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Light and Electron microscopic studies on the sperm host glands in Punjab white quail

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ABSTRACT

The present work was conducted on oviduct of 42 Punjab white quails to observe the histomorphochemical and ultrastructural details of sperm host glands. The tissue samples were collected from the uterovaginal and infundiomagnal junctions of the oviduct in 10% NBF and 2.5% glutaralehyde solutions and processed for histological and electronmicroscopic studies. Fresh tissue samples were collected for cryostat sectioning to demonstrate lipids. It was observed that the UVJ was lined by pseudostratified columnar epithelium with ciliated, non ciliated cells, goblet cells and basal cells. The sperm host glands extended into lamina propria of UVJ as oval, rounded and straight tubules. The proximal part of glandular neck was lined by pseudostratified columnar ciliated epithelium and distal part with non ciliated columnar cells. The ciliated cells showed cilia and microvilli on the apical surface in the neck region, whereas the non ciliated cells present in the distal part of SHG had oval or elongated nuclei in their basal part and were studded with some of the microvilli in their luminal surface. These glands were surrounded by some of the smooth muscle cells and nerve fibers. Histochemical studies revealed a moderate to strong reaction of acid and neutral mucopolysaccharides and lipids whereas reaction for basic proteins was weak in the supranuclear part of SHG.

Key words: Electronmicroscopy, Histochemistry, Histology, Punjab white quail, Sperm host glands.

INTRODUCTION

The spermatozoa are stored in the sperm host glands (SHG) located in the distal half of the oviduct of almost all the avian species and sperms are released from these glands as and when required for fertilization (Bakst, 1993). These were first recognized as sperm nests by Van Drimmelen (1946), vaginal glands by Fuji (1963), sperm glands by Van Krey *et al*. (1967), uterovaginal sperm host glands by Gilbert *et al*. (1968) and sperm storage glands by Burke *et al.* (1972). The spermatozoa are kept viable in these glands for about 30 days in chicken and approximately 7 days in Japanese quail. The fine structure of sperm host glands has been described in fowl (Koyanagi and Nishiyama, 1981), turkey (King *et al.* 1999), chicken (Das, 2003) and domestic duck (Rao and Vijayragvan, 2000), but scanty information is available on the histomorphology of sperm host glands in quail (Bansal *et al*. 2013), so the present study was aimed to elucidate the histomorphochemical and ultrastructral studies on the sperm host glands of Punjab white quail.

MATERIALS AND METHODS

The tissue samples were collected from uterovaginal junction (UVJ) of the oviducts of 42 Punjab white quails (PWQ) of different age groups available at 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). After sacrificing the birds, the tissue samples from infundibulo-magnal junction and utero-vaginal junction were collected in 10% NBF and processed as per Acetone benzene technique (Luna, 1968). The serial sections of 5 µm were cut and stained with hematoxylin and eosin for routine morphology, Masson's trichrome for collagen fibers, Periodic Acid Schiff for neutral mucopolysaccharides and Bromphenol blue for basic proteins. For demonstration of total lipids, cryostat sections were stained with Sudan black B. For electron microscopy, small tissue samples were fixed in Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer solution) for 8-12 hours followed by secondary fixation in 2% osmium tetraoxide for 2 hours. After dehydration, the tissue was embedded in an Epon-Araldite mixture. The ultrathin sections of 70-90 nm thickness were cut and collected on uncoated copper grids. These grids were stained with uranyl acetate for 15 min followed by lead citrate for 10 min and finally examined under transmission electron microscope for detailed study of sperm host glands.

RESULTS AND DISCUSSION

Histology: The epithelium at UVJ was lined by pseudostratified columnar type containing ciliated, non-ciliated, goblet and basal cells. Abundance of tubular glands was present in the lamina propria of mucosal folds with a

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distinct lumen. Sperm-storage tubules were observed in the uterovaginal junction and these tubules were mostly branched and slightly coiled and extended into the lamina propria from the bases of the mucosal folds. Each tubule was lined by a columnar epithelium resting on a basal lamina and was situated in close proximity to blood vessels. These glands contained darkly stained basal nuclei with lightly stained cytoplasm. The staining affinity was more in the older birds. The number of these tubules increased with the age of the quails i.e. these were more at 32 weeks of age than 8 weeks of age (Fig 1 and 2). At infundibulomagnal junction, in between the proprial glands, some lightly stained glands with narrow lumen and morphologically similar to the sperm host glands were also observed. Similar type of sperm host glands were also observed at the infundibulo-magnum junction of domestic quail and their number was higher than in uterovaginal junction (Moraes *et al.*, 2009). But in present study, the number of sperm host glands was more at uterovaginal junction than infundibulomagnal junction as reported earlier by Van Krey *et al.* (1967). This may be related to the sooner contact of oocyte with spermatozoa due to topographic location of ovary as compared to uterovaginal junction, where the spermatozoa takes longer time to reach the infundibulum. Similar findings were reported by Bezuidenhout *et al.* (1995) at uterovaginal junction in ostrich, in hen by Mansori (2004) and Mehta and Guha (2012).

