Ultrastructural variations in canine testes across seasons

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Abstract

A scanning electron microscopic (SEM) study was undertaken on the testes of adult dogs during the summer, monsoon and winter seasons. Testes were collected from adult dogs (mean body weight 20.28 ± 0.65 kg) operated for neutering at the Veterinary Hospital of Municipal Corporation, Junagadh. A SEM approach of the dog testis revealed the seminiferous tubules with germinal epithelium surrounded by a basement membrane, Sertoli cells and interstitial tissue. Sertoli cells had a rough appearance, with two types of processes: club-like and cord-like. The cord-like processes extended from Sertoli cells toward the apical portion. The club-like processes had simple, rough margins surrounding the spermatogonia and spermatocytes. This approach could more clearly distinguish the histoarchitectural details, including lymphatic channels and the structure of spermatozoa. During the summer season, the seminiferous tubules had almost no spermatids and spermatozoa. In contrast, during the monsoon season, the germinal epithelium filled the seminiferous tubules entirely and the lumen was filled with flagella of spermatozoa. During winter, the seminiferous tubules also showed germinal epithelium containing spermatogenic cells of each generation.

Keywords: Dog, testis, SEM

Introduction

Scanning electron microscopy (SEM) remains distinct in its ability to visualise surface topography. (Fischer *et al*., 2024). The development of the scanning electron microscope (SEM) has enabled the recognition of cell and organ surface features and the architectural arrays of cells and tissues (Chemes, 2023). Imaging and molecular technologies have been used to generate diagnostic ailments for reproductive abnormalities and to enhance knowledge about reproductive diseases in domestic dogs. More details are needed to comprehend the basic physiology and structure of dog testicles (Gadariya *et al*., 2025). SEM offers a significant advantage over conventional histological techniques by providing three-dimensional visualisation of surface morphology at high resolution. Unlike light microscopy, SEM enables detailed examination of the ultrastructural organisation of testicular tissue, allowing researchers to observe subtle morphological differences that may result from seasonal changes or environmental factors. Interest in research on canine semen has increased recently (Kumar *et al.,* 2023). However, comprehensive SEM-based studies focused on the adult canine testis, particularly in the Indian climatic context, remain limited.

The present study aimed to elucidate ultrastructural changes in the testes of adult dogs across seasons, focusing on morphological features observable under scanning electron microscopy (SEM). By comparing the ultrastructural changes observed during summer, monsoon and winter, the investigation aimed to elucidate the influence of season on spermatogenesis and overall testicular function. By analysing seasonal variations, the research could provide valuable insights into the reproductive physiology of canids in the Indian context, where climatic fluctuations can significantly affect reproductive cycles. Understanding these microstructural adaptations not only enhances our knowledge of canine reproductive biology but also aids in optimising breeding management and conservation strategies for domestic and wild canids in similar environments.

Materials and methods

Testes were collected from 6 adult dogs (mean body weight 20.28 ± 0.65 kg), two in each season, *viz*., summer, monsoon and winter. The sample collection was done from dogs operated for neutering at the Veterinary Hospital of Municipal Corporation, Junagadh. After collecting small pieces of tissue samples from the testis, the samples were washed using cold 0.1 M phosphate buffer at a pH of 7.2. The tissue samples were then kept in a 2.5% glutaraldehyde solution prepared in 0.1 M phosphate buffer at pH 7.2 for a period of six to eight hours. Once the fixation process was completed, the samples were rinsed three times in 0.1 M phosphate buffer at 4°C, with each rinse lasting 15 minutes. The samples were then dehydrated at 4°C using a series of ascending concentrations of acetone, *viz*., 30%, 50%, 70%, 80%, 90%, 95% and 100% (pure acetone). Following dehydration, the samples were dried using critical point drying with liquid carbon dioxide (CO2). The samples were then placed in a desiccator. To prepare the specimens for examination, they were mounted on aluminium stubs using double-sided carbon conductive tape. An Emitech SC7620 sputter coater was used to apply a thin layer of gold to the samples (Plate 1 - A & B). The SEM observations were recorded on a Zeiss EVO-18 (Germany), at the Department of Biotechnology, Junagadh Agricultural University.

Results

Scanning electron microscopic (SEM) observations revealed that the seminiferous tubules had a convoluted appearance (Plate 2). The basal surface of the basement membrane appeared either smooth or had a slightly undulated appearance. The cross-section of the seminiferous tubules contained a highly compact stratified germinal epithelium, encompassing the population of germinal cells and Sertoli cells. Between the seminiferous tubules, large lymphatic channels were observed that separated the seminiferous tubules from each other. The interstitial tissue possessed Leydig cells (Plate 3).

