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### **Post Hatch Developmental Changes in the Harderian Gland of Chicken**

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#### **Abstract**

The present study was aimed to study histomorphological changes in the Harderian gland of chicken from 3 day of hatch to 24 day old. The gland was compound tubule-acinar type, consisted of capsule, parenchyma and stroma. In post hatch group of chicks, the gland was surrounded by thin connective tissue capsule from which septae extend which subdivide the gland into lobules of varying size. In the parenchyma of the gland, acini situated at the periphery and tubules at the centre of lobule. The acini were lined by columnar epithelial cells of varying height with basally located nucleus and the tubular systems were lined by a single layer of epithelium varying from columnar to cuboidal. Stroma or interstitial tissue consisted of collagen, fibroblasts, blood vessels, nerves and immune cells, plasma cells and lymphocytes. The plasma cells increased with advancement of age. Secretory units of the gland secreted its material by blebs formation on the luminal surface epithelium at early post hatch groups, which subsequently released by disintegration of the cells in the advanced age.

**Key words:** Histomorphological, Harderian gland, Plasma cells, Chicken

#### **Introduction**

The Harderian gland is the major exocrine paraocular gland of the domestic fowl. It lies in the orbit ventral and postero-medial to the eyeball. It is a peripheral lymphoepithelial organ (secondary immune organ) which, together with the spleen, the bursa of Fabricius and the caecal tonsils forms a system of avian organs that determines both general and local immunity (Fix and Arp, 1991 and Shirama *et al.*, 1996). It also represents an important part of the immune barrier - Conjunctiva Associated Lymphoid Tissue (CALT) (Khan *et al.*, 2007, Pawar *et al.*, 1998 and Payne, 1994). Harderian gland of chicken presents macrophages, lymphocytes, granulocytes in the subepithelial layer and lumina of the lobules for the local immunity of the eye orbit (Baba *et al.*, 1990). Therefore, present study was planned to elucidate the histomorphological changes during post hatch development of Harderian gland of chicken.

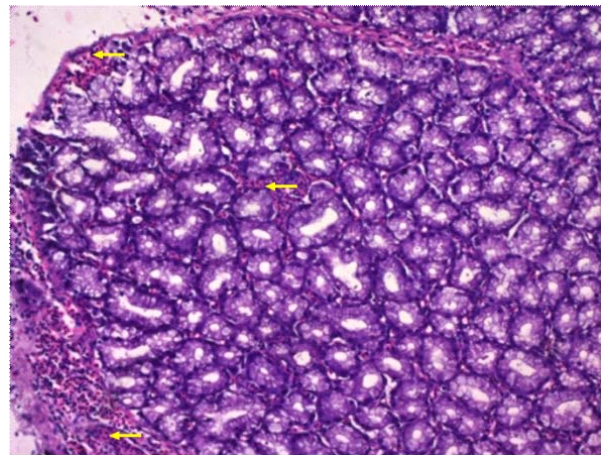
#### **Materials and Methods**

To investigate the structural organization of the harderian gland in post hatch developing chicken, a total of 32 birds were procured from Poultry Farm of Nagpur Veterinary College, Nagpur. The birds were divided into four age groups viz 3, 10, 17, and 24 days, with 8 birds in each group. The

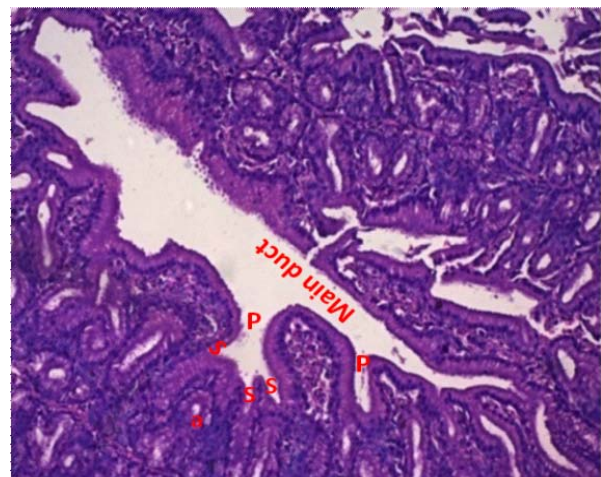
Harderian gland was dissected by removing the periorbital fascia and fixed in 10% neutral buffered formalin for 48 hours. The tissues were washed overnight under running tap water, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax as per method suggested by Drury and Wallington (1980). Sections of 4-5  $\mu$  thickness were cut by Leica Microtome, Japan, and stained with Hematoxylin and Eosin for general histo-architecture. A methyl green pyronin-Y method was applied to identify the plasma cells (Kurnick, 1955).

