Histomorphogenesis of the Chukar Partridge (Alectoris Chukar) Knee Joint

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ABSTRACT

Introduction: There are differences in the pattern of the knee joint formation of the studied species. According to the small number of studies which focused chronologically on the avian knee histomorphogenetic events, its "morphogenetic pattern" is hard to be proposed. The present work was designed to identify the sequential events of the knee formation in the partridge embryo. Regarding to different incubation periods of the partridge and the chick embryos, it is interesting to know if sequential morphogenetic events of the knee joint formation of these two avian models will happen through corresponding days of incubation.

Methods: In this study partridge (AlectorisChukar) embryos were used from day 5 to 23 of incubation. 5 μm thick sections were obtained sagittally and frontally from the knee region. The slides were stained with Hematoxylin-Eosin (HE), green Trichrome Masson (TM) and Alsian Blue–Van Gieson's (ABV) methods and studied by light microscope.

Results: In the knee development of partridge we determined three layered interzone formation, joint cavitation and other morphogenetic events of the knee structures of the partridge.

Conclusion: According to comparison of present results and other similar studies, it seems that birds and mammals follow different timely and sequential pattern of the knee formation.

Key Words:

Embryology, Knee, Joint, Galliformes, Histology

1. Introduction

There are differences in the pattern of the knee joint formation of the studied species [1-6]. If these differences have been randomly happened in mentioned species or relate to the differences of birds and mammals, need to know the "morphogenetic timely pattern" of these two classes. According to the small number of studies which focused chronologically on the avian knee histomorphogenetic events, this pattern is hard to be proposed. So the present work was designed to identify the sequential events of the knee formation in the partridge embryo. Regarding to different incubation periods of the partridge and the chick embryos, it is interesting to know if sequential morphogenetic events of the knee joint formation of these two avian models will happen through corresponding days of incubation [7].

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Developmental events of the involved structures in the knee formation, such as bones and cartilages [8-11], joint capsule [2, 12, 13] and muscles and tendons [14-17] have been studied precisely in different species. During the embryonic period, these events occur sequentially and interact on each other to produce a complex and precise process of joint morphogenesis. In the avian models, knee morphogenesis has been studied in the chicken [6] and quail (in press) but there is no information about the period and the sequences of its development in the partridge. This study aims to describe the knee formation stages in this bird.

2. Materials and Methods

Embryonated partridge (Alectoris Chukar) eggs were incubated at 37.5±0.2ºC with 56% humidity. From day 5 every 24 hours, embryos were removed from eggs and fixed in bouins' solution. The hind limbs of the embryos, at least three specimens per day, were dissected for tissue processing from day 5 to 23. Paraffin blocks were prepared from the knee region and 5 µm thick sections were obtained sagittally and frontally with a MR2258 microtome (HistoLine, Pantigliate, Italy). The slides were stained with Hematoxylin-Eosin (HE), green Trichrome Masson (TM) and Alsian Blue–Van Gieson's (ABV) methods and studied by light microscope. All the slides of different days which were processed for TM and ABV methods preserved and stained synchronously all together.

3. Results

Day 5

At the beginning, the limb bud was lined by simple cuboidal to columnar cells covering a mass of inner undifferentiated mesenchymal cells.

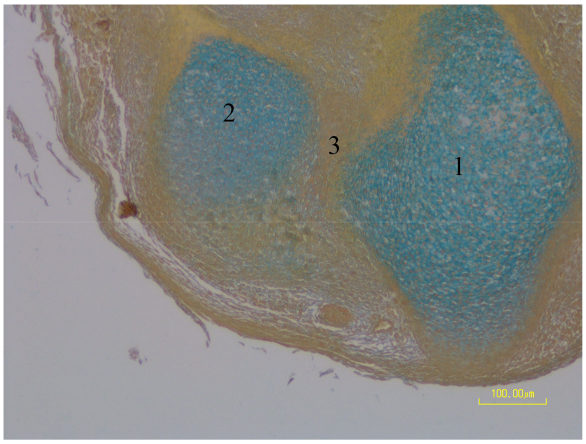

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Figure 1. Knee joint of the 8 days partridge embryo, ABV staining, 1. Tibia, 2. Femur, 3. Articular mesenchyme.

