteoblasts and the development method of cartilage and bone structure in humerus and wing skeleton considering the age of the quail embryo (Lansdown 1969).

Hogg's study that was done on *Gallus domesticus* showed the time of appearance of ossification centers after hatching in different parts of the wing skeleton (Hogg 1980).

In this study the formation process of the ossification centers in the skeletal of pectoral limb girdle and wing quail after hatching with using radiography and histology tests were investigated in all quails during different days.

## Materials and Methods

This study was performed on quail (*Coturnix japonica*) after hatching. Fourteen quails (8 male and 6 female) with the age of 1 day to the end of investigation period were maintained in the same standard conditions such as diet, temperature, humidity (49%) and lighting (12:12). The assigned technique included processing the radiographic stereotype with normal radiography film. The radiography machine was Dean 44 X-Ray machine, KV 40-110 and mAs 0.1-200 and focal-film distance 100 cm.

For radiography of the specimens the lateral and VentroDorsal positions were used. Radiography was performed in the 1st and 7th days and then once at the end of the second, third, fourth, fifth, sixth, seventh, eighth, and ninth week and after the ninth week until the full maturity stage and completion of skeletogenesis, radiography of specimens was done every 14 days once. Subsequent radiography, curing periods of each cage a bird was selected randomly and was euthanatized by sodium pentobarbital and for histological analysis of the ossification centers it was transmitted to laboratory of the Veterinary Faculty of Science and Research Branch of Tehran.

Along with radiology, histopathology examination was spotted for further and accurate study in this issue wherein tissue specimens were prepared and rapidly fixed in neutral buffered formalin 10%. Thereafter, conventional paraffin wax embedding technique was performed in fixed specimens. Then, the sections were cut into 5 microns thickness and were stained by Hematoxyline and Eosin (H&E) and Periodic Acid Schiff (PAS) staining methods. Study of the ossification centers on the specimens continued until 90 days after hatching.

## Results

**The observation of ossification time in**

Table 1. The observation of ossification in the pectoral limb girdle in the radiology.

| Area | Days after hatching | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 84 | 77 | 70 | 63 | 56 | 49 | 42 | 35 | 28 | 21 | 14 | 7 | 1 |
| Scapula | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Clavicle | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Coracoid | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Sternum | + | + | + | + | + | + | + | + | + | + | + | - | - |

Table 2. The observation of ossification in the wing bones based on radiology.

| Area | Days after hatching | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 70 | 77 | 84 |
| Head of humerus | - | - | - | - | - | - | -+ | + | + | + | + | + | + |
| Dorsal tubercle of humerus | - | - | - | - | - | - | - | - | -+ | + | + | + | + |
| Venteral tubercle of humerus | - | - | - | - | - | - | - | -+ | -+ | + | + | + | + |
| Humerus | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Venteral condyle of humerus | - | - | - | - | - | - | - | -+ | -+ | -+ | + | + | + |
| Dorsal condyle of humerus | - | - | - | - | - | - | - | -+ | -+ | -+ | + | + | + |
| Radius | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Ulna | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Radial carpal bone | - | - | -+ | + | + | + | + | + | + | + | + | + | + |
| Ulnar carpal bone | - | - | -+ | + | + | + | + | + | + | + | + | + | + |
| Metacarpus II | - | - | - | - | -+ | -+ | -+ | + | + | + | + | + | + |
| Metacarpus III | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Metacarpus IV | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Proximal phalanx of digit II | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Distal phalanx of digit II | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Proximal phalanx of digit III | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Distal phalanx of digit III | - | + | + | + | + | + | + | + | + | + | + | + | + |
| Phalanx of digit IV | - | + | + | + | + | + | + | + | + | + | + | + | + |

**pectoral limb girdle and sternum (Scapula, Clavicle, Coracoids, Humerus):** In the first day after hatching these bones were not seen in any of the samples because they were cartilaginous and 7 days later were observed in all cases.