## Results and Discussion

Light microscopic examination in general revealed that the Harderian gland is a compound tubulo-acinar gland covered by a thin connective tissue capsule from which the fine septa arises, that divided the parenchyma of the gland into lobules of unequal size. This finding of the present study is in agreement with the earlier reports of Wight *et al.* (1971) in domestic fowl, Maxwell *et al.* (1986) in turkey, Krause and McMenamin (1992) in North American opossum, Kozlu and Altunay (2011) in quail, Mobini (2012) in chicken and Dimitrov (2012) in Mongolian pheasant. Blood vessels and nerve fibres were found to pass through the capsule and its septa. This observation is similar to previous findings of Brobby (1972) in domestic duck, Boydak and Aydin (2009) in domestic geese, Kozlu *et al.* (2010) in osprey. The present study revealed that on the 3<sup>rd</sup> day of hatch, gland had a distinct capsule, septa and parenchyma. The parenchyma was consisted of well developed acini and tubules. The structure of lobule of the gland became apparent. Connective tissue fibres were found to penetrate the gland but gave incomplete lobulations at few places. Blood vessels showed marked increase along connective tissue strands and within the interacinar spaces (Fig. 1). Similar findings were noted by Onyeanus *et al.* (1993) in guinea fowl. At 10<sup>th</sup> day, glandular parenchyma showed distinct lobulations. The interlobular septae made up of connective tissue were thinner than capsule. The acini located at the periphery of the lobules were seen clustered around the secondary collecting tubules, which were connected by tertiary collecting tubules and opened into a main collecting duct (Fig. 2). This observation of the present study is identical to the reports of Wight *et al.* (1971) in domestic fowl, Maxwell *et al.* (1986) in turkey, Indu *et al.* (2014) in White Pekin ducks and Frahm and Mohammadpour (2015) in ostrich. The acini were lined by columnar epithelial cells of varying height with basally located nucleus. The primary as well as secondary excretory ducts were lined by a single layer of epithelium varying from columnar to cuboidal (Fig. 3) Similar observations were noted by Kozlu and Altunay (2011) in quail, Mobini (2012) in chicken and Frahm and Mohammadpour (2015) in ostrich. According to the staining affinity, there were two types of cells



**Fig. 1.** Photomicrograph of the Harderian gland of 3 day old chick showing incomplete lobulation and blood vessels along connective tissue strands as well as within interacinar spaces (H&E X100)

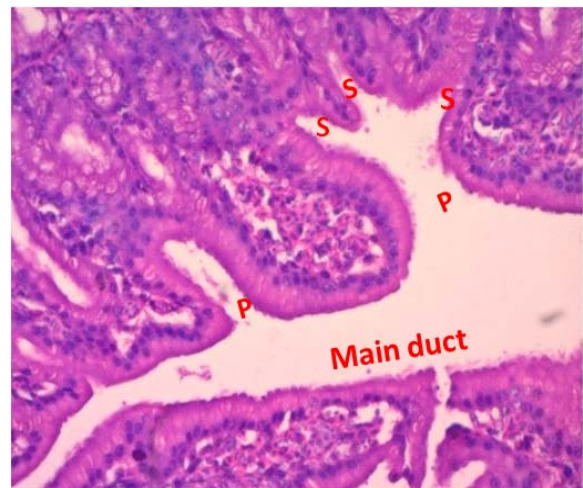


**Fig. 2.** Photomicrograph of the Harderian gland of 10 day old chick showing primary duct (P), secondary duct (S) and main collecting duct at centre and acini (a) situated at the periphery of lobule (H&E X100)

