



## Microscopic Features of Gonadally Inactive Testis of Khaki Campbell Duck (*Anas platyrhynchos domesticus*) in Bangladesh

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### ABSTRACT

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The microscopic features of the testis were studied in gonadally inactive Khaki Campbell duck (*Anas platyrhynchos domesticus*) in Bangladesh. The study was conducted in the Department of Anatomy & Histology, Bangladesh Agricultural University, Mymensingh. Five adult healthy birds of one-year-old were used for this study. The testes were collected immediately after ethical killing of the birds for histological observations. The collected tissue samples were then processed and stained with Hematoxyline & Eosin (H & E) stain for histological observations. The seminiferous tubules showed considerable involution with cessation of spermatogenesis. The basal lamina of the seminiferous tubules was irregular in outline and was invaginated into the germinal epithelium in the form of finger-like plicae or folds. Most of the lumen of the seminiferous tubules was empty and all generation of germ cells were not present in most of the seminiferous tubules. The interstitium showed a relative increase in volume and interstitial tissue consisted of loose connective tissue, interstitial cells (Leydig cells), few connective cells and blood vessels. This study first time described the microscopic features of testis of Khaki Campbell ducks in Bangladesh during inactive phases of the reproductive cycle.

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### Introduction

Cyclical patterns of testicular activity and inactivity has been observed in the most of the avian species with a period of rapid testicular re-growth in mature males just prior to the start of the breeding season (Murton and Westwood, 1977). After breeding, the testes regress and remain inactive until the next breeding season. The size and histological feature of the avian testis varies greatly between the non-breeding and breeding seasons. Vertebrate's reproduction is under endogenous regulation and generally depends also on environmental factors. In birds, the constant seasonal reproductive cycles are influenced by environmental factors such as light, temperature, rainfall index and food availability (Kemp, 1973; Silverin, 1975). The internal morphological changes of the testis reflect the changes in the breeding activity of birds. Although details investigations on the histology of testes have been performed in some domestic poultry such as roosters (Rothwell, 1975; Trefil et al., 2006; Aire and Ozegbe, 2007; Bull et al., 2007), Muscovy ducks (Marchand and Gomot, 1973; Gerzilov et al., 2002; Islam et al., 2013), geese (Mori and George, 1978; Halse, 1985),

turkeys (Noirault et al., 2006; Aire and Ozegbe, 2007; Bakst et al., 2007), ducks (Aire and Ozegbe, 2007; Elbajory et al., 2013), Japanese quails (Lin et al., 1990; Maretta and Marettova, 2004; Al-Tememy, 2010) guinea fowl (Dharani et al., 2017), but investigations on the gonadally inactive testis of Khaki Campbell duck in Bangladesh has not performed yet. On the other hand, the economy of Bangladesh largely depends on agriculture and poultry, is a raising sector now. Among poultry, ducks are considered as a second widespread species following the chickens in Bangladesh (Ahmed, 1986). Nowadays, Khaki Campbell (*Anas platyrhynchos domesticus*) duck is becoming more popular but this species is really less focused. The duck production in Bangladesh turns to be a big industry for supplying the animal protein to the mass people in near future. So, it is high time to in depth knowledge about Khaki Campbell duck. Focusing on reproduction, the present research is worthy in this realm. The structural changes of the testicular cells occur according to age and reproductive cycle (Gerzilov et al., 2002). On the other hand, basic research is decreasing day

by day although we know basic research is the foundation of other applied and clinical research.

Therefore, the present study is aimed to: investigate the microscopic features of gonadally inactive testes of Khaki Campbell duck in Bangladesh and to: compare the histological features of gonadally inactive testes of Khaki Campbell duck with other avian species.

## Materials and Methods

The research was conducted in the post-graduate laboratory of Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh. Guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh, Bangladesh [AWEEC/BAU/2019(30)] were followed during conducting this study.

