IJAE
Vol. 120, n. 2: 127-139, 2015
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Research article - Embryology

Knee joint morphogenesis of the quail (*Coturnix japonica*) embryo

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Submitted December 12, 2014; accepted revised March 19, 2015

Abstract

Knee joint development and its morphogenetic events have been studied in human, chicken and other animal models and differences have been found in the pattern of the knee joint morphogenesis among the studied species. According to the small number of studies which have focused on the chronology of knee morphogenesis, a "morphogenetic timely pattern" is hard to suggest. Quail is an animal model for which there is no information about knee joint morphogenesis. This study was planned to define the time table of the knee joint structures formation in this bird. For this purpose embryonated Japanese quail eggs were incubated for 3 to 12 days. Embryos were removed from their eggs every twelve hours and staged according to Ainsworth et al. The hind limbs of the embryos at the stages 17 to 41 were dissected and 6 μm thick slides were prepared from their knee region. The time of appearance of menisci, ligaments, articular cavity and other knee joint components were identified in the quail embryo. During quail knee morphogenesis we observed the appearance of a three layered interzone, femorotibial cavitation and long bone ossification earlier than in chicken. A hypothesis is presented on the differential role of the flexor and extensor muscles of the knee joint on embryonic knee development in birds as compared with humans.

Key words

Knee, Joint Morphogenesis, Quail Embryology

Introduction

Joint morphogenesis is crucial in limb development and involves several coordinated processes. These processes have been mostly studied individually for bone and cartilage (Fell, 1925; Mitrovic, 1977; Pourlis et al., 1998; Bi et al., 1999; Hartmann and Tabin, 2000), muscles and tendons (Shellswell and Wolpert, 1977; Kardon, 1998; Benjamin and Ralphs, 2000; Schweitzer et al., 2001; Buckingham et al., 2003) and joint capsule (Mitrovic, 1978; Nalin et al., 1995; Bland and Ashhurst, 1997). Developmental events produce, in a precise timely sequence, the "morphogenetic timely pattern" of the joint. In the literature differences were found in this pattern for the knee joint of the studied species (Gardner and 0'Rahilly, 1968; Clark et al., 1983; Bland and Ashhurst, 1997; Merida- Velasco et al., 1997b; Ito and Kida, 2000; Ratajczak, 2000; Roddy et al., 2009). Given the small number of studies which focused chronologically on the knee morphogenetic events, a "morphogenetic timely pattern" is hard to be pro-

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http://www.fupress.com/ijae
DOI: 10.13128/IJAE-17800

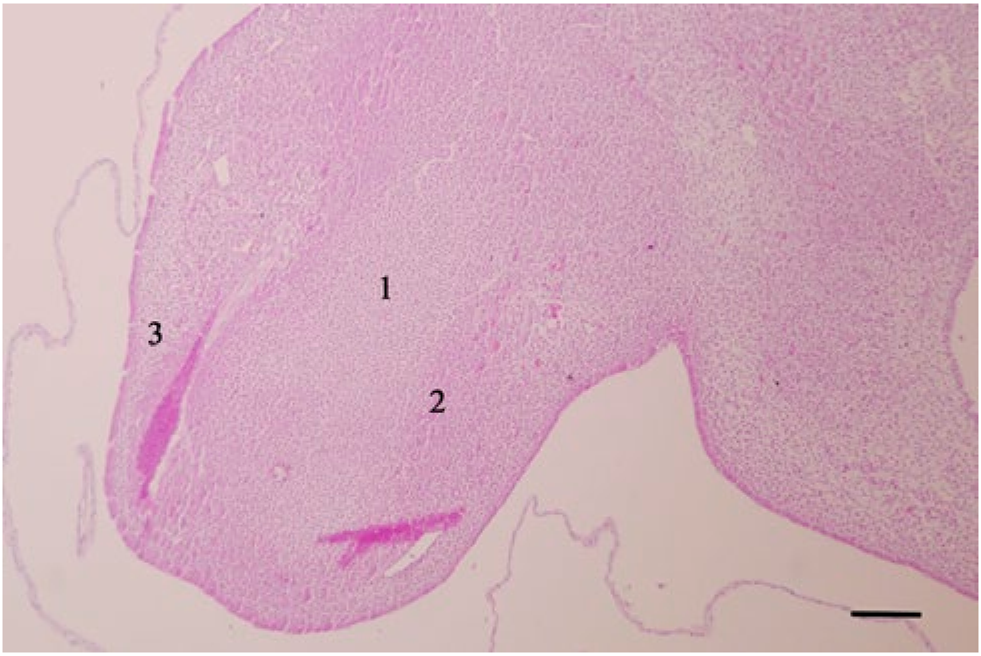

Fig. 1 – Hind limb bud at stage 20, scale bar: 100 microns. 1- Central cells of mesenchymal condensation of the femur, 2- Pripheral cells of mesenchymal condensation of the Femur, 3- None condensed mesenchyme.