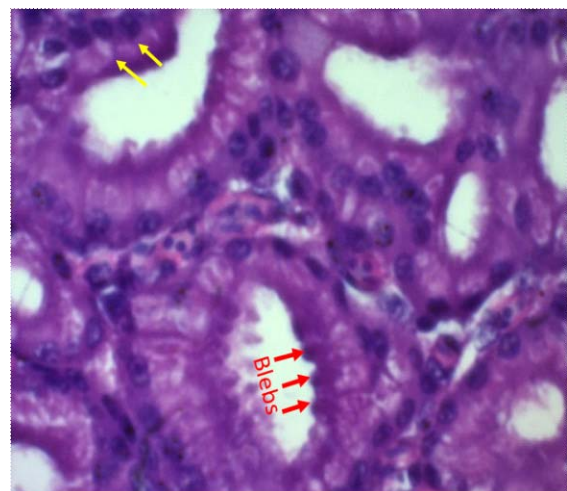


seen in the epithelium i.e. light cells (highly vacuolated) and dark cells (less vacuolated). Blebs of secretory material of the acinar cells were observed at luminal surface of epithelium (Fig. 4). Secretory vacuoles were found to secrete its secretion by exocytosis from the apical portion of cell which indicates the exocrine function of the organ. Secretory mode of cells was apocrine. Lumen of the acini and ducts were filled with the secretory materials. This observation of present study is in agreement with observations recorded by Pradidarcheep *et al.* (2003) in tree shrew and Frahm and Mohammadpour (2015) in ostrich. By day 17, villiform projections protruded into the lumen of ducts and few goblet cells were seen in the epithelial lining of ducts (Fig. 5). The connective tissue of the villi replaced by lymphoid infiltrations along entire course of ducts. This observation of the present study is in agreement with the earlier report of Burns and Maxwell (1979) in domestic fowl. Intra-lobular ducts were lined by single layer of columnar epithelial cells and showed presence of remnants of cells with nuclei in the lumen (Fig. 6). Disintegration of cells denoted holocrine mode of secretion. This observation of the present study is in agreement with the findings of Kozlu *et al.* (2010) in osprey and Hussein *et al.* (2015) in guinea pig. The acini were surrounded by a discontinuous layer of flattened myoepithelial cells in close association with the basal surface of epithelium. Similar findings were reported by Eltony (2009) in female rat. The interstitial tissue of the gland consisted of variety of cells i.e. plasma cells, lymphocytes, fibroblasts, macrophages, mast cells and erythrocytes in which plasma cells were more in number. These cells were diffusely scattered and occupy mostly the central part of the lobules of the gland and interstitial tissue between the surface epithelium lining the main collecting lumina and the acini, while a far smaller number of them were situated at the periphery, between the acini of the gland, connective tissue septa and near blood vessels (Fig. 7). These findings of the present study are in agreement with the observations of Onyeausi *et al.* (1993) in guinea fowl, Bejdic *et al.* (2014) and Pawar *et al.* (1998) in laying birds. The cytoplasm of the plasma cells was pale pink in color with light bluish nuclei noted by methyl green pyronin-Y staining. There was increase in number of plasma cells and the lymphocytes with advancement of the age. The cyst formation was noticed in the lining epithelium of the main collecting tubule at 17 and 24 day old chicks (Fig. 8).

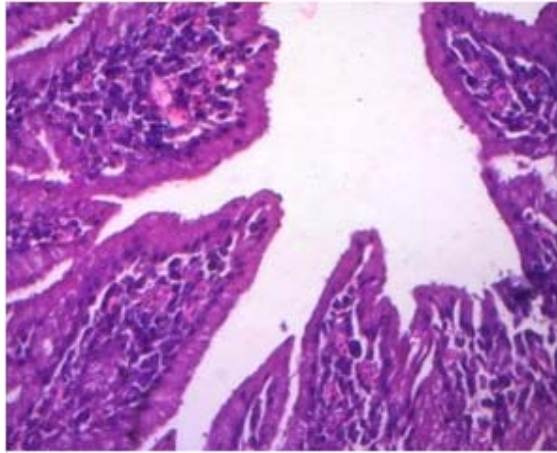
This study concluded that number of the plasma cells increased with increase in age of birds since plasma cells participate in the mechanism of the local immune system because of which Harderian gland acts as important immunopotent organ. The mode of secretion of the gland was apocrine



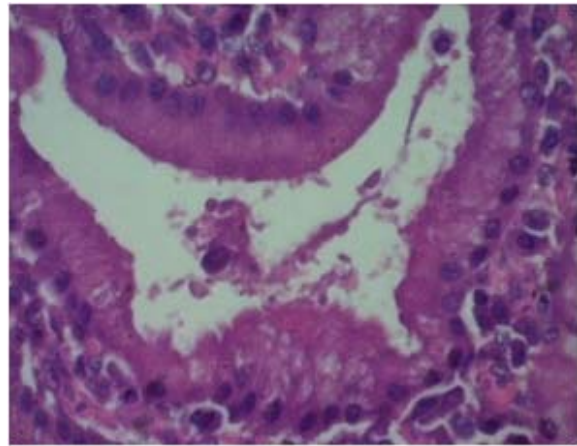
**Fig. 3.** Photomicrograph of the Harderian gland of 10 day old chick showing columnar epithelium in the primary (P) and secondary duct (S). The main duct is lined by high cuboidal epithelium (H&E X400)



