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Pre-hatched Developmental Changes of Harderian Gland in Chicken

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ABSTRACT

The present study aimed to explore histogenesis of the Harderian gland of chicken from the 11th to 20th day of incubation. The Harderian gland was situated in the orbit, postero-medial to the eyeball in the embryo and loosely attached by periorbital fascia to the underlying muscles. The primordial Harderian gland appeared as an aggregation of undifferentiated cells on either side between interorbital septum and the retina, on 11th day of incubation. By the 16th day of incubation, gland was seen as elongated tube with little contortion of the lumen bounded by epithelial cells which subsequently became large on the 18th day of incubation. The gland appeared as branched tubular structure and gave rise to out pocketing in the mesenchyme. At the 20th day of incubation, the Harderian gland was seen to be a branched tubuloacinar type surrounded by thin layer of connective tissue. The acini formation was well defined and intralobular ducts with large lumen were seen in the parenchyma.

Keywords: Histogenesis, Harderian gland, Chicken, Mesenchymal cells

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 (Nasrin *et al*., 2013; Shirama *et al*., 1996). It also represents an important part of the immune barrier CALT- Conjunctiva Associated Lymphoid Tissue (Payne, 1994; Pawar *et al.,* 1998; Khan *et al.,* 2007). Harderian gland of chicken present macrophages, lymphocytes, granulocytes in the subepithelial layer and lumina of the lobules for the local immunity of the eye orbit (Baba *et al.,* 1990). In recent years, great morphological and histological studies have been carried out on the Harderian gland during post hatch period in many avian species (Altunay and Kozlu, 2004; Dimitrov and Nikiforov, 2005; Boydak and Aydin, 2009). Pre-hatch study of the Harderian gland in White Leghorn birds at different incubation was done by Pawar *et al.* (1999). However, relatively scanty information is available regarding the histogenesis of the Harderian gland of chicken. Therefore, the present study was contemplated to elucidate the histomorphological changes during pre-hatched development of Harderian gland of chicken.

MATERIALS AND METHODS

To investigate the structural organization of the Harderian gland in pre-hatched developing chicken, incubated fertile eggs were collected on the 11th, 12th, 13th, 14th, 16th, 18th and 20th day of incubation from poultry farm of Nagpur Veterinary College, Nagpur. The embryos were extracted from the incubated eggs. The collected embryos were washed with normal saline and then fixed in 10 per cent neutral buffered formalin and Bouin's fluid and processed for further study. Due to very small size of the gland, head of the embryo was separated and processed for serial transverse section up to the 16th day of incubation. However, grossly an appreciable size of the gland was seen on the 18th day of incubation on the ventro-medial aspect of the eyeball near the ventral oblique muscle. On the

18th day and 20th day of incubation, gland was dissected and processed for histological sectioning. Sections were cut at 5-7µ thicknesses by Leica Microtome, Japan, and stained with haematoxylin and eosin for general histo-architecture, Masson's trichrome stain for collagen fibers, Verhoeff's stain for elastic fibres and Gomori's silver stain for reticular fibres (Bancroft *et al*., 1996).

RESULTS AND DISCUSSION

The primordial Harderian gland appeared as an aggregation of undifferentiated cells surrounded by a loose connective tissue sheath on either side between interorbital septum and the retina, posterio-medial to the eyeball on the 11th day of incubation. The primordial cells were surrounded by the spindle shaped flattened differentiating mesenchymal cells (Fig. 1A). Pawar *et al.* (1999) reported that the primordium of Harderian gland appeared as an aggregation of undifferentiated cells medial to retina. They further stated that primordial cells were surrounded by mesenchymal cells at the 6th day of incubation in White Leghorn chicken. In agreement with the observations recorded in the present study regarding first appearance of primordial cells of developing Harderian gland on the 11th day of incubation, Salah El-Din and Dakrory (2015) also reported that the Harderian gland originates between the 11th and 12th day of incubation in chicken.

