

LIGHT MICROSCOPIC STUDIES ON THE TESTIS OF THE COMMON COOT (*FULICA ATRA*)

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ABSTRACT

The testis of the common coot was examined histologically and histochemically to establish baseline reproductive characteristics. The birds were dissected and testes were located in the abdominal region. The testicular tissue was removed, washed and sliced into small pieces and fixed in 10% neutral buffered formalin and were processed. Using a semiautomatic microtome, paraffin sections of 5–6 µm thickness were cut and later subjected to histological analysis. The testis was enclosed by a tunica albuginea composed of collagen and smooth muscle fibers, with an outer tunica serosa and an inner tunica vasculosa. Seminiferous tubules were irregularly distributed throughout the parenchyma. The germinal epithelium of the tubules contained two types of spermatogonia, primary and secondary spermatocytes, three stages of spermatids, and Sertoli cells. Myoid cells were observed in the peritubular region, while Leydig cells occupied the interstitial connective tissue. Masson's Trichrome staining demonstrated collagen fibers in the capsule, septa, and peritubular regions. PAS–Alcian Blue staining showed moderate to strong positivity for neutral mucopolysaccharides in testicular tissues. These findings enhance understanding of the gonadal tissue in avian reproductive biology.

Key words: Common coot, histology, testis, seminiferous tubules

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INTRODUCTION

The common coot bird also known as Eurasian coot (*Fulica atra*) is one of the most abundant water birds in Europe

(Jortay, 2002). They breed in Europe, Asia and in Africa. In winters when the waters freeze, they migrate far south and west of Asia. Coots are primarily linked to wet dune slacks with *Phragmites australis* reed beds, and secondarily, to wet dune slacks with *Bolboschoenus maritimus* and channels (Talbi *et. al.*, 2020)

The movement and interactions of common coot between terrestrial and aquatic

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habitats reveals that the bird is one of the important indicators regarding general health of water bodies (Wong and Candolin, 2015). The two testicles are situated in the abdominal cavity and surrounded by a connective tissue capsule lined by mesothelium. The formation of mature spermatozoa from spermatogonia in birds follows the same basic pattern as in mammals, consisting of spermatocytogenesis, spermatidogenesis, and spermiogenesis. Adult testicular function is modulated by a myriad of external factors and orchestrated by numerous hormones that together enable birds to adapt to and breed in diverse habitats worldwide (Pierre Deviche *et al.*, 2011).

MATERIALS AND METHODS

The present research findings were carried out in the Department of Veterinary Anatomy, Veterinary college and Research Institute, Salem. Predator killed two adult coot birds were collected near the water bodies. The birds were dissected and testes were located in the abdominal region. The testicular tissue were removed, washed and sliced into small pieces and fixed in 10% neutral buffered formalin and were processed as per (Bancroft and Stevens, 1996). Using a semiautomatic microtome, paraffin sections of 5–6 μ m thickness were cut and later subjected to histological analysis.

Staining Methods

1. Standard Haematoxylin and Eosin for routine histological observation (Bancroft and Stevens, 1996).

2. Masson Trichrome for connective tissue fibers (Luna, 1968)
3. Combined Alcian blue – PAS method for acid and neutral mucopolysaccharides (Bancroft and Stevens, 1996).

RESULTS AND DISCUSSION

The capsule (Tunica albuginea) of the testis is made up collagen fibres and smooth muscle fibres. The outermost layer to the tunica albuginea is tunica serosa and the innermost layer to the tunica albuginea is tunica vasculosa (Fig.1). This observation is similar to the findings of Aire and Ozegbe (2007) in birds. The tunica vasculosa consists of network of blood vessels where erythrocytes are distributed. The collagen fibres are not much denser. These collagen fibres along with smooth muscle fibres increased the thickness of the capsule. The collagen and smooth muscle fibres interweaved with each other. The collagen fibres along with few smooth muscle fibres is noticed in the septa which penetrated the parenchyma of the testis. The peritubular regions of the tubules are covered by the network of collagen fibres along with few smooth muscle fibres. The seminiferous tubules are distributed in the parenchyma appeared as elongated irregular structures of large sized tubules and also with small irregular shaped structures. The primary spermatocyte appears larger than spermatogonia. The nucleus is comparatively larger than those of other germ cells and is centrally located within the cytoplasm. The cells appear rounded or oval in shape (Fig.2). Spermatogonia are stem cells in the testes that divide by mitosis. Some develop