Five apparently healthy male Khaki Campbell ducks (*Anas platyrhynchos domesticus*) of one-year-old were used in the present study. The ducks were collected from poultry farm of BAU, Mymensingh. After taking live body weight, birds were sacrificed by cervical sub-luxation method. After dissection of carcasses, testes were removed and tissue pieces were collected from different regions of the testis and subsequently fixed in 10% formalin solution. All the tissues were processed for paraffin sections by alcohol-xylene method. Sections were cut at 5-6  $\mu\text{m}$  thickness using a Rotary Microtome (American Optical Spencer Model 820) and stained with (H&E) stain.

Necessary photographs were taken during histological examination for better illustration of the result. The histological pictures were taken with photographic light microscope (AxioCam ERc 5s) and placed for better illustration of the result. The histological parameters were measured by using pre-calibrated ocular micrometer.

## Results

Each testis was covered by testicular capsule which was not uniform in structure and sloughed off in some places

(Figure 1A). The testicular capsule was comprised of dense connective tissue with presence of fibroblasts. The thickness of the testicular capsule was  $72.12 \pm 2.94 \mu\text{m}$ . In examined observation fields, there were no thick connective tissue stripes dividing the testicular parenchyma into lobes and lobules (Figure 1A). In this study, clear regression signs of testicular parenchyma were demonstrated. Seminiferous tubules were atrophic and most of the tubules were collapsed. The seminiferous tubules were irregular in shape and size. They were not closely attached with one another (Figure 1A). The diameter of the seminiferous tubules was also reduced. The mean length and width of the seminiferous tubules was  $468.6 \pm 166.91 \mu\text{m}$  and  $231.46 \pm 41.68 \mu\text{m}$  respectively. The basal lamina of the seminiferous tubule is irregular in outline and commonly thick. In some seminiferous tubules, the basal lamina was invaginated into the germinal epithelium in the form of finger-like plicae or folds (Figure 1B). Anastomoses among the different seminiferous tubules were also noticed. The tubules lumen was relatively large, irregular and most of the lumen was empty (Figure 1B), few were filled with spermatozoa. There was also increased cellular debris in the tubular lumen (Figure 1B). One major change was the presence of sloughed-off cells in the seminiferous tubular lumen (Figure 1B). As a result, most cells were not located within appropriate cellular associations. Generally, fewer germ cells were found in the most of the seminiferous tubules (Figure 2A and 2B), and germ cells beyond the primary spermatocyte stage were mostly affected. There were a few seminiferous tubules in which all generation of germ cells such as spermatogonia, primary and secondary spermatocyte, spermatid and spermatozoa were found (Figure 3A). The sertoli cells were round to slender with irregular outline (Figure 3A). They extended from the basement membrane to the lumen of the seminiferous tubules.

Increased interstitial space was observed in this study. The interstitial cells were numerous. Leydig cells were scattered in the interstitial spaces. They were polygonal in shape with a large spherical nucleus (Figure 3B). Blood vessels were also present in the interstitial space (Figure 1A).

