Liver Histogenesis in Chukar Partridge (Alectoris Chukar) Embryo

Shadi Hashemnia [1*], Bahador Shojaei [1], Hamed Razavi [1]

1. Department of Basic Science, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Shadi hashemnia is assistant professor of veterinary histology and embryology. She teaches histology at Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran. Her research interests are histochemistry and histomorphogenesis.

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ABSTRACT

Introduction: In chick embryo, endodermal component of the liver becomes split into 2 diverticula in a short time. The anterior diverticulum lies to the left of the ductus venosus and the posterior one to its right side. As the liver primordium grows, it is invaded by the embryonic veins. Based on the different incubation time periods and evolutionary origin of avian species, various times for morphogenetic events of the liver are expected. The chukar partridge (Alectoris chukar) belongs to a common family with the chicken and shows 3 to 4 days longer embryonic period. This study was designed to describe normal histological and histochemical events of the liver histogenesis of the chukar partridge. It would be interesting to see how the time and sequence of developmental events of liver histogenesis are similar to those of the chick embryo.

Methods: In this study, partridge (Alectoris chukar) embryos were studied from day 5 to 24 of incubation. In the course of the study, their liver 5-µm thick sagittal and transverse sections were prepared. Then, the sections were stained with hematoxylin-eosin (H&E) and Periodic Acid Schiff (PAS) methods and studied under the light microscope.

Results: During the histogenetic study of the chukar partridge liver, we noted the structures and events such as the dark and light hepatocytes, mitotic divisions, sinusoids and kupffer cells, as well as the glycogen storage in the hepatocytes and their development, also changes due to the time of incubation period.

Conclusion: Although the sequential events of the liver histogenesis of chukar partridge follow the general pattern of the other birds, some differences exist in the time of formation liver components compared to other studied species.

Key Words:
Embryology, Liver, Galliformes, Histology

*** Corresponding Author:**
Shadi Hashemnia, PhD
Address: *Department of Basic Science, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
Tel: *+98 (913) 399 2738* ***Fax:*** *+98 (34) 33257447*
E-mail: *shadihashemnia@uk.ac.ir* *shadihashemnia@yahoo.com*

1. Introduction

The first morphological sign of the embryonic liver is the formation of the hepatic diverticulum, which outgrows from the thickened foregut epithelium. The anterior portion of the hepatic diverticulum gives rise to the liver and intrahepatic biliary tree, while the posterior portion forms the gall bladder and extrahepatic bile ducts [1]. It derives from different populations of medial and lateral endoderm that converge during foregut morphogenesis [2, 3]. Generally, the onset of differentiation of the endoderm coincides with its interaction with mesenchyme [4]. Then, they grow into the septum transversum where they interact with the surrounding mesenchyme to form other cellular components of the liver, including connective tissue, endothelial cells, kupffer cells, and the blood-forming cells characteristic of the embryonic and fetal liver.

The relatively large endodermal cells differentiate into hepatocytes and arranged in rods with intervening sinusoids [5]. In chick embryo, endodermal component of the liver splits into 2 diverticula by stage 17 [6]. The anterior diverticulum lies to the left of the ductus venosus and the posterior one to its right side [7]. As the liver primordium grows, it comes into contact with the body wall and is invaded by the embryonic veins [8].

The histogenesis of the liver has been studied in mammals by several researchers [9-11]. In the avian species, some studies have also conducted on the liver developmental aspects [12-20] whose differences in their length of incubation period and evolutionary origin of avian species result in various time and sequence of the liver morphogenetic events. Chukar partridge (Alectoris chukar) belongs to a common family with the chicken and shows 3 to 4 days longer embryonic period. This study was designed to study normal histological and histochemical events of the liver histogenesis of chukar partridge. It would be interesting to see how the time and sequence of developmental events of liver histogenesis are similar to those of chick embryo.

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, at least 3 eggs were examined to check the embryos vitality by opening a window in the egg shell and direct observation of the blood circulation and or embryo movement. The examined embryos were killed by freezing at -20°C (5- to 14-day-old) or decapitation (15- to 24-day-old) and fixed in Bouin's solution. The liver of at least 3 embryos per day was used for tissue processing from day 5 to 24. Paraffin blocks were prepared from the specimens and 5-µm thick sections were obtained with a MR2258 microtome (Histo-Line, Pantigliate, Italy). The slides were stained with hematoxylin-eosin (H&E) and Periodic Acid Schiff (PAS) (Merck, KGaA, 64271, Darmstadt, Germany) methods and studied under light microscope.