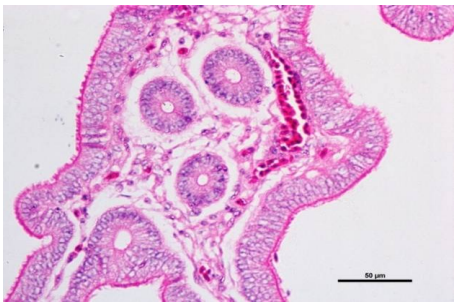

Fig 1: PWQ at 12 weeks of age showing sperm host glands lined by simple columnar type of epithelium. Hematoxylin and eosin stain X 400.

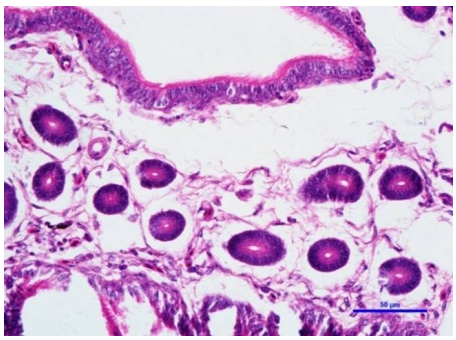

Fig 2: PWQ at 32 weeks of age showing increase in number sperm host glands and different cell types in UVJ epithelium. Hematoxylin and eosin stain X 400.

The glandular grooves could be seen on the surface epithelium of uterovaginal junction. Sperm host glands were interspersed between the uterine glands as tubular invaginations of surface epithelium and were coiled, straight or oval in shape. These glands had proximal and distal parts; the proximal part was lined by pseudostratified columnar epithelium and distal part by non-ciliated simple columnar epithelium (Fig 3). The proximal part was considered as neck region whereas distal part as proper sperm host glands. These findings corroborate well with the observation of Baskt and Bird (1987) in American Kestrel, Frieβ *et al.* (1978) in Japanese quail and Bansal *et al.* (2013) in Punjab white quail. Deka *et al*. (2018) studied the comparative anatomical study of vagina (sperm storage organ) of Pati and Chara-Chemballi ducks during laying periods. They observed that the lining epithelium consisted of pseudostratified columnar epithelial cells with few goblet cells.

Abundance of collagen fibres were observed in the interglandular tissue with fewer amounts of reticular, elastic and nerve fibres. The nerve fibres were demonstrated mostly in the propria submucosa near the sperm host glands as seen by Das (2003) in chicken oviduct. The later author suggested that the innervation to the oviduct plays a functional role in storage and release of spermatozoa in the sperm host glands.

Histochemistry: Histochemical studies revealed moderate to strong reaction of acid and neutral mucopolysaccharides in the supranuclear part of SHG (Fig 4 and 5). Intense activity of neutral mucopolysaccharides was seen in lamina epithelialis and sperm host glands and intense activity acid mucopolysaccharides propria submucosa and basement membrane of sperm host glands. The non-ciliated cells of lining epithelium of uterovaginal junction showed a strong reaction for PAS and AB/PAS as reported in the surface epithelium and sperm storage tubules (SST) in American Kesrel (Baskt and Bird, 1987) and in quail (Bansal *et al.* 2013). Deka *et al*., (2018) found a weak PAS positive reaction in the lining epithelium of vagina of Pati and Chara-Chembali ducks.

The activity bromphenol blue increased with age of the birds and was found to be minimum at 12 week of age and maximum at 32 week of age (Fig 6). In sperm host glands, there was weak reaction for basic protein in epithelial cells which became moderate to strong in supranuclear region of SHG and in cilia lining SHG and utero-vaginal junction. This may be due to synthesis of protein by SHG as observed by Schuppin *et al.* (1984).

At UVJ, the reaction of sudanophilic lipids was weak in lamina epithelialis and negligible to weak in the other layers. Similar findings were reported by Bansal *et al.* (2010) in Punjab white quail and Vijayakumar *et al.* (2016) in duck. However, sperm host glands epithelium showed moderate activity in PWQ observed by Baskt and Akuffo (2007)in

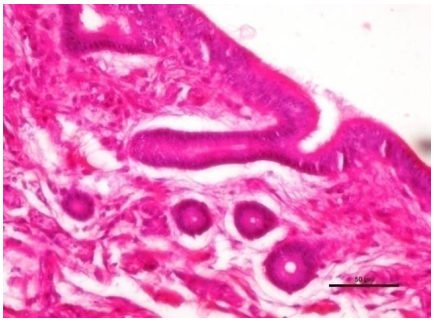

Fig 3: PWQ at 8 weeks of age showing Proximal (P) and distal (D) parts of SHG. Hematoxylin and eosin stain X 400.

