Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(3): 1135-1140

Received: 05-03-2018
Accepted: 06-04-2018

Rabab Saleem
Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Balwinder Singh
Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Khan Idrees Mohd
Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Correspondence
Rabab Saleem
Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India

Scanning electron microscopic studies on male reproductive system of local hill fowl of Uttarakhand (Uttara fowl)

Rabab Saleem, Balwinder Singh and Khan Idrees Mohd

Abstract
The scanning electron microscopic study was conducted on testes, epididymis and ductus deferens of male local Hill fowl of Uttarakhand. The samples were collected from freshly slaughtered birds and fixed using Karnovasky's fluid. The samples were further processed and dried using Critical Point Drier and coated with Gold-Palladium for SEM viewing. The semeniferous tubules were lined by the germinal epithelium and were separated from each other by interstitial connective tissue. Spermatocytes were located in the middle zone of the tubule. In the apical zone, many spermatids often attached to the Sertoli cells. The diameter of spermatogonia was recorded as 8.25x8.65 µm. The efferent ducts consisted of folds which projected prominently into the lumen. They were lined by ciliated and non- ciliated types of cells. The ciliated cells showed tufts of cilia where as the non- ciliated cells free surface was were lined by microvilli. The luminal surface epithelium of both connecting and epididymal duct showed cobbled appearance. The luminal surface of vas deferens was raised at regular intervals giving wavy appearance with ridges and grooves. The cells were non-ciliated in appearance. However, two types of surface cells were identified wherein most of the cells had microvilli on their free surface and the other type of remaining cells were devoid of microvilli.

Keywords: Local hill fowl, male reproductive system, scanning electron microscopy

1. Introduction
Hill fowl an indigenous fowl in the Kumaon region of Uttarakhand, is said to be descended from the Red jungle fowl. The hill fowls are unique in their adaptation to the agro- climatic conditions of their habitat [14]. The reproductive system of male birds consists of pair of testis, epididymis and highly convoluted deferent duct running along the side of the ureter [14]. The production and reproduction traits in birds are directly related to the fertility which in turn is related to the structural and functional status of the reproductive system. This entails a need to gain an insight into the reproductive system of domestic fowl [14]. There is no literature available on the male genitalia of local hill fowl. Therefore, this investigation was proposed to explore the ultra-structural study of male genitalia in the local Hill fowl.

2. Materials and Methods
The experiment was conducted on 18 apparently healthy male local Hill fowl birds belonging to different age groups viz. 20, 24 and 28 weeks. Each group was having 6 birds. The birds were procured from Instructional Poultry Farm, Nagla, GBPUA&T, Pantnagar.
The scanning electron microscopic study was conducted on testes, epididymis and ductus deferens of local Hill fowl as per the method described by, Electron Microscopic manual of AIIMS New Delhi, Malorni *et al.* [15] and Chauhan [7] with some modifications. Representative samples of 1mm size were excised carefully and fixed with 2.5% glutaraldehyde plus 2% paraformaldehyde (Karnovasky's fluid) made in 0.1M sodium phosphate buffer (pH 7.4) by immersion in glass vials for 24 hours. After primary fixation in 2.5% glutaraldehyde, the samples were post fixed in 0.2 M cacodylate buffer for 12 hours. After fixation these tissues were dehydrated through graded acetone from 30% upto absolute acetone to avoid initial osmotic damage to the specimen and critical point dried in liquid CO_2 (Critical point 31.5 degree C at 1100 p.s.i). The dried samples were mounted on aluminium stubs with adhesive tape. The mounted specimens were then gold coated using JFC-1600 Auto Fine Coater gold sputter and were viewed under Scanning Electron Microscope (JEOL JSM-6610 LV).

