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Research article

Seasonal histological effects on the Leydig and Sertoli cells in the testis of indigenous dogs (*Canis Familiaris)*

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Abstract

This study was performed on twenty adult male dogs aged 2-3 years old. Samples of testis tissue were taken during the four seasons in 2016 in Iraq. Specimens were fixed in 10% formalin. Routine histological techniques were carried out for light microscopy. H&E and PAS stains were used. During active seasons (spring and autumn) the study revealed a significant increase in the number and diameter of Leydig and Sertoli cells compared to other inactive seasons (summer and winter). Cytological indications of mitotic events of Leydig cells were clearly observed, whereas, Sertoli cells never showed any mitotic divisions. The basement membrane of seminiferous tubules appears wavy with impacts of irregular gates of cellular penetrations. It was characterized by dynamic, flexible structure, and can respond easily to the cellular mechanical pressure. The new generation of Leydig cells was derived either from the differentiation of interstitial fibroblast-like cells or from the mitosis of Leydig cells themselves; whereas the new generation of Sertoli cells was provided only by the differentiation of interstitial fibroblast-like cells. The study suggested that, after stimulation, the compensatory fibroblast-like cells can penetrate the basement membrane and then differentiate to Sertoli cells, as the similarity between the two cells were very large. It was concluded firstly that the proliferation of Sertoli cells within the seminiferous tubules was done only by the penetration and differentiation of the interstitial fibroblast-like cells and not by mitosis of Sertoli cells themselves.

Keywords: Testis, Seasonal, Leydig cells, Sertoli cells.

Introduction

The fibroblast has abundant and irregularly branched long cytoplasmic processes that extend to adjacent cells. Its nucleus was ovoid, large, and pale staining with fine chromatin and prominent nucleolus. Its cytoplasm is rich in rough ER and well developed Golgi apparatus. Fibroblast is reservoir of growth factors that influence cell growth and differentiation (1). Fibroblast can divide when the testis requires additional fibroblasts and, under stimulation, can give rise to other cell types and converted to another state. Myofibroblast, a cell with properties of both fibroblasts and smooth muscle cells have the most morphological characteristics of fibroblasts. The fibrocyte can be micro environmentally stimulated to return to a more metabolically active state (2). Many researches referred to the effect of age and season on Sertoli population in the testes of stallions (3, 4, 5, 6). The junctions between the basolateral membranes of adjacent Sertoli cells give rise to the tightest blood-testis barrier (7, 1). (8) reported that basement membrane can be penetrated by the decidual cells that may aid in its

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disintegration. In active states, the areas of penetration of basement membrane appear irregular and tortuous and changing the usual linear appearance of the basement membrane.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 420

A total of 20 clinically healthy local breed adult male dogs were used in this study. The study was performed in 2016 in Iraq. Based on season, animals were divided into four groups (5 of each). Spring season (March to May), summer (June to August), autumn (September to November) and winter (December to February).All animals were euthanized by intra-venous injection of over dose of sodium pentobarbital (100 mg/kg) (9). Testes were collected and opened longitudinally then washed with normal saline. To investigate the seasonal variation in testicular tissue, two representative specimens of 1 mm^3 from each sample were collected and fixed with neutral buffered formalin for 72 hrs. The specimens were processed by routine histological processing method to obtain histological sections of (5-7) µm thickness (10). H&E and PAS stains were used in this study (11).The general histological picture of testis especially the cellularity of the interstitium in each season was studied. Other micro morphometrical parameters were recorded in this study, namely the number and diameter of Sertoli cells; the number, location and cellular properties of Leydig cells, the thickness of basement membrane of seminiferous tubules, the presence or absence of vacuoles in the interstitial tissue. All the above parameters were set in tables to achieve comparison among different seasons.

Results

Generally, the present study revealed that the cellular activity of the testes in winter time was similar to that of summer time; and in spring time was similar to that of autumn time. Table revealed that spring and autumn times showing the highest reproductive activity of the dog testis whereas, in winter time, this activity was decreased, and then subsides in summer time.