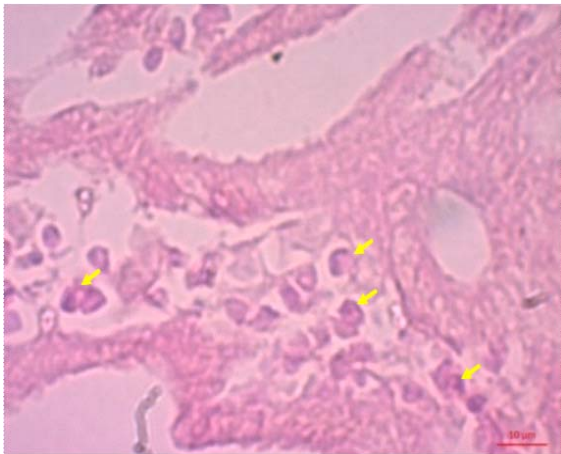
**Fig. 4.** Photomicrograph showing dark and light cells (arrow) in the epithelium and cytoplasmic blebs on the luminal surface epithelium of acini in the Harderian gland of 10 day old chicks (H&E X1000)



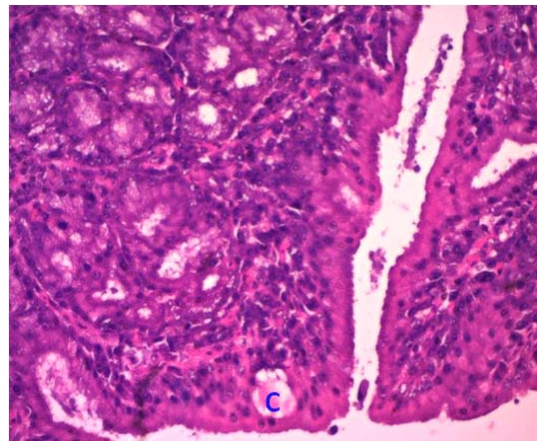
**Fig. 5.** Photomicrograph of the Harderian gland of 17 day old chick showing villiform projection of the tubule at the centre of lobule (H&E X400)



**Fig. 6.** Photomicrograph of the Harderian gland of 17 day old chick showing intralobular duct lined by columnar epithelium and containing remnant of cell with its nuclei in lumen (H&E X 1000)



**Fig. 7.** Photomicrograph of the Harderian gland of 24 day old chick showing plasma cells in subepithelial region of the duct (Methyl green pyronin-Y X 1000)



**Fig. 8.** Photomicrograph of the Harderian gland of 24 day old chick showing cyst (C) at the tip of the tubule (H&E X 400)

as well as holocrine. Apocrine mechanism of the secretory units was observed in earlier post hatch developing gland while it become holocrine with advancement of the age.

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**Conflict of Interest:** All authors declare no conflict of interest.

### References:

- Baba, T., Kawata, T., Masumoto, K. and Kajikawa, T. (1990). Role of the Harderian gland in immunoglobulin A production in chicken lacrimal fluid. *Res. Vet. Sci.* **49**: 20-24.
- Bejdic, Pamela, Avdic, Rizah, Amidzic, Ljiljana, Cutahija, Velida, Tandir, Faruk and Hadziomerovic,

Nedzad (2014). Developmental changes of lymphoid tissue in the Harderian gland of laying hens. *Mac. Vet. Rev.* **37**(1) : 83-88.

Boydak, M. and Aydin, M.F. (2009). Histology of the Harderian gland of domestic geese (*Anser domesticus*). *Acta. Vet. Brno.* **78**: 199-204.

Brobbly, G.W. Snr. (1972). On the Harderian Gland of the Domestic Duck (*Anas platyrhynchos*) Morphological and Histochemical Investigations. *Z. Zellforsch.* **133**: 223-230.

Burns, R.B. and Maxwell, M.H. (1979). The structure of the Harderian and lacrimal gland ducts of the turkey, fowl and duck. A light microscope study. *J. Anat.* **128**(2): 285-292.

