Testosterone role during seasons changes in the dogs testes

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Summary

This study was conducted to evaluate the effect of seasons on serum testosterone level, testis histology examination and testosterone receptors by using cytoimmunochemistry techniques. Samples of blood and both testes were collected from 24 local dogs aged between 2-3 years and weighted 18-22 kg. This study was carried out at 2015, and divided into four seasons. No differences in testosterone level were observed between seasons. However, histological examination were characterized by significant ($P<0.01$) changes during seasons in tubular diameters, germinal thickness and tubular area. Cytoimmunochemistry investigation showed differences between seasons also; there was a distribution of testosterone hormone receptors in the Leydig cells, Sertoli cells and in germinal epithelium during spring and autumn. At the same time the winter season showed a distribution of testosterone hormone receptors in Leydig and Sertoli cells only. The summer season is characterized by diffuse of receptors in Leydig cells only. In conclusion there was a significant effect of season on testis of dog in Iraq, the summer season was the lowest in reproductive activity while the spring and autumn season was the highest of reproductive activity of dog in Iraq.

Keywords: Male dog, Seasons, Testosterone, Dog testes histology, Cytoimmunochemistry.

Introduction

Dogs have an important value in Iraq; it took parts in many errands (1). The reproductive pattern of the canids is different from that of other mammalian species and the reproductive physiology of it was restricted by a current lack of knowledge (2). Testosterone was responsible for male secondary sexual development, positive bone formation, skin and cardiovascular effects (3). As well as it also takes a part for changes within basolateral nuclear group of the amygdaloid body associated with aggression (4). The spermatogenesis depends on the action of testosterone. It had been known that testosterone hormone diffuses directly into the seminiferous tubules or indirectly through binding to albumin carrier which took it into seminiferous tubules, and high level of this hormone found surrounding the seminifrous tubules due to presence of Leydig cells (5). Testosterone was converted to estrogen in male dogs and the regulation of androgens levels was controlled by the pituitary-gonadal axis via gonadotropic releasing hormone (GnRH), interstitial stimulating hormone (ICSH) and spermatogenesis stimulating hormone (SSH) (6 and 7). There could be a seasonal effect upon fertility, as it often lowers in summer with decreasing of normal morphological spermatozoa (8). However, in most domestic species, the female only shows clear seasonal cyclic whereas the male displays an evidence of a seasonal variation (9). The reproductive analysis of the dog could be done by physical examination, semen evaluation, endocrine parameters, testicular biopsy, epididymal markers and sperm aspiration (10). No information are available concerning the effect of breeding season of male dog in Iraq, so this study could be considered the first done to know the effect of seasons on serum testosterone hormone in Iraq as well as histological observations and cytoimmunochemistry investigation of dog testes.

Materials and Methods

Twenty four adult male dogs aged 2-3 years and weighted 18-22 kg were obtained from different regions of Baghdad city, Iraq. The study was carried out through out four seasons in the year 2015, during spring (March-May), summer (June-August), autumn (September-November) and winter (December-February). Blood samples (10 ml) were collected from the

right cephalic vein of each dog, centrifuged at 3000 rpm for 15 minutes and the serum was stored at -20ºC until hormone analysis. Enzyme immunoassay (ELISA) kits for testosterone (Megacor Company) were used for estimation of testosterone hormone; all samples were assayed in duplicate tests. Both testes were taken from the dogs by orchidectomy operations according to the procedures cited by (1). Histological preparation was done according to procedure described by (11), and stained by Periodic Acid Schiff (12). Ordinary light Microscope used examination and measurement were achieved by using at ocular micrometer. The tubular diameters, germinal cell thickness and tubular area were determined (1). Paraffin-embedded sections were used for all immunohistochemical analyses. Sections were incubated with 10% normal goat serum to reduce background staining caused by the second antibody. The sections then incubated with primary antibody (1:1500). The sections were then incubated with a second antibody. Finally, the reacted sections were counterstained with haematoxylin solution. The primary antibody was detected by using sequencing of genes (13 and 14). The mean value ± SE of parameters were calculated and analyses in one way ANOVA and the LSD was used to determine the differences between mean groups (15).

Results and Discussion

Serum testosterone levels showed no differences with seasonal changes. The high hormonal level were during autumn (1.75±0.1 ng/ml) and during summer (1.6±0.9 ng/ml), whereas the lowest level of this hormone (0.7±0.1 ng/ml) was examined in spring and winter (Table, 1).

Table, 1: Testosterone hormone level (ng/ml) during different seasons in dog.

Seasons	Spring	Summer	Autumn	Winter
mean±SE no=6	0.7±0.1	1.6±0.9	1.75±0.1	0.7±0.1

*Mean±SE.

The testosterone levels (Table 1) were lower than the means value reported by (2), also the authors found no differences between the testosterone level in both spring and summer seasons. This was in agreement with our finding, the mean values of testosterone levels were close to mean levels as had been previously reported (10, 16 and 17). Testosterone level in serum was significantly elevated in summer and autumn as compared with winter and spring, similar finding were recorded by (16). (17-20) reported that serum testosterone level was not affected by environmental conditions during different seasons, and by lighting time zone (21). However significant variations in serum testosterone levels of dogs during summer, spring and autumn were recorded (22) Moreover that there were a significant differences in the testosterone concentration between summer than that both of spring and autumn, and they added that the combined effect of temperature and rainfall index was the important cause of this differences, so the high temperature and high rainfall resulted in substantial increases in humidity, which as a result may be the major factors.