Day 6

Mesenchymal cells condensed to shape the femur and tibia precursors. They consisted of two types of cells: compact layer of peripheral, elongated cells and central, transversely arranged cells. Undifferentiated mesenchyme of the knee linked mesenchymal condensations of the femur and tibia.

Day 8

Mesenchymal condensations were consisted of larger and clearer cells in their medullary region. Muscle primordia were identified on both sides of femur. Blood vessels and nerve fibers reached close to the mesenchymal condensations. Cartilaginous matrix was seen in the epiphyses of both femur and tibia which was confirmed in the Alcian Blue staining (Figure 1).

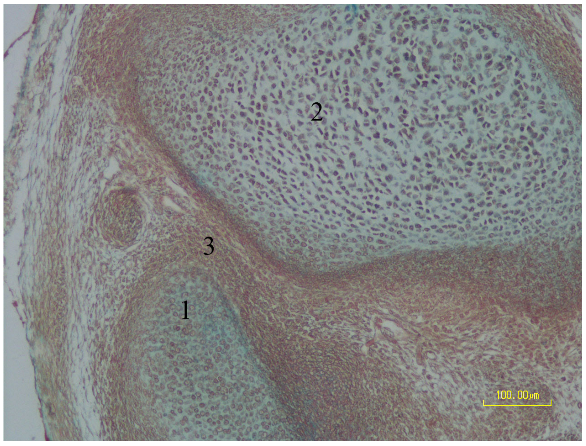

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Figure 2. Knee joint of the 10 days partridge embryo, TM staining, 1. Femur, 2. Tibia, 3. Three layered interzone.

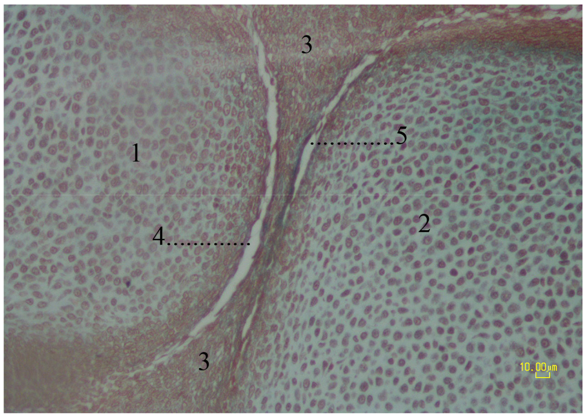

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Figure 3. Knee joint of the 12 days partridge embryo, TM staining, 1. Femur, 2. Tibia, 3. Medial meniscus, 4. Femoromeniscal and 5. Meniscotibial articular cavities.

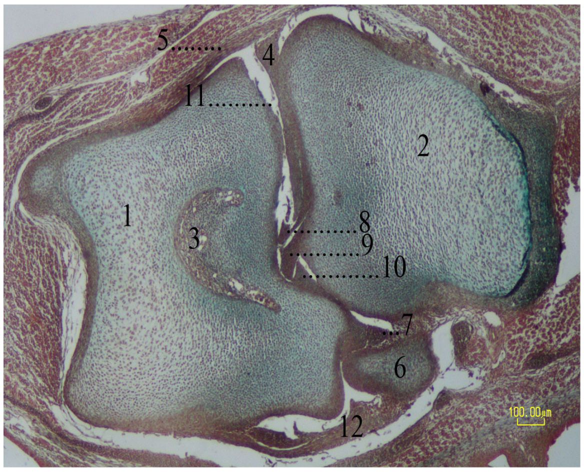

Figure 4. Knee joint of the 13 days partridge embryo, TM staining, 1. Femur, 2. Tibia, 3. Epiphyseal vessels, 4. Medial meniscus, 5. Medial collateral ligament, 6. Fibula, 7. Tibiofibular ligament, 8. Caudal cruciate ligament, 9. Cranial cruciate ligament, 10. Lateral meniscus, 11. Femoromeniscal articular cavity, 12. Lateral collateral ligament.

