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Microstructure and Histochemical Localization in the Testis of Aseel Cross Birds

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Abstract

The present study elucidated the histological, histochemical and histoenzymatic characteristics of the adult avian testis. The tunica albuginea was composed of predominantly dense collagen fibers intermingled with smooth muscle fibers and collagenous septa extended inward from the capsule to surround and support the seminiferous tubules within the testicular parenchyma. The seminiferous tubules had exhibited active spermatogenesis and contained well-defined stages of germ cells such as spermatogonia, primary and secondary spermatocytes, spermatids and mature spermatozoa which were supported by Sertoli cells. The Myoid cells were observed around the seminiferous tubules whereas the Leydig cells had occupied the interstitial connective tissue between the tubules. Histochemically, the PAS–Alcian blue staining had demonstrated strong PAS positivity in the tunica albuginea, connective tissue septa and germinal epithelium due to the presence of abundant neutral mucopolysaccharides and glycoconjugates associated with structural support and cellular metabolism. Histoenzymatic studies revealed strong alkaline phosphatase activity in spermatozoa and moderate activity in other germinal cells due to active membrane transport and metabolic functions during spermatogenesis. Acid phosphatase activity was moderate within the germinal epithelium and mild in the surrounding interstitial regions due to lysosomal activity associated with cellular turnover and maturation processes. Thus, these findings had provided the valuable information on the structural, functional and metabolic organization of the adult avian testis and contributed to a better understanding of reproductive physiology in birds.

Key words: *Microstructure, Histochemical Localization, Testis, Aseel Crossbred, Native chicken*

Introduction

The testes of Aseel cross birds are located intra-abdominally and positioned cranial to the kidneys and ventral to the lungs which is typical in avian species. The mammalian testes are located within the scrotum to maintain the lower temperature require for the spermatogenesis whereas the avian testes are functional within the abdominal cavity in spite of the comparatively higher core body temperature of birds. In birds, the spermatogenesis occurs efficiently at body temperatures (40–42°C) due to unique physiological and cellular adaptations that enable the normal sperm production under the elevated thermal conditions (Lake, 1981; Aire, 2007). The absence of a scrotum and the maintenance of testicular function at higher temperatures distinguish the avian reproductive physiology from that of mammalian species.

The Aseel crossbred birds are well known indigenous poultry strains characterized by multi-coloured plumage, compact and muscular body conformation, strong legs and a short curved beak. These birds are highly valued for their hardiness, adaptability to the diverse environmental conditions and resistance to the climatic stress. In addition, the Aseel birds are renowned for their superior meat quality, which is characterized by desirable flavour, firm texture, high nutritive value and comparatively lower fat content than the commercial broiler chickens (Rajkumar *et al*., 2017). Due to these characteristics, the Aseel and its crosses are extensively utilized in rural poultry production systems as well as in breeding programmes aimed to improve the meat quality and adaptability traits in poultry sector.

The testis is the principal male reproductive organ which is responsible for the spermatogenesis and endocrine regulation in the birds. It performs the dual functions by producing spermatozoa within the seminiferous tubules and synthesizing the steroid hormones especially the testosterone through the interstitial Leydig cells.

Testosterone plays an important role in the development of secondary sexual characteristics, maintenance of reproductive behaviour and regulation of spermatogenesis (Johnson, 2000). Therefore, the structural integrity and functional organization of the testicular tissue are important to maintain the normal fertility and reproductive efficiency in avian species.

The histological and histochemical investigations of the testis provide valuable information on the cellular architecture, physiological activity and biochemical composition of reproductive tissues. The seminiferous tubules of birds are lined by stratified germinal epithelium consists of Sertoli cells and various stages of spermatogenic cells such as spermatogonia, spermatocytes, spermatids and spermatozoa. The Sertoli cells provide structural and nutritional support to the developing germ cells and regulate the microenvironment necessary for spermatogenesis (Aire, 2007). Surrounding the seminiferous tubules are peritubular myoid cells which contribute to the tubular contraction and movement of spermatozoa.

The interstitial connective tissue is located between the seminiferous tubules contains Leydig cells, blood vessels, lymphatics and connective tissue fibers. The Leydig cells are responsible for the steroidogenesis and secretion of testosterone which is required for the initiation and maintenance of spermatogenic activity (Etches, 1996). The histochemical studies further help to identify the distribution of glycoconjugates, enzymes and other biochemical components associated with the cellular metabolism, secretory activity and tissue organization within the testis. Localization of phosphatase enzymes, mucopolysaccharides and connective tissue components provides the important information about the reproductive activity and functional status of the gonads.