3. Results

Day 5 of incubation

The liver was covered by a single layer of cuboidal cells called mesothelium. Its parenchyma contained numerous large and irregular spaces with immature blood cells. Hepatocytes formed cord and acinus arrangements. Two types of hepatocytes existed, the light cells, which were seen mostly in the peripheral area of the liver and the dark ones in the center. Hepatocytes were in contact with the bile canaliculi and sinusoids through their apex and base, respectively. Their nuclei were large and round with 1 or 2 distinct visible nucleoli. Numerous cells in different mitotic division stages and binuclear cells, especially in the peripheral zone were seen (Figure 1).

Day 6 of incubation

The liver bud had occupied the right half of the abdominal cavity, ventral to the nephric system and in the right side of the gut. The mesothelium was still composed of simple cuboidal epithelium. Two groups of hepatocyte arrangement were observed in this day; acini arrangements with pyramidal cells and cord arrangements with cuboidal or columnar hepatocytes. The mitotic figures, especially in the peripheral cells were seen. The sinusoids were large and irregular with small amount of immature blood cells (Figure 2).

Day 7 of incubation

The mesothelium was almost composed of simple squamous cells, but simple cuboidal cells were still partially seen. Mitotic division was visible in a large number of hepatocytes. The cord arrangement of the hepatocytes was more than the acinar formation. The number of immature blood cells in the large sinusoids had increased (Figure 3).

Day 8 of incubation

All the cuboidal mesothelial cells had turned into simple squamous form. They had formed the Glisson capsule with the thin underlying connective tissue. The hepatocytes had grown and both light and dark cells were visible. Sinusoids had become narrower compared to the previous day and the endothelial and kupffer cell nuclei were identifiable in their walls. The number of immature blood cells had increased (Figure 4).

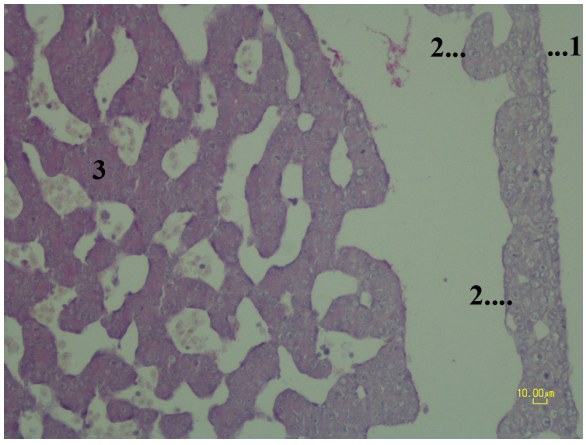

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Figure 1. Liver on day 5. PAS staining, 1- Mesothelium 2- Light cells with mitotic figures, 3-Dark cells.

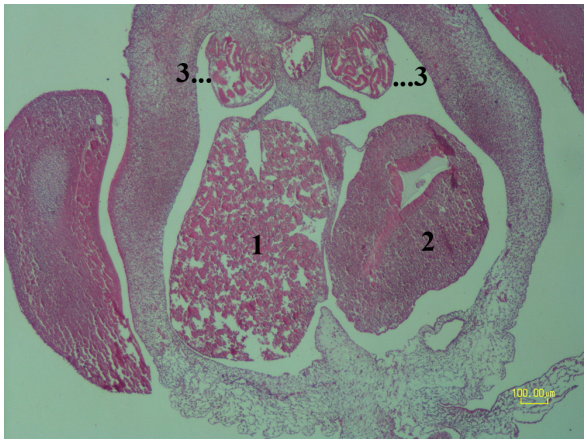

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Figure 2. Liver on day 6. H&E staining, 1- Liver, 2- Gut, 3- Nephric system.

Day 9 of incubation

No difference was seen in comparison to the previous day. Mitotic figures were identifiable in the hepatocytes. The PAS reaction was still negative.

Day 10 of incubation

The light cells were limited to the periphery of the liver. The dark cells were in the center with a large round nucleus and visible nucleoli and acidophilic cytoplasm. The sinusoids were seen narrower with endothelial and some kupffer cells in their wall as well as immature blood cells and a small amount of mature blood cells among them. A very weak PAS positive reaction as small red granules was identifiable in the cytoplasm of some central cells.

Day 11 of incubation

The liver parenchyma was generally composed of dark cells but small amount of light cells were seen in the periphery. There was an increase in the amount of mature red blood cells in the sinusoids. Bile canaliculi were visible between the hepatic cords. The PAS reaction was the same as the previous day.