3. Results and Discussion

3.1 Testis

In present study, it was observed that the bulk of the testis of local Hill fowl consisted of numerous seminiferous tubules (Fig. 1, 2, 3, 4). These tubules were lined by the germinal epithelium and were separated from each other by interstitial connective tissue. Similar findings were observed in mice by Mehraein and Negahdar [16]. The spermatogonia lined the basement membrane along with the sertoli cells (Fig. 2, 3). The diameter of spermatogonia was recorded as 8.25 x 8.65 µm (Fig. 6).There were no slender processes of sertoli cells in this area, whereas a number of Sertoli processes grew in a cluster in the upper area or towards the middle portion of seminiferous tubules. These observations were similar to those of Siews *et al.* [21] in human testis, Elftman [11] in rats and Gravis [12] in Syrian hamster. Spermatocytes were located in the middle zone of the tubule. In the apical zone, many spermatids often attached to the Sertoli cells. In this area, the spermatids at the maturation phase invaded the Sertoli cell only with their heads. There were numerous tails of spermatozoa projecting into the lumen (Fig. 3, 5). Similar findings were observed in dogs by Choudhary *et al.* [8]. The spermatids were elongated cells pointed at one end. The interstitial tissue was primarily composed of Leydig cells, blood vessels and few other cell types such as macrophages and fibroblasts. Seminiferous tubules appeared in the form of cylinders which were stacked with their long axes parallel. Therefore, two types of free space continuous with each other were produced: open, triangular interstices between three adjacent cylinders, and flat, biconcave interstices between two adjacent cylinders (Fig. 1, 3, 4). Similar findings were observed in rat and dog by Clark [9] and Connell [10] respectively.

3.2 Epididymis

Epididymis of local Hill fowl composed of a rete testis, efferent duct, connecting and the epididymal duct. Similar findings were observed in domestic fowl, turkey, Japanese quail and guinea fowl by Tingari [21], Budras & Sauer [6], Hess *et al.* [13], Aire [1], Aire *et al.* [2] respectively. The efferent ducts were lined by ciliated and non- ciliated types of cells (Fig. 7). The ciliated cells showed tufts of cilia where as the non- ciliated cells free surface was were lined by microvilli (Fig. 8, 9). Similar findings were observed in domestic fowl by Budras and Sauer [6] and in turkey by Bakst [4]. However, Aire & Soley [3] in ostrich observed several non-ciliated cells showing single long cilium which projected into the ductal lumen. The luminal surface epithelium of both connecting and epididymal duct showed cobbled appearance. The spermatozoa were present in the lumen of all ducts of the epididymis with their heads attached to the epithelial surface (Fig. 10). Similar findings were observed in ostrich by Aire & Soley [3].

3.3 Ductus deferens

The luminal surface of ductus deferens of local Hill fowl showed numerous longitudinal folds. They were raised at regular intervals giving it a wavy appearance with ridges and grooves (Fig. 11). The apical surface of the epithelial cells appeared slightly dome-shaped with irregularly polygonal boundaries which were marked by shallow grooves (Fig. 12, 13). Every groove presents in the midline a straight ridge due to the overlapping of the adjacent cell membranes. Similar findings were observed by Orlandini *et al.* [20] in human vas deferens. The cells were non-ciliated in appearance. However, two types of surface cells were identified wherein most of the cells had microvilli on their free surface and the other type of remaining cells were devoid of microvilli (Fig. 14). Globular apical protrusions were a typical feature which characterized the epithelium of ductus deferens (Fig. 13, 15). It was in accordance with the findings of Brueschke *et al.* [5] in humans and dogs, Nowell and Faulkin [17] in man, Orlandini and Pacini [18, 19] in rats and humans. The lumen of ductus deferens also showed presence of abundant spermatozoa (Fig. 16).

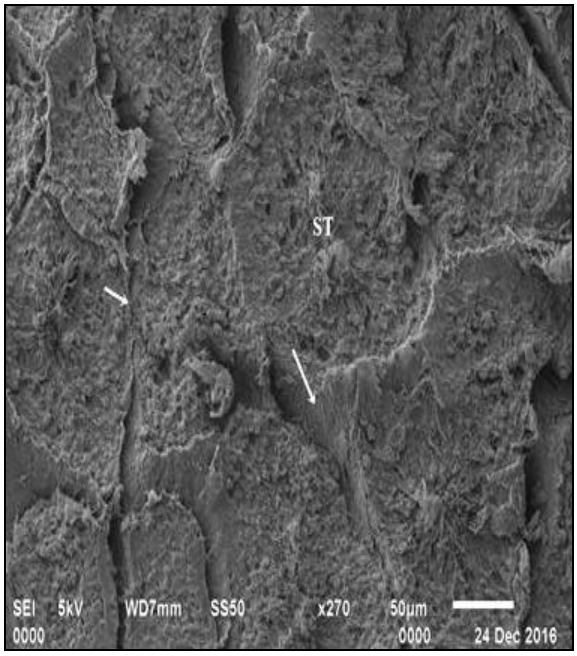

Fig 1: Scanning electron micrograph of testis of local Hill fowl showing seminifero