The Sertoli cells were rough in appearance, with many visible processes. The Sertoli cells were observed with two types of processes, club-like and cord-like processes. The club-like processes had simple, rough margins originating from Sertoli cells, surrounding the surfaces of spermatogonia and spermatocytes (Plate 4). Spermatogenic cells were not easily differentiated from each other. Numerous spermatogonia were observed at the basal portion of the seminiferous tubules and other spermatogenic cells were noted towards the apical portion. At the lumen, numerous spermatids and spermatozoa were observed.

During the summer season, the seminiferous tubules consisted of a clear lumen, which had almost nil spermatids and spermatozoa. A greater number of spermatogonia were observed in the basal portion. Toward the lumen, very few spermatids and a few spermatozoa were observed. These cells were degenerating cells, with flagella observed in the lumen at higher magnification (Plate 5).

In contrast, during the monsoon season, the germinal epithelium completely filled the seminiferous tubules. In the tubules, cord-like processes of Sertoli cells were observed from the basal compartment to the luminal compartment (Plate 6). At the lumen, where the spermatid or head of spermatozoa attached to the Sertoli cells, the club-like processes were observed. (Plate 7). As the spermatozoa become older or aged, the apical Sertoli

cell processes change in range, ultimately releasing the spermatozoa head; thus, the spermatozoa detached from the Sertoli cell were found freely in the lumen of seminiferous tubules (Plate 8).

During the winter season, no noticeable differences were observed in the ultrastructure of the dog testis when compared with that of the monsoon season. As observed in the monsoon, the seminiferous tubules showed Sertoli cells and germinal epithelium containing each generation of spermatogenic cells (Plate 9). With the higher magnification in the luminal compartment, the spermatozoa were observed. The head of the spermatozoa was attached to the club-like process of the Sertoli cell. The head, middle piece and flagella of spermatozoa were well defined and distinguished (Plate 10). As observed in the monsoon season, the lumen was filled with flagella of spermatids or spermatozoa (Plate 11).

Plate 1, A & B: Photograph showing specimens mounted on aluminium stubs with double-sided carbon conductivity tape

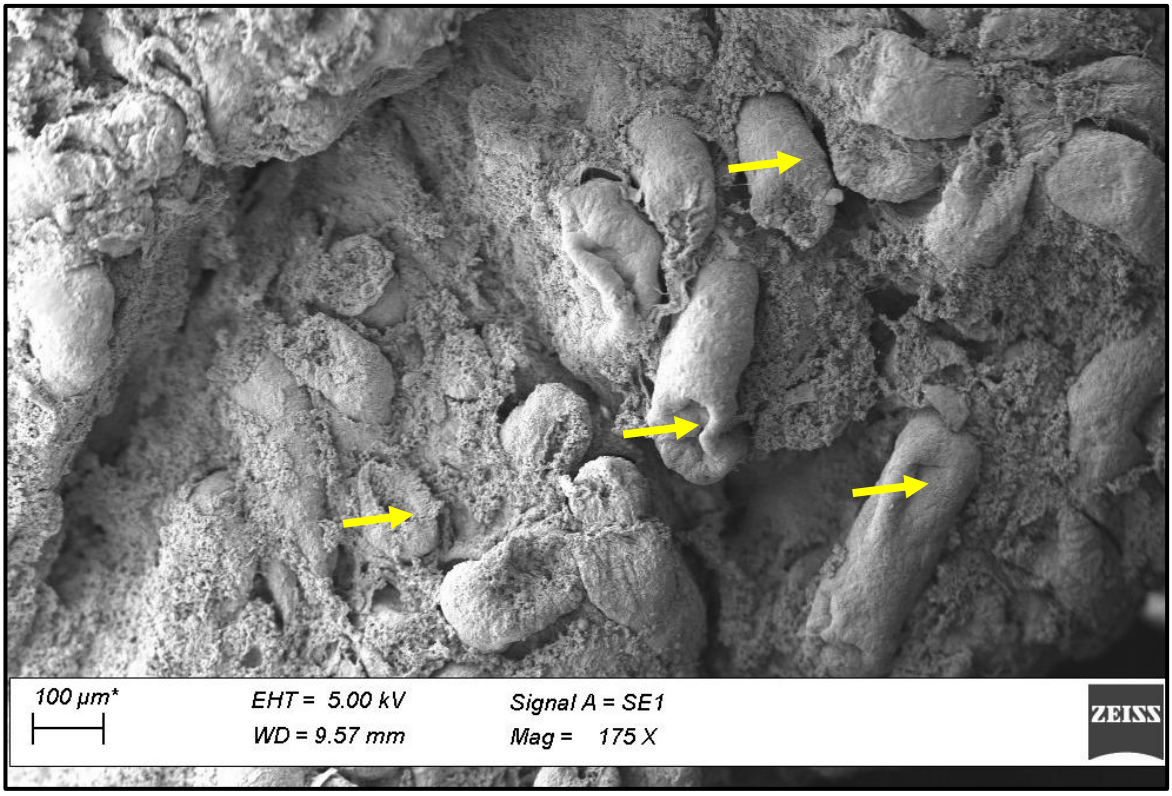

Plate 2: Scanning electron photomicrograph of the testis showing seminiferous tubules (arrow). X 175.