Day 10

In this day the knee joint was grossly visible in the limb bud. The interzone of the knee included some isolate unoccupied spaces. Mesenchymal cells were shaping the cnemial crest on the anterior side of the proximal tibial epiphysis. The knee interzone began to differentiate into three distinct layers: two compact marginal layers, precursors of the articular cartilage, and a vacuolated central layer with aggregated precursor cells of intra articular structures. Patella precursor was seen as an unclear aggregation attached to the quadriceps muscle primordium (Figure 2).

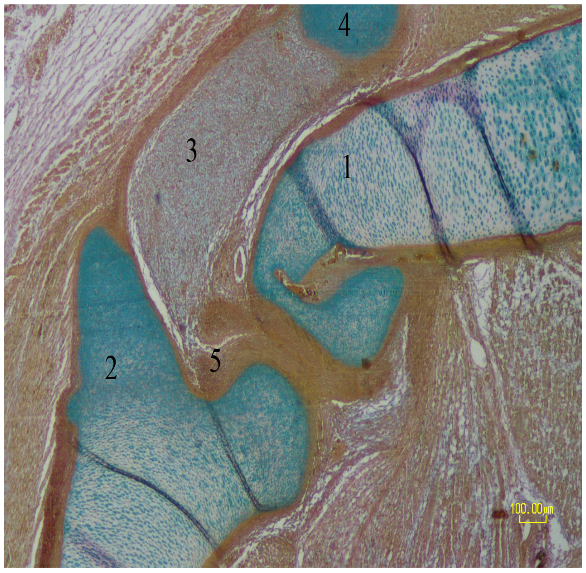

Figure 6. Knee joint of the 15 days partridge embryo, ABV staining, 1. Femur, 2. Tibia, 3. Infrapatellar fat pad, 4. Patella, 5. Cranial cruciate ligament.

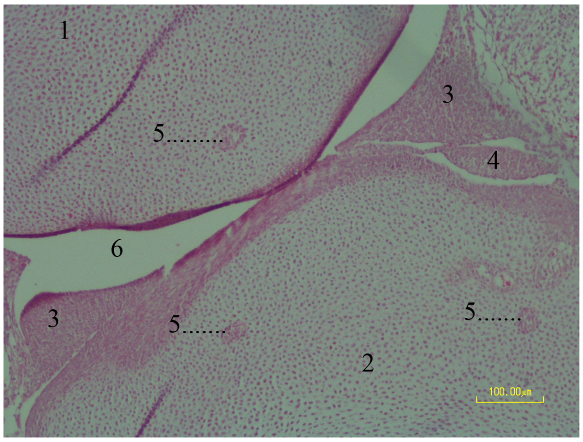

Figure 5. Knee joint of the 14 days partridge embryo, HE staining, 1. Femur, 2. Tibia, 3. Medial meniscus, 4. Meniscal ligament, 5. Epiphyseal vessels, 6. Joint cavity.

Day 11

Patella precursor was seen more clearly. At its distal end, patellar ligament precursor extended to the cnemial crest.

Day 12

At the posterior side of the knee, cell condensation of the articular capsule was seen. At the medial side, medial meniscofemoral and meniscotibial cavities were formed, but the respective lateral cavities were not clear yet. The Trichrome Masson stained slides showed the formation of the medial meniscus with few fine collagen fibers. As seen in the Alcian Blue staining, chondirfication progressed at the bones epiphysis (Figure 3).

Day 13

Ligaments primordia of tibiofibular, lateral and medial collaterals and cranial and caudal cruciates were seen clearly as mesenchymal structures. Lateral meniscus was shaped at the lateral side of the knee interzone. Epiphysial vessels were seen at the deep sections of the bones epiphysis (Figure 4).

Day 14

In the distal femural and proximal tibial epiphyses, epiphysial canals were seen. The concentration of the blue color was decreased in AB slides as the canals began to form. The content of the collagen fibers of the ligaments and menisci increased compare to the day 12 (Figure 5).