into primary spermatocytes, starting the process of sperm formation. Two types of spermatogonia were observed. The first type was characterized by a large, lightly stained rounded or oval nucleus, while the second type displayed a large, densely stained nucleus (Fig.2). The Sertoli cells extended from the basal lamina and appeared as irregular shaped with faint nucleus with prominent nuclei, irregular cytoplasm. These findings are consistent with those reported by Jayachitra *et. al.* (2019) in the testis of Soviet chinchilla rabbit. The secondary spermatocyte appeared smaller than the primary spermatocytes. The nucleus appears rounded and is situated in the middle of the cell. The cells appeared rounded or oval shaped. These cells are distributed towards the lumen and also found among the primary spermatocytes. Compared to other cell types, spermatids are smaller and positioned near the lumen of the tubules. Three types of spermatids are noticed (Fig.2). Type 1 appeared with pale nucleus. These are newly formed. The type 2 spermatid with rounded dark smaller nucleus. Type 1 spermatid a relatively larger nucleus than type 2. These findings are similar to the findings of Obeid *et al.* (2021) in Domestic duck testis (*Anas Platyrhynchos*). The Type 3 spermatid with more condensed, smaller, eccentrically placed nucleus. This type is found inside the lumen and they are in the transition phase of spermiogenesis. The spermatozoa are located within the seminiferous tubules, where they are found distributed in the lumen of the seminiferous tubules. These cells exhibit strong basophilic staining, giving them a dark appearance under microscopic examination. The peritubular

regions contain myoepithelial cells, which are usually organized in a two-layered pattern (Fig.2). The interstitial connective tissue is moderate and contains Leydig cells with dark-staining nuclei and eosinophilic cytoplasm (Fig.3).

The spermatogonia, primary spermatocyte, secondary spermatocyte and spermatids (germinal cells) were moderately positive for neutral mucopolysaccharides. (Fig.4). This observation was in contrast to the findings of Gopikrishna *et. al.* (2017) in the testis of adult ram. Whereas the Sertoli cells were also moderately positive for neutral mucopolysaccharides. The peritubular connective tissue showed strong positive reaction for PAS as reported by Deshmukh *et. al.* (2014). The myoid cells showed strongly positive reaction for neutral mucopolysaccharides (Fig.4). Intense positive staining for neutral mucins was observed in Leydig cells (Fig. 4), similar to the results reported by Gopikrishna *et. al.* (2017). This finding was not in accordance with the statement of Deshmukh *et. al.* (2014) who reported weak reaction in the Leydig cells of the testis in Aseel and Vanaraja breeds of poultry. The capsule was moderately positive for the neutral mucopolysaccharides (Fig.5). Whereas Rajendranath *et. al.* (2017) reported intense PAS reaction in testicular capsule in the testis of Emu.

CONCLUSION

Histological observations revealed distinct stages of spermatogenesis along with Sertoli, myoid, and Leydig cells. Histochemical analysis using PAS–

Alcian Blue staining demonstrated moderate to strong positivity for neutral mucopolysaccharides in germinal cells, Sertoli cells, myoid cells, Leydig cells, and the testicular capsule. These findings provide

baseline information on the testicular histology and histochemistry of the common coot and contribute to the understanding of reproductive biology in aquatic birds.

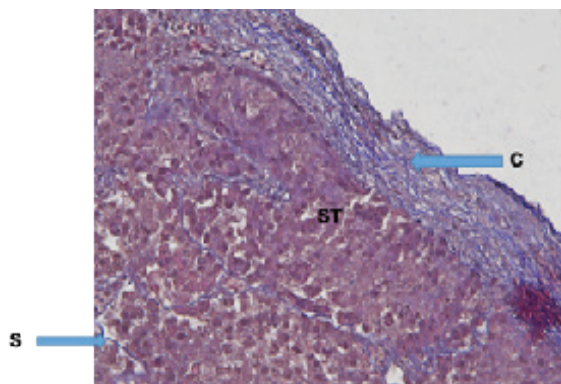


Fig.1. Photomicrograph of histology of testis of Coot bird showing C-Capsule S-septa ST – Seminiferous tubules (Masson Trichrome x 400)