Day 12 of incubation

The amount of both light cells and visible mitotic divisions had greatly decreased in the peripheral area. Blood vessels, including large veins were visible in the parenchyma. The hepatocytes had arranged as 2-row hepatic cords around a central vein. There was an increase in the number of sinusoids but their size had shrunk. Both mature and immature red blood cells were identifiable in the sinusoids and vessels. Bile canaliculi were more obvious between the hepatocytes and had secretions. The PAS reaction, similar to previous day, was detectable as few small red granules in sporadic cells (Figure 5).

Day 13 of incubation

The kupffer cells had increased and more mature red blood cells were present in the sinusoids and vessels. Strong PAS positive reaction, which did not have uniform distribution in the liver, was seen for the first time in the cytoplasm of some hepatocytes (Figure 6).

Day 14 of incubation

The number of immature blood cells had decreased. The PAS reaction was not as strong as the previous day. Small bile ducts with simple cuboidal cholangiocytes were seen on this day for the first time (Figure 7).

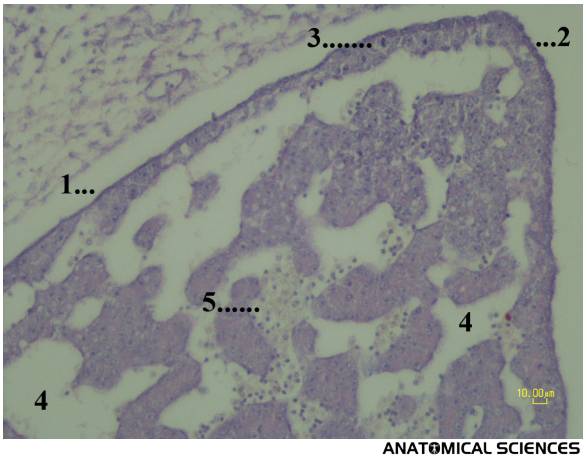

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Figure 3. Liver on day 7. PAS staining, 1- Mesothelium with simple squamous cells, 2- Mesothelium with simple cuboidal cells, 3- A dividing hepatocyte, 4- Sinusoids, 5- Immature blood cells.

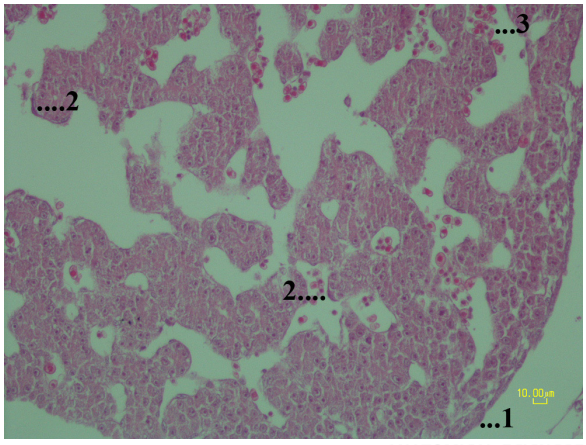

Figure 4. Liver on day 8. H&E staining. 1- Glisson capsule, 2-Kupffer cells 3-Immature red blood cells.

Day 15 of incubation

The liver parenchyma comprised of large and dark hepatocytes, central veins, and lots of narrow sinusoids. A few immature blood cells were still visible. The secretion in the bile canaliculus was seen more clearly. The PAS reaction was the same as before.

Day 16 of incubation

The liver parenchyma was more compact and hepatocytes were arranged more regular around the central vein. There was a decrease in the number of immature blood cells and an increase in the central veins. The portal triad was seen more clearly in this day.

Day 17 of incubation

Immature blood cells were seen very rarely at this time. The PAS reaction was observed in more hepatocytes than the previous days.

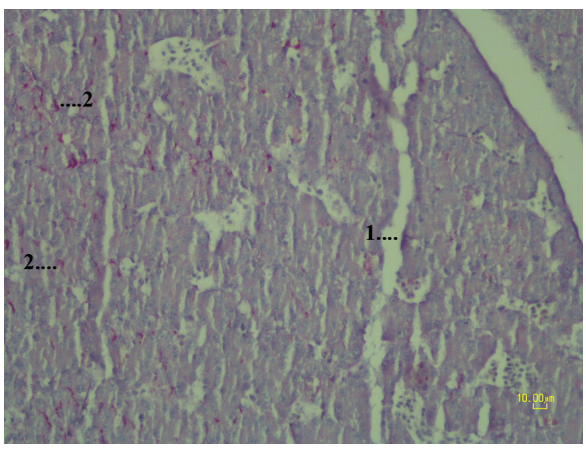

Figure 6. Liver on day 13. PAS staining, 1-Kupffer cell, 2-PAS positive red granules.

