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Histological examination of testicular cell development in khaki Campbell ducklings (*Anas Platyrhynchos Domesticus*)

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Abstract
Duck has great prospects for sustainable development of the economy of the poultry industries in Bangladesh. The current research was designed to investigate the histological changes of testes of Khaki Campbell ducklings. The study was conducted in the Department of Anatomy & Histology, Bangladesh Agricultural University, Mymensingh. The experimental Khaki Campbell ducks (*Anas Platyrhynchos Domesticus*) were collected from poultry farm of Bangladesh Agricultural University, Mymensingh, having apparently good health and devoid of any external deformities. The ducklings were from three age groups (Total 15 ducklings): day one (1), one (1) month and three (3) months, having five ducklings in each group. The testes were collected immediately after ethical killing of the birds for histological observations. The collected tissue samples were then processed and stained with Hematoxylene& Eosin (H & E) stain for histological observations. The histological observations revealed that at day 1 and 1 month old, spermatogonia and sertoli cells were present within the tubiform shaped seminiferous tubules. Slightly distinct seminiferous tubules were observed at 3 months of age which contained spermatocytes along with spermatogonia. The present study indicates that development of seminiferous tubules up to 3 month is age related which was similar to that in other avian species. This study first time described the developmental features of testicular cells/tissues of Khaki Campbell duckling in Bangladesh.

Keywords: Ducklings, development, testes, histology, Khaki Campbell duck

Introduction
Testes are the structural and functional unit of the male reproductive system having two functions: they produce the male gametes or spermatozoa, and they produce male sexual hormone, testosterone, which stimulates the accessory male sexual characteristics. Investigations on the testicular development from hatching to sexual maturity have been of great interest to those in the poultry industry and other investigators (Parker, 1942; Bennet, 1947) [7, 2]. The seminiferous epithelium consists of two cell types: fixed, somatic cells, represented by the Sertoli cells, and temporary and mobile germ cells, consisting of a series of differentiating cells (spermatogonia, primary and secondary spermatocytes and spermatids). The basal cell is the spermatogonium which divides repeatedly to form larger cells in successive stages of meiotic division and maturity. Spermatogenesis in birds, as in mammals, involves a series of divisions of spermatogonia, resulting in primary spermatocytes and secondary spermatocytes, both of which undergo meiotic divisions, resulting in the evolution of spermatids (Jamison B, 2007) [5]. The poultry sub-sector plays an important role in the context of agricultural growth and improvement of diet for the people in Bangladesh. Khaki Campbell duck may become an important poultry species can contribute efficiently in increasing egg and meat production. They are highly productive, and suitable for commercial production for both meat and eggs. The main advantages of raising Khaki Campbell ducks is that they yield meat and eggs which are very tastier having huge demand and price in the market. Many investigations have already been performed by the researchers on the testes of chicken, duck, pigeon, quails and many other birds. But in Bangladesh, the research on testes of Khaki Campbell ducklings is no longer conducted before. So this research results will be valuable to compare findings of Khaki Campbell ducklings with other species. Comparatively the production and profitability of duck farming is high than other poultry farming. In this view, the research findings have possibility to compare with the other avian species to identify the structural variations of the testes. Usually the structural changes of the testicular cells occur according to age and reproductive cycle. The basic histological structure of the testis at different ages carries some important information about reproductive performance. So the determination of the histological development of the testes will be valuable for the poultry industry, students and researchers. Considering all these aspects the present study was conducted to:

- Investigation on the developmental features of testicular cells/tissues of Khaki Campbell duckling in Bangladesh.
- Comparing the developmental features of testicular cells/tissues of Khaki Campbell duckling with other avian species.

Materials and Methods
A total of 15 male Khaki Campbell ducklings of day 1, 1 month and 3 months ages were used in the present study. After taking live body weight, birds were killed ethically. 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 µm thickness using a Rotary Microtome and stained with Haematoxylin and Eosin stain. The details histological study was completed using a high power light microscopy. The measurements of the thickness of the tunica albuginea, diameter of the seminiferous tubule were done by using ocular micrometer.

