



Histoarchitecture of Harderian Gland in Turkeys at Pre-pubertal Age


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 0009-0006-2412-1490

ABSTRACT

The study was conducted during August, 2021 to June, 2022 at the Department of Veterinary Anatomy, Veterinary College and Research Institute, Namakkal, Tamilnadu, India to elucidate the histological features to provide reference to physiology, immunology and pathology. The harderian gland samples were collected from Broad Breasted Bronze varieties of turkeys immediately after slaughter. Those were collected from turkeys at 5 months of age before sexual maturity, earlier in the year 2021. The Harderian gland of turkey was compound tubulo-acinar type covered by connective tissue capsule. The inter-lobar septa from the capsule divided the glandular parenchyma into many variously sized and shaped lobes filled with secretory units. The capsule and septa showed collagen, elastic and reticular fibers with numerous fibrocytes and fibroblasts. Each lobe had a secretory component and a lymphoid component. The secretory part was lined by pyramidal cells in acini and columnar cells in tubular units and both had spherical nucleus at base. The lymphoid component was constituted by various populations of lymphoid cells such as lymphoblasts, small, medium and large lymphocytes. It also showed numerous plasma cells. Each lobe had a central duct surrounded by several acinar and tubular secretory units. Each secretory unit was lined by closely attached pyramidal cells in acini and columnar cells in tubular units. Myoepithelial cells were noticed at the base of secretory units. The central duct of each lobe was wide and irregular in shape with many crypts and was drained into a large main duct which extended from the posterior to anterior end of the gland.

KEYWORDS: Harderian gland, histoarchitecture, puberty, turkey

Citation (VANCOUVER): Jayachitra and Sivagnanam, Histoarchitecture of Harderian gland in Turkeys at Pre-pubertal Age. *International Journal of Bio-resource and Stress Management*, 2023; 14(10), 1454-1459. [HTTPS://DOI.ORG/10.23910/1.2023.4833a](https://doi.org/10.23910/1.2023.4833a).

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.



1. INTRODUCTION

Turkey is a large gallinaceous bird belongs to North America, highly adaptive to local climatic conditions and is an important source of animal protein in many parts of the world. Furthermore to having been utilized as a food source, turkeys were also valued for their bones and feathers, which had both practical and symbolic uses. Domestic turkeys are purposefully developed to become larger than wild turkeys for their meat. Although turkeys are resistant to most of the diseases compare to chicken, they are affected by few economically important diseases. Marek's disease infection and infectious bronchitis are relatively uncommon. Only an infrequent form of Coccidiosis and Ranikhet sickness exist. The most prevalent ailments include round worm infections, mycoplasmosis, blue comb, fowl cholera, fowl typhoid, and fowl pox. Hence, greater emphasis should be given to prevention which needs thorough understanding of immune organs to develop prevention strategies like vaccines. In quail, the periorbital fascia served as an attachment point for the Harderian gland, which was situated at the ventro-medial portion of the orbit (Kozlu and Altunay, 2011). While in capercaillie, the Harderian gland was located between the medial straight muscle, the pyramidal third eyelid muscle, and the ventral oblique muscle at the ventro-medial part of the orbit, near to the interorbital septum (Nawrot et al., 2016). In both flying and non-flying birds, Beheiry et al. (2020) observed the Harderian gland in the ventro-medial aspect of the eyeball, which extended posteriorly from the optic nerve to the anterior section of the eyeball. According to Rana et al. (2020), the Harderian gland in chickens was situated ventral to the eyeball and had a constriction in the middle. The harderoian gland functions are diverse which plays a role in lubrication of the eye and its nictitating membrane, it forms immune responses in birds, it has a role of photoreception in rodents as a part of the retinal-pineal axis, produces of pheromones and thermoregulatory lipids. It also helps in osmoregulation in some reptiles and production of growth factors and saliva in some chelonians. The Harderian gland of chicken also plays an important role in adaptive immune responses upon ocular exposure to avian pathogens such as avian influenza. Hence, to determine the role of harderian gland in generating immunity, chickens were immunized ocularly with an adenovirus (Ad5) vector expressing the AI hemagglutinin H5 gene (Van Ginkel et al., 2009). The harderian gland is a bursa dependent organ which forms the major head associated lymphoid tissue and it influence the B lymphocyte activation, proliferation and differentiation. In addition to its immunological role in production of humoral antibody response against infection, it is also responsible for lubrication of eye as