Winter season:

Sertoli cells were found as elongated cells with oval to triangular pale nuclei and prominent dark nucleoli. The cytoplasm and boundaries of Sertoli cells were difficult to be recognized by routine stains, but the limits could be detected easily only during the accumulation of mature spermatids at the cytoplasmic processes of these cells. The results showed that the least number of Sertoli cells per tubule was recorded in winter season. Its long axis was less than those of spring and autumn but there was no significant difference in its nuclear diameter compared with other seasons (Table 1) and (fig.1).The cytoplasm of Leydig cells was poorly acidophilic and its cellular boundaries were identified difficulty, their nuclei were obviously dark, rounded, with small nuclear diameter and one central or eccentric prominent nucleoli Figure (2). Based on season, Leydig cells occupied two locations, either near the seminiferous tubules (peritubular Leydig cells) or around the blood vessels (perivascular Leydig cells). Lipoid vacuoles were present in the blood stream Figure (3). The cytoplasm of Leydig cells containing ill-defined small vacuoles but, relatively large clear vacuoles were recorded in the interstitial tissue near the accumulations of Leydig cells. In this season and summer times, the number of Leydig cells in the interstitial tissue was less than that of spring and autumn Table (1). The basement membrane appeared unwary (or

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nearly straight) with a mean thickness higher than those of spring and autumn .

Spring season:

In active spring season, the current study revealed a significant elevation in the number of Leydig and Sertoli cells. Prominent Sertoli cells showing apparent cytoplasmic processes and large prominent euchromatic nuclei with obvious (1-2)nucleoli.The highest number of Sertoli cells was recorded in this season which appeared well developed and its cytoplasmic processes reach to the lumen of seminiferous tubules with highest long axis. Despite there was increase in the size and cytoplasmic processes, the nuclear diameter of Sertoli remains intact, as well as, some of Sertoli cells had two nucleoli in active spring season Table (1) and Figure (5).In the interstitial tissue, the highest number of Leydig cells was recorded in spring season with higher nuclear diameter. There was direct proportional increase of Leydig and Sertoli cells. In the beginning of mitosis the offspring nuclei of Leydig cells were smaller, and then they reach their natural size in the mature Leydig cells. Most of Leydig cells were of peritubular type as they located closely near the seminiferous tubules. Binucleated Leydig cells were clearly observed with only one nucleolus Figure (6). The basement membrane of seminiferous tubules appeared thin and wavy. The related myoid cells that surrounding the seminiferous tubules appeared stretched flattened or elongated Figure (7).

Summer season:

Sertoli cells had fewer number, and shorter long axis than winter. On the other hand, most of Leydig cells were found closely near or around blood vessels in the interstitial tissue (perivascular type) and many mitotic figures were recorded in this season Figure (8). The nuclei of Leydig cells were pale in color and have smallest diameter if compared with other seasons, as well as, their number was less than that of spring and autumn but similar to that of winter. The basement membrane appeared straight with a mean thickness higher than those of spring and autumn.

Autumn season:

The histological features of testis were nearest to that of spring season. Number of Sertoli and Leydig cells in the interstitial tissue was higher than those of winter and summer but less than that of spring with higher nuclear diameter. The basement membrane of seminiferous tubules appeared wavy in some autumn section with narrow thickness Table (1) and Figure (9).