**Sternum:** It was not observed until the seventh day in all of the samples. After the 14th day, it was observed in all specimens. The beginning of ossification in the pectoral girdle is shown in Table 1.

**The observation of Ossification time in the wing bones (Head of humerus):** It was not observed in all of the specimens until the 35th day. It was observed at day 42 in more than 90% and after day 49 in all specimens.

**Dorsal tubercle of humerus:** In the all of specimens, ossification time was not observed until the 49th day. Instead, it was observed at day 56 in more than 90% of specimens and after the 56th day in all specimens.

**Ventral tubercle of humerus:** The ossification time was not seen in all of the specimens until the 42nd day wherein it was observed at day 49 in 80%, day 56 in 95% and after day 63 in all specimens.

**Humerus:** In the first day after hatching this bone was not observed in all of the samples because it was cartilaginous and was observed in all specimens after the 7th day.

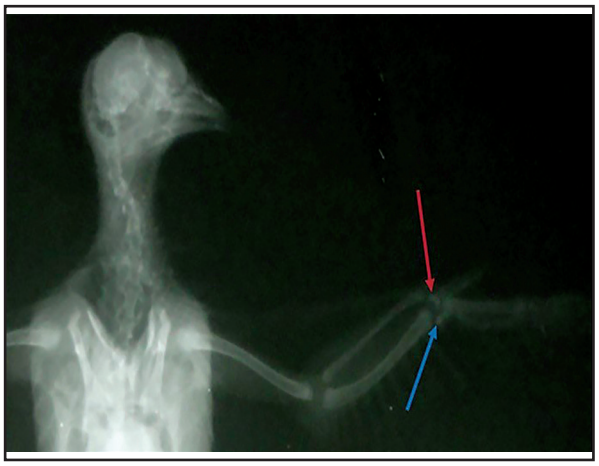

Figure 1. Radial carpal bone (Red arrow) and Ulnar carpal bone (Blue arrow) in 21st day.

Figure 2. Metacarpal Bone: Metacarpus II (Blue arrow), Metacarpus III (Red arrow), Metacarpus IV (Green arrow) in 21st day.

**Ventral condyle of humerus:** In all of the samples, the ventral condyle was not observed until the 42nd day in the humerus. The ossification time at day 49 was 60% and at day 56 was 85%.

**Dorsal condyle of humerus:** Until the 42nd day, the dorsal condyle of humerus was not clarified in all of the specimens. It was observed at day 49 in 60%, day 56 in 85%, the 63rd day in 95%, and after the 70th day in all of the samples.

**Radius:** The radius was not observed after hatching in the first day in the all of samples owing to it being cartilaginous wherein it was observed in all specimens after the 7th day.

**Ulna:** Until the 7th day of hatching, the ulna was not seen and afterward it was detected through radiology in all of the samples.

**Ulnar carpal bone:** The ulnar carpal bone was not observed until the 7th day in all of the samples. Thereafter, the forenamed bone was observed in more than 95% of samples and after the 21st day in 100% of the specimens (Fig. 1).

**Metacarpus II:** The metacapus II was not observed in the all of samples until the 21st day. It was observed at day 28 in 50%, at day 35 in 80%, at day 42 in 95%, and after the 49th day in all of the samples.

**Metacarpus III:** In the first day after hatching, the metacarpus III was not determined based on radiology in all of the specimens due to cartilaginous structure and it was observed in all specimens after the 7th day.

**Metacarpus IV:** In all specimens, metacarpus IV was not seen in the first day after hatching because it was cartilaginous and it was observed in all specimens after day7 (Fig. 2).

**Proximal and distal phalanx of digit II:** In the first day after hatching these bones were not observed in any of the samples because they were cartilaginous and after the 7th day they were observed as joined together, but after the 21st day they were separable.

**Proximal and distal phalanx of digit III:** The forenamed bones were not observed during the first day after hatching in all of the specimens owing to their structure which was cartilaginous, but after the 7th day they appeared as joined together. Finally, after day 21 they were recognizable.