Dimitrov, D.S. (2012). Histometric investigation of the third eyelid gland in Mongolian Pheasants (*Phasianus Colchicus Mongolicus*). *Bulgarian Journal of Veterinary Medicine* **15**(3): 160 – 165.

Drury, R.A.B. and Wallington, E.A. (1980). Carleton's Histological Technique. 5th edn., Oxford University Press, New York.

Eltony Sohair A.M. (2009). A comparative study of the Harderian gland in the female rat and female rabbit (A Histological, Histochemical, Scanning Electron Microscopic and Morphometric Study) *Egypt. J. Histol.* **32**(1): 46-65.

Fix, A.S. and Arp, L.H. (1991). Morphologic characterization of conjunctiva associated lymphoid tissue in chicken. *Am. J. Vet. Res.* **52**: 1852–1859.

Frahmand, S. and Mohammadpour, A.A. (2015). Harderian gland in Canadian ostrich (*Struthio camelus*): a morphological and histochemical study. *Anat. Histol. Embryol.* **44**(3): 178-185.

Hussein, Ola A., Elgamal, Dalia A. and Elgayar, Sanaa A.M. (2015). Structure of the secretory cells of male and female adult guinea pigs Harderian gland. *Tissue and Cell* **47**: 323-335.

Indu, V.R., Lucy, K.M., Sreeranjini, A.R., Maya, S., Ashok, N. and Chungath, J.J. (2014). Gross and Histological Studies on the Harderian gland of White Pekin Ducks (*Anas platyrhynchos*). *Indian Journal of Veterinary Anatomy* **26**(1): 25-26.

Khan M.Z.I., Jahan, M.R., Islam, M.N., Haque, Z., Islam, M.R. and Kon, Y. (2007). Immunoglobulin (Ig)-containing plasma cells in the Harderian gland in broiler and native chickens of Bangladesh. *Tissue and Cell* **39**: 141-149.

Kozlu, T., Bozkurt, Y.A., Altunay, H. and Sari, E.K. (2010). Histological and histochemical studies on the Harderian gland of the osprey (*Pandion haliaetus*). *Journal of Animal and Veterinary Advances* **9**(13): 1875-1879.

Kozlu, T. and Altunay, H. (2011). Light and Electron Microscopic Studies of the Quail (*Coturnix coturnix*) Harderian Gland. *Journal of Animal and Veterinary Advances* **10**(7): 932-938.

Krause, W.J. and McMenamin, P.G. (1992). Morphological Observations on the Harderian Gland of the North American Opossum (*Didelphis virginiana*). *Anatomy and Embryology* **186**: 145-152.

Kurnick, N.B. (1955). Pyronin Y in the methyl green pyronin histological stain. *Stain Technol.* **30**: 213-230.

Maxwell, M.H., Rothwell, B. and Burns, R.B. (1986). A fine structural study of the turkey Harderian gland. *J. Anat.* **148**: 147-157.

Mobini, B. (2012). Histological and histochemical studies on the Harderian gland in native chickens. *Veterinarni Medicina* **57**(8): 404–409.

Onyeasuasi, B.I., Ema, N.I., Ezeokoli, C.D. and Onyeasuasi, J.C. (1993). The structure of the Harderian Gland of the Guinea Fowl at embryonic and post embryonic stages. *Anat. Histol. Embryol.* **22**: 183-190.



Pawar, A., Venkatkrishnan, A., Vijayaragavan, C. and Ramkrishna, V. (1998). Histological study of the Harderian gland of White Leghorn with reference to plasma cells. *Indian J. Anim. Sci.* **68**: 141-142.

Payne, A.P. (1994) The Harderian gland: a tercentennial review. *J Anat.* **185**: 1-49.

Pradidarcheep, W., Asavapongpatana, S., Mingsakul, T., Poonkhum, R., Nilbu-nga, S. and Soman, R. (2003). Microscopic Anatomy of the orbital Harderian gland in the common tree shrew (*Tupaia glis*) *Journal of Morphology* **255**(3):328–336.

Shirama, K., Satoh, T., Kitamura, T. and Yamada, J. (1996). The avian Harderian gland: morphology and immunology. *Microsc. Res. Techniq.* **34**: 16-27.

Wight, P.A.L., Burns, R.B., Rothwell, B. and Macken, G.M. (1971). The Harderian gland of the domestic fowl I. Histology, with reference to the genesis of plasma cells and Russell bodies. *J. Anat.* **110**(2): 307-315.

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