its structural framework is formed by exocrine tubulo-acinar units (Maslak and Reynolds, 1995). The birds are often used as experimental subjects who act as vital and precious model for exploring the fundamentals of immune system in immunological research (Khan et al., 1996). But, the histology of immune organs are variable among avian species (He et al., 2015) and only microanatomy of primary lymphoid organs in birds is broadly investigated in various species of birds especially focused in chicken. The histological structure of hardeian gland is changeable before and after sexual maturity. But the data on complete histoarchitecture of hardeian gland in prepubertal turkey is negligible. Hence, this study was formulated to elucidate the histological features to provide reference to physiology, immunology and pathology.

2. MATERIALS AND METHODS

The research on histology of harderian gland in turkey was conducted at the Department of Veterinary Anatomy, Veterinary College and Research Institute, Namakkal earlier in the year August 2021 to June 2022. The study was carried out in six numbers of Broad Breasted Bronze varieties of turkey birds reared in an organized turkey farm at Dharmapuri district of Tamil Nadu and were slaughtered at five months of age before sexual maturity. During slaughter, the whole head of a turkey was collected; they were thoroughly washed in water followed by normal saline solution to remove all the blood and tissue debris. Further, a sagittal section of the head was done through the dorsal midline of skull by using scalpel to locate the Harderian gland. The harderian gland which lies against the interorbital septum was carefully dissected out, washed in normal saline and fixed in 10 per cent neutral buffered formaldehyde solution for 48 hours. After complete fixation, the tissues were processed by routine alcohol-xylene -paraffin method. The fixed tissues were trimmed to the size of about 3 cm and were allowed for washing in running tap water for overnight. Subsequently, they were transferred to ascending grades of iso - prophyll alcohol from 50 per cent to 100 percent concentration, each for one hour. Further, the tissues were shifted to xylene solution for thirty minutes of three changes. The xylene completely removed the alcohol from the tissues, afterwards it is allowed for paraffin impregnation with paraffin- ceresin wax at 60°C for 5- 7 hours. After complete impregnation the tissues were made into paraffin blocks by using pure paraffin- ceresin wax (Bancroft and Stevens, 1996). The paraffin sections of 3-5µm thickness were cut from the tissue blocks by using a Leica microtome (RM-2145) and were air dried. The dried tissue sections were subjected to Standard Haematoxylin and Eosin staining method to explore its histoarchitecture. The observations on histoarchitecture were recorded and photomicrographs



were taken by Leica trinocular light microscope with an image analyzer.

3. RESULTS AND DISCUSSION

The Harderian gland was a compound tubulo-acinar type of gland covered by connective tissue capsule with adipose tissue encircled around it. The dense connective tissue capsule of the gland showed numerous collagen, reticular and few elastic fibres. The inter-lobar septa from the capsule divided the glandular parenchyma into many variously sized and shaped lobes (Figure 1) filled with secretory units as observed by Jahan *et al.* (2006) in broiler and native chickens, Kozlu and Altunay (2011) in quail, Mobini (2012) in native chicken and Bejdic *et al.* (2014) in laying hen. But, as stated by these authors, the lobes were not subdivided into lobules. Whereas, Altunay and Kozlu (2004) in ostrich, Boydak and Aydin (2009) in geese and Khayoon *et al.* (2019) in turkey reported that the Harderian gland was tubulo-alveolar type. The inter-lobar septa seen throughout the glandular parenchyma displayed numerous collagen, reticular fibres as in the capsule. Numerous capillaries, connective tissue cells and few lymphocytes were also observed in the connective tissue component of the gland.