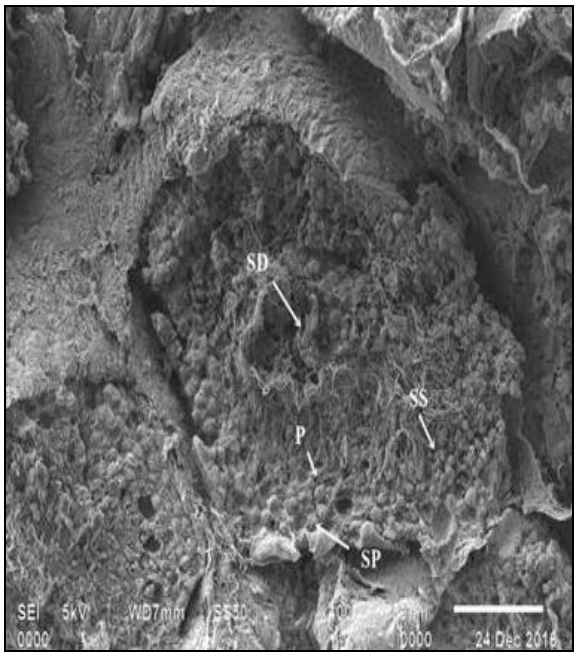

Fig 2: Scanning electron micrograph of testis of local Hill fowl showing spermatogonia (SP), primary spermatocyte (P), secondary spermatocyte (SS) and spermatids (SD) in seminiferous tubule. X 400

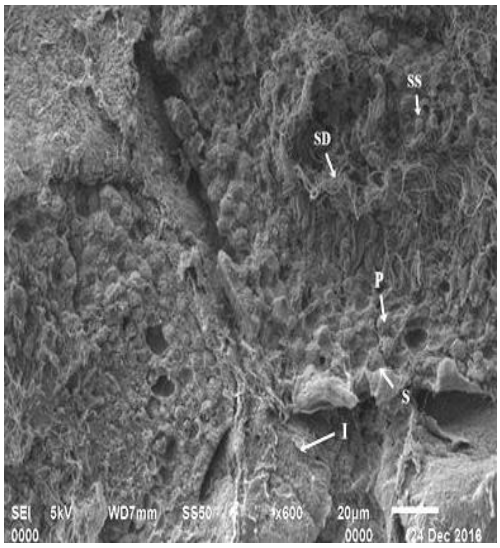

Fig 3: Scanning electron micrograph of testis of local Hill fowl showing spermatogonia (S), primary spermatocyte (P), secondary spermatocyte (SS), spermatids (SD) and interstitial tissue (I). X 600

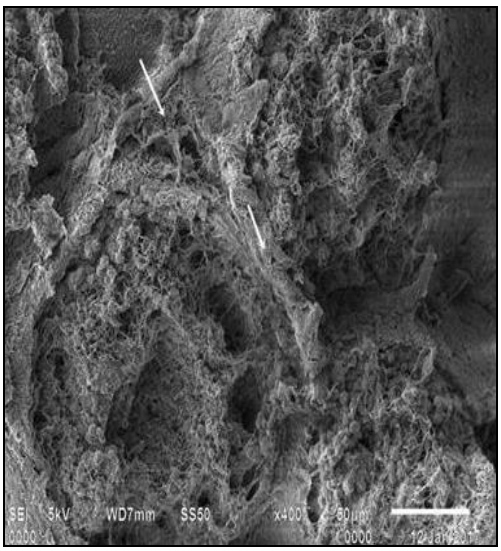

Fig 2: Scanning electron micrograph of testis of local Hill fowl showing spermatogonia (SP), primary spermatocyte (P), secondary Spermatocyte (SS) and spermatids (SD) in seminiferous tubule. X 400