On the 12th day of incubation, the glandular blastema was found elongated in the form of a primitive tube. Epithelial growth was observed with advanced central canalization. Primordial cells were seen in the form of island, surrounded by mesenchymal cells (Fig. 1B). This primitive island became primitive acini at the periphery bounded by thin connective tissue sheath and joined with primitive tubules. This observation of the present study corresponds with the observation recorded by Pawar *et al.* (1999) in white leghorn chicken. They reported that the primordial cells of the primitive Harderian gland were in the form of island on the 12th day of incubation. These islands were surrounded by mesenchymal cells which became primitive acini joined by primitive tubules.

On the 13th day of incubation, the gland became elongated and consisted of few small opened tubules. The epitheloid cell mass was seen inner to the eye ball, surrounded by thin connective tissue on the 14th day of incubation. Few cells from these clusters of epitheloid cells were shifted at the periphery which became developing acini and vacuole formation found in the centre of cell mass by the degeneration of the mesenchymal cells, later it got converted into the lumen for peripheral epitheloid cells. During this stage, the acinar epithelium was observed to differentiate into glandular epithelium surrounding a lumen and basal epithelial cells layer (Fig. 2A). This

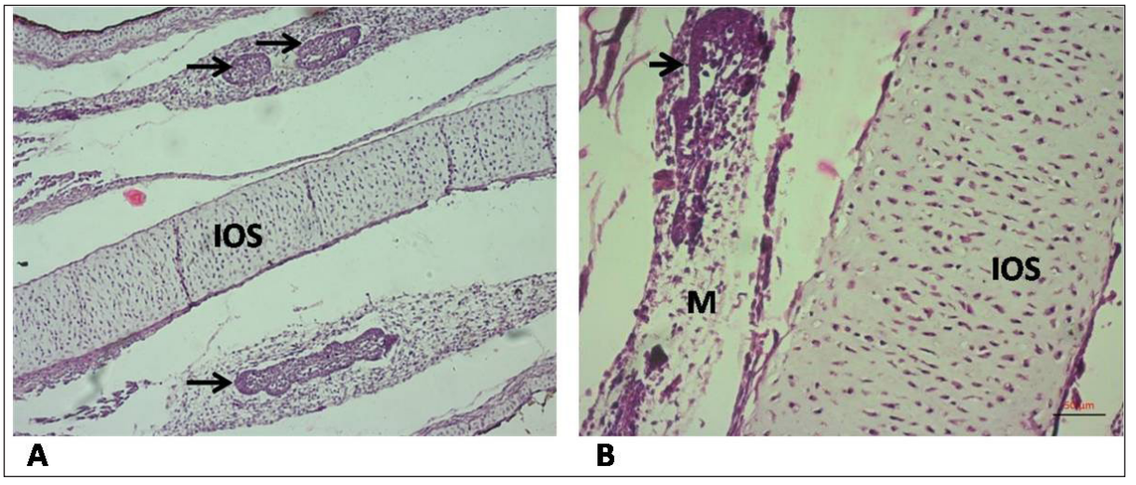

Fig. 1: A: Photomicrograph of transverse section of head region on the 11th day of incubation of chick, showing aggregation of primordial cells of Harderian gland (Arrow) on both side of interorbital septum (IOS) (H&E × 100); **B:** Photomicrograph of transverse section of head region on 12 day incubation of chicken, showing blastema of Harderian gland in the form of primitive tube in the mesenchyme (M) (H&E × 200).

finding of the present study is supported by Baccari (1996) in birds. Similarly in agreement with the findings of the present study, Niedorf and Wolters (1978) reported that the development of Harderian gland can be divided into two phases. They further stated that the acinar epithelium differentiates into a glandular epithelium surrounding lumen and a separate layer of basal epithelial cells between the 13-17 days of incubation in chicken.

By the 16th day of incubation, gland was seen as elongated tube with little contortion of the lumen bounded by epithelial cell layer within the mesenchyme. At the base of the tubules, collection of epithelial cells was seen depicting an acinar gland (Fig. 2B).