posed. Quail is an animal model on which many developmental studies are carried out. According to different incubation periods of the Japanese quail and the chick embryo, it is interesting to know if morphogenesis of the knee joint of these two birds will commence and progress through corresponding stages of incubation (Hamburger and Hamilton, 1951; Ainsworth et al., 2009). Knee morphogenesis has been studied in the chicken (Roddy et al., 2009), but there is no information about the period and the sequences of its development in the quail. This study was planned to define the time table of the knee joint structures formation in this bird.

Material and Methods

Embryonated Japanese quail (*Coturnix japonica*) eggs were incubated at 37.3 ± 0.2ºC with 70% humidity for 3 to 12 days. Embryos were removed from eggs every twelve hours and fixed in 10% formalin. 24 hours later the formalin was changed and after four days of fixation the embryos were staged according to Ainsworth et al. (2009).

The hind limb of the embryos at the stages 19 to 41 (at least three specimens per stage) were macroscopically studied and then dissected for tissue processing. Paraffin blocks were prepared from the knee region and 6 μm thick sections were obtained with a MR2258 microtome (HistoLine, Pantigliate, Italy). The slides were stained with hematoxylin-eosin and analyzed by light microscopy.

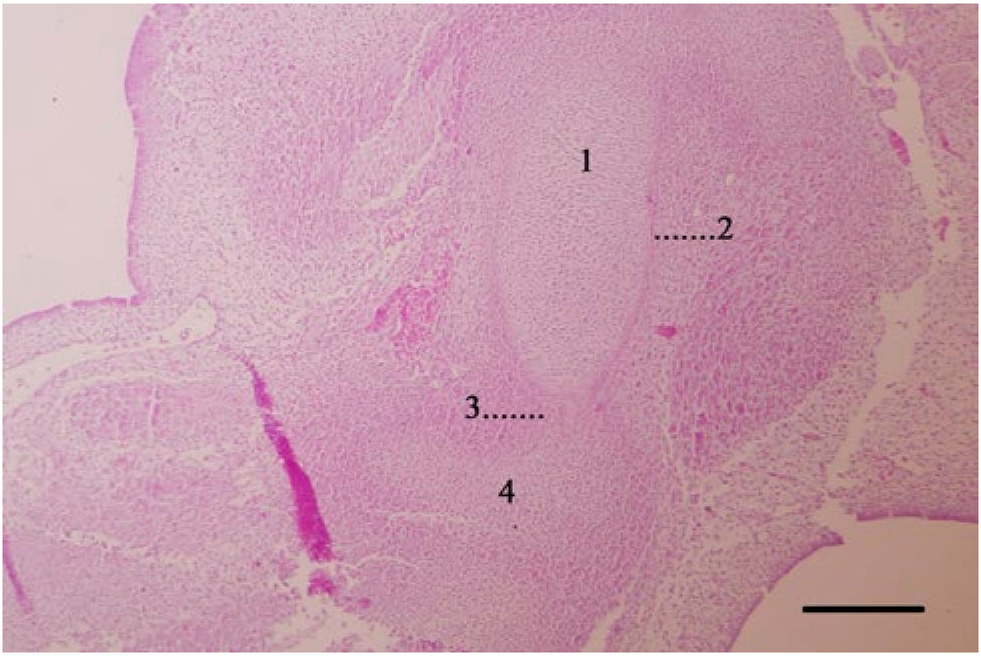

Fig. 2 – Knee joint at stage 23, scale bar: 100 microns. 1 and 2- Central and peripheral cells of the mesenchymal condensation of the Femur, 3- Knee interzone, 4- mesenchymal condensation of the Tibia.

Results

Stage 19

Microscopically, the limb bud consisted of two types of cells; outer simple cuboidal to columnar and inner undifferentiated mesenchymal cells.