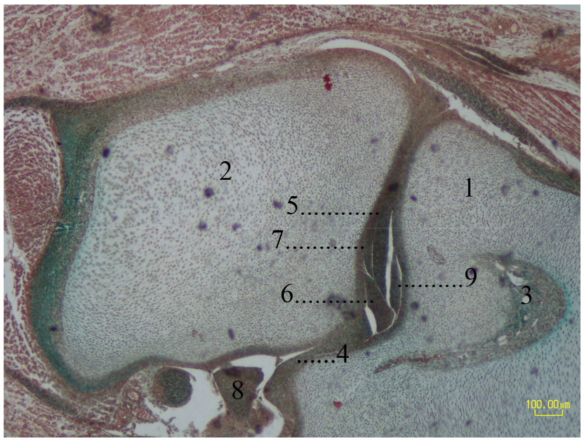

Figure 7. Knee joint of the 16 days partridge embryo, TM staining, 1. Femur, 2. Tibia, 3. Epiphyseal vessels, 4. Lateral meniscus, 5. Medial meniscus, 6. Caudal cruciate ligament, 7. Meniscal ligament, 8. Fibula, 9. Cranial cruciate ligament.

Day 15

Differential concentration of the blue stain showed that histogenesis of the patella and cnemial crest did not progress as much as other bony elements. Cartilaginous patella and infrapatellar fat pad were clearly distinguishable from all other adjacent tissues. Cellular trabeculae still existed in the femoropatellar articular cavity (Figure 6).

Day 16

The knee structures had been formed completely but a few cellular debris were still seen in the articular cavities. The green stain due to collagen fibers of the ligaments and menisci increased in the Trichrome Masson staining compare to the day 14 (Figure 7).

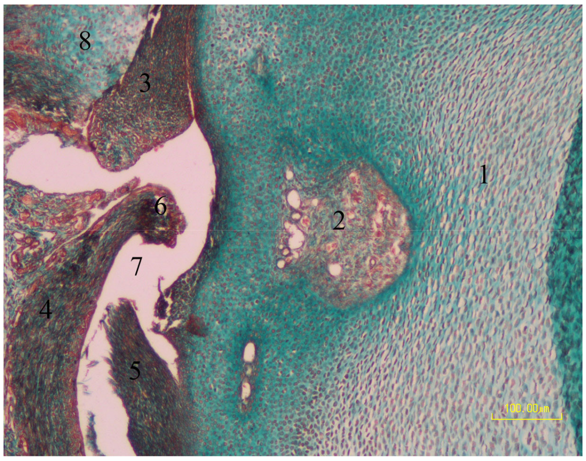

Figure 9. Knee joint of the 18 days partridge embryo, TM staining, 1. Femur, 2. Epiphyseal vessels, 3. Medial meniscus, 4. Lateral meniscus, 5. Cranial cruciate ligament, 6. Caudal cruciate ligament, 7. Knee joint cavity, 8. Tibia.

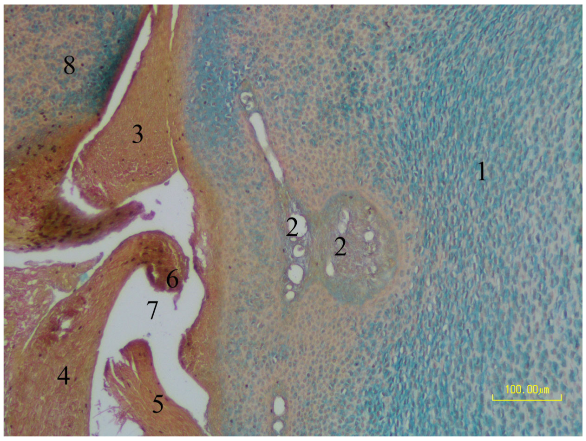

Figure 8. Knee joint of the 18 days partridge embryo, ABV staining, 1. Femur, 2. Epiphyseal vessels, 3. Medial meniscus, 4. Lateral meniscus, 5. Cranial cruciate ligament, 6. Caudal cruciate ligament, 7. Knee joint cavity, 8. Tibia.