Despite the economic and genetic importance of Aseel birds, the detailed histological and histochemical information about their reproductive organs were limited. Therefore, comprehensive studies on the structural and biochemical organization of the testis are required to understand the reproductive physiology which in turn improve the breeding and conservation strategies in the indigenous poultry breeds.

Materials and Methods

The present study was conducted on healthy adult Aseel crossbred birds of 18 weeks of age. The testes were collected immediately after slaughter from a local poultry slaughterhouse. After careful dissection, the testes were separated from the surrounding tissues and gently washed in normal physiological saline to remove the adhering blood and debris. Representative tissue samples were trimmed into small pieces and processed for histological, histochemical and histoenzymatic investigations.

For routine histological and histochemical studies, the tissue samples were fixed in 10% neutral buffered formalin to preserve the structural integrity of the cellular and connective tissue components. The fixed tissues were processed by standard paraffin embedding procedures involving dehydration through ascending grades of alcohol, clearing in xylene and embedding in paraffin wax. Paraffin sections of 5–6 µm thickness were prepared with use of rotary microtome and mounted on clean glass slides for microscopic examination. For histoenzymatic studies, fresh tissue samples were processed under cold conditions to preserve enzymatic activity and cryosections of 15–25 µm thickness were obtained with use of cryostat microtome.

The prepared sections were subjected to various histological, histochemical and histoenzymatic staining procedures. Routine histological examination was carried out with use of Haematoxylin and Eosin (H & E) staining following the method described by Bancroft and Stevens (1996), which facilitated the study of general tissue architecture and cellular organization. Masson's Trichrome staining technique was employed for the demonstration of connective tissue fibers especially the collagen fibers according to the method of Luna (1968).

For histochemical evaluation, the combined Alcian blue–Periodic Acid Schiff (AB–PAS) staining method was used for the localization and differentiation of acidic and neutral mucopolysaccharides following the procedure described by Bancroft and Stevens (1996). Alcian blue specifically stains acidic mucosubstances blue whereas PAS demonstrates neutral mucopolysaccharides and glycogen as magenta-colored substances. Histoenzymatic

localization of alkaline phosphatase (ALP) and acid phosphatase (ACP) activities was carried out with use of Gomori's lead nitrate method according to Singh and Sulochana (1996). These enzymes are important indicators of cellular metabolism, absorptive activity and lysosomal function within reproductive tissues. The stained sections were examined under a light microscope and the intensity as well as distribution of histological, histochemical and histoenzymatic reactions were recorded systematically.

Results and Discussion

The tunica albuginea of the testis in adult Aseel cross birds was composed predominantly of dense collagen fibers intermingled with smooth muscle fibers (Figure 4). The abundance of collagen fibers had contributed to the increased thickness and structural strength of the capsule (Figure 2). Similar observations were reported by Khalaf *et al*. (2024) in pigeon and by Ahlam *et al*. (2024) in Hoopoe bird (*Upupa epops*), where the tunica albuginea was described as the thick and collagenous in nature. The collagen fibers of the capsule had extended inward into the testicular parenchyma as connective tissue septa, which divided and supported the seminiferous tubules. The septa were richly distributed with collagen fibers whereas the smooth muscle fibers were largely absent within these regions. The collagenous septa had surrounded the seminiferous tubules and provided the mechanical support to the testicular tissue.

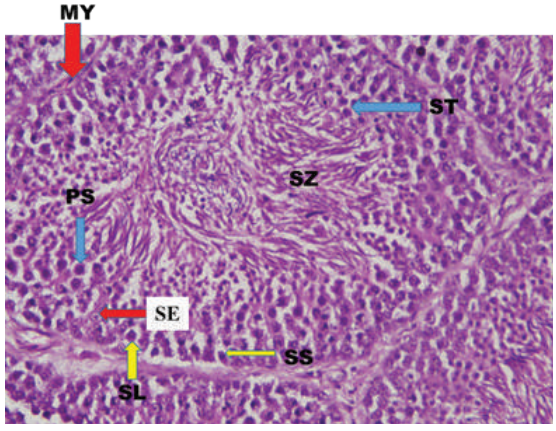

Fig. 1. Photomicrograph of Aseel testis showing marked profiles of Seminiferous tubules. SZ: Spermatozoa, ST: Spermatid, SS: Small Spermatogonia, SL: Large Spermatogonia, SE: Sertoli cells, PS: Primary Spermatocyte, MY: Myoid cells (H & E ×400)

