Hhistological aspects of thymus in japonese quail (*Coturnix coturnix japonica*) from one to 180 days of age

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Abstract
Thymus, described from antiquity, has long time been considered a mysterious organ because its role in the body was unknown. The first microscopic description of the thymus belongs to Hassal, and in the first half of the 20th century the thymectomy was performed to treat "myasthenia gravis." In 1962, the role of thymusin the production of T-lymphocytes which are responsible for the cell mediated immunitywas revealed. Bird's thymus represents 2% of the body weight at hatching and continues to develop until the time of sexual maturity. Coturnix japonica is a species used in the laboratory as an experimental animal, and it is economically important because of the nutritional value of the eggs which are recommended to be consumed in various diseases. The japonese quail reaches sexual maturity at the age of 7 weeks when signs of thymic involution should be observed. The present study aimed to describe some histological particular features of thymus in the japonese quail. In the study, 25 quail wereeuthanized at the age of 1, 7, 15, 24 and 60 days for thymus prelevation. The fragments were prepared by paraffin embeding and stained HEA, Giemsa and PAS. This has highlighted some of the particularities of this species. In most species thymus regresses under physiological conditions due to sex hormones action at puberty. The histological aspects obtained in this study demonstrated that in this species the thymus involution was not present even in the individuals sacrificed at the age of 6 months. The dimensions of the cortical and medullary areas of the thymus at 24 and 60 days wereapproximately constant.
Key words: *Coturnix japonica,thymus, histology*

Introduction

The Japanese quail (*Coturnix coturnix japonica*) is a popular laboratory animal model since the middle of the last century (Huss et al., 2008). Genetic studies regarding the chromosomal and mitochondrial DNA of this species have been conducted (Nishibori et al., 2001, Shibusawa et al., 2004). Because thequail’s eggs are used in human alimentation the effects of different diets on fertility, embryonic viability, egg mass and production rates have been studied (Abedi et al., 2017, Mills et al, 1997, Sahin et al., 2003) . The influence of diets on lymphoid organs such as thymus, spleen, Fabricius Bursahas also been studied (Sahin et al., 2002, Sahin et al., 2004, Wang et al., 2009). Since thymus is essential for T lymphocytes maturation which are considered to be the cellular immune effectors, the increase in naive B and T cell populations in secondary lymphoid tissues are correlated with increased immunity (Miller, 2004, Wang et al., 2001, Li et al., 2007). In most species the thymus atresia at puberty is determined by sex hormone secretion and the occurrence of necrose areas after estrogen administration was observed in some studies (Razia et al., 2006).The avian thymus is placed around the jugular vein, and histologically, a lobe has a dark cortical area and alight medullawith several thymic corpuscle (Solcan, 2011).Here are double positive cells which after selection and maturation will become CD4 + or CD8 + single positive lymphocytes (Wang et al., 2001). These cells leave the thymus via the blood vessels and reach the peripheral immune system (Bidere et al., 2006, Miller, 2002).

The aim of this paper is to highlight the evolution of histological aspects of thymus in *C. japonica* at various ages.

Materials and methods

Histological observations were made on several thymuses obtained from 25 quails(*Cortunix japonica*). These were grown in the University Biobasis according to the specific technology. The slaughtering was performed at one day, 7, 15, 24 and 60 days of age, when thymus and various organs were collected. Thymus fragments from 4 birds from each age category were processed by paraffin embeding and stained by HEA, Giemsa and PAS methods.Image capture and measurements were performed with a CX41 Olympus microscope.

Results and discussions

The histological study of the thymus specimens collected from individuals with different ages allowed the observation and in time evolution. Thus, in certain age groups, particular features of this organ were found in C. japonica.

The CX41 Olympus microscope used for image capture also allowed the size measurement of thymus structures presented by age in Table 1.

Table 1. Dimensions of of thymus structures according to age

Age /Thymic Structure (µm)	Day 1		Day 7	Day 15	Day 24	Day 60
Thymic lobe	2090-3389		2190-3478	1698-3700	1689-3701	1001-3780
Cortex	750-812		802-1139	425-619	358-620	358-507
Medulla	583-2094		568-2186	581-990	547-989	740-1110
Hassal corpuscle	-		45-334,2	201-210,2	101-209	107-208

The capsule made up of semi-orientated connective tissue showed infiltrations of adipose tissue no matter the age at which the prelevation was performed. Regardless the age, capsulae derived septa with nearly constant diameter splitted the parenchyma of the thymus into lobules.

At day one, thymic lobules showed a polygonal appearance, being bound by conjunctival septa with a diametre of 10-12µm. Cortex (Fig. 1a), consisting of young lymphocytes and epithelial reticulum cells, was intensely colored. At the boundary between cortex and medulla and especially in medulla small congestion areas appeared (Fig. 1, arrows).

The medulla (Fig. 1b) was less colored due to the lower lymphoid population compared with the cortex. The epithelial reticular cells from the medulla showed a polygonal appearance,those located peripherically being smaller than the central ones (Fig. 2). The Hassal corpuscles were atypical, identified as cell clusters without the characteristic concentric arrangement of the cells (Fig. 2, arrow).