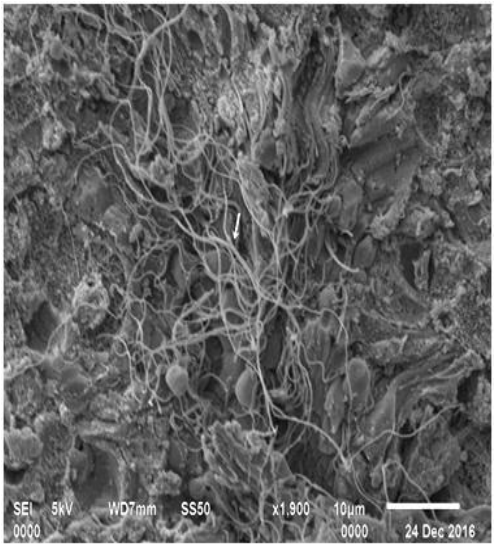

Fig 5: Scanning electron micrograph of testis of local Hill fowl showing spermatids (→). X 1900

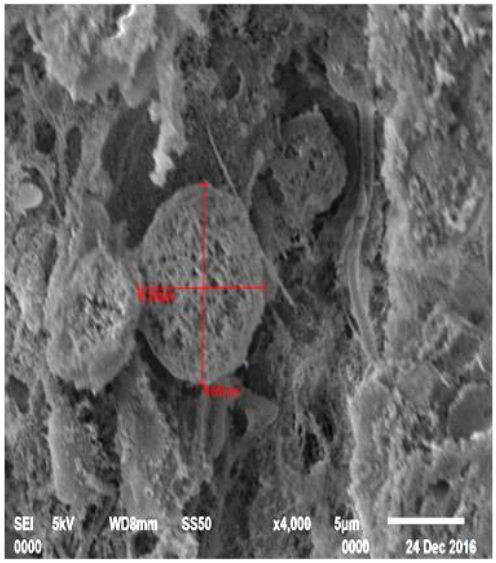

Fig 6: Scanning electron micrograph of spermatogonia showing its average diameter. X 4000

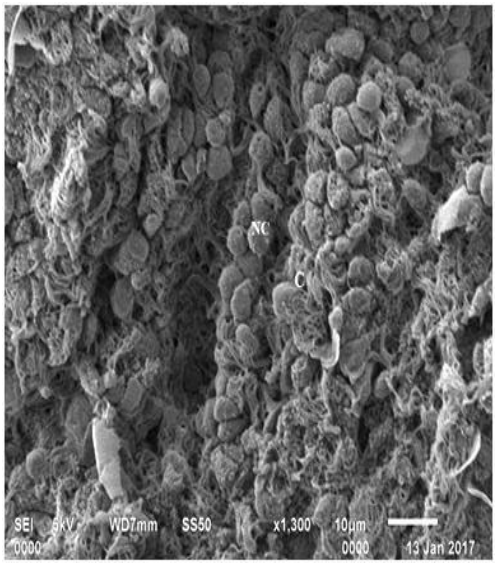

Fig 7: Scanning electron micrograph of efferent duct local Hill fowl showing ciliated (C) and non-ciliated cells (NC). X 1300

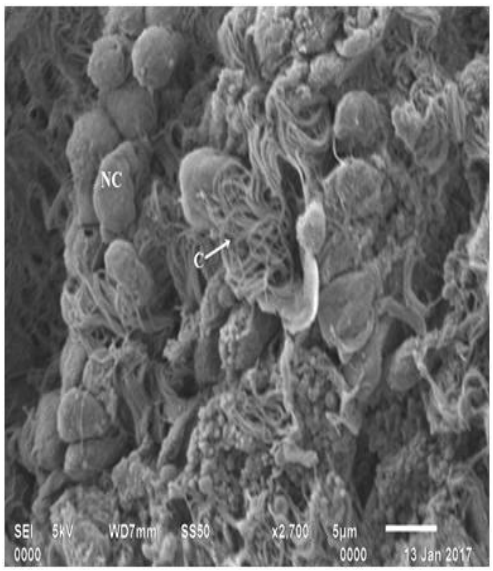

Fig 8: Scanning electron micrograph of efferent duct showing ciliated cells with tufts of cilia (C) and non-ciliated cells lined by microvilli (NC). X 2700