Results

The testis of day 1 old duckling was covered by testicular capsule (tunica albuginea) (Figure 1A). The tunica albuginea was comprised of dense connective tissue with presence of fibroblasts and was thin. There was no thick connective tissue septa dividing the testis into lobules (Figure 1A).Major portion of the testicular parenchyma was covered by seminiferous tubules. In one day old duckling, the structure of seminiferous tubules (ST) was not distinct. They were slightly curved and tubular in morphology. Their wall consisted of basement membrane, surrounded by peritubular tissue. Anastomoses among the different seminiferous tubules were noticed in some places within the observation fields (Figure 1A). Lumenwas absent in the seminiferous tubules (Figure 1A).In day old ducklings, Sertoli cells were densely packed and formed the wall of seminiferous tubules. Their nuclei were small, pale and oval contained darkly stained nucleolus. Only relatively large spermatogonia and big round nuclei were irregularly arranged within the seminiferous tubules. Other spermatogenic cells are absent in one day old duckling(Figure 1A).The interstitial space contained connective tissue with fibroblasts and blood vessel (Figure1A).Leydig cells were absent.

In 1 month old duckling, the structure of seminiferous tubules (ST) was similar to that in day 1 old duckling but in some areas, a cleft-like lumen has appeared (Figure 1B).There were no difference in the seminiferous tubular cells between day 1 and 1 month old duckling.

The thickness of testicular capsule (tunica albuginea) was increased in 3 months old duckling compare to day 1 and 1 month old ducklings (Figure 2A).

At 3 months of age, the curvature of seminiferous tubule was increased and some seminiferous tubule had a lumen. Their wall consisted of well-developed basement membrane surrounded by peritubular tissue (Figure 2B). The cells in the seminiferous tubule were arranged in two rows; a peripheral row one, next to basement membrane was formed by spermatogonia and. The second layer was formed by spermatocytes situated near to the lumen. The spermatocytes were large, with oval nuclei and acidophilic cytoplasm. Their nuclei were stained deeply and their chromatin showing a net-like appearance (Figure 2C). At three months of age, the interstitial space contained distinct groups of leydig cells with oval nucleus, blood vessel, connective tissue fibers etc. (Figure 2D). The mean thickness of testicular capsule (tunica albuginea) was 12.92 ± 1.55 µm, 17.32 ± 1.84 µmat day 1, 1 month and 3 months respectively.

The mean length of the seminiferous tubules (ST) was 35.64 ± 7.42 µm, 40.68 ± 5.23 µm, 54.72 ± 7.44 µm day 1, 1 month and 3 months respectively.

The mean width of the seminiferous tubules (ST) was 17.88 ± 8.02 µm, 20.42 ± 4.60 µm, 40.32 ± 3.15 µm day 1, 1 month and 3 months, respectively.

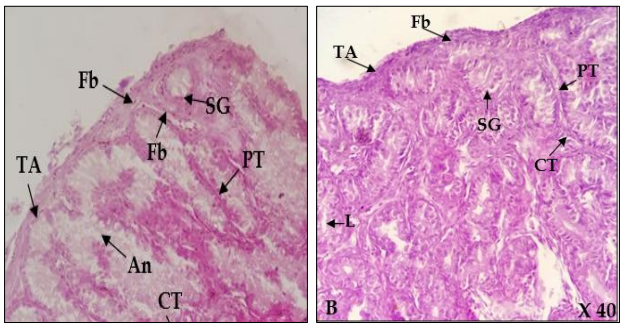

Fig 1: A-B. Photomicrographs of the testesof Khaki Campbell duck at day 1 old (A) and 1 month old (*Anasplatyrhynchosdomesticus*) showing tunica albuginea (TA) with fibroblasts (Fb), slightly curve seminiferous tubules (ST) without lumen, basement membrane surrounded by peritubular tissue (PT), anastomsed seminiferous tubules (An), spermatogonia (SG), interstitial space contained connective tissue fibers (CT) with fibroblasts (Fb), blood vessel, H & E stain (A-B, X40).