In the 7-day-old chickens, thymic lobules (Fig. 3) showed the same cortex and medulla aspects as in one day-old ones, with the difference that some of the etipthelial reticular cells underwent necrosis with the presence of a colloidal central substance, flattened appereance and concentric arrangement characteristic for Hassal corpuscles (Fig. 4).

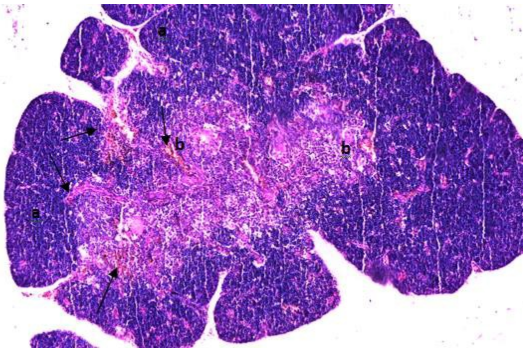


Fig. 1. Thymus in one day old quail chicks. The thymic lobe. Intensely dark cortex (a) with immature T-lymphocytes; light stained medulla (b) with mature T lymphocytes and non-lymphocyte support cells. Lobules are delimited by conjunctive septae. Giemsa x 60

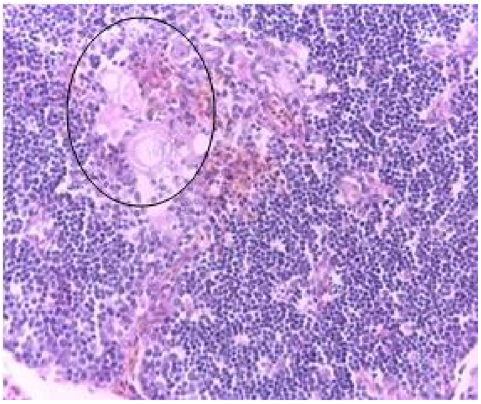


Fig. 2. Thymus in one day old quail chicks. Enlarged epithelial reticular cells in the medulla. PAS x400.

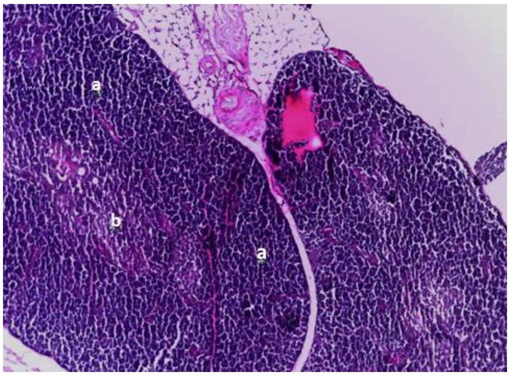


Fig. 3. Thymus in 7 days age quail. The cortex of the thymic lobe (a) is evident, dark colored. In the medulla (b) there are clusters of epithelial reticular cells. Giemsa x60.

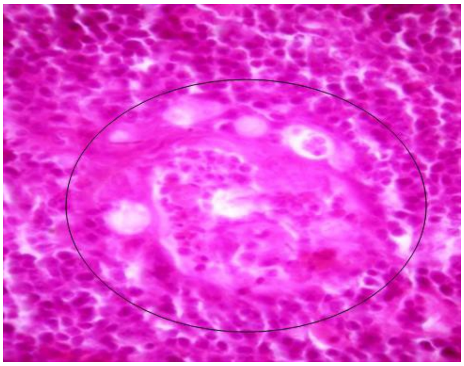
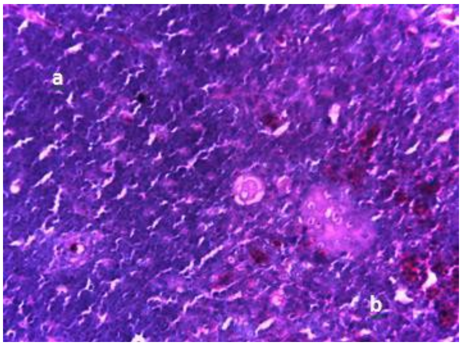


Fig. 4. Thymus in 7 days aged quail. The cortex of the thymic lobe is dark colored. In the medulla the Hassal corpuscle consisting of epithelial reticular cells in a concentric arrangement with central lymphocytes. PAS x400.

In quail chickens of 15 days (Fig. 5 and 6) and 24 days of age (Fig. 7) histological aspects are similar to those previously described in the 7 days old subjects, but both the cortex and the medulla were smaller in size.