**Phalanx of digit IV:** In the first day af-

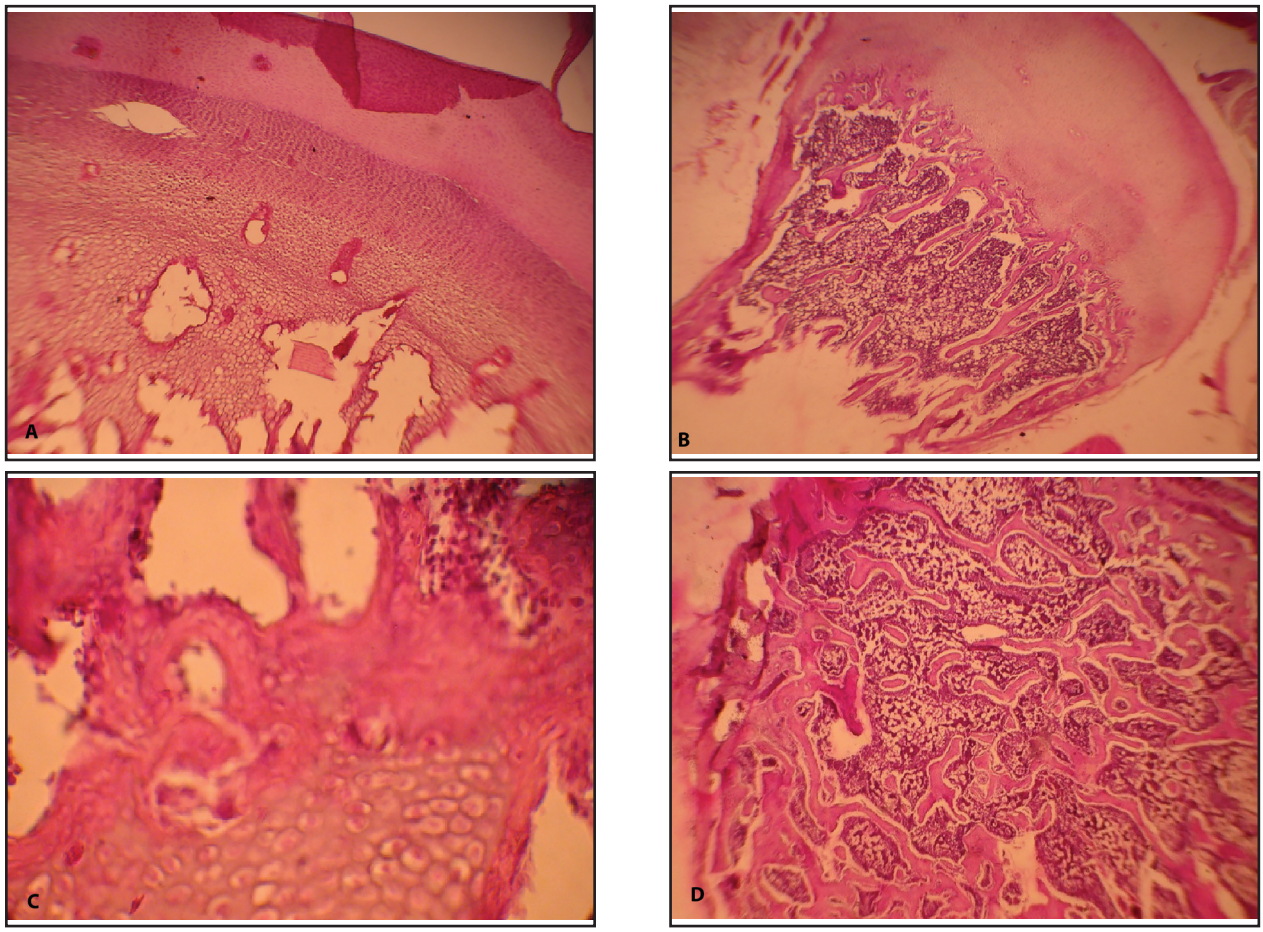


Figure 2. aFigure 3. The histological section of the proximal humerus. A. Conversion of cartilage into bone area. 21 d. B. Epiphyseal plate has been shown in this micrograph. 28 d. C. Conversion of cartilage into bone area. 42 d. D. Cancellous bone area. 56 d. (40×H&E).