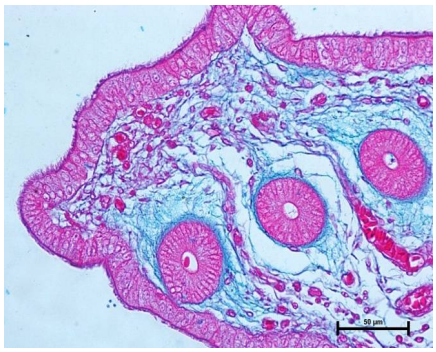

Fig 4: PWQ at 12 weeks of age showing moderate to strong PAS reaction in the epithelium of sperm host gland and lamina epithelialis. Moderate to strong alcian blue reaction was observed in the basement membrane of sperm host glands and propria submucosa layer. PAS-AB stain X 400.

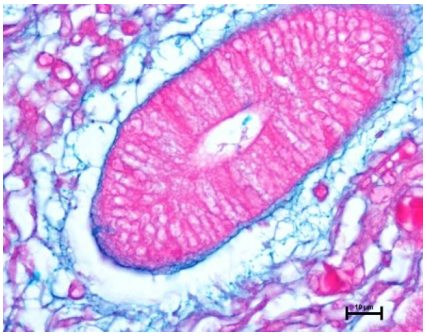

Fig 5: PWQ at 12 weeks of age showing moderate to strong intense PAS reaction in the epithelium and alcian blue reaction in the basement membrane of sperm host glands. PAS-AB stain X 1000.

the SST epithelium chicken, turkey and JQ. This may be due to the fact that these glands are responsible for maintaining the sperm plasmalemma and protection against oxidative damage.

Electron microscopy: Scanning electron microscopy showed that mucosa of utero vaginal junction of Punjab white quail and Japanese quail were thrown into broad mucosal folds and grooves. In these grooves opening of sperm host glands were seen. At places, clumps of secretory material were

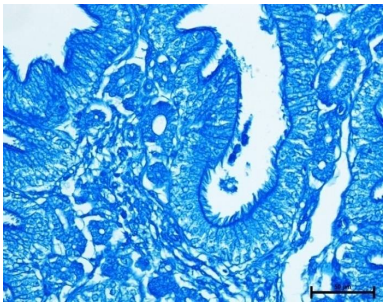

Fig 6: PWQ at 12 weeks of age showing moderate to strong in ciliated epithelium and sperm host glands. PAS-AB stain X 400.

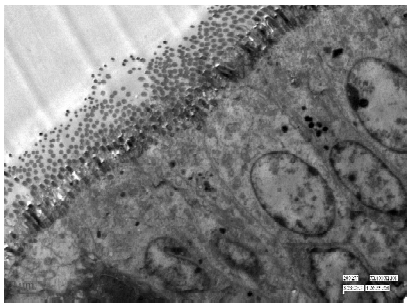

Fig 7: Electron microphotograph showing ciliated and non ciliated cells at uterovaginal epithelium. X 1100.

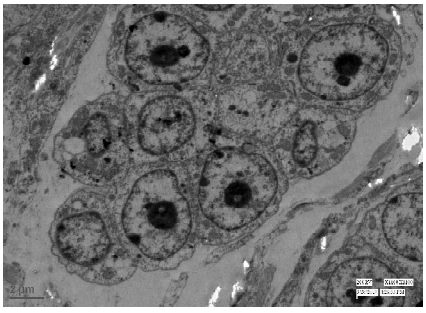

Fig 8: Electron microphotograph showing epthelium of sperm host gland with pyramidal cells having larger nucleus X 1100.

observed. At higher magnification the surface of lining cells were seen having microvillus projections. Ibrahim *et al.* (2015) studied utero-vaginal junction of Japanese quail and showed transition of mucosal folds from longitudinal folds of uterus to complex interconnecting folds of the vagina. The mucous membrane of this area was covered densely with cilia.

The transmission electron microscopy revealed that the sperm host glands were lined with cuboidal type of epithelial cells opening into centrally located lumen. Nuclei of the cells were round in shape with distinct nucleoli. The luminal border of the cells was laden with secretory vesicles. The secretory vesicles were both of dense and opaque types. In between these granules Golgi complex strands of rER and mitochondria were observed. The peripheral part of these glands rested on the basement membrane and was in contact with capillary and migratory cells and mesenchymal cells.

At higher magnification, the dilated lamellae of Golgi complex were clearly visible. The peripheral portion contained rER (Fig 7 and 8). The cells were pyramidal in shape with broad base and narrow apex, a narrow apices of these cells were united together to form lumen of the gland. Based on electron density lining epithelium was consisted of dark and light cells. Apical cytoplasm of these cells was filled with electron dense secretory granules.

From the present study, it may be suggested that these tubules may play a functional role in storage and release of spermatozoa from the SHG in response to oviposition or ovulation.

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