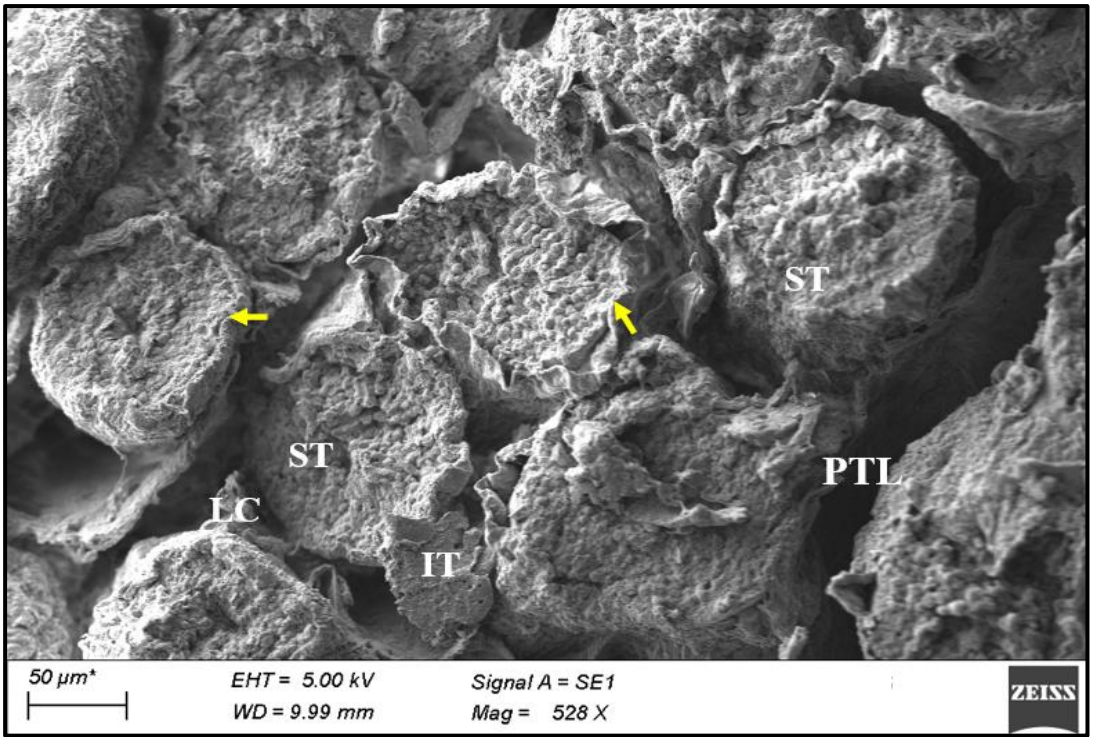

Plate 3: Scanning electron photomicrograph showing seminiferous tubules (ST), basement membrane (arrow), peritubular lymphatic channel (PTL), interstitial tissue (IT) and Leydig cells (LC). X 528.

