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Research Article

HISTOMORPHOCHEMICAL STUDIES ON THE MAGNUM OF PUNJAB WHITE QUAILS

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ABSTRACT

The present work was conducted on the magnum of 42 Punjab white quails from 8 weeks to 32 weeks of age to study histological, micrometrical and histochemical details. The tissue samples were fixed in 10% Neutral Buffered Formalin, processed and stained to observe histomorphological and histochemical details. The results revealed that there were mainly tall and broader primary folds but occasionally secondary folds from 8 weeks onwards. The lamina epithelialis was comprised of simple columnar cells with goblet cells. The nucleus of the ciliated cells were oval and occupied central to apical region of the cell whereas those of globlet cells were round and located at the base. The height of epithelium increased from the 8 to 24 weeks, while decreased at 28 and 32 weeks. The propria submucosa contained long branched coiled tubular glands from 8 week onwards which increased in thickness with age. The proprial glands were of three types depending upon the phase of activity i.e. regenerating, secretory and resting. The tunica muscularis was made up of inner circular and outer longitudinal layers of smooth muscles, whereas serosa was composed of loose connective tissue lined by mesothelium. Histochemical observations depicted moderate PAS-AB and sudanophilic reaction in the epithelium whereas proprial glands showed strong PAS positive activity.The activity of bromphenol blue was reported to be weak in lamina epithelialis and moderate to strong in propria submucosa. The present study showed that the histomorphological and histochemical observations on the magnum of Punjab white quails can be correlated with the reproductive status at different age groups.

Keywords: Histochemistry, Histomorphology, Magnum, Micrometry, Punjab white quail

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The avian oviduct is divided into the five segments i.e. infundibulum, magnum, isthmus, uterus or shell gland and vagina. The developing egg remains for 9 hours in the proximal part (infundibulum, magnum or isthmus) and for 39 hours in the distal part of the oviduct (uterus) during the egg laying cycle (Sharaf *et al.*, 2012). Magnum is the longest region of the avian oviduct and is concerned with the secretion of albumen. The lining epithelium of magnum is generally simple columnar with variable numbers of mucus-secreting and ciliated cells. The lamina propria is filled with long, branched and coiled tubular glands along with small amount of loose connective and diffuse lymphatic tissue (Mirhish and Nsaif, 2013). The findings available on the histomorphochemical studies have been conducted on the magnum of Japenese quail (Lucy and Harshan, 2000), Turkey hens (Mirhish and Nsaif, 2013) and ostrich (Sharaf *et al.*, 2012), but few reports are available on the magnum of Punjab white quail (Bansal *et al.*, 2010). So, the present study was aimed to observe the normal histoachitecture of magnum in Punjab white quail at different age groups.

MATERIALS AND METHODS

The tissue samples were collected from magnum of 42 Punjab white quails (PWQ) of different age groups from poultry farm, GADVASU, Ludhiana. Based on the age, the birds were divided into seven groups (six in each) as 8 weeks (Group I), 12 weeks (Group II), 16 weeks (Group III), 20 weeks (Group IV), 24 weeks (Group V), 28 weeks (Group VI) and 32 weeks (Group VII) as earlier described (Sukhadeve *et al.*, 2019). The tissue samples were collected in 10% NBF and processed as per routine acetone benzene schedule (Luna, 1968). The paraffin sections of 5-6 µm were obtained on glass slides with the help of rotary microtome and were stained with Haematoxylin and Eosin for general histomorphology, Masson's Trichrome for collagen fibres, Gridley's for reticular fibres, Verhoeff's for elastic fibres and Holmes's for neuronal elements. For histochemical studies, Periodic acid Schiff (PAS) and Alcian blue (AB) at pH 2.5 was performed for demonstration of neutral and acid mucopolysaccharides, and bromphenol blue for basic proteins (Chayen *et al.*, 1969). Fresh tissue samples were subjected to cryostat sectioning at 10 µm thickness and incubated to demonstrate the sudanophilic lipids (Pearse, 1972). Micrometrical parameters were recorded on Hematoxylin and Eosin stained sections by means of standard method of micrometry using Nikon 80 i camera mounted microscope with the help of image J software.

RESULTS AND DISCUSSION

Histomorphology: The histological observations showed that magnum was made up of four layers viz tunica mucosa, propria submucosa, tunica muscularis and tunica serosa as described by the Banks (1993) in fowl.

Tunica Mucosa: The mucosal folds were less in number and branched in the magnum than the infundibulum.