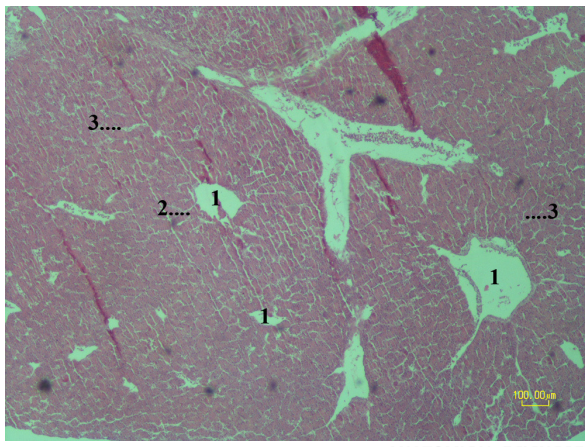

Figure 5. Liver on day 12. H&E staining. 1- Central vein, 2-Hepatocytes in 2-row arrangement, 3-Narrow sinusoids.

Day 18 of incubation

At this day, almost all blood cells were mature. A strong PAS positive reaction was present in most hepatocytes in a way that the peripheral cells had small red granules and the central hepatocytes consisted of larger and more granules. Other structures were similar to those of the previous day.

Day 19 of incubation

Hepatocytes were big and well developed so the sinusoids were hardly observed among the hepatic cords. Bulk of mature blood cells was visible inside the vessels.

Day 20 of incubation

The number of large blood vessels with wide lumen had decreased and small veins with narrow lumen in transverse and longitudinal sections were present. Other observations were similar to previous day.

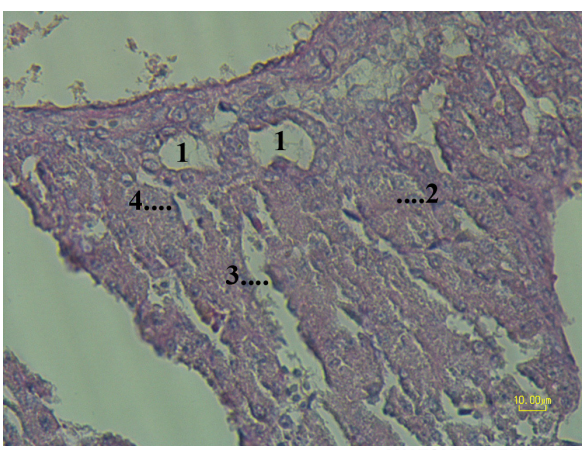

Figure 7. Liver on day 14. PAS staining, 1- Bile ducts, 2- PAS positive reaction, 3- Kupffer cell 3- Endothelial cell.

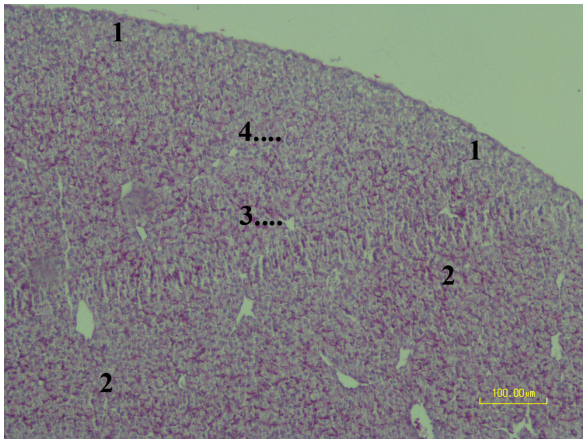

Figure 8. Liver on day 21. PAS staining, 1- Peripheral thin strip of cells with moderate PAS positive reaction, 2- Strong PAS positive central hepatocytes, 3- Central vein, 4- Narrow sinusoid.

Day 21 of incubation

A peripheral thin strip of hepatocytes showed moderate PAS reaction while the rest of hepatocytes were strongly positive with numerous dark red granules. These granules were seen all over the cytoplasm but their density was much more in the apical and basal part which were adjacent to the bile canaliculi and sinusoids, respectively. Sinusoids were much narrower compared to previous days (Figure 8).

Day 22 of incubation

There was no new histogenetic event in the liver from this day to the end of incubation period and all the observations were the same as previous day.

4. Discussion

The avian liver had been the subject of histogenetic studies and most of them focused on the chicken embryo. Wong and Cavey (1993) studied the liver development in the chick embryo with light and electron microscope. They observed that the liver appeared on stage 30 of HH (Hamburger and Hamilton) and its development completed almost by stage 40 or day 14 [16]. The histogenetic process of Dandarawi chicken liver was also investigated. In that study, the specimens were collected on the odd days of incubation period and histomorphometric observations were reported according to light and electron microscopy [20].