Stage 20

Mesenchymal condensation of the femur was seen. It consisted of two types of cells: a compact layer of peripheral, elongated cells and central, transversely arranged medullary cells. Blood vessels and nerve fibers reached close to the mesenchymal condensation (Fig. 1).

Stage 23

Hind limb bud grew and its distal extremity leaned caudally. Knee undifferentiated mesenchyme linked femur and tibia mesenchymal condensations together (Fig. 2). In the middle of condensations, central cells were larger and clearer than those in the two extremities. Peripheral elongated cells were seen in several rows. In sagittal sections, muscle primordia were identified on both sides of femur. Blood vessels and nerve fibers were found more developed around condensations.

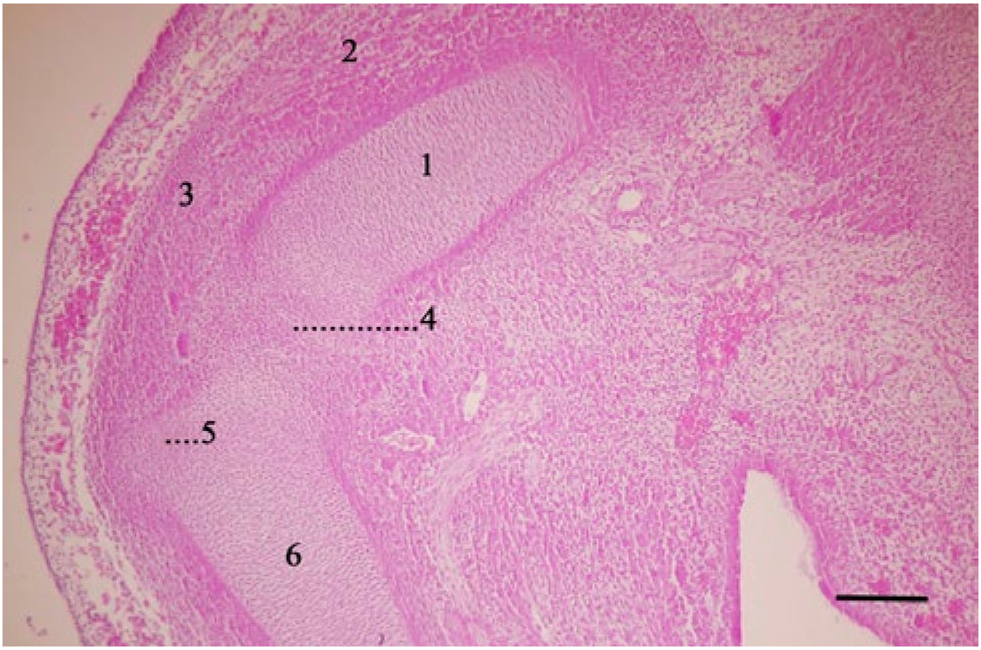

Fig. 3 – Knee joint at stage 25, scale bar: 100 microns. 1- Femur, 2- Quadriceps muscle precursor, 3- Patella precursor, 4- Knee interzone, 5- Cnemial crest precursor, 6- Tibia.

Stage 25

In the limb bud, the knee joint area was macroscopically detectable. The cells central to bone primordia were: (A) undifferentiated mesenchymal cells in the epiphysis; (B) elongated mesenchymal cells perpendicular to the longitudinal axis of the diaphysis, near each epiphysis, and (C) a small number of cartilage cells in the middle of the diaphysis. Peripheral cells were seen more elongated and condensed than before. Patella precursor cells were seen as an unclear aggregation attached to the quadriceps muscle primordium (Fig. 3). Knee interzone contained cells similar to central cells of earlier stages. Mesenchymal cells were shaping the cnemial crest on the anterior side of the proximal tibial epiphysis.

Stage 27

Articular capsule was seen as a cell condensation at the posterior side of the knee. Chondroblasts were seen in the middle of the shaft of long bone primordia. Calcium deposition was seen under the perichondrium of the femur and tibia primordia. Medial meniscus and cranial cruciate ligament primordia were seen as mesenchymal condensations.

