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Original Article

Histoarchitectural Observation of the Vagina in Pre-laying and Laying Japanese Quail (*Coturnix Coturnix Japonica)*

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Abstract *- The oviduct was observed histomorphologically in the current study at several age groups during the pre-laying and laying stages. (Age weeks 4 and 5 are prelaying, while weeks 6 and 7 are laying). The tunica mucosa, tunica muscularis, and tunica serosa are the three layers that make up the vaginal wall in birds of all ages, arranged histomorphologically from inside to exterior. Pre-laying birds' vaginal tunica mucosa developed tall, narrow primary mucosal folds that were longitudinally oriented, along with a few minor secondary and tertiary folds. The vaginal mucosal folds in laying birds, however, were large and had tertiary folds. A thin layer of loose connective tissue was known as tunica serosa in all age groups.*

Keywords *- Histomorphology, Histoarchitecture vagina, Quail pre-laying, Laying.*

1. Introduction

The quail's oviduct will be the preferred organ for studying the secretion mechanism of the calcium and protein involved in egg production. Because each quail maintains its wild bird nature and has a unique color pattern on its eggshell, this organ is fascinating even for those studying comparative anatomy. In order to create the calciferous porous egg that is unique to each bird species, the oviduct serves as the last assembly area where albumin and egg envelopes are attached. Information about this organ's histology is therefore crucial.

2. Materials and Methods

The current study was conducted in the Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Parbhani. Using standard quail management practices, 48 female Japanese quail birds (Coturnix coturnix japonica) were raised on the poultry farm of the Department of Poultry Science, College of Veterinary and Animal Sciences, Parbhani, after being purchased from an authorized supplier for the current study.

Twelve birds had their oviducts removed at the end of the fourth, fifth (pre-laying), sixth, and seventh weeks. Using an abdominal laprotomy and sternal displacement in the skull, the whole oviduct—from the infundibulum to the vagina—was extracted from the killed birds. The gathered materials were separated into their component parts and cleansed using regular saline.

The vaginal tissue fragments were preserved in 10% formal saline and 10% neutral buffered formalin. The tissues were then normally paraffin-embedded using the technique described by Drury and Wallington (1980). Sections of 5 m thickness were cut with a rotary microtome and prepared for staining in order to conduct histomorphological analyses.

3. Result and Discussion

In the present study, the histological wall of the vagina showed three layers, viz. tunica mucosa, tunica muscularis, and tunica serosa.

It was observed that in 4th and 5th-week-old birds, tunica mucosa of the vagina formed longitudinally oriented tall and narrow primary mucosal folds with small secondary folds.

The tertiary mucosal folds were few in 4th and 5th week age birds. However, in the 5th week of age, primary mucosal folds were slightly thicker compared to mucosal folds in the 4th week of age (Figures 1 and 2).

In the 6th and 7th weeks of age, the vaginal mucosal folds were extensive with tertiary folds (Figures 3 and 4). It was also observed that in 6th and 7th week birds, some of the vaginal mucosal folds were highly vascularized and, at places, showed blunting with increased cellularity. Also, the epithelial cells were found to be intensely basophilic with a loss of integrity.

These changes in some of the epithelial folds may be ascribed to the adaptive response against physiological adjustment.

In agreement with the present findings, Bansal *et al.* (2010) in Punjab white quail reported primary, secondary and tertiary folds in the vaginal mucosa. Sharaf *et al.* (2013) in ostrich mentioned a few secondary and tertiary vaginal mucosal folds in the pre-laying stage and extensive secondary and tertiary folds in the laying stage. In line with present observations, Das and Biswal (1968), Sari *et al.* (2014) in Duck, Fujii (1981), Bacha and Bacha (2000), Wani *et al.* (2017), Mahajan *et al.* (2021) in chicken, Parizzi *et al.* (2008) in Rhea, Ghule *et al.* (2010) in Japanese quail, Mirhish and Nsaif (2013) in Turkey, Vijayakumar *et al.* (2016) in Emu, Alshammary *et al.* (2017) in Geese reported the similar morphology of vaginal mucosal folds.

The increase in the number and height of vaginal mucosal folds recorded with the advancement of age in the present study may be indicative of the continuation in the growth of vaginal mucosa till the 7^{th} week of age studied in the present work.

In all age groups of birds under the present study, the vaginal mucosal surface was lined by pseudostratified columnar ciliated epithelium. The lining epithelium was composed of four types of cells viz. ciliated cells, non-ciliated cells, goblet cells with basal cells. The ciliated cells were the predominant cells, whereas the goblet cells were very few in the lining epithelium.

The nuclei of the ciliated cells were large, spherical to oval, elongated, and apically placed, whereas the nuclei of non-ciliated cells were central to basal in position. The basal cells were smaller in size, with small spherical to oval nuclei.