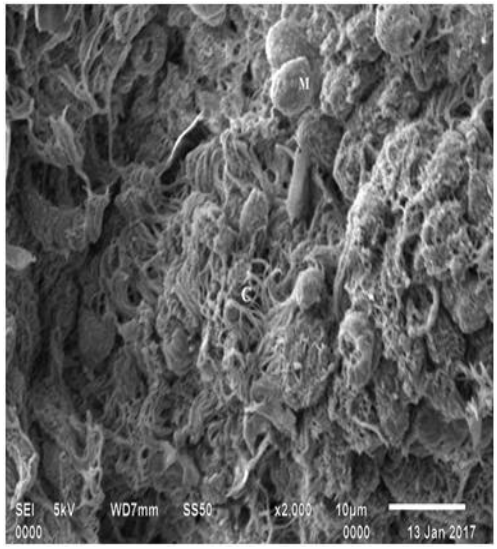

Fig 9: Scanning electron micrograph of efferent duct showing ciliated cells with tufts of cilia (C) and non-ciliated cells lined by microvilli (M). X 2000

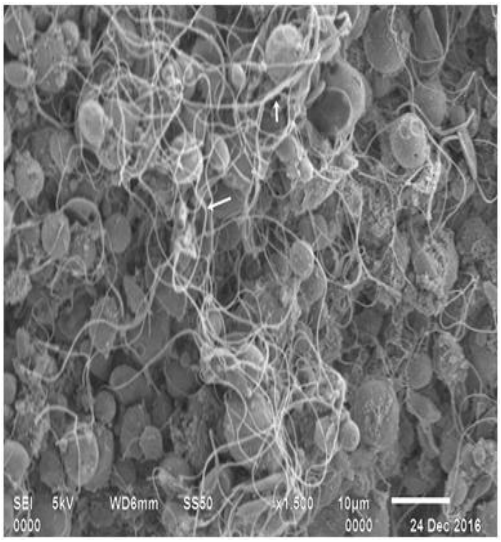

Fig 10: Scanning electron micrograph of ductus epididymis Showing spermatozoa in the lumen with their heads attached to the epithelial surface (→). X 1500

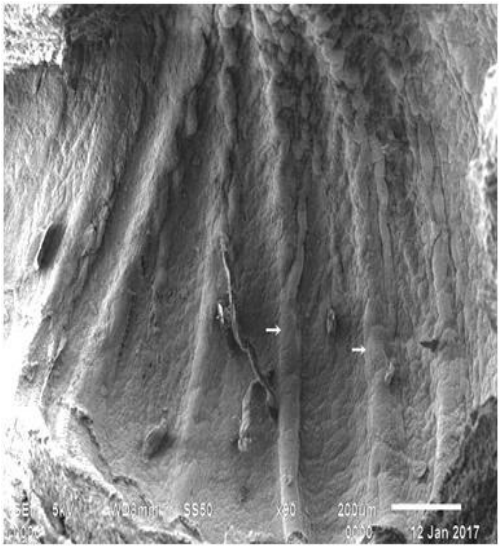

Fig 11: Scanning electron micrograph of luminal surface of ductus deferens of local Hill fowl showing numerous longitudinal folds raised at regula r intervals giving a wavy Appearance (→). X 90

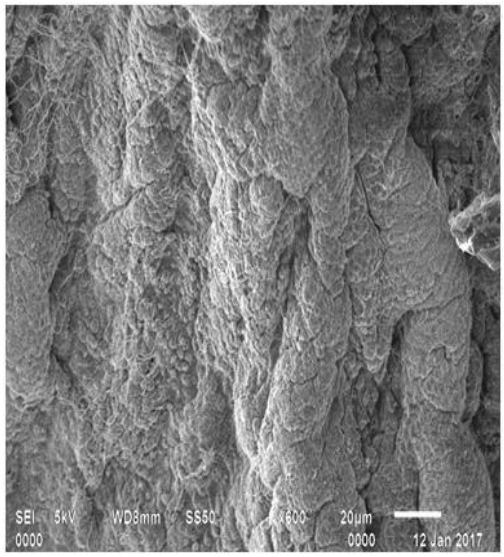

Fig 12: Scanning electron micrograph of apical surface of the epithelial cells of ductus deferens showing dome-shaped irregular polygonal boundaries marked by shallow grooves. X 600