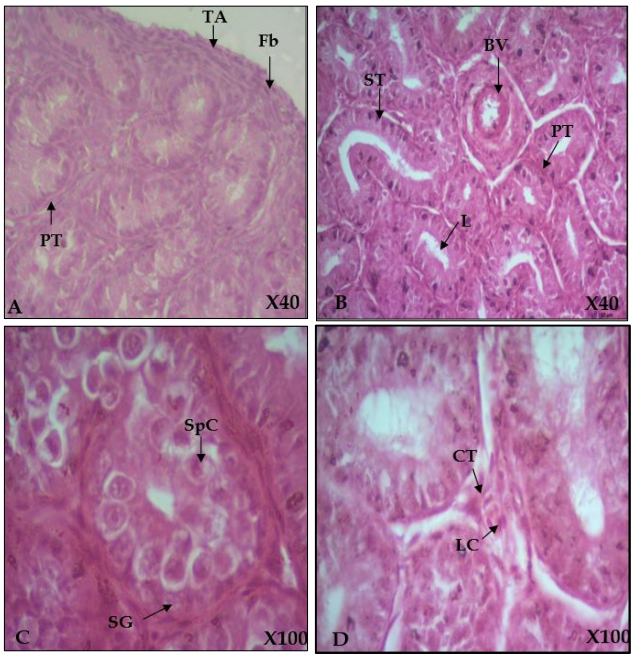

Fig 2: A-D. Photomicrographs of the testesof Khaki Campbell duck (*Anas Platyrhynchos Domesticus*) at three months old showing tunica albuginea (TA) with fibroblasts (Fb), curved seminiferous tubules (ST) with lumen (L) in most seminiferous tubule, basement membrane surrounded by per tubular tissue (PT), spermatogonia (SG), spermatocyte (SpC) in seminiferous tubules, interstitial space contained connective tissue fibers (CT), leydig cell (LC), blood vessel (BV), H & E stain (A-B, X40; C-D, X100).

Discussion

The testicular capsular structure in Khaki Campbell ducklings was similar to that of other birds reported by Aire (1997) [1]; Jamieson B., (2007) [5]; Salwa *et al.*, (2013) [9] in Sudanese duck; Razi *et al.*, (2010) [8] in White Rooster, Gerzilov *et al.*, (2016) [4] in Muscovy duck. There were no septa, dividing the organ into lobule. This findings are similar to those found by Razi *et al.*, (2010) [8] White Rooster; Gerzilov *et al.*, (2016) [4] in Muscovy duck; Salwa *et al.*, (2013) [9] in Sudanese duck; Razi *et al.*, (2010) [8] in White

Rooster; Khadem (2014) [6] in indigenous duck. The thickness of testicular capsule increased with ages.

In day old ducklings, the structure of seminiferous tubules (ST) were not distinct. They were slightly curved and tubiformed. No lumen in the seminiferous tubules was observed. The similar study was reported by Bochukov *et al.*, (1995) [3] in Japanese quail, Gerzilov *et al.*, (2016) [4] in Muscovy duck. In one month old ducklings, cleft-like lumen has appeared in few seminiferous tubules which were also reported by Gerzilov *et al.*, (2016) [4] in Muscovy duck. The structure of seminiferous tubules in 3 months old was more distinct and most of the seminiferous tubules contains empty lumen.This observation is similar to the Gerzilov *et al.*, (2016) [4].

In day 1 old to 3 months old ducklings, Sertoli cell nuclei were small, pale and oval contained darkly stained nucleolus. This feature is similar to the findings made by the Gerzilov *et al.*, (2016) [4] in Muscovy duck

Only relatively large spermatogonia and big round nuclei were irregularly arranged within the seminiferous tubules in day 1 old and 1 month old duckling, spermatogonia and spermatocytes were present in 3 months old duckling. This histological picture has been observed in Muscovy duck (Gerzilov *et al.*, 2016) [4].

In day1 old and 1 month old of ducklings the interstitial connective tissue was with rich cellular content with prevalence of fibroblasts. In 3 months old the interstitial space contained leydig cells, fibroblasts, connective tissue fiber, blood vessel etc. These results are in total in agreement with the findings of (Gerzilov *et al.*, 2016) [4].

The thickness of testicular capsule and diameter of the seminiferous tubules were gradually increased from day 1 to 1 month old then reduced in one year old. Gerzilov *et al.* (2016) [4] also observed similar feature in Muscovy duck.

Conclusions

The results of the present study revealed that at day 1 and 1 month old, spermatogonia and sertoli cells were present within the seminiferous tubules and spermatocytes along with spermatogonia were present at 3 months of age. Other testicular cells like spermatid and spermatozoa were not observed within the testis of the ducklings of experimental ages indicating that the testicular development occurs slowly in Khaki Campbell duckling. The results of our studies also pointed out that the structure of testes of Khaki Campbell ducklings was similar to that in other avian species. This study described the developmental features of testicular cells of Khaki Campbell ducklings which may provide some valuable information to the researcher, students and autonomous learner.

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