On the 18th day of incubation, the gland was seen distinctly outlined with the formation of large contorted lumina bounded by epithelial cells. The external boundary was delineated by thin fibrous connective tissue accompanying with the fibroblasts cells (Fig. 2C). Similar observations were reported by Onyeanusi *et al.* (1993) in guinea fowl, Liman and Gulmez (1996) in geese and Pawar *et al.* (1999) in White Leghorn chicken. The gland appeared as branched tubular structure and gave rise to out pocketing in the mesenchyme or connective tissue which later formed the duct (Fig. 2D). Some of the clusters of the cells surrounded by connective tissue were also seen in the mesenchyme. A very few thin strands of collagen, elastic

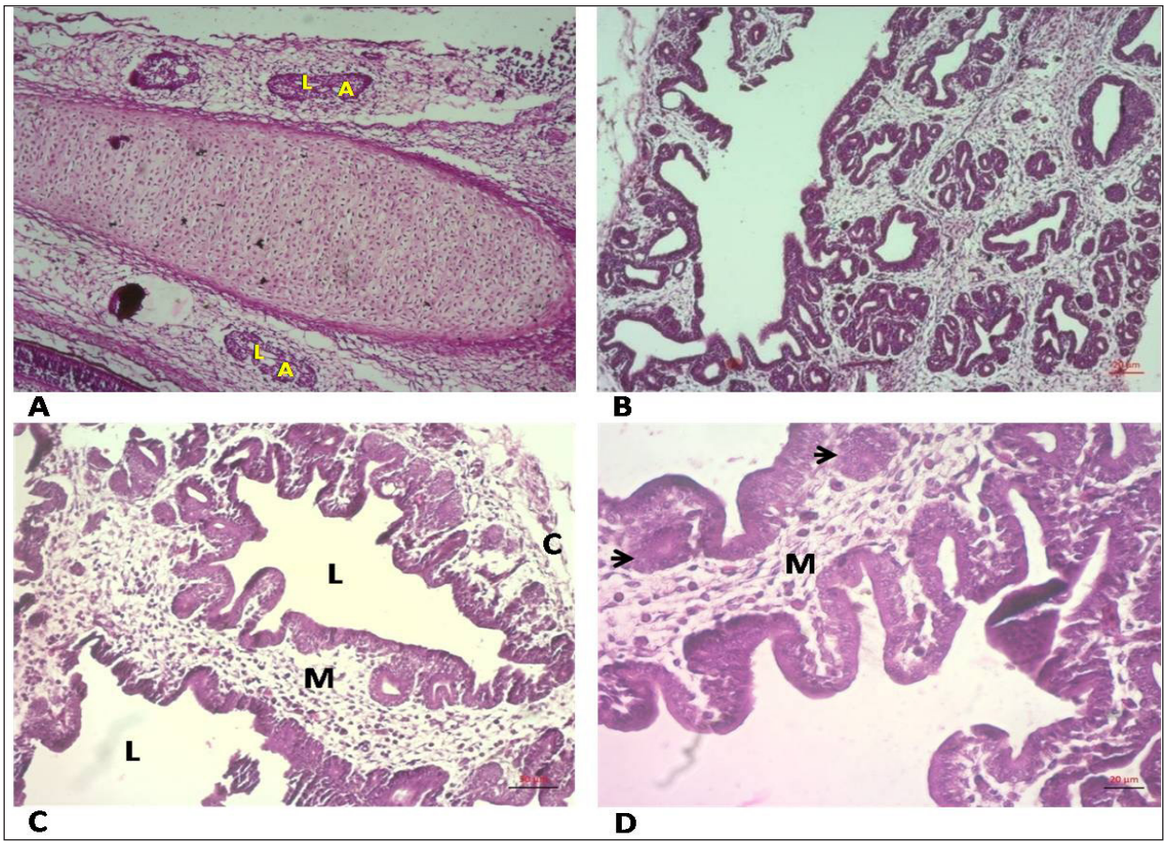

Fig. 2: A: Photomicrograph of transverse section of head region on the 14th day incubation of chicken, showing acinar epithelial cells at periphery (A) and lumen (L) in the center of gland (H&E × 100); **B:** Photomicrograph of transverse section of head region on 16 day of incubation of chicken, showing elongated tube with little contortion of the lumen bounded by epithelial cell layer forming Harderian gland (H&E × 200); **C:** Photomicrograph of Harderian gland of 18 day old embryo of chicken, showing contorted lumina (L) of the epithelial cells in the mesenchyme (M) delineated by connective tissue sheath (C) (H&E × 200); **D:** Photomicrograph of Harderian gland of 18 day old embryo of chicken, showing branched tubular structure (Arrow) that gave out pocketing in the mesenchyme (M) (H&E × 400).