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Mostly the primary folds were observed which were tall and broader but occasionally secondary folds were also seen in the birds from 8 weeks onwards. Similar type of foldings were observed in Punjab white quail (Bansal *et al.*, 2010), in Aseel and RIR birds (Lata, 2007), in chicken (Bharti and Gautam, 2013) and in RIR birds (Naragude *et al.*, 1999). Mucosal folds of magnum in Punjab white quail was measured in 8 weeks, 12 weeks, 16 weeks, 20 weeks, 24 weeks, 28 weeks and 32 weeks as 1318.04 ± 18.73 µm, 1436.59 ± 12.48 µm, 1482.61 ± 11.39 µm, 1543.38 ± 15.04 µm, 1592.81 ± 19.77 µm, 1636.57 ± 16.89 µm and 1687.80 ± 13.74 µm, respectively. The mucosal folds length was highest among all the segments of oviduct which increased from the 8 weeks to 28 weeks of age and then decreased after 28 weeks of age. Similar findings have been reported in PWQ by Bansal *et al.* (2010), however Vijyakumar *et al.* (2016) observed lesser mucosal fold length in emu as compared to present observations. This may be due to difference in the flying and non flying birds.

The lamina epithelialis mucosae comprised of simple columnar ciliated type with few goblet cells upto 16 weeks of age (Fig. 1). The epithelium was tall columnar type from 20 weeks of age onwards. The nucleus of the ciliated cells were oval and occupied central to apical region of the cell whereas the nucleus of the globlet cells were round and located at the base of the cell (Fig. 2). Similar type of lining epithelium of magnum was reported in Punjab white quails by Bansal *et al.* (2010), turkey by Parto *et al.* (2011) and in fowl by Eurell and Frappier (2006) and Bharti and Gautam (2013). Punjab white quail measured epithelial height at 8 weeks, 12 weeks, 16 weeks, 20 weeks, 24 weeks, 28 weeks and 32 weeks was 24.62 ± 8.36 µm, 25.47 ± 3.72 µm, 26.28 ± 7.29 µm, 26.53 ± 4.87 µm, 26.92±6.03 µm, 28.16±9.34 µm and 30.12 ± 5.75 µm, respectively. The height of epithelium increased from the 8 weeks to 24 weeks which reduced at 28 weeks and 32 weeks. Similar findings on the epithelial height of magnum were reported in Japanese quail by Mahmud (2014) and Punjab White quail by Bansal *et al.* (2010).

Propria submucosa: This layer contained long branched coiled tubular glands from 8 week onwards. The glands were lined by simple cuboidal epithelium with well-defined lumen filled with secretory material (Fig. 1). With the increase in age, the boundaries of proprial glands were observed and basal nuclei which was shrunken and deeply stained but the cytoplasm was lightly stained (Fig. 3). The proprial glands were highly developed in this region due to which the thickness of lamina mucosa has increased to many folds as reported in pigeon (Mohammadpour and Keshtmandi, 2008), Punjab white quails (Bansal *et al.*, 2010), turkey (Parto *et al.*, 2011) and ostrich (Sharaf *et al.*, 2012). According to the phase of activity, three types of tubular glands were present in the magnum i.e. regenerating, secretory and resting stage as reported by Wani *et al.* (2017) in Kashmir Faverolla Chicken. The glands at secretory stage showed pyknotic and basal nuclei with strongly acidophilic cytoplasm. Whereas that of resting stage had frothy appearance with ill developed lumen (Fig. 4). Similar type of classification has been reported in fowl in the magnal glands by Bacha and Bacha (2012). It was observed that the size of the proprial glands reduced at 28th and 32nd week of age as the amount of connective tissue in lamina propria increased with the age of bird. The increase the interglandular space may be due to the regression process in the oviduct which was in accordance with the Mahmud (2014) in Japanese quail, Bharti and Gautam (2013) in chicken.

Tunica Muscularis: It was made up of inner circular and outer longitudinal smooth muscle layers which were separated by myentric plexus seen in all age groups (Fig. 5). The thickness of tunica muscularis of magnum in Punjab white quail was observed at 8 weeks, 12 weeks, 16 weeks, 20 weeks, 24 weeks, 28 weeks and 32 weeks as 20.76±7.27 µm, 21.82±5.63 µm, 21.57±3.82 µm, 23.20± 4.19 µm, 24.31±6.17 µm, 26.62±5.53 µm and 27.53± 6.98 µm, respectively. Similar findings have been reported in Japanese quail by Mahmud (2014) and Punjab White quail by Bansal *et al.* (2010).