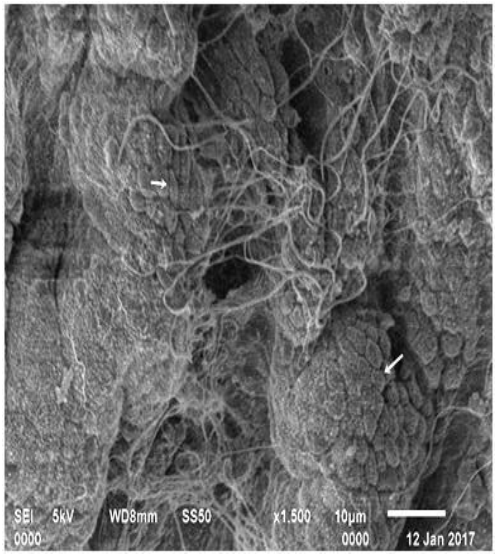

Fig 13: Scannin g electron micrograph of apical surface of the epithelial cells of ductus deferens showing dome-shaped irregular polygonal boundaries marked by shallow grooves (→). X 1500

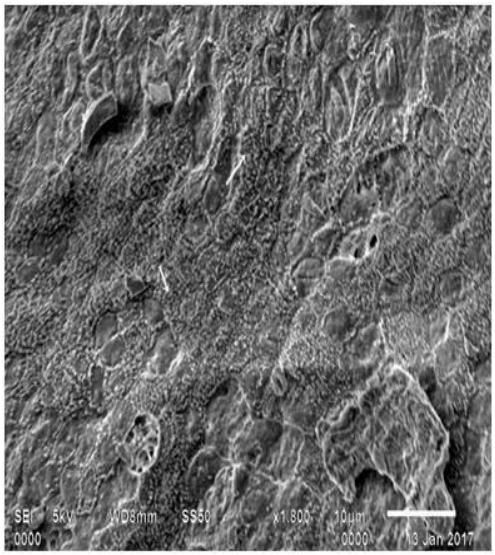

Fig 14: Scanning electron micrograph of ductus deferens of local Hill fowl showing non ciliated cells with microvilli (→). X 1800

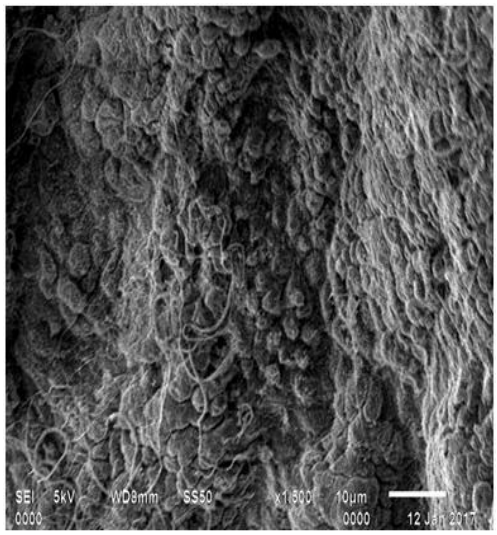

Fig 15: Scanning electron micrograph of ductus deferens of local Hill fowl showing globular apical protrusions. X 1500

Fig 16: Scanning electron micrograph of ductus deferens of local Hill fowl showing presence of abundant spermatozoa in the lumen (→). X 1500

4. Conclusion

The semeniferous tubules were lined by the germinal epithelium and were separated from each other by interstitial connective tissue. The spermatogonia lined the basement membrane along with the sertoli cells. The diameter of spermatogonia was recorded as 8.25 x 8.65 µm. In the apical zone, many spermatids often attached to the Sertoli cells. The efferent ducts consisted of folds which projected prominently into the lumen. They were lined by ciliated and non- ciliated types of cells. The luminal surface of vas deferens was raised at regular intervals giving wavy appearance with ridges and grooves. The cells were non-ciliated in appearance. Globular apical protrusions were a typical feature which characterized the epithelium of ductus deferens. The lumen of ductus deferens also showed presence of abundant spermatozoa.