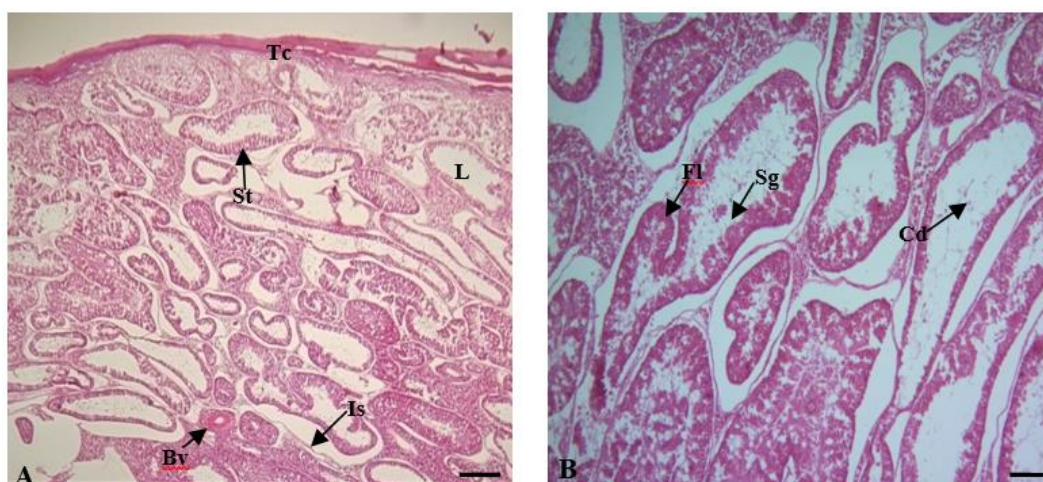


Figure 1. Photomicrographs of the inactive testis of Khaki Campbell duck showing testicular capsule (Tc), irregular shaped seminiferous tubule (St), finger like folding of seminiferous tubule (Fl), irregular and empty lumen (L), sloughed off cells (Sg), cellular debris (Cd) within the lumen of some seminiferous tubules, increased interstitial space (Is), blood vessel (Bv) in interstitial space. Scale bar: 100  $\mu\text{m}$  (A), 50  $\mu\text{m}$  (B).

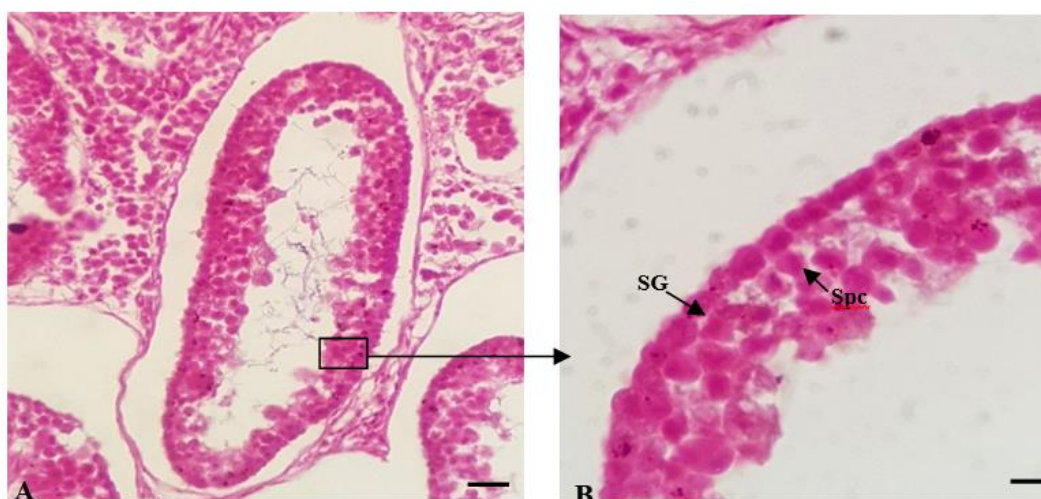


Figure 2. Photomicrographs of the inactive testis of Khaki Campbell duck showing regressed seminiferous tubule with few germ cells generation (A), Spermatogonia (SG), Spermatocyte (Spc). Scale bar: 10  $\mu$ m (A), 5  $\mu$ m (B).