These observations of the present study are in line with the reports made by Bansal *et al.* (2010) on Punjab white quail. They mentioned the ciliated, non-ciliated, goblet, and basal cells in the vaginal pseudostratified ciliated columnar epithelium. Sharaf *et al.* (2013) in ostrich reported ciliated, non-ciliated and basal cells in the lining pseudostratified ciliated columnar epithelium of the vagina.

The presence of pseudostratified ciliated columnar cells with few goblet cells in vaginal mucosa was also reported by Bacha and Bacha (2000) in chicken, Priedkalns and Leiser (2006), Samuelson (2007) in avian and Parizzi *et al.* (2008) in Rhea. The findings of the present study are in agreement with the earlier reports of Ghule *et al.* (2010) in Japanese quail, Mirhish and Nsaif (2013) in Turkey, Sari *et al.* (2014), Essam *et al.* (2016) in Duck, Vijayakumar *et al.* (2016) in Emu and Alshammary *et al.* (2017) in Geese. These researchers stated that the vaginal mucosa was lined by pseudostratified ciliated columnar epithelium. The present observation also matches with the reports of Fujii (1981), who reported more ciliated cells in the vaginal epithelium of chicken.

The observations recorded in the present study are partially in range with the reports made by Bansal *et al.* (2010) on Punjab white quail and Deka *et al.* (2018) on Pati and Chara Chemballi duck.

In all age groups of birds under the present study, it was observed that the lamina propria-submucosa formed the core of mucosal folds. The lamina propria-submucosa was composed of loose connective tissue with collagen fibers, reticular fibers, elastic fibers, fibroblasts, lymphocytes, plasma cells, leucocytes, and blood vessels with few smooth muscle fibers.

In all age groups of birds**,** the collagen fibers were predominant fibers in the loose connective tissue of lamina propria-submucosa. The collagen fibers were arranged around the blood vessels and also formed a layer below and along the mucosal epithelium.

The intermingled wavy reticular fibers in the vaginal lamina propria- submucosa formed a distinct layer below the mucosal lining epithelium and were observed in the wall of blood vessels. The reticular fibers were found to be decreased in the lamina propria-submucosa with the advancement of age. However, the collagen fibers were found to be increased with the advancement of age. The elastic fibers were few and loosely arranged in vaginal lamina propria-submucosa in all age groups of birds.

In the present study, increased vascularisation in the lamina propria-submucosa was observed with the advancement of age. The glands were found to be absent in the vaginal lamina propria-submucosa in all age groups of birds in the present study.

In accordance with the present observations, Priedkalns and Leiser (2006) in Avian reported that the vaginal lamina propria-submucosa was composed of loose connective tissue with lymphocytes, plasma cells, and granulocytes. Samuleson (2007) in Avian and Mirhish and Nsaif (2013) in Turkey reported the presence of immune cells in the lamina propria-submucosa of the vagina. In line with present findings, Vijayakumar *et al.* (2016) in Emu, Deka *et al.* (2018) in Duck reported similar composition of vaginal lamina propria-submucosa. Sharaf *et al.* (2013) in ostrich stated that the vaginal lamina propria-submucosa was highly cellular with dense collagenous connective tissue without glands.

Present findings agree with the earlier reports made by Bansal *et al.* (2010) in Punjab white quail, Essam *et al.* (2016), Deka *et al.* (2018) in Duck, Alshammary *et al.* (2017) in Geese, Vijayakumar *et al.* (2016) in Emu, Parto *et al.* (2011)

in Turkey and Samuelson (2007) in avian. They reported that the vaginal lamina propria-submucosa was devoid of glands.

The present observations disagree with the reports of Lucy and Harshan (2011) on Japanese quail and Saber *et al.* (2009) on ostrich. They reported the lymphoid aggregates in the vaginal lamina propria-submucosa. This difference may be attributed to the immune status of birds.

The presence of a distinct layer of reticular fiber below the mucosal lining epithelium confirms the reticular lamina, the integral part of the epithelial basement membrane.

The invaginating mucosal surface and the later formed tubular structure were initially composed of a pseudostratified type epithelial layer, which later on transformed into the unilaminar lining epithelium of the sperm host gland.

In all age groups of birds under the present study, the tunica muscularis was well-developed and thicker than other segments of the oviduct. The tunica muscularis was composed of inner circularly and outer longitudinally arranged smooth muscle fiber with interposed collagen reticular and elastic fibers with blood vessels. The inner circular layer of tunica muscularis was thicker with compactly arranged smooth muscle fibers than the outer longitudinal layer. The outer longitudinal layer showed the presence of large blood vessels (Figures 1 to 4).