Table 1

Histochemicalobservations on different layers of magnum in Punjab white quail

Histochemical moieties	Tunics of oviduct			
	Lamina epithelialis	Propria submucosa	Tunica muscularis	Tunica serosa
Neutral Mucopolysaccharides	-	+/++	-/+	-/+
Acidic Mucopolysaccharides	+++/++++	+	-/+	-/+
Basic proteins	-/+	++/+++	+/++	+/++
Sudanophilic lipids	++/+++	+/++	-/+	-/+

-No reaction, +mild reaction, ++moderate reaction, +++Strong reaction, ++++Intense reaction

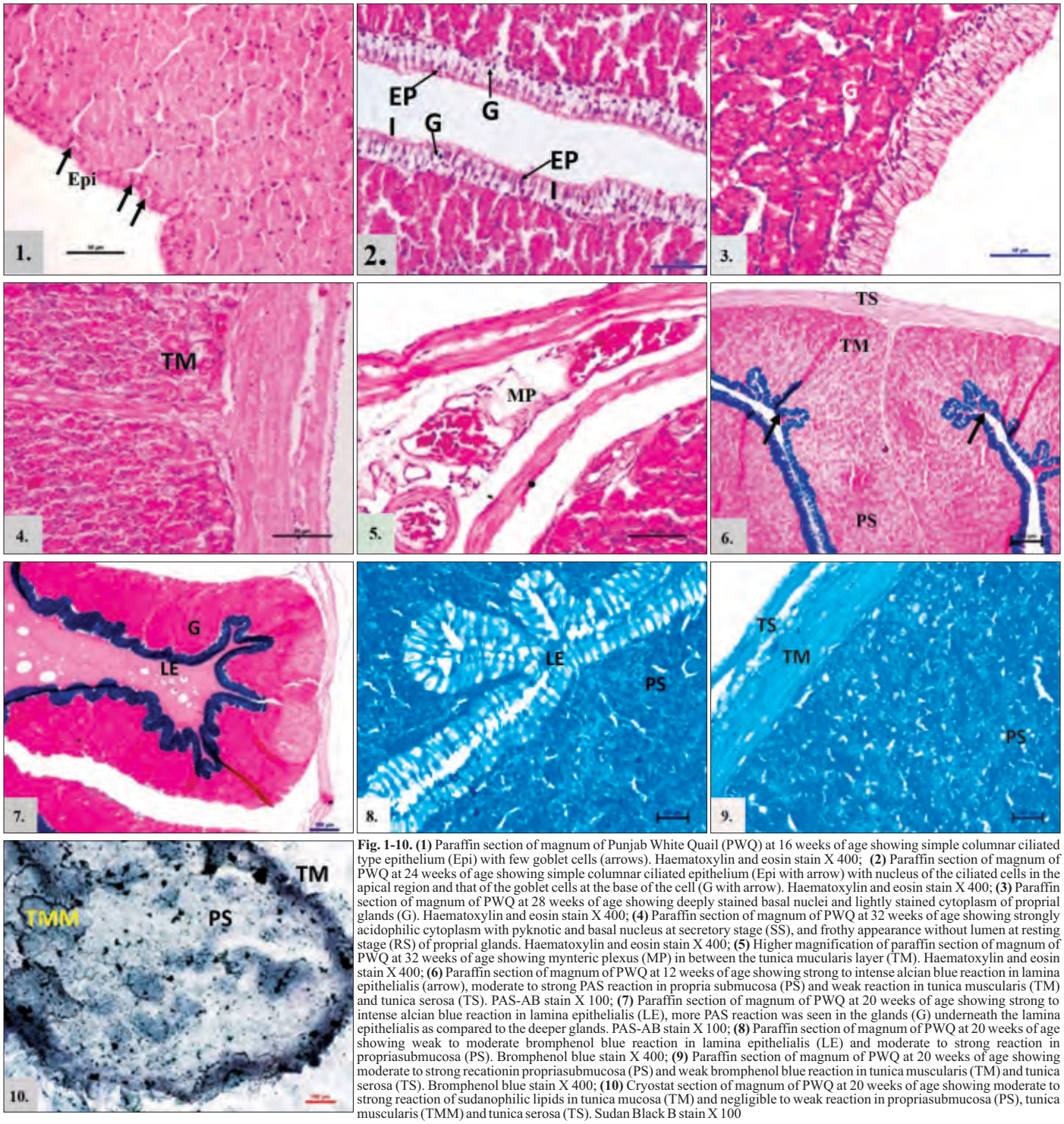

Fig. 1-10. (1) Paraffin section of magnum of Punjab White Quail (PWQ) at 16 weeks of age showing simple columnar ciliated type epithelium (Epi) with few goblet cells (arrows). Haematoxylin and eosin stain X 400; **(2)** Paraffin section of magnum of PWQ at 24 weeks of age showing simple columnar ciliated epithelium (Epi with arrow) with nucleus of the ciliated cells in the apical region and that of the goblet cells at the base of the cell (G with arrow). Haematoxylin and eosin stain X 400; **(3)** Paraffin section of magnum of PWQ at 28 weeks of age showing deeply stained basal nuclei and lightly stained cytoplasm of proprial glands (G). Haematoxylin and eosin stain X 400; **(4)** Paraffin section of magnum of PWQ at 32 weeks of age showing strongly acidophilic cytoplasm with pyknotic and basal nucleus at secretory stage (SS), and frothy appearance without lumen at resting stage (RS) of proprial glands. Haematoxylin and eosin stain X 400; **(5)** Higher magnification of paraffin section of magnum of PWQ at 32 weeks of age showing mynteric plexus (MP) in between the tunica mucularis layer (TM). Haematoxylin and eosin stain X 400; **(6)** Paraffin section of magnum of PWQ at 12 weeks of age showing strong to intense alcian blue reaction in lamina epithelialis (arrow), moderate to strong PAS reaction in propria submucosa (PS) and weak reaction in tunica muscularis (TM) and tunica serosa (TS). PAS-AB stain X 100; **(7)** Paraffin section of magnum of PWQ at 20 weeks of age showing strong to intense alcian blue reaction in lamina epithelialis (LE), more PAS reaction was seen in the glands (G) underneath the lamina epithelialis as compared to the deeper glands. PAS-AB stain X 100; **(8)** Paraffin section of magnum of PWQ at 20 weeks of age showing weak to moderate bromphenol blue reaction in lamina epithelialis (LE) and moderate to strong reaction in propriasubmucosa (PS). Bromphenol blue stain X 400; **(9)** Paraffin section of magnum of PWQ at 20 weeks of age showing moderate to strong recationin propriasubmucosa (PS) and weak bromphenol blue reaction in tunica muscularis (TM) and tunica serosa (TS). Bromphenol blue stain X 400; **(10)** Cryostat section of magnum of PWQ at 20 weeks of age showing moderate to strong reaction of sudanophilic lipids in tunica mucosa (TM) and negligible to weak reaction in propriasubmucosa (PS), tunica muscularis (TMM) and tunica serosa (TS). Sudan Black B stain X 100