Table (1): Micromorphometric measurements of different parameters in dog testis at different seasons (µm)

Season Parameters	Winter	Spring	Summer	Autumn
No. of leydig cells/ mm²	361.87± 13.4 A	415.88± 17.1 B	337.57± 15.9 A	407.78± 20.3 B
No. of sertoli cells/ tubule	15.25±0.366 A	24.37±±0.49 B	16.5±0.42 A	19.87±0.398 B
Basement membrane thickness/µm	3.7±0.08 A	3.1±0.12 B	3.8±0.06 A	2.9±0.09 B
Nuclear diameter of Leydig cell/ µm	5.1±0.02 A	5.6±0.01 A	4.9±0.08 A	5.5±0.07 B
Nuclear diameter of Sertoli cell/ µm	7.9±0.4 A	8.03±0.1 A	8.1±0.09 A	8.06±0.08 A
Length of Sertoli cell	20.3±1.01A	33.8±2.1B	17.2±1.29A	29.4±1.7 B

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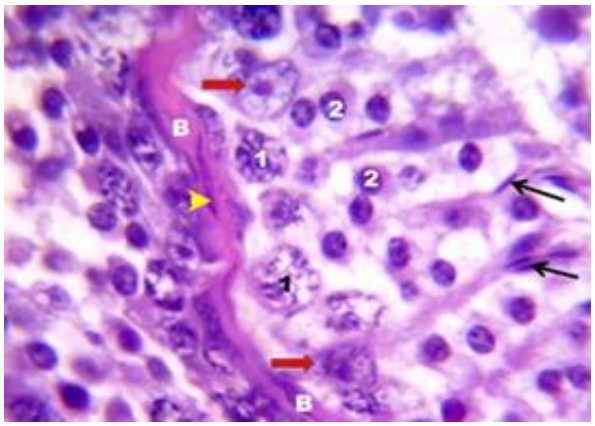


Figure (1): Microphotograph of seminiferous tubule in spring season showing wavy basement membrane (B) and related myoid cell (yellow arrowhead), Sertoli cells with its cytoplasmic processes (red arrows), primary spermatocytes (1), secondary spermatocytes (2) and late spermatids (black arrows). H&E stain, X1000

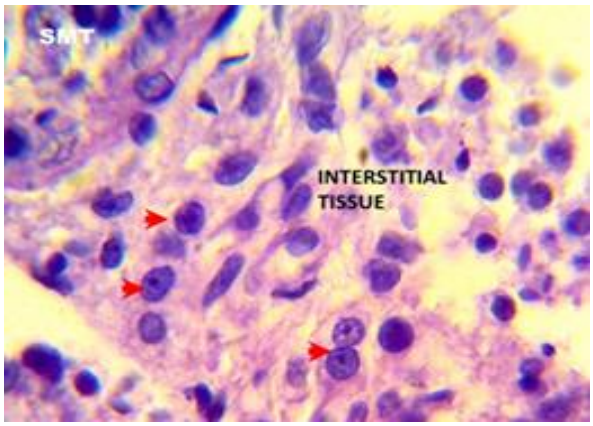
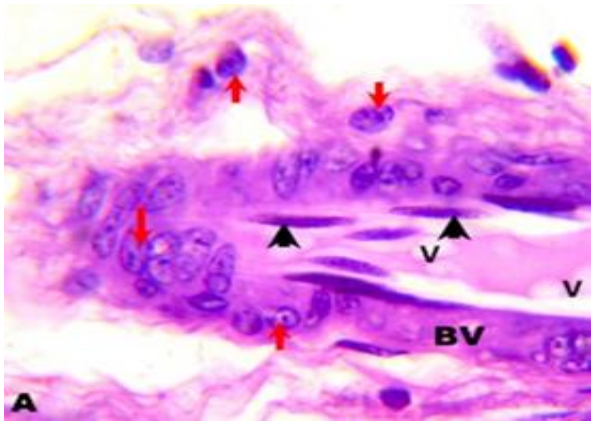


Figure (2): Microphotograph of interstitial tissue of testis near seminiferous tubule (SNT) in winter season showing newly formed dark nucleated leydig cells (red arrows). Note the central or eccentric prominent nucleoli. H&E stain, X1000