The findings of the present study are in agreement with the Priedkalns and Leiser (2006), Samuelson (2007), Ghule *et al.* (2010), Vijayakumar *et al.* (2016), Alshammary *et al.* (2017), Wani *et al.* (2017) and Deka *et al.* (2018). They reported that the tunica muscularis of the vagina was composed of inner circular and outer longitudinal layers of smooth muscle fibers in different avian species. In accordance with present findings, Bacha and Bacha (2000) in chicken and Parto *et al.* (2011) in Turkey stated that the inner circular layer of tunica muscularis in the vagina was strongly developed. Similar reports were made by Das and Biswal (1968) in Duck and Parizzi *et al.* (2008) in Rhea Bird. They reported that the tunica muscularis in the vagina was greatly developed and thicker.

The tunica serosa was a thin layer composed of loose connective tissue with collagen, reticular, elastic fibers, with blood vessels. The observations of the present study agree with the reports of Priedkalns and Leiser (2006), Samuelson (2007) in avian, Ghule *et al.* (2010) in Japanese quail, Essam *et al.* (2016), Deka *et al.* (2018) in Duck and Alshammary *et al.* (2017) in Geese. These researchers stated that the tunica serosa was composed of loose connective tissue.

4. Summary and Conclusion

Histomorphological observations of the vagina were made in the current study across a range of age groups during the pre-laying and laying stages. The oviduct, which extended from the cranial to the caudal aspect, was comprised of the uterus, vagina, magnum, isthmus, and infundibulum. Its diameter varied.

According to histomorphology, the vaginal wall in birds of all ages was made up of three layers: the tunica mucosa, tunica muscularis, and tunica serosa, which were arranged from interior to exterior.

The tunica mucosa was lined with simple columnar ciliated to pseudostratified columnar ciliated with secretory goblet cells in various oviduct segments. The mucosal folds ranged in shape from low to high and had subfolding. The lamina propria and submucosa's loose connective tissue served as the mucosal folds' central structure. At different age groups, the thickness of the tunics varied in different areas of the oviduct.

Prior to laying, the tunica mucosa vagina generated tall, narrow primary mucosal folds that were longitudinally oriented, accompanied by modest secondary folds and few tertiary folds. The vaginal mucosal folds in laying birds, however, were large and had tertiary folds.

The number and height of the mucosal folds in the vagina significantly increase with age, which may indicate that the mucosa continues to grow and improve physiological functioning until the final age week examined in this work.

All age birds had pseudostratified columnar ciliated with non-ciliated and secretory cells lining the neck portion of the vagina. The loose connective tissue that made up the lamina propria and submucosa included collagen and reticular fibers, fibroblasts, lymphocytes, plasma cells, and blood vessels with only a small number of elastic fibers.

Invagination and proliferation of surface epithelial cells resulted in the formation of glands within the submucosa and lamina propria's underlying connective tissue. The tall, simple columnar epithelium at the uterovaginal junction bordered the tubular coiled sperm host glands across the lamina propria-submucosa of mucosal folds, with a conspicuous nucleolus and round to oval basally positioned nuclei.

The loose vascularized connective tissue in the vagina separated the inner circular and outer longitudinal layers of the tunica muscularis. In the vagina, the tunica muscularis was thickest. The continuing growth of the organ until the seventh week of life may be the cause of the gradual rise in tunica muscularis thickness with age. Additionally, it may provide support for a robust, regular contraction of the tube wall.

In all age groups, tricuspid serosa was a thin layer of loose connective tissue in the vagina.

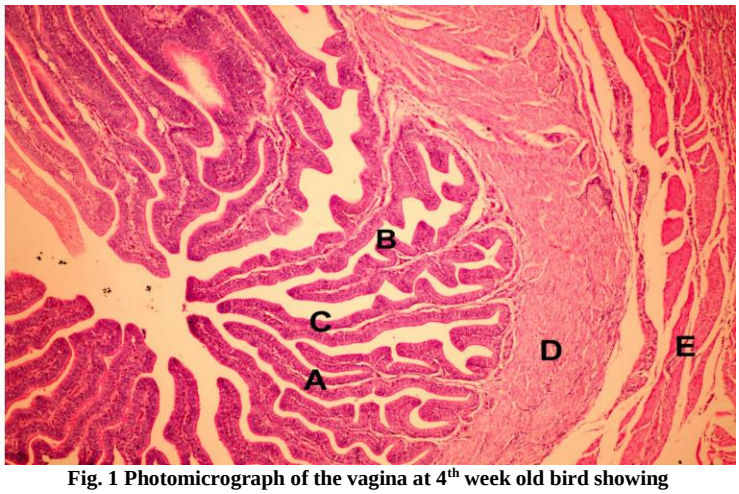

Fig. 1 Photomicrograph of the vagina at 4th week old bird showing
A. Primary mucosal folds, B. Secondary mucosal folds, C.Tertiary mucosal fold, D. Inner circular layer of tunica muscularis, E.Outer longitudinal layer of tunica muscularis (Hematoxylin and Eosin, X 100)