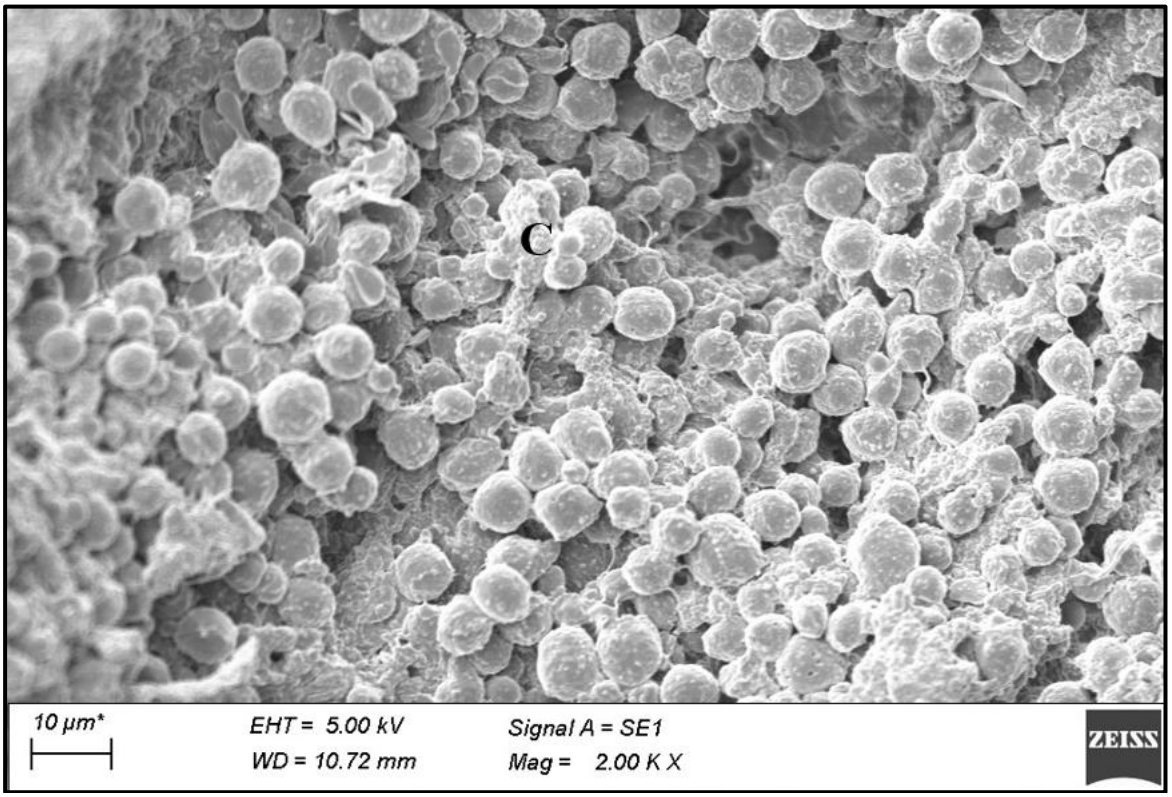

Plate 4: Summer season. Scanning electron Photomicrograph showing germinal epithelium and club-like processes (C) of Sertoli cells. X 2.00 K.

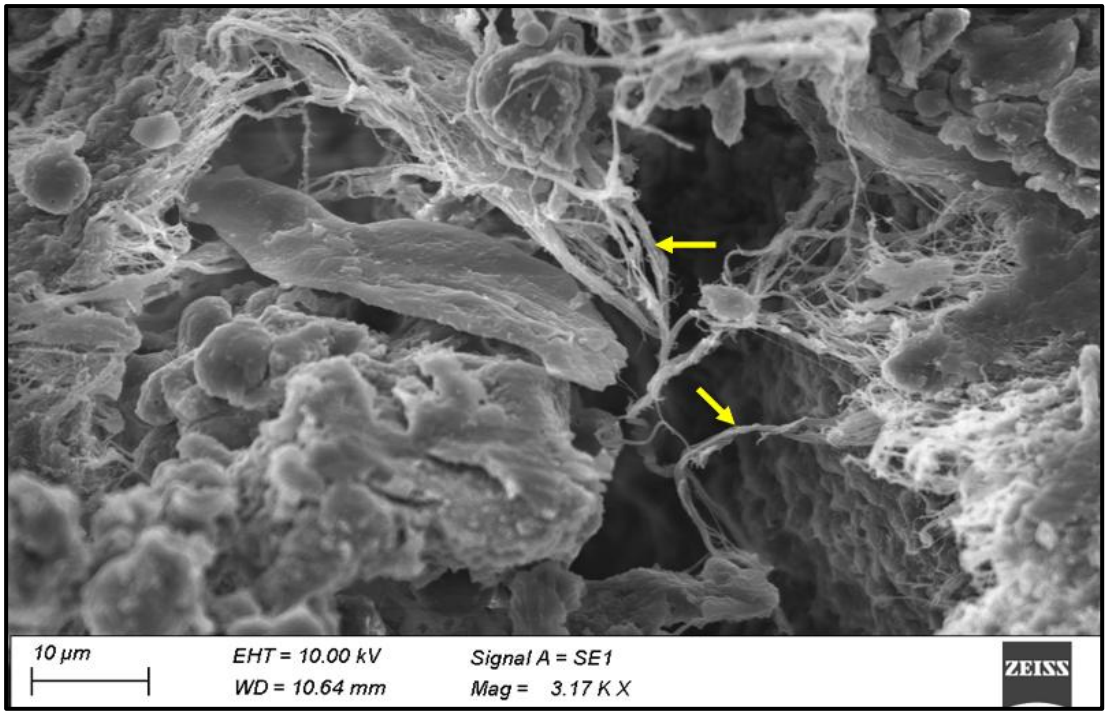

Plate 5: Summer season. Scanning electron photomicrograph showing degenerating flagella (arrow) in the lumen. X 3.17K.