Tunica Serosa: It was composed of loose connective tissue lined by mesothelium seen at 8 week of age onwards (Fig. 4). The thickness was measured at 8 weeks, 12 weeks, 16 weeks, 20 weeks, 24 weeks, 28 weeks and 32 weeks as 3.72±0.9 µm, 3.62±0.6 µm, 3.97±0.17 µm, 4.28±0.5 µm, 4.67±0.11 µm, 5.38±0.26 µm and 5.43±0.3 µm, respectively. Similar findings have been reported by Naragude *et al.* (1999) in RIR birds.

Thin and long collagen fibres formed a thin narrow strand in the centre of the core of the mucosal folds extending from the tunica muscularis and in propria submucosa. Few reticular, elastic fibers and fine neuronal elements were seen in the in the propria submucosa, muscularis and serosal layer. Similar findings were also reported by Garg (2006) with thick collagen fibers extending into the core of the mucosal folds. Lata (2007) in RIR and Assel hen and Bharti and Gautam (2013) in chicken also demonstrated similar fibrous components.

Histochemistry: The histochemical reaction of acid and neutral mucopolysachharides, lipids and proteins in different layers of magnum are shown in table 1.

Mucopolysaccharides: The lamina epithelialis showed strong reaction for alcian blue in the magnum of PWQ from 8 weeks of age onwards (Fig. 6) however, PAS reaction could not be demonstrated in lamina epithelialis of magnum of any stage of development. This indicates the presence of acid mucopolysaccharides in the lining epithelium in the magnum (Wani *et al.*, 2017). Proprial glands of magnum showed weak to moderate PAS reaction in the initial age (12 week) whereas activity increased from 20th week of age. The PAS activity was more in the glands underneath the lamina epithelialis as compared to the deeper glands from 16 weeks of age onwards (Fig. 7). Similar findings were reported by Bansal *et al.* (2010) in Punjab white quail, Lata (2007) in Asseel and RIR birds and by Ozen *et. al.* (2009) in Pekin duck.

Basic proteins: The activity of bromphenol blue was reported to be weak in lamina epithelialis, moderate to strong in propria submucosa and weak to moderate in tunica muscularis and tunica serosa (Figs. 8 and 9) at initial stage of development, however it increased in different layers of magnum from 20th week onwards. The lamina epithelialis showed weak activity for basic proteins in the grooves between mucosal folds particularly in supranuclear region (Fig. 8). The yolk present in the lumen, proprial glands, tunica muscularis and tunica serosa showed strong activity for basic proteins from 20th week onwards. Similar activity of proteins was noticed in the cell lining the grooves of mucosal folds in magnum of emu birds by Vijayakumar *et al.* (2016) and Ozen *et al.* (2009) in pekin duck.

Sudanophilic lipids: The lamina epithelialis showed moderate to strong reaction whereas propria submucosa had weak reaction while tunica muscularis and tunica serosa had negligible to weak sudanophilic lipids (Fig 10). The reaction was more in the supranuclear part of lamina epithelialis and secretory blebs which may be due to presence of granules in supranuclear region.

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