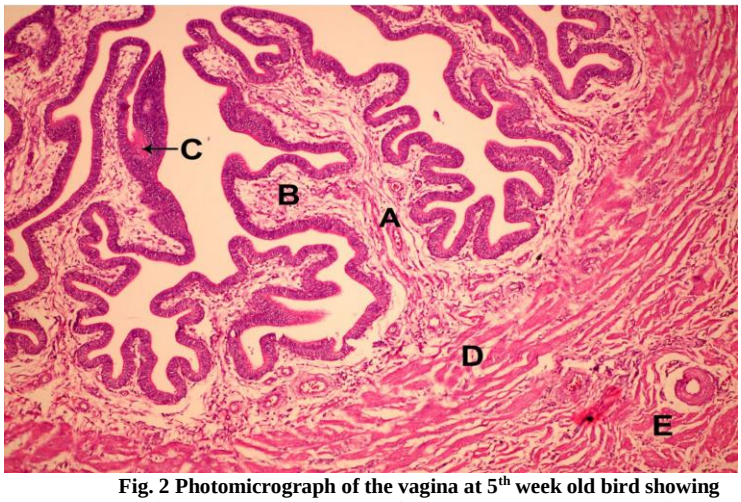

Fig. 2 Photomicrograph of the vagina at 5th week old bird showing
A. Primary mucosal folds, B. Secondary mucosal folds, C. Tertiary mucosal fold, D. Inner circular layer of tunica muscularis, E.Outer longitudinal layer of tunica muscularis. (Hematoxylin and Eosin, X 100)

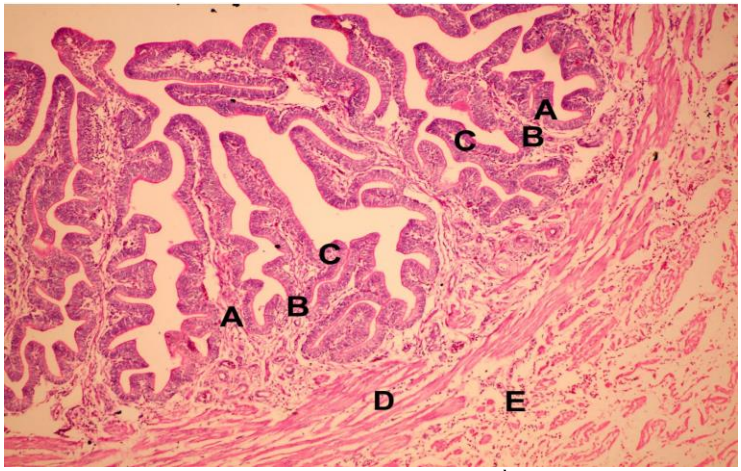

Fig. 3 Photomicrograph of the vagina at 6th week old bird showing
A. Primary mucosal folds. B. Secondary mucosal folds.C. Tertiary mucosal fold, D. Inner circular layer of tunica muscularis, E. Outer longitudinal layer of tunica muscularis (Hematoxylin and Eosin, X 100)

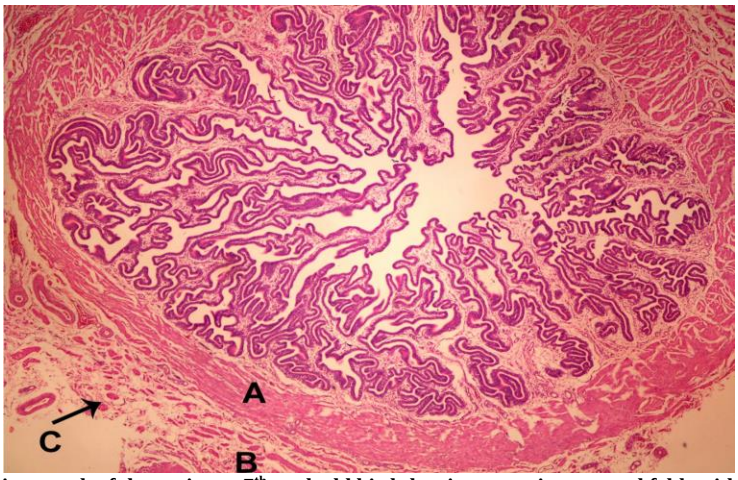

Fig. 4 Photomicrograph of the vagina at 7th week old bird showing extensive mucosal folds with tertiary folds
A. Inner circular layer of tunica muscularis, B. Outer longitudinal layer of tunica muscularis, C. Tunica serosa (Hematoxylin and Eosin, X 40)

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