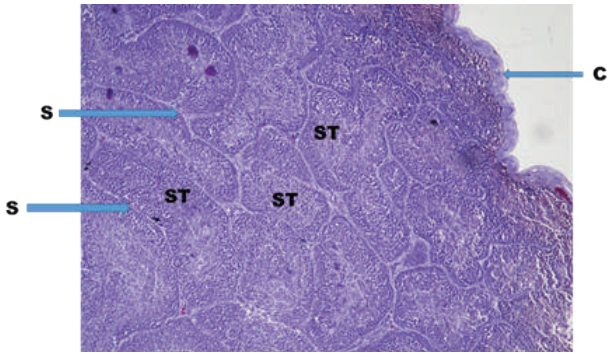

Fig. 2. Photomicrograph showing Parenchyma of Aseel testis. C: Capsule, S: Septa, ST: Seminiferous tubules (Masson Trichrome ×100)

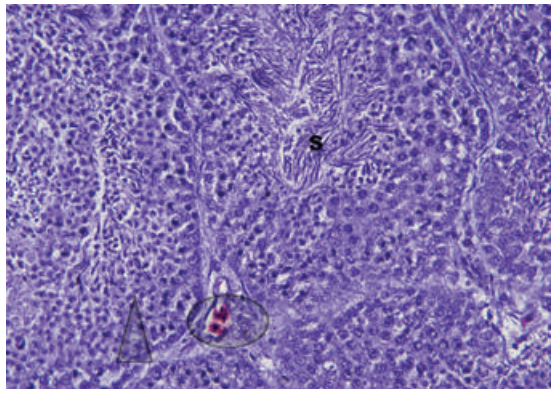

Fig. 3. Photomicrograph of Aseel testis showing Seminiferous tubules and interstitial tissue. S: Spermatozoa, circled part showing Leydig cells, Triangled marked portion showing sertoli cells (Masson Trichrome ×400)

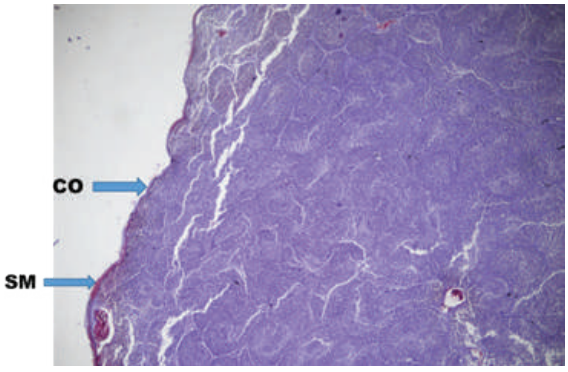

Fig. 4. Photomicrograph of Aseel testis showing Collagen and Smooth muscle fibres in the capsule. CO: Collagen fibres, SM: Smooth Muscle fibres (Masson Trichrome × 40)

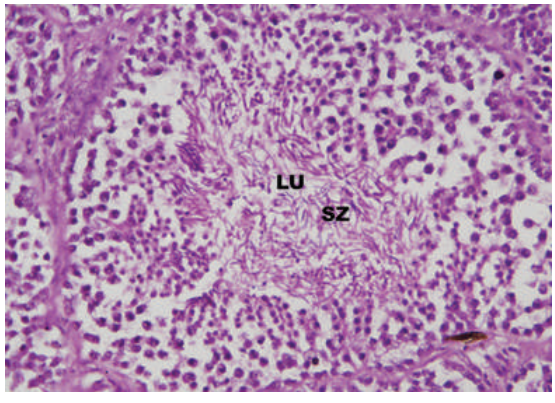

Fig. 5. Photomicrograph of Aseel testis showing Seminiferous tubules. LU: Lumen, SZ: Spermatozoa (H & E ×400)

The seminiferous tubules had appeared as irregular in shape and varied considerably in size (Figure 2). The germinal epithelium lining the seminiferous tubules consisted of Sertoli cells and various stages of spermatogenic cells as similar to the observations reported by Dhuha Adel Kareem *et al*. (2020) in adult ducks. The spermatogonia were distributed near the basement membrane at the peripheral region of the seminiferous tubules. Two distinct types of spermatogonia such as smaller and larger spermatogonia were identified based on their size and nuclear morphology (Figure 1). The spermatogonia had appeared spherical with deeply

stained nuclei due to the active mitotic activity. Among the spermatogonia, the Sertoli cells were identified as pale-staining cells possessing rounded or oval nuclei (Figure 3). The Sertoli cells had provided the structural and nutritional support to the developing germ cells and regulate the spermatogenesis within the seminiferous tubules.