and reticular fibrils were found in the connective tissue sheath. These appearances later on gave rise to primitive lobule formation within the gland. The epithelial cells of the duct appeared in two layers or pseudostratified in most of the areas. Acini and tubules were lined by simple columnar epithelium with basally located nucleus. These observations of the present study corroborates with the findings reported by Onyeanusi *et al.* (1993) in guinea fowl and Pawar *et al.* (1998) in White Leghorn chicken.

At the 20th day of incubation, Harderian gland was more developed compared to the preceding stage. The Harderian gland was seen to be a branched tubuloacinar type surrounded by thin layer of connective tissue. These connective tissue trabeculae were invaginated inside the parenchyma and incompletely divided the Harderian gland (Fig. 3A). The connective tissue capsule of the Harderian gland was made up of collagen, elastic and reticular fibers (Fig. 3B and 3C). These fibers were

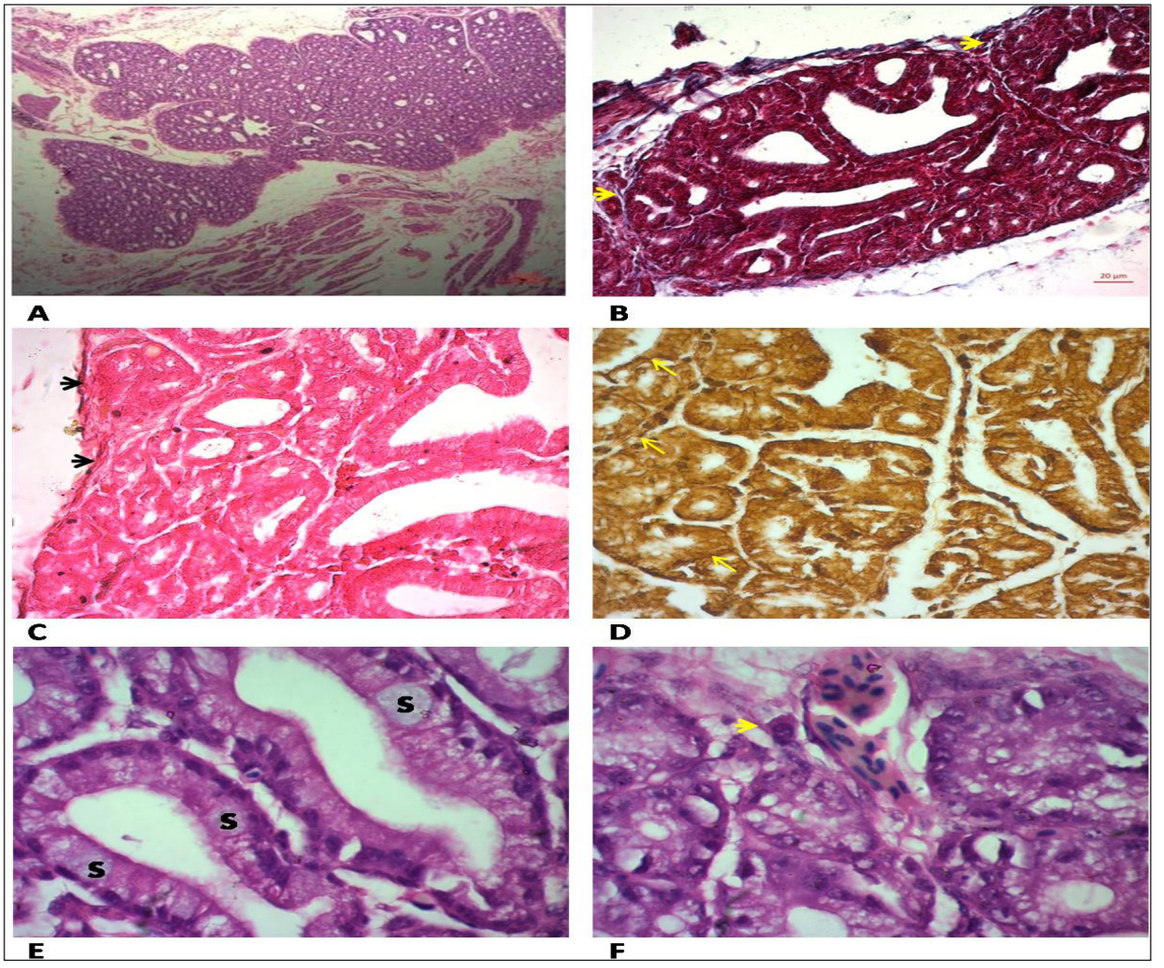

Fig. 3: A: Photomicrograph of Harderian gland of 20 day old embryo of chicken, showing branched tubuloacinar structure surrounded by capsule (H&E × 40); **B:** Photomicrograph of Harderian gland of 20 day old embryo of chicken, showing collagen fibers in the capsule and septa (Masson's trichrome stain × 200); **C:** Photomicrograph of Harderian gland of 20 day old embryo in chicken, showing very thin elastic fibers (Arrow) in the capsule (Verhoeff's stain × 400); **D:** Photomicrograph of Harderian gland of 20 day old embryo of chicken showing thin, short reticular fibrils (Arrow) in the interstitium of gland (Gomori's silver stain × 400); **E:** Photomicrograph of Harderian gland of 20 day old embryo of chicken, showing secretory vesicles (S) in between the epithelial lining of acini (H&E × 1000); **F:** Photomicrograph of Harderian gland of 20 day old embryo of chicken, showing single plasma cell (Arrow) near to the blood vessel (H&E × 1000).

very less in amount. Thin, scanty reticular fibrils were also seen in the interstitium of glandular unit (Fig. 3D). Apparent lobulations were seen. The acini formation was well defined and lined with the single layer of cuboidal epithelial cells. Vacuolation of the epithelial cells was conspicuously observed within their cytoplasm. Some of the large secretory vesicles filled with secretory material were