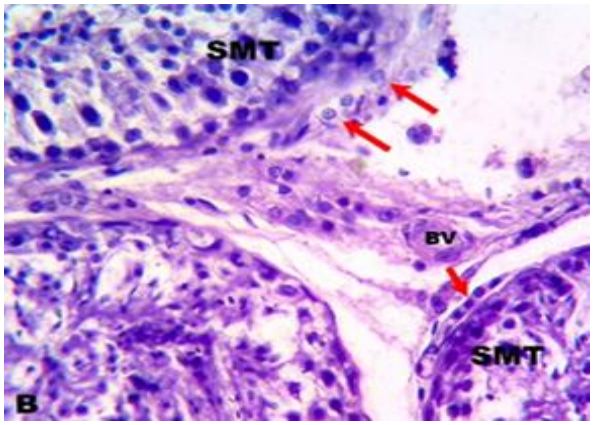


Figure (3): Microphotograph of interstitial tissue of testis showing perivascular Leydig cells(red arrows) located around blood vessel (BV) in winter season (A) while they located peritubular in spring season (B) H&E stain, (A)X1000 , (B) X400.SMT= seminiferous tubule , V= endothelial cells.

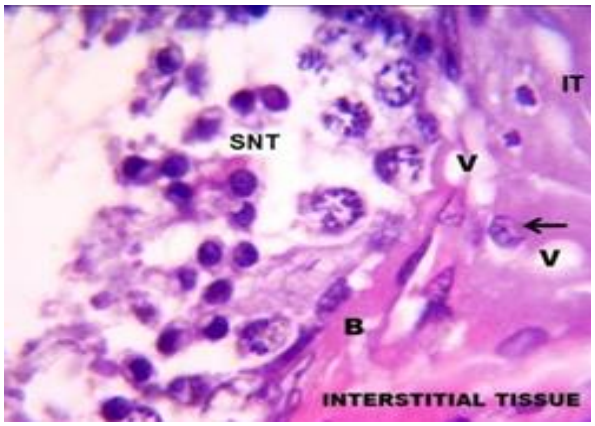


Figure (4): Microphotograph of testis showing (SNT) and interstitial tissue (IT) in winter season. Note the large vacuoles (V) near leydig cell (arrow) in the interstitial tissue. H&E stain, X1000.

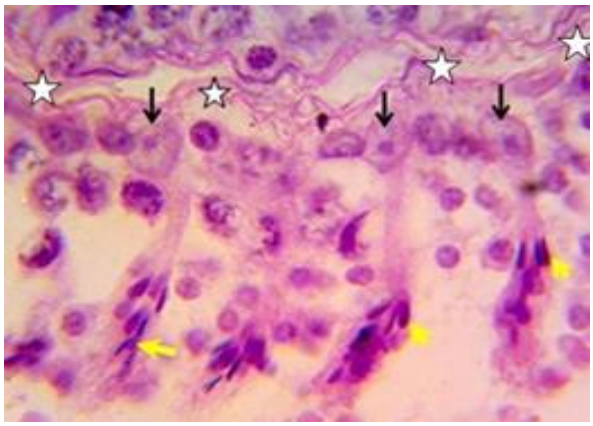


Figure (5): Microphotograph of seminiferous tubule in spring season showing wavy basement membrane (stars) and Sertoli cell nuclei (black arrows) with its long

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cytoplasmic processes, which harbor mature spermatids (yellow arrows). H&E stain, X1000