The small spindle-shaped cells with centrally located nuclei were observed at the periphery of the seminiferous tubules within the septal regions. These cells were identified as myoid cells (Figure 1) as similar to the findings of Deshmukh *et al*. (2014) in Aseel and Vanaraja breeds of poultry. The Myoid cells are contractile in nature and assist in the movement of spermatozoa and tubular fluid through the seminiferous tubules. The primary spermatocytes were observed above the spermatogonial layer towards the middle region of the seminiferous epithelium. The spermatids were distributed near the luminal region of the seminiferous tubules whereas the mature spermatozoa were densely concentrated within the lumen (Figure 3) due to the active spermatogenesis in the adult birds.

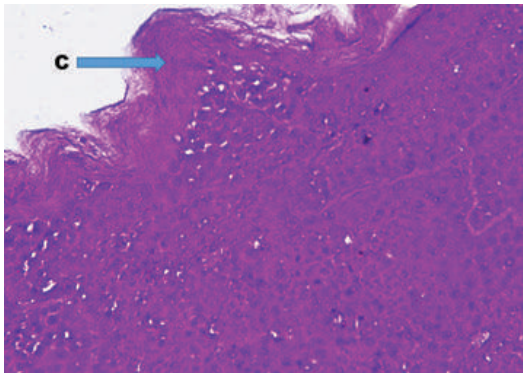

Fig. 6. Photomicrograph showing C-Capsule of the testis (Combined PAS –Alcian blue ×400)

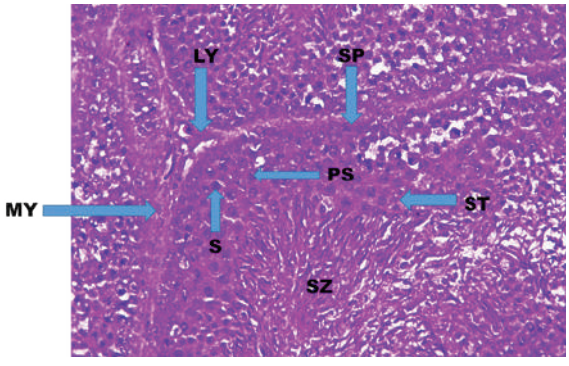

Fig. 7. Photomicrograph of Seminiferous tubules and Interstitial connective tissue. SP: septa, S: spermatogonia, PS: Primary Spermatocyte, ST: Spermatid, SZ: Spermatozoa, LY: Leydig cells, MY: Myoid cells (Combined PAS –Alcian blue ×400)

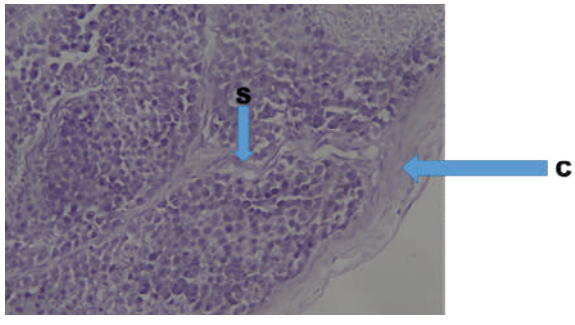

Fig. 8. Photomicrograph showing C-Capsule and S-Septa of Aseel testis (Alkaline phosphatase Gomori's method ×400)

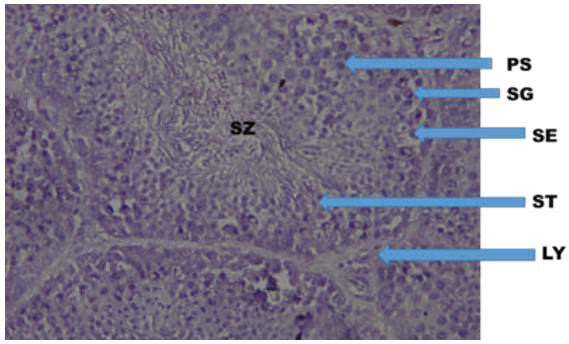

Fig. 9. Photomicrograph showing Seminiferous tubules of Aseel testis. SG: Spermatogonia, PS: Primary Spermatocyte, SE: Sertoli cells, ST: Spermatocyte, SZ: Spermatozoa, LY: Leydig cells (Alkaline phosphatase Gomori's method ×400)