Fig. – 4 Knee joint at stage 30, scale bar: 100 microns. 1- Quadriceps muscle precursor, 2- Patella precursor, 3- Femoropatellar joint cavity, 4- Femur, 5- Muscle groups, 6- Femoromeniscal joint cavity, 7- Medial meniscus, 8- Meniscotibial joint cavity, 9- Cnemial crest, 10- Tibia.

Stage 30

All three kinds of cells seen at stage 25 in femur, tibia and fibula primordia were still present, but in the middle of the body of these bones chondroblasts had changed into quite large chondrocytes trapped within their lacunae in the cartilage matrix. Distal to the patellar condensation, infrapatellar fat pad was forming. Quadriceps tendon had crossed over the patellar surface to become the patellar ligament. Embryonic perichondrium appeared in the diaphysis around hyaline cartilage. Knee interzone differentiated into three distinct layers: two marginal layers, precursors of the articular cartilage, and a central layer which started to contain cavities and to form intra-articular structures. The lateral parts of menisci and, in some sections, their laminae were seen respectively as triangular and linear cell masses. Medial meniscofemoral and meniscotibial cavities were also seen, but the respective lateral cavities were yet unclear. Muscle groups were quite distinct (Fig. 4).

Stage 32

The femur was covered by a bilayered perichondrium. The inner layer consisted of chondroprogenitor cells and the outer one was fibrous. Blood vessel had extended towards both epiphyses. Ambiens tendon primordium was seen crossing over patella. Cells and fibers were still observed in the synovial sheath around this tendon. The articular cavity was better defined and the articular cartilage had its final form. The

Fig. 5 – Knee joint at stage 34, scale bar: 100 microns. 1, Patella, 2- Infrapatellar tissue, 3- Femoropatellar joint cavity, 4- Femur, 5- Thick part of the medial meniscus, 6- Tibia.

cranial cruciate ligament was more obvious than at previous stage. Precursor of the caudal cruciate ligament appeared as a stripy mesenchymal condensation. The cell density reduced between the patella and femur primordia and some spaces between cells of this area were seen indicating the onset of articular cavity formation. Cnemial crest mesenchymal cells, compared with the previous stage, were more homogeneous to cells of tibial epiphysis. Blood vessels began to penetrate into the body of the tibia and the related periosteal band (bony collar) was formed.

Stage 34

In tibia and femur diaphyses, blood cells were seen between chondrocytes. In the periosteal band region, the two layers of perichondrium were completely split. Patellar condensation was distinguished from infrapatellar tissue, but the latter was not demarcated from the anterior extension of menisci (Fig. 5). Femoropatellar joint cavity was identified, but still contained a relatively large number of mesenchymal cells. Femorotibial joint cavity was more mature, but there were still scattered cells inside it. Menisci and their corresponding spaces were seen more clearly than before. Cnemial crest was still mesenchymal but even more homogeneous to tibial epiphysis.

Stage 35

In long bones, bone marrow and bone trabeculae were observed. Cartilaginous epiphysial canals were seen. With the evolution of the joint cavity, articular capsule

Fig. 6 Knee joint at stage 36, scale bar: 100 microns. 1- Quadriceps muscle, 2- Femur, 3- Patella, 4- Ambiens tendon and its synovial sheath, 5- Patellar ligament, 6- Caudal cruciate ligament, 7- Cranial cruciate ligament, 8- Medial meniscus and its attachment to the femur, 9- Tibia.

reached its final form. Meniscotibial space was seen as a thin slit. Femoropatellar and cruciate ligaments were seen more clearly. Ligaments connecting the fibula to the tibia and femur were observed. Cnemial crest cells density was similar to that of tibia.

Stage 36

The primary ossification center was extending toward the ends of the long bones. In the distal femural and proximal tibial epiphyses, epiphysial canals had developed. Patellar primordium was clearly distinguished from surrounding tissues. Its central mesenchymal cells were bigger and more distant from each other, while the surrounding cells had smaller size and higher density. Synovial sheath of ambiens tendon was not completely clear of cell debris. Femoromeniscal, meniscotibial and femoropatellar articular cavities were respectively fairly clear, with few cells and rich of cells (Fig. 6). Cruciate ligaments and menisci were observed more clearly. Tibial and femural chondroblasts reached close to epiphyses. In long bones epiphysis, from surface to depth three types of mesenchymal cells were observed: most superficial, quite dense cells that were articular cartilage precursors; deeper, less dense cells among which blood vessels entered; and the deepest, least dense layer, with the largest cells.

