Research Paper: Histomorphological Study on Prenatal Development of Spleen in Partridge (Alectoris Chukar)

Sohrab Heydarian[1], Iraj Pousty[1*], Hamid Reza Moradi[2]

1. Department of Basic Sciences, Faculty of Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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Dr. Sohrab Heydarian is Assistant Professor at the Department of Basic sciences, Faculty of Veterinary Medicine, Islamic Azad University of Kermanshah, Iran. He teaches veterinary histology, embryology and anatomy courses and his research field is Histology.

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ABSTRACT

Introduction: Phasianidae is a non-migratory group of birds belonged to partridges family. One of the well-known species of partridge is Alectoris chukar, which is a medium-sized bird, larger than quails and smaller than pheasants. They are native to Asia, Africa, Europe and the Middle East and belong to the category of gallinaceous birds. Understanding the physiology and immunology of the lymphoid system is incomplete without knowledge of its basic structure that we are going to examine it in these birds.

Methods: Histomorphological studies were conducted on the spleen of 60 fertilized eggs from the healthy Alectoris chukar birds. Eggs were opened and the spleens of Embryonic Day (ED) 10 to 22 were dissected by dissecting microscope. Gross morphological parameters were studied immediately after collection of samples and then the spleen samples were fixed in Bouin's solution. After tissue processing, spleens were embedded in paraffin and the prepared sections (5–6 µm) were stained using Hematoxylin and Eosin (H&E) method.

Results: Based on our findings the capsule of spleen was formed by a thin and single layer of the mesothelial cells, on ED 12. At this time, macrophages were observed beneath the endothelium of sinusoids for the first time. The early distinction between the red and white pulp had been obvious on ED 14. The central artery was seen in white pulp of the spleen at ED 20. Reticular cells with large euchromatin nucleus and specified nucleolus were observed in the white pulp. At ED 22, there were Billroth cords in the spleen. Trabecular artery was observed in spleen of the partridge embryos at this time.

Conclusion: This study was the first study on histogenesis and histological of spleen were performed in prenatal partridge.

Key Words:
Histomorphology, Spleen, Partridge, Prenatal

*** Corresponding Author:**
Iraj Pousty, PhD
Address: *Department of Basic Sciences, Faculty of Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.*
Tel: *+98 (21) 44865179*
E-mail: *sohrabheydariyan@yahoo.com*

1. Introduction

Phasianidae is a family of non-migratory birds belonged to partridges family. One of the well-known species of partridge is Alectoris chukar, a medium-sized bird larger than quails and smaller than pheasants. They are native to Asia, Africa, Europe and the Middle East and belong to the category of gallinaceous birds [1].

Knowing the anatomy and histology of the lymphoid tissues is the key to understand the immunology and physiology. Epithelial anlages are the origin of bursa of the Fabricius and thymus. Moreover, mesenchymal anlages will develop into the spleen, lymph nodes and bone marrow [2]. Spleen is regarded as 'peripheral or secondary' lymphoid tissues. It filters blood and mediate the immune responses. In addition, phagocytosis of foreign agents, removal of old blood cells, and storage of platelets are other spleen functions [3, 4]. Spleen is round to oval in gallinaceous birds, ducks and psittacines; whereas, spleen in charadriiformes and passeriformes is more elongated. In proportion to the body weight, the spleen is smaller in birds than mammals. Diameter of a normal chicken spleen is approximately one quarter the length of the proventriculus [5]. The trabeculae in the dove [6] and goose [7] spleens were poorly formed and the white and red pulps could not be distinguished from each other as is also the case in the chicken spleen. Review of literature reveals no information about prenatal development of partridge spleen. Therefore, the present study was designed to investigate the anatomy and histology of the spleen of partridge (Alectoris chukar) during their prenatal stages of development.

2. Materials and Methods

Embryos

In this study, 60 fertilized eggs from the healthy Alectoris chukar birds were used. The following steps were performed: At first, very light weight (small) and very heavy (large) eggs were excluded; The eggs were kept for 6 days at 15°C; The eggs were disinfected with formaldehyde gas and incubator supplied with 55% relative humidity at 37.5°C (relative humidity in 3 final days was 60% at 36.5°C); Then, four partridge eggs were randomly used of incubation per day. Eggs were opened and the spleens of Embryonic Day (ED) 10 to 22 were dissected by dissection microscope. In the present study, a total of 52 partridge embryos were used.

Evaluations of morphology were carried out in accordance with the guidelines laid down by the National Institute of Health (NIH) in the USA and in accordance with Iran Veterinary Council (IVC) regulations.