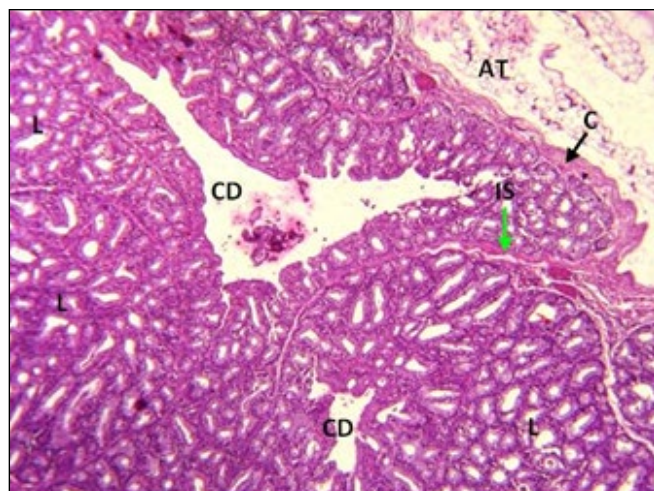


Figure 1: Photomicrograph showing the capsule and lobes of Harderian gland in turkey (H&E x 100); C: Capsule; IS: Inter-lobar septa; L: Lobe; CD: Central duct; AT: Adipose tissue

Each lobe had secretory component (Figure 2) and lymphoid component (Figure 3) formed by tubulo-acinar secretory units and lymphatic cords respectively as mentioned by Bejdic *et al.* (2014) in laying hen. The secretory part of the Harderian gland revealed many closely packed acini and each acini was completely surrounded by loose connective tissue network. In this stroma, numerous reticular fibres were identified. The connective tissue stroma around the acini was the site of lymphoid

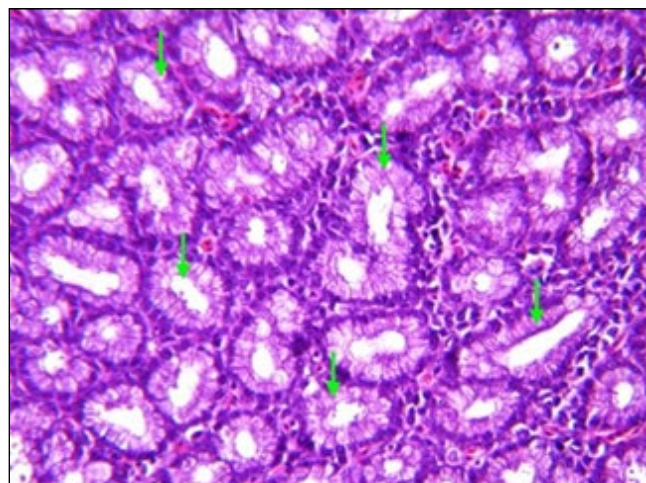


Figure 2: Photomicrograph showing the secretory component (arrows) of Harderian gland in turkey (H&E x 400)

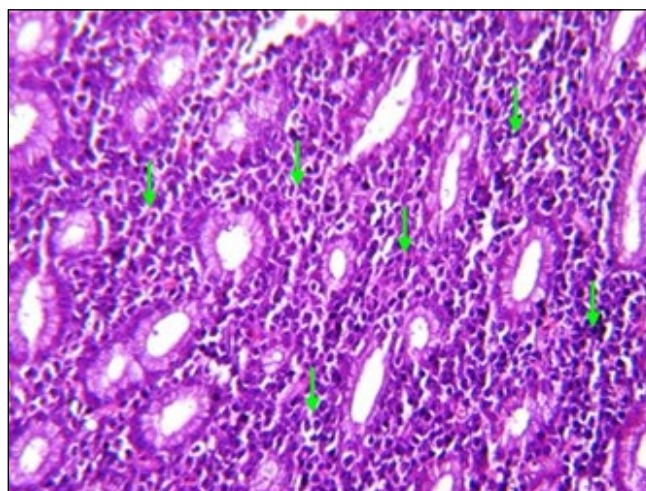


Figure 3: Photomicrograph showing the lymphoid component (arrows) of Harderian gland in turkey (H&E x 400)

cell infiltration to constitute the lymphoid component. The exocrine component of Harderian gland had a role in lubrication of nictitating membrane and the immune cells had a role in immunological reactions (Maslak and Reynolds, 1995; Beheiry *et al.*, 2020).