Stage 37

Bone formation in the diaphysis developed toward epiphysis. Osteoblasts were attached to calcified trabeculae. In the middle of the shaft, trabeculae were thicker

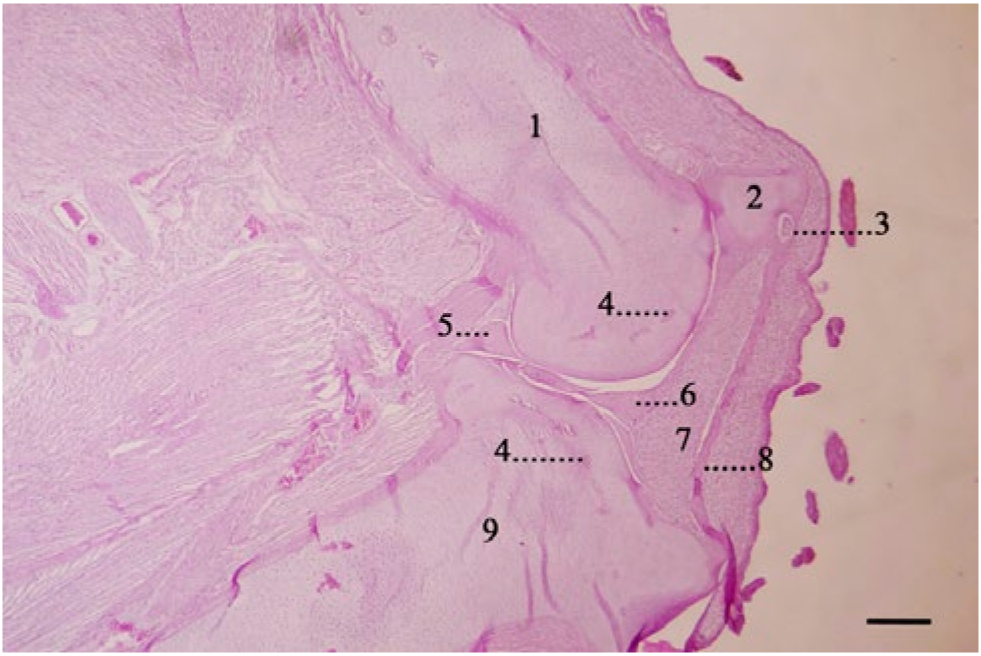

Fig. 7 Knee joint at stage 39, scale bar: 100 microns. 1- Femur, 2- Patella, 3- Ambiens tendon and its synovial sheath, 4- Epiphysial canals, 5 and 6- Caudal and cranial hornes of the medial meniscus, 7- Infrapatellar fat pad, 8- Patellar ligament, 9- Tibia.

and contained many osteocytes. Osteoclasts were seen in some areas. A periosteal band developed on both epiphyses. Epiphyseal blood vessels increased compared to the previous stage and got closer to each other. Medial meniscus was quite distinct, but scattered cells were still observed in the meniscotibial articular cavity. The menisci were lined by synovial layer observed clearly. Patella was more developed but its central and peripheral cells still were different for size and density.

Stage 39

Ossification developed and meniscotibial joint cavity was completely clean. Femoropatellar cavity still contained scattered cells. The central large mesenchymal cells of the patella extended towards periphery. The synovial sheath surrounding ambiens tendon still included some mesenchymal cells (Fig. 7).

Stage 40

Chondrification started in femural and tibial epiphysis, so a mesenchymal growth plate was identified between the epiphysis and diaphysis of long bones.

Table 1 – Major events of knee morphogenesis related to Ainsworth et al. (2010) stages.

Stage	Major morphogenetic events
19	Limb bud with two cell types
20	Appearance of the mesenchymal condensations of femur
25	Appearance of the mesenchymal condensations of patella, cnemial crest and knee interzone
27	Identification of the joint capsule, appearance of chondroblasts in the diaphysis of long bones, identification of the medial meniscus and cranial cruciate ligament
30	Appearance of chondrocytes in the diaphysis of long bones, interzone differentiation into 3 layers, appearance of the lateral meniscus, cavitation of the femorotibial joint, penetration of blood vessels into the femur
32	Onset of cavitation of the femoropatellar joint, identification of the caudal cruciate ligament, penetration of blood vessels into the tibia and fibula
35	Appearance of epiphyseal canals
37	Appearance of the synovial layer
39	Completing of the femorotibial joint cavity
40	Identification of the mesenchymal growth plate
41	Beginning of ossification process of the patella

Stage 41

Cartilage cells continued to replace mesenchymal ones in the long bone primordia. Blood vessels entered the patella from its apex. Fat pad cells with their vacuoles became larger. The synovial sheath of ambiens tendon was cell free. The knee joint components and its articular cavities were fully formed.