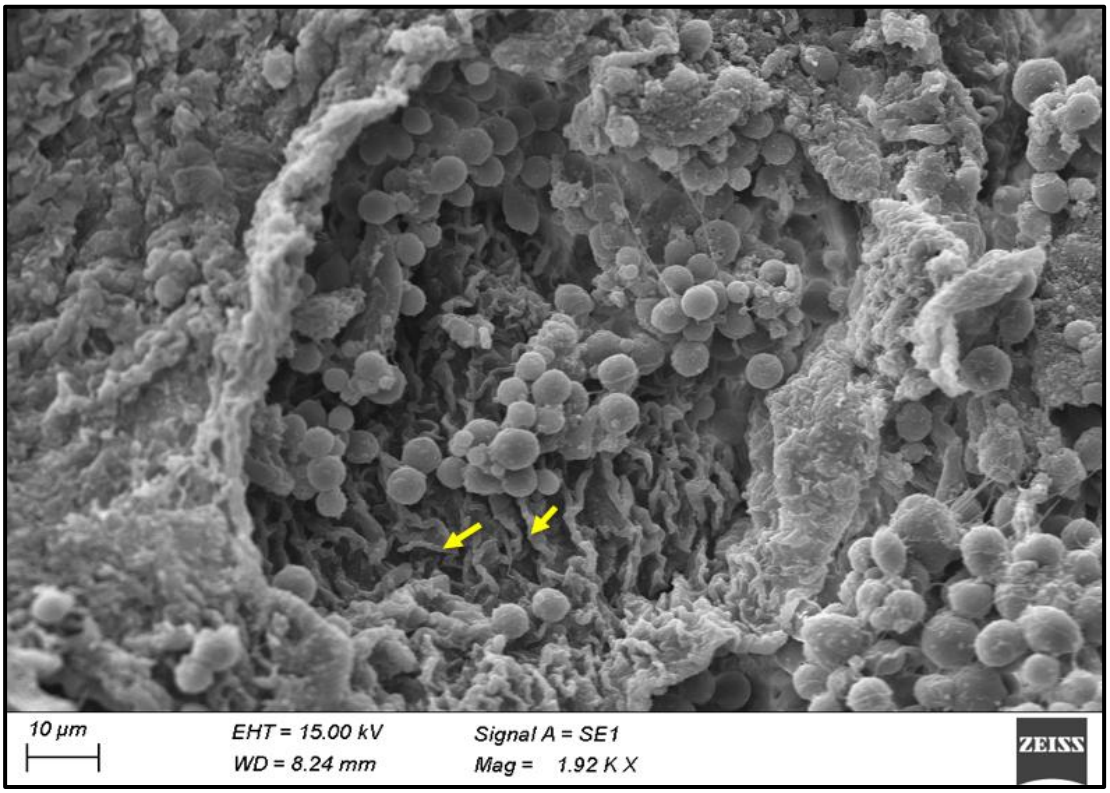

Plate 6: Monsoon season. Scanning electron photomicrograph showing cord-like processes (arrow) of Sertoli cells and germinal epithelium with a lumen containing spermatids and spermatozoa. X 1.92K.