Each lobe had single wide central duct which was surrounded by several acinar and tubular secretory units (Figure 4) as described by Boydak and Aydin (2009) in geese, Nawrot *et al.* (2016) in capercaillie, Khayoon *et al.* (2019) in turkey and Beheiry *et al.* (2020) in duck, goose and ibis. This observation differs from Kozlu and Altunay (2011) in quail who explained that the central duct of the gland was divided into first and second part which was designated as primary and secondary duct respectively. The secretory units were completely demarcated from each other by interstitial connective tissue as in duck, goose and ibis (Beheiry *et al.*, 2020).

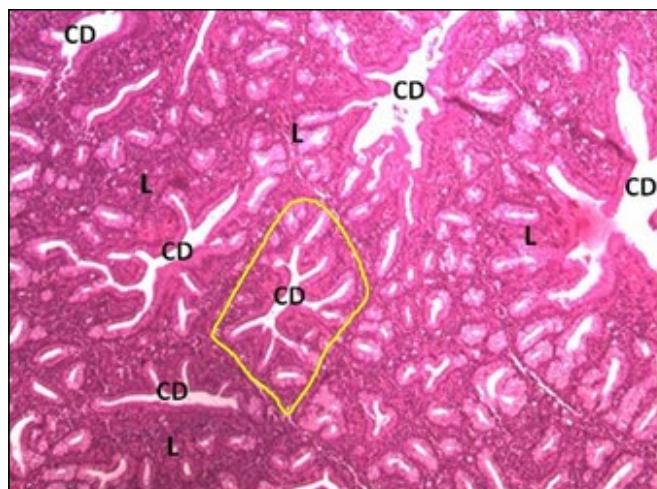


Figure 4: Photomicrograph showing the lobes with central duct in harderian gland in turkey (H&E x 40); CD: Central duct; L: Lobe

Each secretory unit was lined by closely attached pyramidal cells in acini and columnar cells in tubular units and both had spherical nucleus at the base (Figure 5). The cytoplasm of epithelial cells was basophilic and granular whereas Nawrot et al. (2016) in capercaillie observed only pyramidal epithelium in all the secretory units with oval nucleus at base and in duck, goose and ibis, the secretory acini and tubules were lined by simple columnar epithelium with round nucleus at the base (Beheiry et al., 2020). The basophilic cytoplasm indicated the domination of mucinous secretory nature of the gland. The distinct cell boundaries were noticed in all epithelial cells which showed merocrine mode for release of its secretion. Dissimilar to this, the acini were lined by simple columnar epithelium with indistinct boundaries in native chicken (Jahan et al., 2006). The glandular epithelium with large nuclei at the base

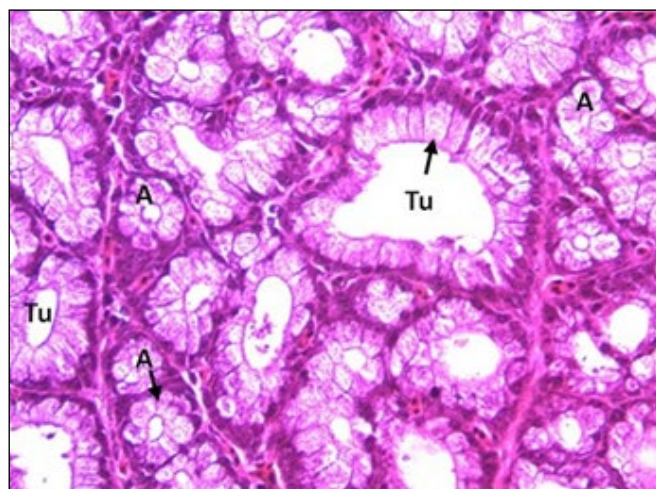


Figure 5: Photomicrograph showing the epithelium of secretory units in turkey (H&E x 400); A: Acinus, Tu: Tubular unit

and secretory granules in cytoplasm with disturbed apical cell membrane showed apocrine mode of release in geese (Boydak and Aydin, 2009) but, Kozlu et al. (2010) in osprey stated that the glandular epithelium of Harderian gland showed holocrine type of secretion. In contrast, Mobini (2012) in native chicken reported that the apical portion of secretory cells was darkly stained with serous secretion and basal portion was lightly stained with mucous secretion.