5. Acknowledgments

I am highly thankful to my advisor Dr. Balwinder Singh Professor Department of Veterinary Anatomy College of Veterinary Sciences G.B.P.U.A.T, Pantnagar for his guidance. I am also thankful to Dr. Khan Idrees Mohd for being side to side during my research work and his ever willingness for help.

6. References

1. Aire TA. The epididymis of the Japanese quail. Acta Anat. 1979; 103:305-312.
2. Aire TA, Ayeni JS, Olowo- Okorun MO. The structure of the excurrent ducts of the testis of the guinea fowl. J Anat. 1979; 129:633-647.
3. Aire TA, Soley JT. The surface features of the epithelial lining of the ducts of the epididymis of the ostrich (Struthio camelus). Anat. Histo. Embryol. 2000; 29(2):119-126.
4. Bakst MR. Luminal topography of the male chicken and turkey excurrent duct system. Scan Electron Microsc. 1980; 3:419-425.
5. Brueschke EE, Zaneveld L, Rodzen R, Berns D. Development of a reversible vas deferens occlusive device. 111. Morphology of the human and dog vas deferens: a study with the scanning electron microscope. Fertil. Steril. 1974; 25:687-702.
6. Budras KD, Sauer T. Morphology of the epididymidis of the cock (Gallus domesticus) and its effect upon the steroid sex hormone synthesis. Anat. Embryol. 1975; 148:175-196.
7. Chauhan RS. Veterinary Laboratory Diagnosis IBDC, Lucknow, 2003, 293-297.
8. Choudhary OP, Dhote BS, Bharti SK. Scanning Electron Microscopic Studies on Sertoli Cell of Dog (*Canis lupus familiarisi*). Indian Vet. J, 2015; 92(5):56-59.
9. Clark RV. Three-dimensional organization of testicular interstitial tissue and lymphatic space in the rat. Anat Rec. 1975; 184(2):203-225.
10. Connell CJ. A scanning electron microscope study of the interstitial tissue of the canine testis. Anat Rec. 1976; 185(4):389-401.
11. Elftman J. Sertoli cells and testis structure. Amer. J. Anat. 1963; 113:25-32.
12. Gravis CJA. Scanning electron microscopic study of the Sertoli cell and spermiation in the Syrian hamster. Amer. J Anat. 1978; 151:21-38.
13. Hess RA, Thurston RJ, Biellier HV. Morphology of the epidiymal region and ductus deferens of the turkey. J Anat. 1976; 122:241-252.
14. Kaur N, Kumar S, Singh B, Pandey AK, Somvanshi SP. Morphological characterization of feathered shank local hill fowl of Central Himalayan Region of India. Indian J Anim Sci. 2010; 80(9):934-936
15. Malorni W, Faris S. Fiorentini. Morphological aspects of Apoptosis. Apoptosis: Laboratory Manual of Expt. Methods (Andrea Cossoriza and Diana Boraschi, Ed.). Purdue University, West Hafayette, USA. 1998.
16. Mehraein F, Negahdar F. Morphometric Evaluation of Seminiferous Tubules in Aged Mice Testes after Melatonin Administration. Cell Journal. 2011; 13(1):1-4.
17. Nowell JA, Faulkin LJ. Internal topography of the male reproductive system. Scan Electron Microsc. IIT Research Institute, Chicago. 1974; 111:639-646.
18. Orlandini GE, Pacini P. Osservazioni a1 microscopio elettronico a scansione sulla super ficie interna del canale deferente di ratto intero e castrato. Arch. Ital. Anat. Embriol. 1976; 81:391-398.
19. Orlandini GE, Pacini P. Scanning electron microscopic observations on luminal surface of vas deferens. Acta Anat. 1977; 99:344.
20. Orlandini GE, Pacini P, Holstein AF. Scanning electron microscopy of human male genital genital tract. Folia Morph. (In press), 1979.

21. Siew S, Troen P, Nankin HR. Scanning electron microscopy of the adult human testis. Scan Electon Microsc. 1980; 4:189-196.
22. Tingari MD. On the structure of the epididymis and ductus deferens of the domestic fowl. J Anat. 1971; 109:423-435.