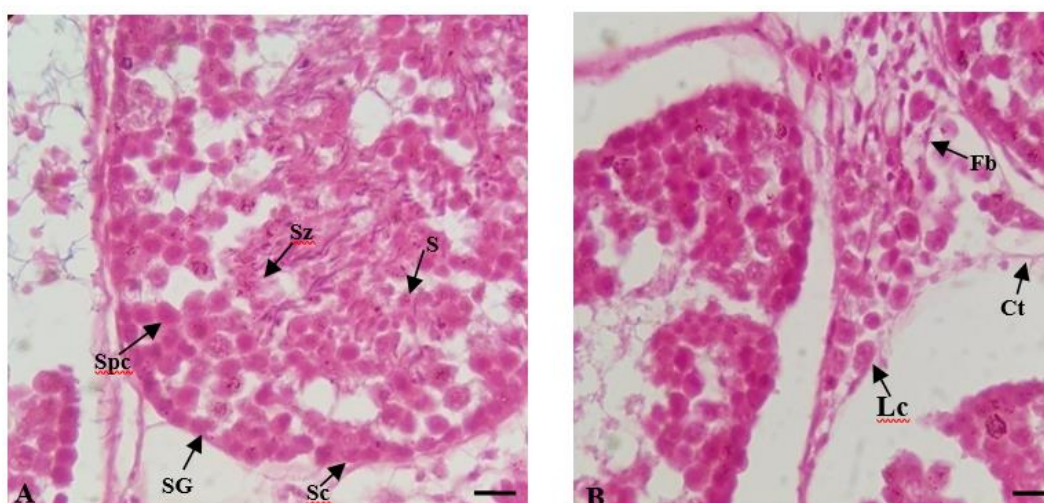


Figure 3. Photomicrographs of the inactive testes of Khaki Campbell duck showing seminiferous tubule with spermatogonia (SG), spermatocyte (Spc), round spermatid (S), spermatozoa (Sz), sertoli cell (Sc), interstitial space contained connective tissue fibres (Ct), fibroblasts (Fb), leydig cell (Lc). Scale bar: 5  $\mu$ m (A & B).

## Discussion

The microscopic features of the testicular capsule in Khaki Campbell ducks were nearly similar to that of other birds reported by Aire (1997); Jamieson (2007), in Sudanese duck by Aire and Ozegebe, (2007) and Salwa et al. (2013), in white rooster by Razi et al. (2010), in Muscovy duck by Gerzilov et al. (2016) with the exception of sloughing of testicular capsule in some places found in present study. This variation may be due to species differences and state of reproductive inactivity of testis. No lobulation of testicular parenchyma was observed in present study which was similar to those found in white rooster by Razi et al. (2010), Muscovy duck by Gerzilov et al. (2016), Sudanese duck by Salwa et al. (2013), indigenous duck by Khadem (2014). The thickness of testicular capsule that was found in this study was lower than that has been reported by Aire and Ozegebe (2007) in birds, by Hodges (1974) in domestic fowl that was measured in gonadally active birds. The specific reason for this difference in testicular capsule thickness is not clearly known, but could be due to age of the subjects, species difference and possibly but not probably reproductive activity.

The irregular shape of the seminiferous tubules, decreased diameter of the seminiferous tubules, finger like folding of basal lamina, presence of cellular debris in the tubular lumen, degenerating germ cells and collapse of seminiferous tubules observed in the present study also reported by Victor and Wilbor (1974) in Japanese quail (*Coturnix coturnix japonica*), by Gumulka and Rozenboim (2015) in gander (*Anser anser domesticus*), by Abdul-Rahman et al. (2015) in guinea fowl, by Simoes et al. (2017) in domestic duck. Whereas complete seminiferous tubular structure with presence of all generation of germ cells was observed in gonadally active bird reported by Razi et al. (2010) in white rooster, by Salwa et al. (2013) in Sudanese duck, by Gerzilov et al. (2016) in Muscovy duck.

In present study, it was observed that the interstitial space was increased. Numerous interstitial cells with scattered polygonal shaped leydig cells were found in the interstitial spaces. Blood vessels were also present in interstitial space. Similar findings were reported by Aire (1997) in domestic fowl, duck, quail and guinea fowl, Dharani et al. (2017) in guinea fowl.

## Conclusion

From the above study, it can be concluded that the histological feature of the testis of the Khaki Campbell duck varies greatly between the active and inactive period of the reproductive cycle. These basic histological features will provide valuable information for morphometric analysis of testicular cells and quantitative assessment of the inactive testis of Khaki Campbell duck to the anatomist, poultry or duck researchers, poultry farmers, veterinarians and autonomous learners.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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