As stated by Altunay and Kozlu (2004) in ostrich and Beheiry et al. (2020) in duck, goose and ibis the myoepithelial cells were noticed at the base of secretory units (Figure 6). They were observed as flattened cells with elongated processes and had small elongated vesicular nucleus. The processes of adjacent myoepithelial cells were connected with each other around the acinus which is similar to the findings of Nawrot et al. (2016) in capercaillie. The myoepithelial cells were oval shaped cells with pale nuclei in Harderian gland of quail (Kozlu and Altunay, 2011).

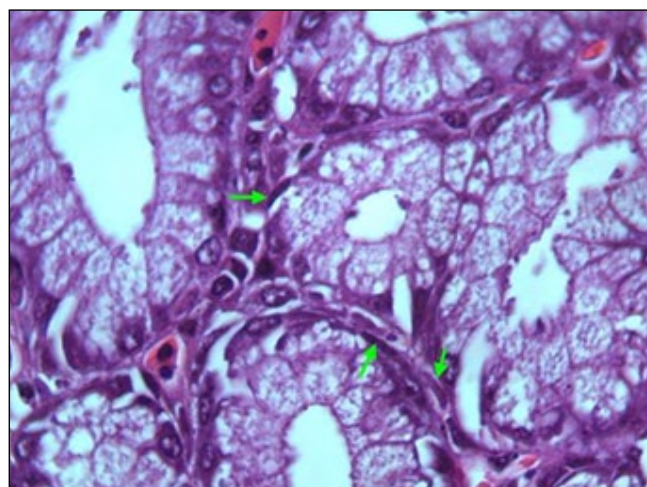


Figure 6: Photomicrograph showing the myoepithelial cells (arrows) in harderian gland in turkey (H&E x 1000)

The lymphoid component was formed by the cords of lymphocytes and were noticed within the interstitium between secretory units as observed by Jahan et al. (2006) in broiler and native chickens, Kozlu et al. (2010) in osprey, Nawrot et al. (2016) in capercaillie and Beheiry et al. (2020) in duck, goose and ibis. The macrophages and plasma cells were also identified in the lymphoid aggregation. The increased accumulation of lymphoid cells in the interstitium was noticed and it resulted in displacement of acinar and tubular secretory units to the periphery. This also caused the less number of secretory units within the lymphoid component.

The lymphoid aggregation around central duct was seen as mentioned by Jahan et al. (2018) who noticed largely aggregated lymphocytes around the central duct in each lobule. Bejdic et al. (2014) in laying hen also observed more

cellular population in the central part and less number of cells in the periphery.

The duct system of the harderian gland was mainly formed by the numerous acinar ducts, central duct and main duct. The acinar duct was indistinguishable from the secretory units which was also secretory in nature. The central duct of each lobe was wide, irregular in shape with many invaginations which appeared as crypts (Figure 7). It was lined by simple columnar epithelium with basal oval nucleus which was also secretory in nature. It concurs with Jahan et al. (2006) in broiler and native chickens, Nawrot et al. (2016) in capercaillie and Khayoon et al. (2019) in turkey.