ter hatching this bone was not observed in any specimen because it was cartilaginous and after day 7 it was observed as joined to proximal of digit III. After the 21st day, these two were separable. The ossification starting time of wing bones is shown in Table 2.

In the histopathology examination, the formation of bone marrow was not observed until the 7th day in all of the samples, but it was formed after day 7. Furthermore, the growth plate was not seen in all of the histopathologic samples and this issue is in accordance with radiographic examination. In addition, the bones lengthening seems to commence from epiphysis cartilage (Figs. 3, 4).

## Discussion

Several studies have been performed with respect to skeletal development in the birds such as *Gallus domesticus*, (Blom and Lilja 2004, Maxwell 2008) but there is not any published evidence regarding skeletal development in quail; hence, we try to compare the development of wing skeleton in this bird with *Gallus domesticus* in this study. The general formula in digit ossification in birds is 1:2:1.(Maxwell and Larsson 2009) But the formula observed in this study was usually 2:2:1 that was observed in further studies of past researchers about chicken (Seki, Kamiyama et al. 2012). It seems that only Bellairs et al (1960) uses

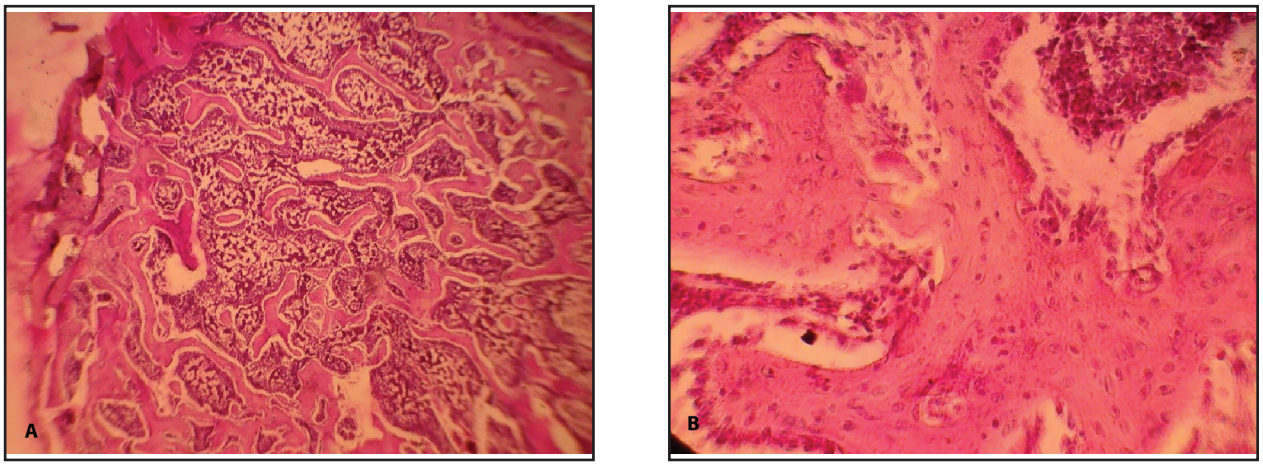


Figure 4. The histological sections of the proximal humerus. A. The cancellous bone area. B. There are a lot of active osteoblasts in this micrograph. 70 d. (40×H&E).

this formula specifically for *Gallus domesticus* and it was confirmed in few birds studied in this investigation (Bellairs and Jenkin 1960). On the other hand, the formula 2:3:2 is also discussed for *Gallus domesticus* that was not seen in the specimens of this study, although it may be mentioned for the inferior row of phalanges that exists as an extra and in the embryo but it is synthesized with the proximal row later.