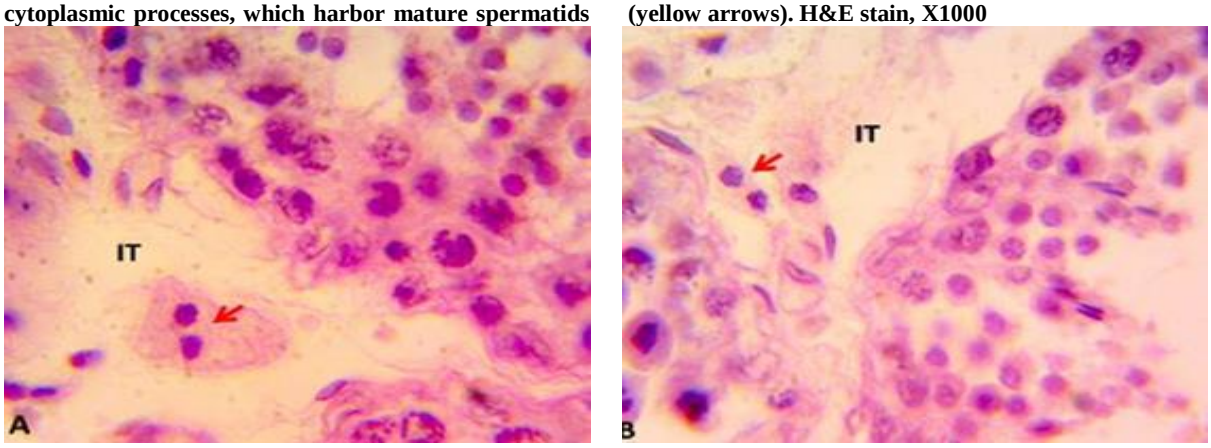

Figure (6): Microphotograph of interstitial tissue (IT) of testis in spring season showing Binucleated Leydig cells (red arrows). H&E stain, X1000

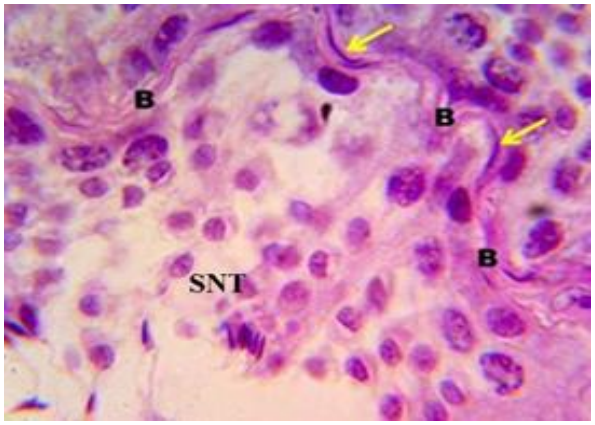

Figure (7): Microphotograph of (SNT) in spring season showing wavy basement membrane (B) and related stretched myoid cells (yellow arrows). H&E stain, X1000

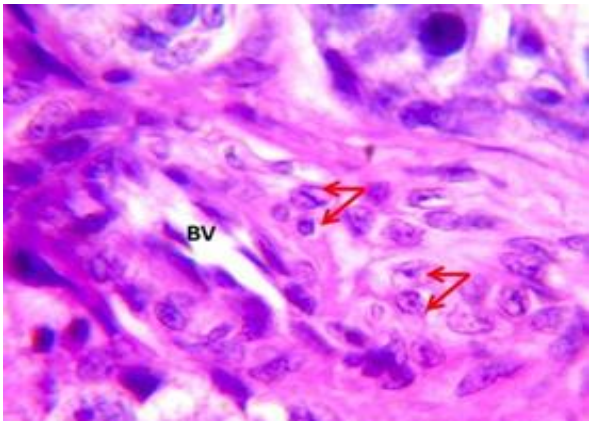

Figure (8): Microphotograph of interstitial tissue of testis in summer showing many newly formed small-size Leydig cells (red arrows) around blood vessel (BV). H&E stain, X1000