Fukuda-Taira (1981) examined the chronological changes in the hepatic induction potency of the 'cardiac' mesoderm [14]. It is unclear whether the proximity or duration of contact with the cardiac mesoderm controls the molecular factors needed for hepatic induction [21]. The present work was conducted to study the liver histogenesis of chukar partridge (Alectoris chukar) for the first time to compare it with a few studied avian species. In order to find the minor histogenetic changes, daily collection of the embryos were done and the developmental characters of the liver, according to the incubation period and 2 staining methods, were studied. The liver bud was detected in the right half of the abdominal cavity, ventral to the nephric system and fully developed at the 18th day of incubation.

In this study, liver parenchyma consisted of two types of hepatocytes: Light cells and dark ones. At first there were numerous light cells, especially in the peripheral part and dark cells were almost in the center. On day 7, there was an increase in the light cells number. On the 11th day of incubation, the light cells were completely limited to the peripheral part of the liver and from day 12, they decreased in number and finally disappeared on the following days.

These findings were similar to the results of the liver histogenesis of Dandarawi chicken. In that research, light cells were observed on days 5 and 7 as clumps at the periphery of the liver, while large groups of dark cells were present toward the center. Then, the light cells decreased in number from day 9 to 13 and finally disappeared. They reported this observation as an indication of differentiation of dark cells from light ones and stated that further research on these cells must be conducted [20]. In fact, there have been several controversial ideas on the light and dark parenchymal cells during liver development.

Ganote and Moses (1968) stated that the light cells may be just an artifact resulting from fixation by the immersion method [22]. Other researchers have argued that these 2 cell types occur naturally in the liver and injury or pathogens can alter their proportions [23-25]; however, Wong and Cavey (1993) declared that dark and light cells are seen throughout hepatic development, and the former always outnumber the latter [16]. It has also been suggested that light and dark cells represent different stages of hydration [24] and dark cytoplasm is an indicator of imminent cell death [25]. The ultrastructural studies of these cells in the avian liver showed that the light cells have a paler cytoplasmic matrix, which contains little or no granular but defined smooth ER [16, 26, 27]. The presence of smooth ER in the light cells and rough ER in the dark cells indicates different functions of these 2 cells [16, 28].

Mitotic divisions, which were visible in the liver parenchyma of chukar partridge from the first day of study, were seen with great abundance on day 7. From day 12 of incubation, the number of visible cells with mitotic division reduced. Similarly, Doaa et al. (2013) reported that clear mitotic divisions are more concentrated in the peripheral part of the liver than in the central one. They described it as an indication of the progressive growth of liver at day 7 as liver growth depends on cell proliferation and concluded that the slower mitotic division occurred from day 9 to the end of incubation period that may afford more time for cell growth [20].

The hepatoblasts are bipotential cells; those deeper in the parenchyma become hepatocytes, whereas those residing next to the portal vein mesenchyme are induced to form biliary epithelial cells [21]. The formation of bile canaliculi has been seen at day 6 of incubation in chick embryo [12, 20]. In spite of longer incubation period of chukar partridge compared to chicken, in the present work, bile canaliculi appeared earlier. They were seen more clearly on days 11 and 12 when they had secretions. These secretions increased on day 15 which was similar to Dandarawi chicken [20]. Difference in the appearance of bile structures was also seen in the small bile ducts. These structures with their simple cuboidal epithelium were first seen on day 14 of incubation in chukar partridge. Two days later, portal area was identifiable for the first time while both of these observations took place in one day (day 15) in Dandarawi chicken liver [20].

The PAS reaction was negative in this study, until day 10 when few small red granules were observed in the cytoplasm. This slight PAS positive reaction indicated the initiation of glycogen storage in the hepatocytes. On day 13, a strong PAS positive reaction was identified in the cytoplasm of some hepatocytes with irregular distribution in the liver. This reaction was less strong in the next days but from day 17 there was another increase in the number of PAS positive hepatocytes and stronger reaction on day 18 which continued to the end of incubation period. The decrease in red granules number in the hepatocytes or in other words attenuating of PAS reaction on day 14 between two intensifications on days 13 and 17 was also reported in another study on chicken liver. In this research, the PAS reaction in day 11 had lower intensity compared to days 9 and 13 of incubation [20]; the point that had previously been mentioned [29]. The relation between the amount of glycogen storage and the rate of hematopoiesis may associate with mitotic activity in early hemopoiesis [16]; The fact that was interestingly seen in the present study at day 13 of incubation, when the strong PAS reaction happened one day after the reduction of the mitotic activity.

Acknowledgments

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