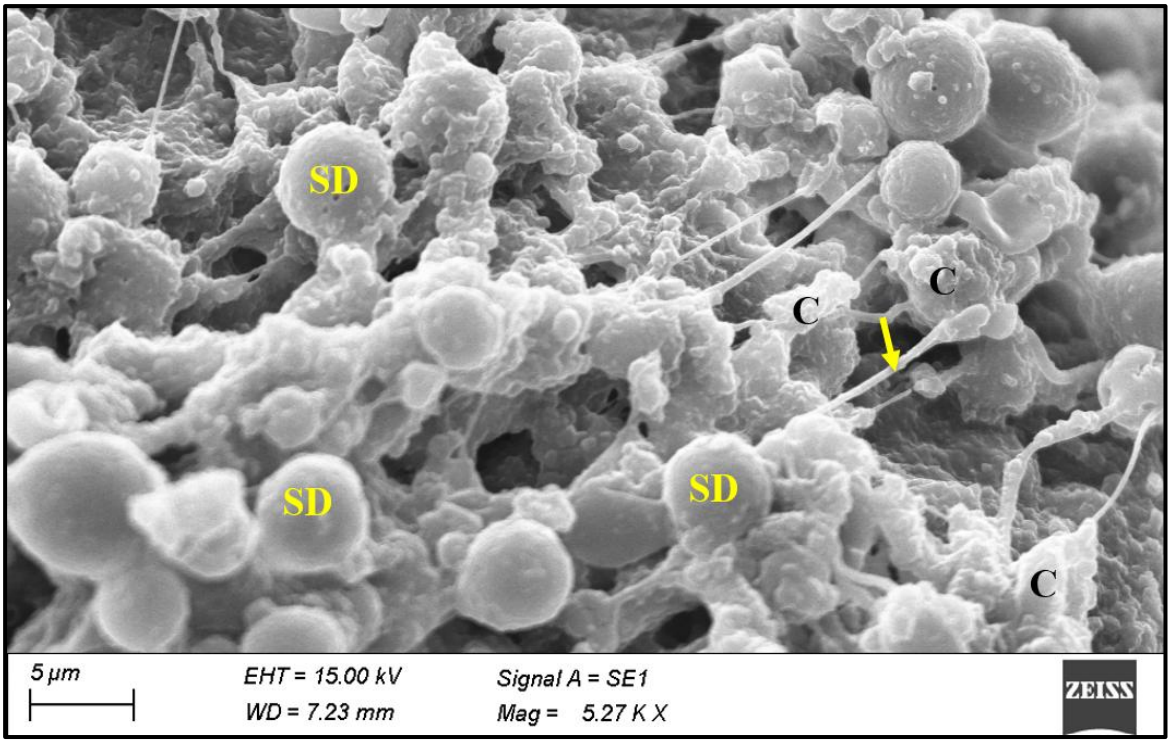

Plate 7: Monsoon season. Scanning electron photomicrograph showing club-like (C) processes of Sertoli cells with numerous spermatids (SD) and flagella of spermatozoa (arrow). X 5.27K.

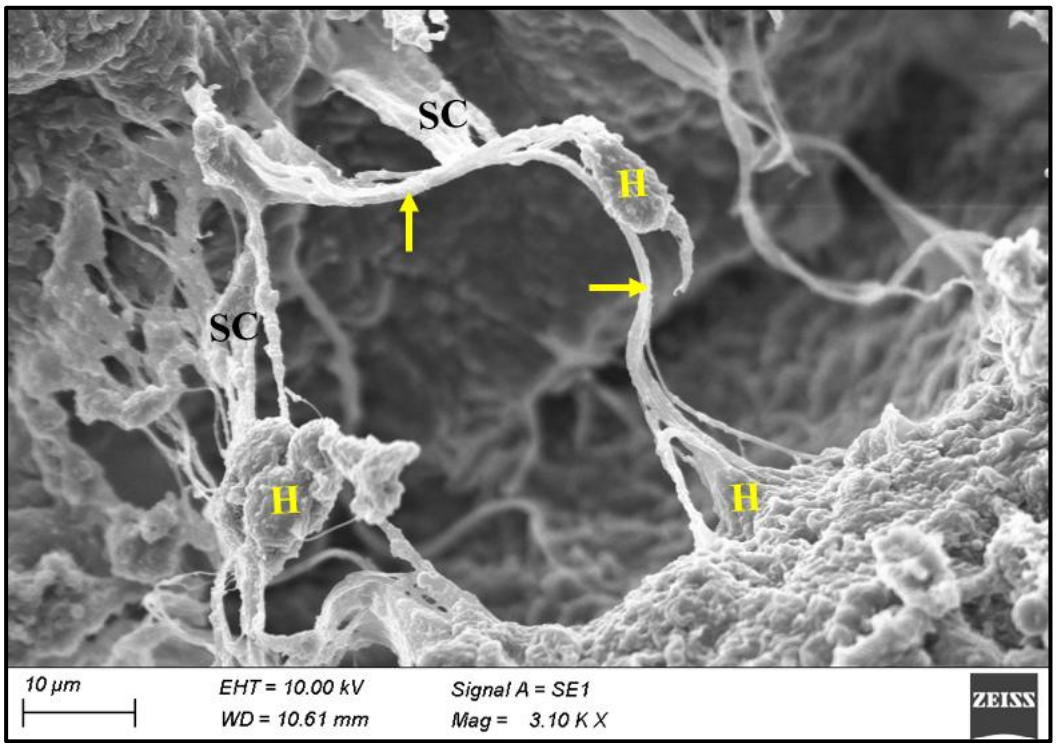

Plate 8: Monsoon season. Scanning electron photomicrograph showing Sertoli cells (SC), head of spermatozoa (H) and tail of spermatozoa (arrow). X 3.10K.