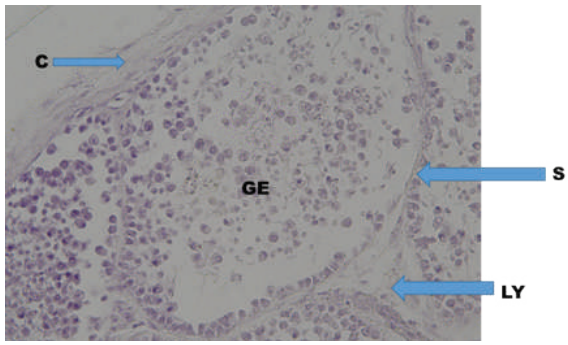

Fig. 10. Photomicrograph showing C-Capsule and S-Septa GE-Germinal epithelium, LY-Leydig cells of Aseel testis (Acid Phosphatase Gomori's method ×400)

The interstitial connective tissue between the seminiferous tubules had contained Leydig cells characterized by comparatively larger size and darker nuclei (Figure 3). The Leydig cells are responsible for steroidogenesis and testosterone production, which are essential for the maintenance of spermatogenesis and secondary sexual characteristics. Due to the adult age of the birds, some seminiferous tubules had exhibited loosely arranged germinal epithelium with fewer germ cells (Figure 5) whereas the majority of the tubules had showed densely packed spermatogenic cells representing active reproductive status.

Histochemically, the combined PAS–Alcian blue staining had demonstrated the strong PAS positivity in the tunica albuginea and connective tissue septa (Figure 6) due to the presence of abundant neutral mucopolysaccharides and glycoproteins associated with collagen fibers and connective tissue components. The germinal epithelium of the seminiferous tubules also had exhibited the strong PAS positivity due to active carbohydrate metabolism and the presence of glycoconjugates which are essential for the spermatogenic activity. The Leydig cells had showed moderate PAS positivity whereas the myoid cells had exhibited moderate PAS reaction (Figure 7). The PAS positivity within the seminiferous epithelium were related to glycogen utilization and glycoprotein synthesis associated with germ cell maturation and sperm production.

The alkaline phosphatase (ALP) activity was moderately localized in the tunica albuginea and septal connective tissue (Figure 8). The spermatogonia, spermatocytes and spermatids had exhibited the mild to moderate ALP activity due to active membrane transport and metabolic processes during spermatogenesis. These findings were differed from the observations of Rajendranath *et al*. (2017) who had reported comparatively different ALP localization patterns in emu testis. Strong alkaline phosphatase activity was observed in spermatozoa due to higher metabolic and membrane-associated enzymatic activity which are necessary for

sperm maturation and motility. The Sertoli cells had exhibited mild ALP activity whereas the Leydig cells had showed only weak positive reactions (Fig. 9).

The acid phosphatase (ACP) activity was mildly distributed in the tunica albuginea and connective tissue septa. The germinal epithelium of the seminiferous tubules had exhibited the moderate ACP activity due to lysosomal activity associated with cellular turnover, degeneration and spermatogenic maturation processes. This finding contrasts with the observations of Gopi Krishna *et al*. (2017) who had reported the strong ACP activity in the seminiferous tubules of adult ram testis. The interstitial connective tissue and Leydig cells had showed the mild ACP activity (Figure 10) which was consistent with the findings of Gopi Krishna *et al*. (2017) in adult ram. The moderate ACP activity within the germinal epithelium was due to active intracellular digestion and recycling processes associated with spermatogenesis.

The adult testis of Aseel crossbred birds had exhibited well-developed seminiferous tubules with active spermatogenesis and abundant collagen fibers within the tunica albuginea and connective tissue septa. The histochemical and histoenzymatic observations had demonstrated the strong PAS positivity and differential phosphatase enzyme activity within the germinal epithelium and interstitial tissues due to active metabolic, absorptive and reproductive functions of the testicular tissue. These findings had provided the valuable information about the structural and functional organization of the avian testis and contributed to better understanding of the reproductive physiology in indigenous poultry breeds.

Conclusion

The adult testis of Aseel crossbred birds had exhibited the well-developed seminiferous tubules with active spermatogenesis due to normal reproductive and functional maturity. The tunica albuginea and connective tissue septa were richly distributed with collagen fibers, providing structural support to the testicular parenchyma. Histochemical observations had demonstrated the strong PAS positivity in the capsule, septa and germinal epithelium due to the presence of abundant glycoconjugates and metabolically active cellular components. The histoenzymatic localization of alkaline and acid phosphatases had revealed differential enzymatic activity within the seminiferous epithelium, spermatozoa and interstitial tissues due to active metabolic, absorptive and cellular turnover processes associated with spermatogenesis. Thus, the present findings had provided the valuable baseline information on the structural, histochemical and functional organization of the adult avian testis which in turn contributed to better understanding of the reproductive physiology and breeding biology in the indigenous poultry breeds.

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