Tissue preparation for histomorphological studies

The spleen samples were fixed in Bouin's solution. After tissue processing (dehydration, clearing, infiltration), spleens were embedded in paraffin (52°C-58°C). Serial sections, 5 to 6 µm thickness, were cut. After being deparaffinised and hydrated, the sections were stained with method of Hematoxylin and Eosin (H&E) for histomorphological studies. The figures were prepared using digital Dino-Lite lens and Dino-capture 2 software.

3. Results

Microscopic studies

In this study, the defined structure was not observed in the partridge embryos before the 10th day. Histological evaluation of the spleen at ED 10 revealed that the repertoire of undifferentiated mesenchymal cells act as a cellular basement. In this stage, squamous mesothelial cells were seen around of the spleen. They will probably be the precursor cells of splenic capsule (Figure 1a). More proliferation of undifferentiated mesenchymal cells was seen at ED 11 and

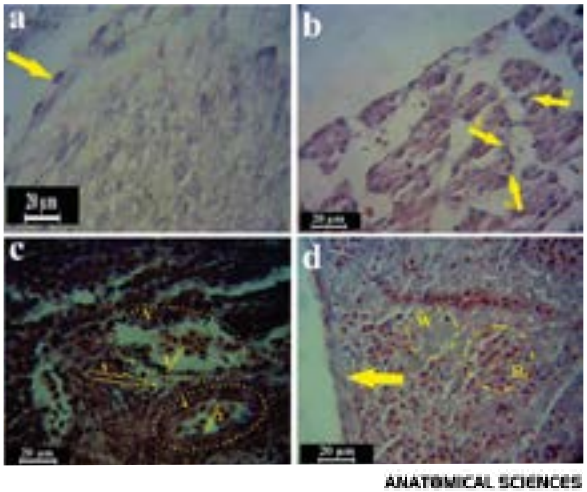

Figure 1. Histological sections of embryonic spleen
(a): Precursor cells of splenic capsule (arrow) and creation of a basement of mesenchymal cells of spleen at ED 10;
(b): The presence of abundant sinusoids containing endothelial cells (E) and macrophages with triangular-shaped nuclei (M) in the parenchyma of the spleen at ED 12;
(c): Large blood vessels (A and V) with red blood cells (1) and surrounding loose and thin connective tissue in spleen at ED 14;
(d): Complete development of capsule (arrow) and distinct the red pulp (R) and white pulp (W) in spleen at ED 16

12. At ED 12, the capsule of spleen was observed as a thin and single layer of the mesothelial cells. The presence of abundant sinusoids was one of the prominent features of the spleen development at this time. Macrophages were observed beneath the endothelium of sinusoids for the first time at ED 12 (Figure 1b). The early distinction between the red and white pulp had been obvious at ED 14. Red pulp with sinusoids along with abundant red blood cells was observed. The presence of the large blood vessels was one of the prominent features at this stage (Figure 1c). At ED 16, the development of splenic capsule was seen completely. The splenic capsules were formed by mesothelial cells multilayer (Figure 1d). With aging, final structure of the spleen was appeared. In partridge embryos, trabeculae had not been obvious in the spleen. The central artery was seen in white pulp of the spleen at ED 20 (Figure 2a). Reticular cells with large euchromatin nucleus and specified nucleolus was observed in the white pulp (Figure 2b). At ED 22, there were specified Billroth cords in the spleen (Figure 2c). Also, trabecular artery was observed in spleen of the partridge embryos at this time (Figure 2d).

Macroscopic studies

Macroscopically, spleen was not observed at ED 10–13. It can be seen as a light red bud at ED 14 or 15. Furthermore, as a pear-like light red, spleen showed clearly with 1 mm of length which lies beside the left lobe of liver between the proventriculus and gizzard at ED 16. At this time, spleen of the partridge was enclosed by a thick capsule (Figure 3).