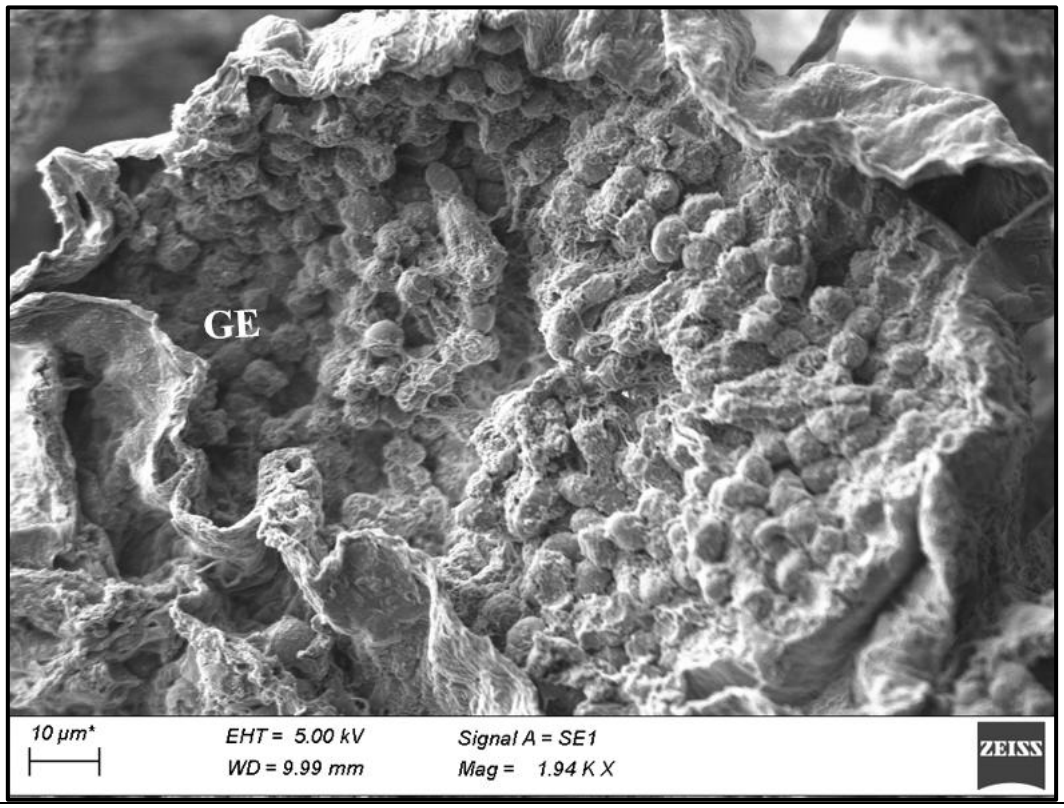

Plate 9: Winter season. Scanning electron photomicrograph showing germinal epithelium (GE) with a lumen containing spermatids and spermatozoa. X 1.94K.

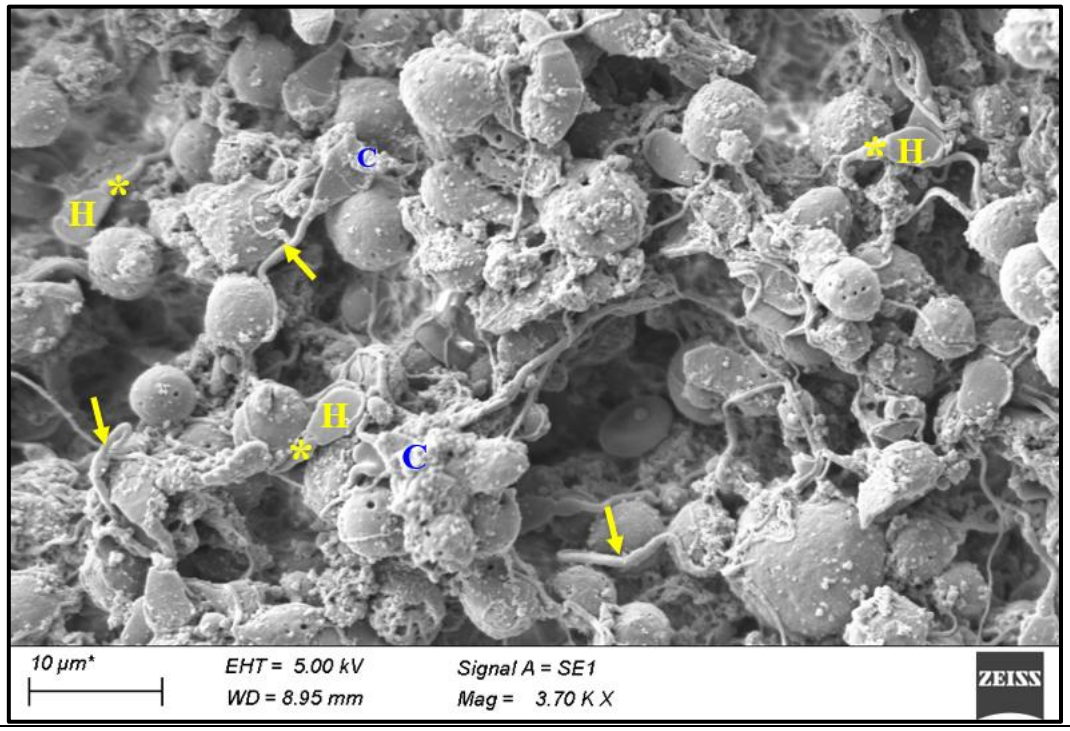

Plate 10: Winter season. Scanning electron Photomicrograph showing spermatozoa head (H), middle piece (asterisk *), tail (arrow) and club-like process (C) of Sertoli cell. X 3.70K.