For adult birds the formula 2:3:2 is also mentioned. Maybe the fuse time of the carpus and manus is after hatching which is in contrast with the findings of past researchers (Yasuda 2004). According to Schinz et al (1973) about 1 or 2 months after hatching the chicken metacarpus ossifies which is at the end of the 7$^{th}$ day for metacarpus III and IV and the end of the seventh week for metacarpus II in quail in all specimens (Mitgutsch, Wimmer et al. 2011).

It seems that less attention is paid to the development of the digits in birds, however, it can be said that the first center of ossification in digits area of wing skeleton in quail is about the end of the first week.

It is probable that the development of skeletal in distal portions of quail wing happens around the end of the first week with special mention of development in metacarpus, and digit area. Such a speed can have little result in the development of the carpus area.

The formation process of pectoral limb girdle and wing bones in quail (*Coturnix japonica*) and chicken have few differences but the growth pattern is similar in both. In this study, the ossification of the long bones of the wing was begun after the 7$^{th}$ day and the ossification was finished at the end of the 63rd day. The growth plate was not observed in the pectoral limb girdle and wing bones. According to this study it seems time, which could be as the completion of the ossification process and the formation of all parts of the pectoral limb girdle and wing is 70 days after hatching.

## Acknowledgments

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# ارزیابی رادیولوژی و بافت شناسی مراکز استخوان سازی اندام سینه‌ای در بلدرچین

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## چکیده

**زمینه مطالعه:** رشد و تمایز اسکلت کمربند سینه‌ای، بال و مراکز استخوان‌سازی این نواحی در زمان پس از هچینگ در بلدرچین مورد بررسی قرار گرفت. **هدف:** هدف از این مطالعه تعیین سن بلوغ جسمی و ارزیابی رادیولوژی و بافت‌شناسی مراکز استخوان‌سازی اندام سینه‌ای در بلدرچین بود. **روش کار:** ۱۴ بلدرچین متعاقب هچینگ در شرایط همسان و استاندارد پرورش یافتند و هر ۷ روز یکبار تا ۹۰ روزگی نمونه‌برداری انجام شد. **نتایج:** بر اساس نتایج رادیولوژی و بافت‌شناسی تمایز اسکلت بال در بلدرچین با ظاهر شدن مراکزی از غضروف‌های نابالغ در دیافیز استخوان بازو، رادیوس و اولنا در پایان ۷ روزگی و در نواحی کارپ از ۱۴روزگی به بعد آغاز گشت. توالی رشد در استخوان بازو، رادیوس، اولنا، کارپ، متاکارپ و انگشتان در طی مراحل مختلفی دیده شد و بیشترین رشد این استخوان‌ها به دوره‌های حداکثر فعالیت غضروفی و مراحل استخوانی شدن آنها مربوط بود و استخوان بازو، ارتباط رشد خود را با طول تمام بال و اسکلت بال ثابت نگاه می‌دارد، اگرچه از ۲۱ روزگی به بعد میزان رشد آن تقلیل یافت. نتایج بافت‌شناسی بر اساس بررسی مقاطع بافتی انتهای فوقانی استخوان بازو بود. نمونه‌های بافتی در یک روزگی فاقد مغز استخوان کامل بودند و مغز استخوان از ۷ روزگی به بعد شروع به تشکیل شدن می‌کند. در هیچ یک از نمونه‌ها صفحه رشد دیده نشد و این تکمیل کننده اطلاعات حاصل از بررسی رادیوگرافی می‌باشد. **نتیجه‌گیری نهایی:** بر اساس این مطالعه، تکمیل روند استخوان‌سازی و تشکیل همه قسمت‌های کمربند سینه‌ای و بال بلدرچین، ۷۰ روز بعد از هچینگ می‌باشد.

**واژه های کلیدی:** بافت شناسی، مراکز استخوان سازی، بلدرچین، رادیوگرافی، بال

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