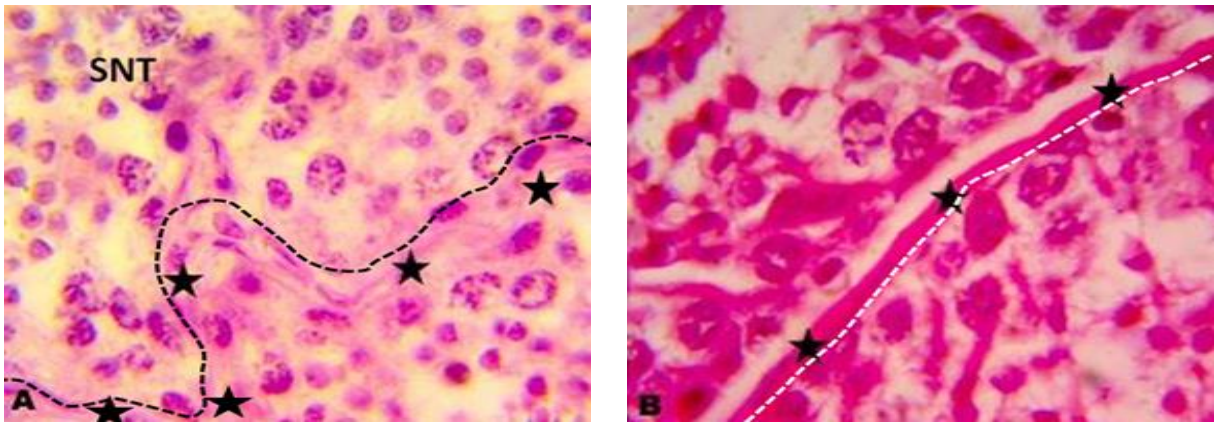

Figure 9: Microphotograph of (SNT) showing wavy basement membrane(stars) in spring season(A)H&E stain, 1000Xand non-wavy basement membrane (stars) in summer season (B). PAS stain, X1000.

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Discussion

Leydig cells compensation can be done either by the proliferation of leydig cells themselves or by differentiation of fibroblast-like cells that present in the interstitium of the testes (12). On the other hand, and despite that Sertoli cells were undividable cells, the result of the present study revealed an increase in the number of these cells in the seminiferous tubules in active seasons. This was partly similar to the findings of Johnson and (5) in stallions who reported that Sertoli cell population can affected by age and season. (1) Reported that the seminiferous epithelium consists of two types of cells; non-dividing supporting (sustentacular) cells (Sertoli cells) and proliferative cells of the spermatogenic lineage. The present study referred to the capability of fibroblast-like cells to penetrate the basement membrane to differentiate into Sertoli cells in order to compensate the seasonal functional demand of the process of spermatogenesis. This was confirmed with (1).The herein study was agreed with (8) regarding the possibility of basement membrane to be penetrated. (12) Indicated that fibroblast-like cell can be converted into Leydig cell in the season of involution or active season. The wavy appearance, the thin basement membrane, and the stretched myoid cells during active season may be due to the mechanical pressure of the penetrating interstitial fibroblast-like cells in order to enter toward the lumen of the seminiferous tubules. Heterochromatic nuclei of leydig cells monitors the elevation of the processes of mitosis or apoptosis, whereas, euchromatic nuclei referred to the elevation of the processes of protein synthesis in leydig and Sertoli cells (7), when Leydig cell divides, the nucleus appears dark and small and the nucleolus is not appeared so that protein synthesis stopped or delayed. The present study was in variance with the findings of (6) who declared that under certain conditions, Sertoli cells of adult stallions could re-enter the cell division cycle. The current study revealed that Sertoli cell never divides; otherwise, the mechanism of blood-testis barrier will be broken. The study declared that the interstitial fibroblast-like cells can penetrate through the gates of basement membrane, then remodeled, and converted to Sertoli cells, as the similarities between the two were very large. This was confirmed by the presence of traces of irregular gates only in the active seasons. The study declared that the basement membrane was characterized by dynamic, flexible structure, and can respond easily to the cellular mechanical pressure. It was concluded that despite the increase of Sertoli cells within the lumen of seminiferous tubules during active season, Sertoli cells never proliferate. These cells were part of the blood-testis barrier and if these cells divide this mechanism will be broken.

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