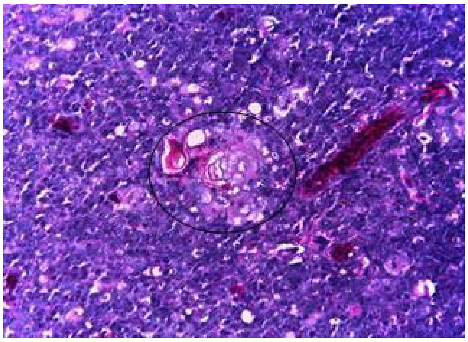
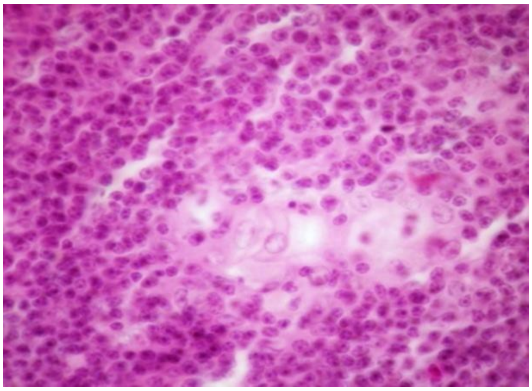


Fig. 5. Thymus in 15 days old quail. The cortex of the thymic lobe is dark colored (a). Hassal corpuscle in the medulla(b) consisting of epithelial reticular cells. Capillaries lined with simple cubic epithelium. Giemsa x400.

Fig. 6. Thymus in 15 days old quail. Dark cortex. In the medulla Hassal corpuscle composed of flattened, degenerated large central cells, with a concentric arrangement.Giemsa x400.

The thymic medulla of the 60-days-old quails contained Hassal corpuscles with areas of necrosis and glycosaminoglycan infiltrations (Fig. 8 and Fig. 9).



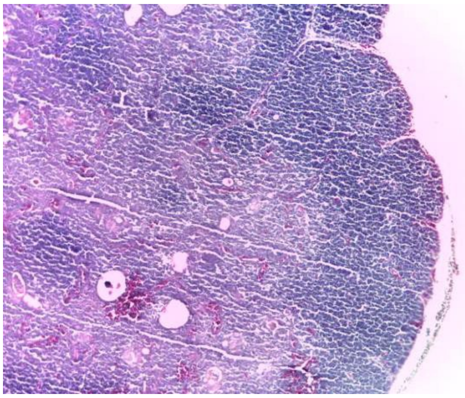


Fig.7. Thymus in 24-day-old quail. In the medulla Hassal corpuscle composed of flattened, degenerated large central cells, with a concentric arrangement and a few central lymphocytes. PAS x400

Fig. 8. Thymus in 60 days old quail. The thymic cortex is obviously dark colored, covered by the delicate capsule infiltrated by fat cells. HEA x400

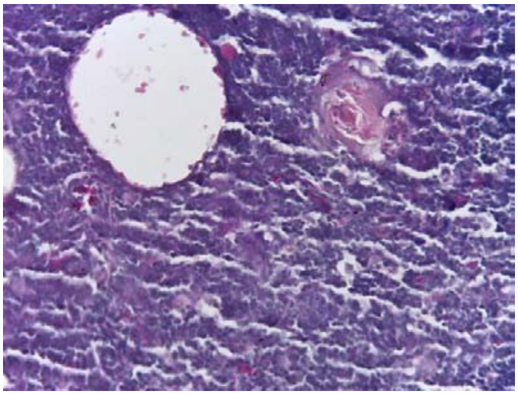


Fig. 9. Thymus in 60 days old quail. Hassal corpuscle in the medulla consisting of flattened concentric arranged epithelial reticular cellswith glycosaminoglycans deposites and lymphocytes. In addition, an area of necrosis is observed. HEA x400

The thymic lobe presented a dense dark cortex with young,large lymphocytes even in the 60 days old quails contrary to other species where involution occurs (Kim et al., 2015). The medial area was lighter colored, consisting in smaller, mature lymphocytes and epithelial reticular cells organized in Hassal corpuscles with various appereances, aspects also described in the literature (Gordon & Manley, 2011, Junqueira & Carneiro, 2005).They consisted of epithelial reticular cells but did not presented the typical concentric arrangement in one day-old quails. The characteristic arrangement was observed only after the age of 7 days. Hassal corpuscles showed specific areas of necrosis and colloidal cystic formations that suggested apoptosis processes at this level which are physiological for intrathymic homeostasis (Huang et al., 2015).

The presence of this phenomenon was highlighted by caspases, proteinases incriminated in inflammation and apoptosis (Deneker et al., 2008, Pozzesi et al., 2013). Causes of thymocytes death were either due to negative selection or due to stress or aging (Godfrey et al., 2000). Researches has shown that T-lymphocytes produced in thymus are apoptosis-oriented if they are not compatible with their future immune function (Alam et al., 1999, Gronsky et al., 2004).

Conclusions

The thymus in quails younger than 7 days contains Hassal corpuscles with an atypically arrangement of the epithelial reticular cells. The fact that the decrease of the cortex and medulla has not been recorded in thymus collected from the 60 days old individuals and the appropriate values for the 15, 24 days old individuals suggests that the physiological involution of the thymus does not occur in this species.

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