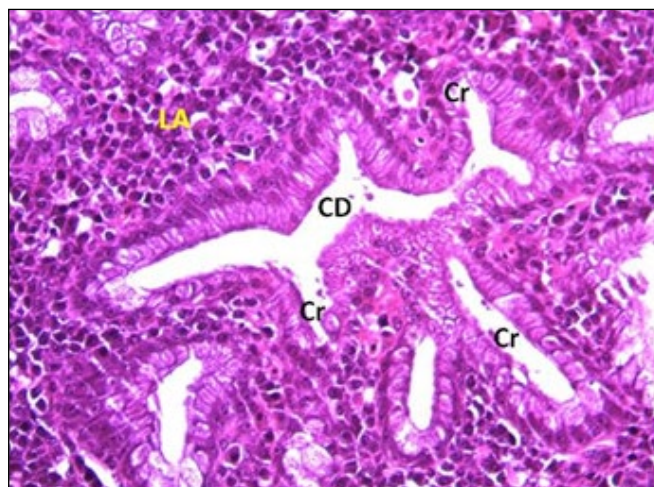


Figure 7: Photomicrograph showing the central duct of harderian gland in turkey (H&E x 400); CD: Central duct; Cr: Crypt; LA: Lymphoid aggregation

Mobini (2012) in native chicken and Beheiry et al. (2020) in duck, goose and ibis also described the presence of crypts in the central duct. In disagreement to this, the central canal was lined by low cylindrical or cuboidal epithelium with microvilli in White leghorn chicken (Olah et al., 1996), varied heights of columnar epithelium with myoepithelial cells at the base in ostrich (Altunay and Kozlu, 2004) and multilayered basal polygonal and apical cuboidal with goblet cells in geese (Boydak and Aydin, 2009). The lymphocytes which formed the nodule like aggregation in the region between the crypts of central duct were also noticed. The lymphocytes and macrophages which undergone apoptosis were appeared as pyknotic and vacuolated cells in the interstitial tissue.

All the central ducts were drained into large main duct which extended from posterior to anterior end of the gland (Figure 8). The main duct of the gland was also lined by simple columnar epithelium with sub-epithelial connective tissue. The sub-epithelial region of main duct also showed accumulation of lymphocytes and its stroma was constituted by fine reticular fibres. It is not accorded with

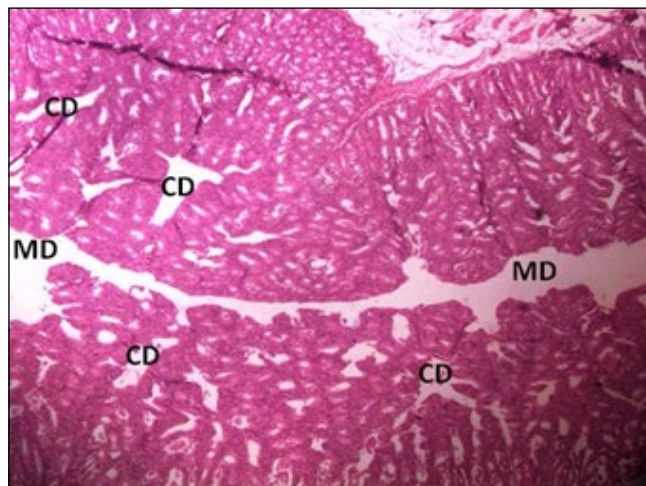


Figure 8: Photomicrograph showing the main and central ducts of harderian gland in turkey (H&E x 40); MD: Main duct; CD: Central duct

the Kozlu and Altunay (2011) who stated that the primary and secondary ducts were lined by simple columnar and cuboidal epithelium respectively in quail. As described by Mobini (2012) in native chicken and Beheiry et al. (2020) in duck, goose and ibis, the solitary lymphatic nodules were not noticed adjacent to crypts of central duct. The clear duct system of Harderian gland was not evidently presented in any of the literature reviewed for the present study.

The right and left Harderian glands were not revealed any significant differences, both revealed same exocrine and lymphoid architecture (Jahan et al., 2018) and also no histological dissimilarities was observed between the sexes as noticed by Kozlu and Altunay (2011) in quail and Mobini (2012) in native chicken.

4. CONCLUSION

The histological structure of harderian gland in turkey was constituted by secretory exocrine and non-secretory lymphoid component in prepubertal age. The exocrine secretion was drained by central duct from each lobe which was united to form main duct system. The lymphoid component was formed by lymphocytes and plasma cells. The detailed histoarchitecture of harderian gland will provide reference to understand its physiology, immunology and pathology.

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