Days 17 to 23

Histogenesis of the menisci, ligaments and bones was in progress. Cartilaginous matrix and collagen fibers concentration decreased and increased respectively to the end of incubation period around the spreading epiphysial canals. The histogenesis of the patella and cnemial crest (collagen production and dechondrification) progressed with a delay of 2.5 to 3 days compared to the femur and tibia (Figures 8 to 10).

4. Discussion

Interzone formation is the first histomorphologic event in the knee joint development. The mesenchymal tissue between bony elements of the knee which is named interzone, builds the structures such as ligaments and menisci through the histomorphogenetic changes. It also rises to the joint cavity with physical separation of the future skeletal elements [18, 19]. In this study the mesenchymal tissue of the interzone appeared about two days later than that reported for the chicken embryo, where this happens at day 5.5 [6]. So, if the morphogenetic processes of the knee would be the same in these two species, it may be predictable that other differentiation steps of the interzone in the partridge must be later in time in comparison with that of the chick embryo. In the partridge embryo, we observed a three layered interzone, femorotibial cavitation, and beginning of cnemial crest and patella formation 2.5, 2, 2 and 2 days, respectively, later than in the chick embryo [6].

More detailed information of the exact time and sequential morphogenesis of the internal structures of the chick embryo

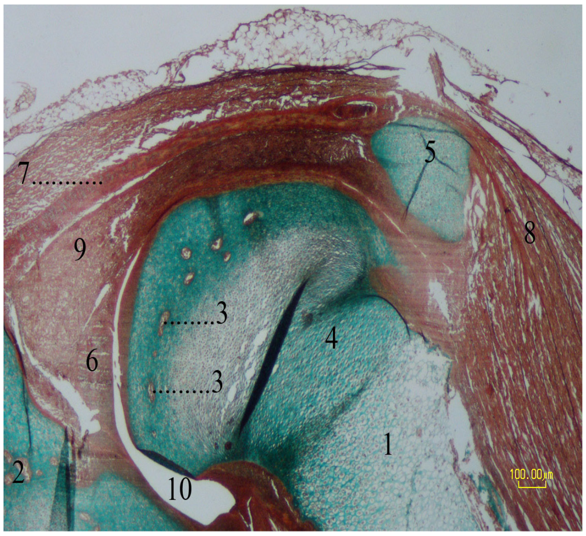

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Figure 10. Knee joint of the 20 days partridge embryo, TM staining, 1. Femur, 2. Tibia, 3. Epiphyseal vessels, 4. Growth plate, 5. Patella, 6. Medial meniscus, 7. Patellar ligament, 8. Quadriceps femoris, 9. Infrapatellar fat pad, 10. Knee joint cavity.

knee were not reported by Roddy et al. [6] to compare with present results, but according to other studies [20-23], regardless of difference in the incubation period, sequential events of the knee formation show similar pattern in studied avian species. The human knee joint morphogenesis follows a different pattern compared to the mentioned avian models [21, 24-26].

In the present study, mesenchymal condensation of the patella was observed at the same day as three layered 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 [24, 25]. In the human fetus, also, formation of the lateral meniscus has been observed earlier than that of the medial one [26] opposite to the chicken [21] and partridge (this study). The same thing occurs for the sequential formation of the cranial and caudal cruciate ligaments [20, 25]. The differences may be the results of different contribution of the flexor and extensor muscles of the knee in these two vertebrate classes [27, 28] or other factors are being involved, which need to be studied more. Comparison of the results of the present study and other similar studies shows that the birds and mammals follow different timely pattern of the knee formation.

Knowing a certain morphogenetic process in various classes of vertebrates and discussing its differences and similarities between them, can lead to introduce evolutionary patterns which help to describe the reason of the normal events and also anomalies [29]. Fukasawa et al. (2009) stated that the earlier morphogenesis of the lateral meniscus than the medial one in human knee may predispose it to discoid meniscus anomaly [26], but the results of the present and Gilanpour's et al. (2000) [21] studies show that in birds the formation of the lateral meniscus takes place after the medial one. It is interesting that in these species the lateral meniscus is discoid and the medial one is crescent-like [30]. It seems that the knowledge of the normal differences of "sequential morphogenetic pattern" in different species, can lead us to better detection of the etiology of abnormalities.

Acknowledgment

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