Fig.2. Photomicrograph of Coot testis showing elongated seminiferous tubules. 1- Type 1 spermatid 2- Type 2 spermatid 3- Type 3 spermatid SP-Spermatogonia with pale nucleus SD – spermatogonia with darker nucleus PS –Primary Spermatocyte SS – secondary Spermatocyte SZ – Spermatozoa M- Myoid cells L– Lumen of the tubule (H&E x400)

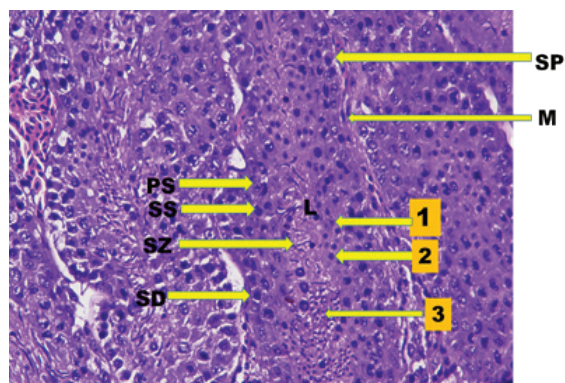
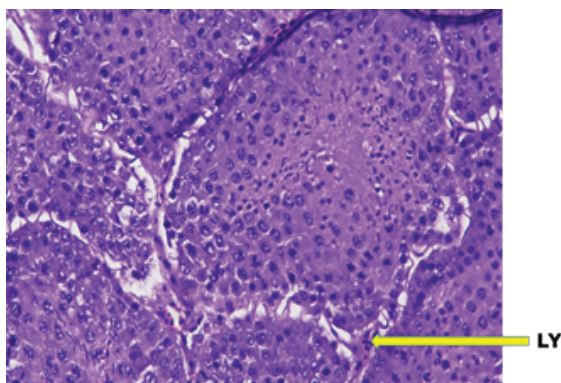


Fig.3. Photomicrograph of Coot testis showing seminiferous tubules and interstitial tissue LY – Leydig cells (H&E x400)



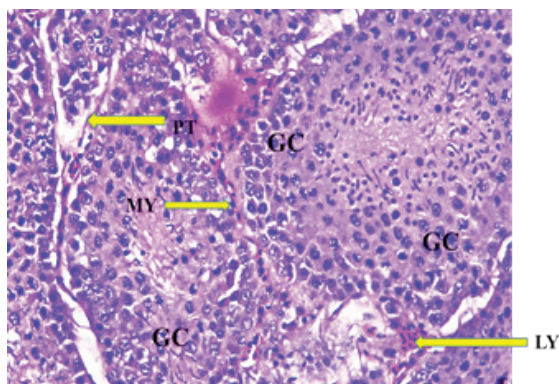
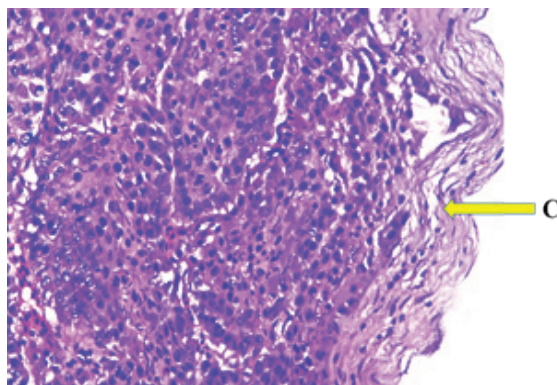


Fig.4. Photomicrograph of Seminiferous tubules and Interstitial connective tissue showing positive PAS reaction in the testis of coot bird GC- Germinal cells LY – Leydig cells MY- Myoid cells PT –peritubular tissue (Combined PAS –Alcian blue x 400)

Fig.5. Photomicrograph showing capsule in the testis of coot bird C- Capsule (Combined PAS –Alcian blue x 400)



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