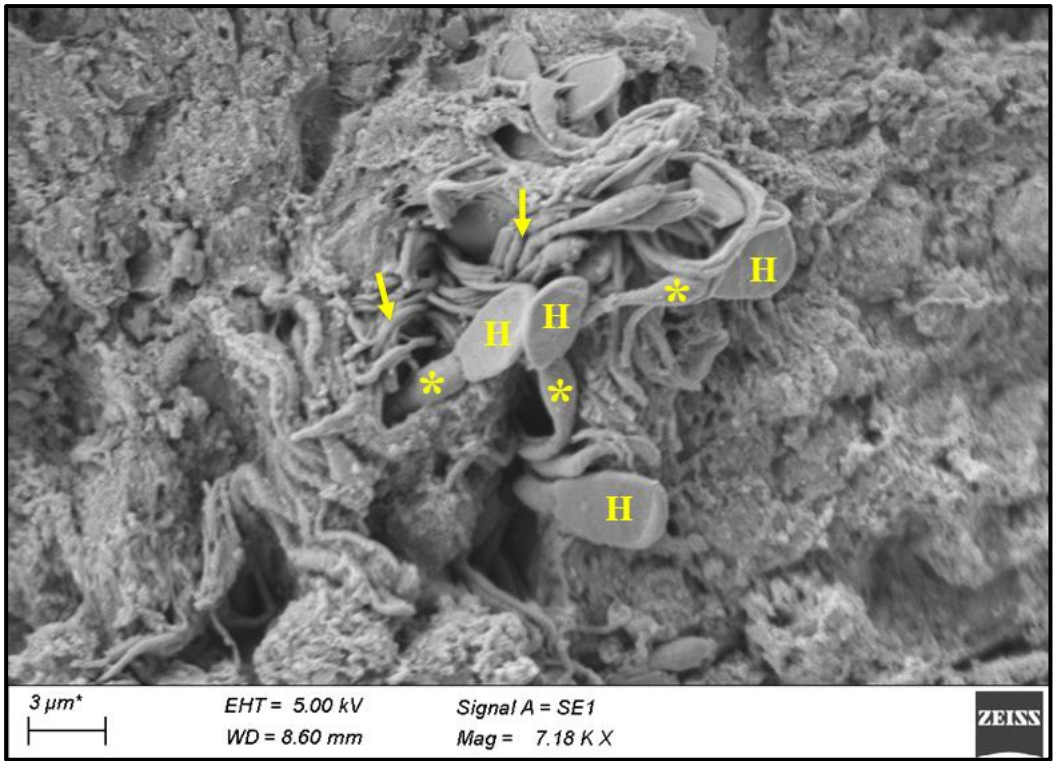

Plate 11: Winter season. Scanning electron photomicrograph showing spermatozoa head (H), middle piece (asterisk *) and tail (arrow). X 7.18K.

Discussion

The current findings on the histoarchitecture of dog testis through a scanning electron microscopic approach were consistent with the results of Connell (1976), who reported that the seminiferous tubules made up the canine testis and in the enlarged intertubular area, interstitial tissue with clusters of Leydig cells was present. Johnasan *et al*. (1978), in their SEM study on the stallion testis, found that the principal parts of spermatid flagella expanded into the tubular lumen, which supports the present results. Moreover, the spongy, web-like cytoplasm of the Sertoli cells or germ cells covered the whole height of the germinal epithelium, which was not observed in the current study. Young *et al.* (1993) observed three types of Sertoli cell processes, *viz.*, club-like, cord-like and sheet-like, in their SEM study on dog testis.

The current findings regarding round spermatids, attachment of the head of spermatozoa and free spermatozoa were endorsed by the observations of Choudhary *et al*. (2014). In the present study, only two types were noted: club and cord-like processes were observed. Derbalah *et al.* (2017) reported sheet and cord-like processes in the bull testis. Our findings from the study on Sertoli cell processes during the monsoon and winter seasons suggest active spermatogenesis in these seasons which is supported by Villagra *et al*. (2018), who reported that Sertoli cells act as a structural support for germ cells during spermatogenesis.

Conclusions

Scanning electron microscopic (SEM) approach of dog testis revealed the seminiferous tubules and interstitial tissue. Sertoli cells were rough in appearance, with two types of processes: club-like and cord-like. The cord-like processes extended from Sertoli cells toward the apical portion. The club-like processes had simple, rough margins surrounding the surfaces of spermatogonia and spermatocytes. The SEM approach could more clearly distinguish the histoarchitecture, including lymphatic channels and the detailed structure of spermatozoa. During the summer season, the seminiferous tubules had almost nil spermatids and spermatozoa. Whereas, in the monsoon and winter seasons, the germinal epithelium filled the seminiferous tubules and the detailed structure of spermatozoa was observed in the lumen.

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