found in between the epithelial lining of the acini (Fig. 3E). Intralobular ducts with large lumen were seen in the parenchyma. The epithelial lining and the tunica propria formed folds, which projected into each lumina and gave branching of the tubule. Very few lymphocytes were seen in the interacinar spaces. Occasionally, single or quite few plasma cells were observed near to the blood vessels along the septa of Harderian gland (Fig. 3F). Contrary to the present finding, Wight *et al.* (1971) and Survashe and Aitken (1978), Onyeanusi *et al.* (1993) and Bejdic *et al.* (2014) found that only after hatch, plasma cells commences first in the Harderian gland of birds.

These observations of the present study indicated that the structural development of the Harderian gland get completed by 20th day of incubation.

In agreement with the findings of the present study, Niedorf and Wolters (1978) reported that glandular epithelium of Harderian gland was found to undergo secretory transformation between 17 and 20 days of embryonic life in chicken that indicated secretory activity of the gland. Onyeanusi *et al.* (1993) also reported similar observations and noted clear formation of acini at 20 days of embryonic life in guinea fowl. They stated that the gland was branched tubulo acinar type and extended deep in to the parenchyma along with some blood vessels near the luminal end. Most of the acini were without lumen surrounded by many epithelial cells with numerous small vesicles within their cytoplasm. However, they did not report the occasional presence of plasma cells near the blood vessels along the septa of Harderian gland which was observed during present study.

CONCLUSION

The present study concluded that the primordial cells as seen to be cluster of epithelial cells in early embryonic stage. The gland contained thin capsule and parenchyma. Parenchyma of the gland consisted of developing acini and highly contorted tubules in the mesenchyme on the 18th day of incubation. The formation of acini and tubules during histogenesis indicate branched tubuloacinar type of gland. The structural development of the Harderian gland gets completed up to the 20 days of incubation. It can be inferred that the functional activity presumably initiated few days before hatching but the immunological function starts only after hatching.

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