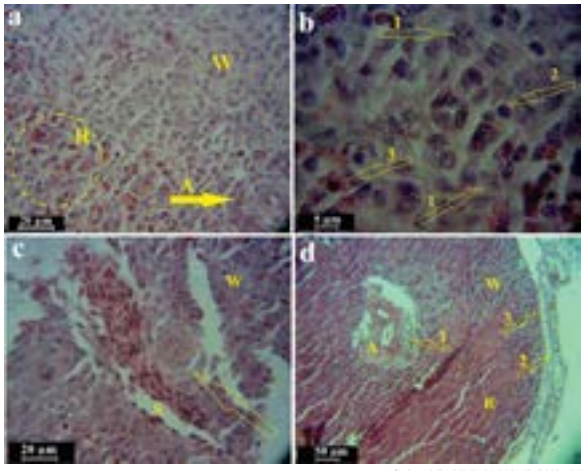

Figure 2. Histological sections of embryonic spleen
(a): The presence of central artery (A) in spleen at ED 20;
(b): White pulp contains reticular cells (1) and lymphocytes (2). So, red blood cells (3) are observable in spleen;
(c): Red pulp contains Billroth cord (arrow) and sinusoids (S) at ED 22;
(d): Surrounding loose connective tissue (1) of trabecular artery (A), mesothelium (2), capsule (3), red (R) and white (W) pulps are observable in spleen at ED 22.

4. Discussion

The spleen is the major secondary lymphatic organ in body, which involves in filtering the blood and mounting immune responses against blood antigens. Erythropoiesis is a major function of the fetal spleen [8]. The present study revealed that spleens were covered with mesothelium at 10th to 12th days of incubation in partridge spleen. Although it has been reported that capsule was formed at the 10th to 14th days of incubation in native chickens (Gallus domesticus) of Bangladesh [3] and spleen was covered with mesothelium at the 10th day of incubation in quail [5]. On day 12 of incubation, abundant venous sinusoids were lined with endothelial cells and red blood cells were observed in the sinusoids. Macrophages were seen beneath the endothelium of sinusoids. In contrast to quail, granulopoiesis and erythropoiesis could be activated on the 12th day of incubation in partridge spleen, this result is in agreement with findings of Ogata and associates [9]. It has been reported that the erythropoiesis and granulopoiesis began before the 10th day of incubation in quail [5].

On day 14 of incubation, the large blood vessels were seen and capsule was observed in two layers in partridge spleen. Trabeculae were not observed on the 14th day, but there is relatively loose connective tissue around some large blood vessels. It has been reported that trabeculae is formed in native chickens (Gallus domesticus) of Bangladesh on the 14th day of incubation [3]. Islamkhan et al. showed that broiler spleen is enclosed by a thick capsule and a few number of trabeculae. [10]. On the 15th day of incubation, walls of the trabecular were quite distinct in quail spleen [5]. Red pulp and white pulp were distinctive and cells were proliferated in white pulp on the 14th day of incubation in partridge spleen. These results are inconsistent with findings of Seres and associates [4]. They reported that white pulp was appeared as diffuse lymphatic tissue; whereas, the splenic red pulp com-

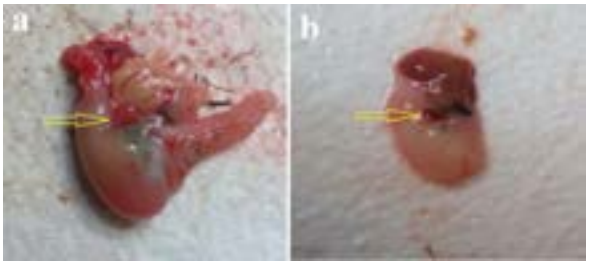

Figure 3. Macroscopic view of embryonic spleen
Arrows show spleen of the partridge at ED 21 (a) and ED 17 (b)

posed of venous sinuses and anastomosing cord of reticular cells, macrophages, lymphocytes and blood cells [11]. The white pulp in indigenous ducklings of Bangladesh spleen is a normal site of the network of reticular cells and reticular fibers, with a number of lymphocytes and plasma cells [12]; these results are in agreement with our study results.

In consistent to quail, sinusoids were grown and greater influxes of blood cells were observed on the 16th day of incubation in partridge spleen [5]. In the present study, large artery and vein in hillus of spleen and reticular cells with euchromatin and prominent nucleolus were seen on the 18th to 20th day of incubation. The central artery was covered by a layer of squamous cells, for the first time. Splenic cords were formed in white pulp on the 21st to 22nd day of incubation. At this time, the capsule is composed of dense irregular connective tissue, which is enclosed by mesothelium cells. The cords of Billroth (splenic cords) are found in the red pulp. All results of the present study agree with the results of other studies in the last days [4, 5]. In the present study, spleen was not seen macroscopically until ED 13. It was observed at ED 14 or 15 and clearly lies between the proventriculus and gizzard at ED 16. Macroscopic observations of the current study are similar to the study of Akter and associates [11].

In conclusion, this study constitutes the first study of histogenesis and histological spleen performed in prenatal partridge. It is expected that the results of our research help further studies on prenatal birth lymphoid organs.

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Conflict of Interest

The authors declared no conflicts of interest.

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