Synopsis

The morphogenetic events of the quail knee joint development are outlined in table 1.

Discussion

The first morphological sign of knee joint development is the appearance of the interzone. The interzone is a mesenchymal tissue which through morphogenetic processes will lead to the formation of structures such as ligaments and menisci. It also will rise to the joint cavity with physical separation of the future skeletal elements (Archer et al., 2003; Khan et al., 2007). In this study the knee interzone appeared as a mesenchymal tissue three stages earlier than that reported for the chicken embryo, where this happens at stage 28 (Roddy et al., 2009). So, if the morphogenetic processes of the knee would be the same in these two species, it may be predicted that other differentiation steps of the interzone in the quail should be reached earlier than in the chick embryo. In the quail embryo, indeed, we observed a three layered interzone, femorotibial cavitation, joint capsule formation and beginning of long bone ossification 2, 4, 3 and 2 stages, respectively, earlier than in the chick embryo (Roddy et al., 2009). More detailed information of the exact time and sequential morphogenesis of the internal structures of the chick embryo knee have not been presented by Roddy et al. (2009) to compare with present results. In human knee joint morphogenesis some of these morphogenetic events take place with a different sequential pattern (Walker, 1991; Merida-Velasco et al., 1997a; Gilanpour et al., 2000; Fukazawa et al., 2009). In the present study, mesenchymal condensation of the patella was observed at a same stage as knee interzone formation. In the human fetus formation of the patellar condensation has been reported at a later stage, after the formation of menisci and ligaments (Walker, 1991; Merida-Velasco et al., 1997a). In the human fetus, also, formation of the lateral meniscus has been observed earlier than that of the medial one (Fukazawa et al., 2009) opposite to the chicken (Gilanpour et al., 2000) and Japanese quail (this study). The same occurs for the sequential formation of the cranial and caudal cruciate ligaments (Merida-Velasco et al., 1997a; Shojaei et al., 2000).

Whether morphogenesis of a structure affects its final shape is under controversy. Fukasawa et al. (2009) have stated that the earlier morphogenesis of the lateral meniscus than the medial one in human knee may predispose it to discoid meniscus anomaly, but the results of the present and Gilanpour's et al. (2000) studies show that in birds the formation of the lateral meniscus takes place after the medial one, yet also in these species the lateral meniscus is discoid and the medial one is crescent-like (Nickel et al., 1977; Shojaei and Jalalinejad, 2012).

In avian embryo, movement is important for joint formation. Association between the emergence of joint shape, cell proliferation and embryonic muscle contraction has been described (Roddy et al., 2011a). On the other hand, muscle paralysis may cause failure of joint cavitation and change in the patterning of internal structures of the knee interzone (Fell and Canti, 1934; Drachman and Sokoloff, 1966; Murray and Drachman, 1969; Persson, 1983; Osborne et al., 2002; Roddy et al., 2011b). It has been reported that early limb movements (about 54 days) may contribute to joint cavitation also in human embryos (Walker, 1991). We could not find any report dealing with differential contribution of flexor and extensor muscle contraction in knee morphogenesis. As it is possible that patella, cranial cruciate ligament and tibial tuberosity formation is related to knee extensor contraction; the formation of these structures occurs earlier in the studied birds (Shojaei et al., 2000; Roddy et al., 2009; present study) than in humans (Clark et al., 1983; Merida-Velasco et al., 1997a), which may reveal earlier or more effective contraction of the knee extensors compare to flexors in birds. In this regard, the cnemial crest of the turkey, partridge and quail in comparison to its mammalian counterpart, tibial tuberosity, contributes more to the formation of tibial articular surface than tibial tuberosity in mammals (unpublished data). It may be proposed that, compared with mammals, the knee extensors exert more strength than flexors during the embryonic period of mentioned birds.

Acknowledgment

This research was financially supported by the Research Council of Shahid Bahonar University of Kerman.

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