The tubular diameter showed a significant ($P<0.05$) differences during different seasons. The winter season (190±0.7) µm was the highest as compared with spring and autumn seasons (122±2.0 and 126±1.2) µm respectively, (Table, 2). The germinal thickness showed a significant ($P<0.01$) differences between spring and summer seasons (57±0.6 and 33±0.3 µm) respectively. The autumn and winter showed no differences compared with spring and summer seasons (Table, 3). Tubular area (Table, 4) showed significant ($P<0.01$) differences during winter season (310±2.1) µm compared with other seasons.

Table, 2: Tubular diameter (µm) during different seasons in dog.

Seasons	Spring	Summer	Autumn	Winter
mean±SE no=6	122±2.0 b	158±2.6 ab	126±1.2 b	190±0.7 a

*Mean±SE.

Table, 3: Germinal thickness (µm) during different seasons in dog.

Seasons	Spring	Summer	Autumn	Winter
mean±SE no=6	57.0±0.6 a	33.0±0.3 b	44.0±0.4 ab	47.5±0.5 ab

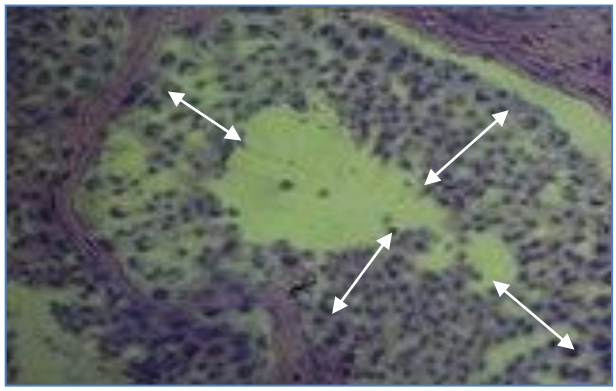
*Mean±SE.

Table, 4: Tubular area (µm) during different seasons in dog.

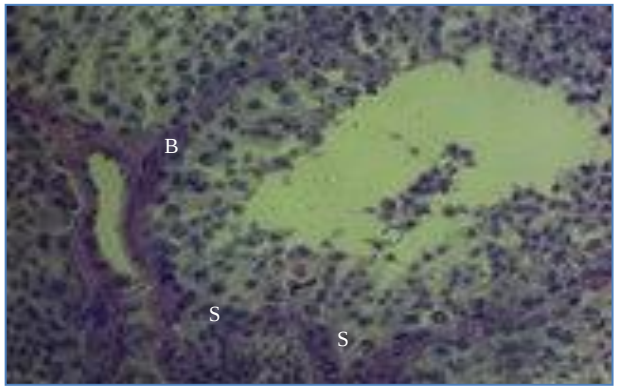
Seasons	Spring	Summer	Autumn	Winter
mean±SE no=6	223±1.5 b	195±1.1 b	216±1.1 b	310±2.1 a

***Mean±SE.**

The study of light microscope on testis tissue of the dog in Iraq revealed that the most types of cells in the seminiferous epithelium were the spermatogenic cells (Fig. 1). Sertoli cells lie next to the basement membrane of the seminiferous epithelium (Fig. 2).

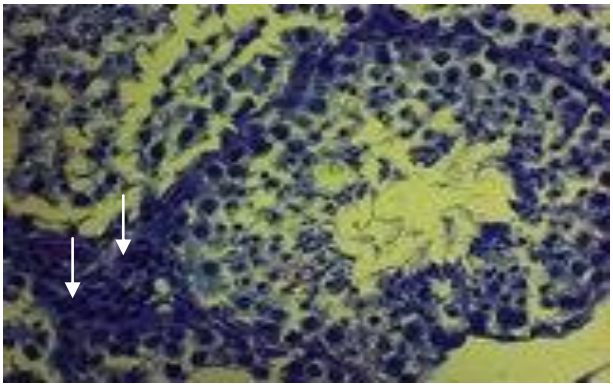


Figure, 1: Spermatgenic cells (double arrows) inside seminiferous tubules during spring season. PAS X400.

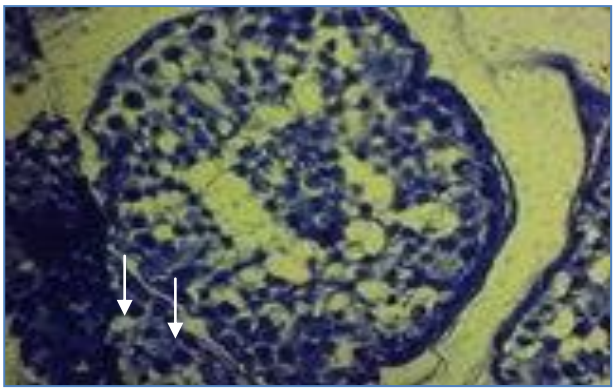


Figure, 2: Sertoli cells (S) and basement membrane (B) inside seminiferous tubules during autumn season. PAS X400.

The seminiferous tubules were also padded by Sertoli cells and the sperms were attached the Sertoli cells or within the lumen of seminiferous tubule. Also the Sertoli cells extended from the basement membrane to the tubular lumen and the location of the Sertoli nucleus was toward the basement membrane and it look like oval in shape. Whereas the Leydig cells were found inside the interstitial tissue of the testis encircling the seminiferous tubules of the dog (Fig. 3). It had one nucleus and acidophilic cytoplasm (Fig. 4).



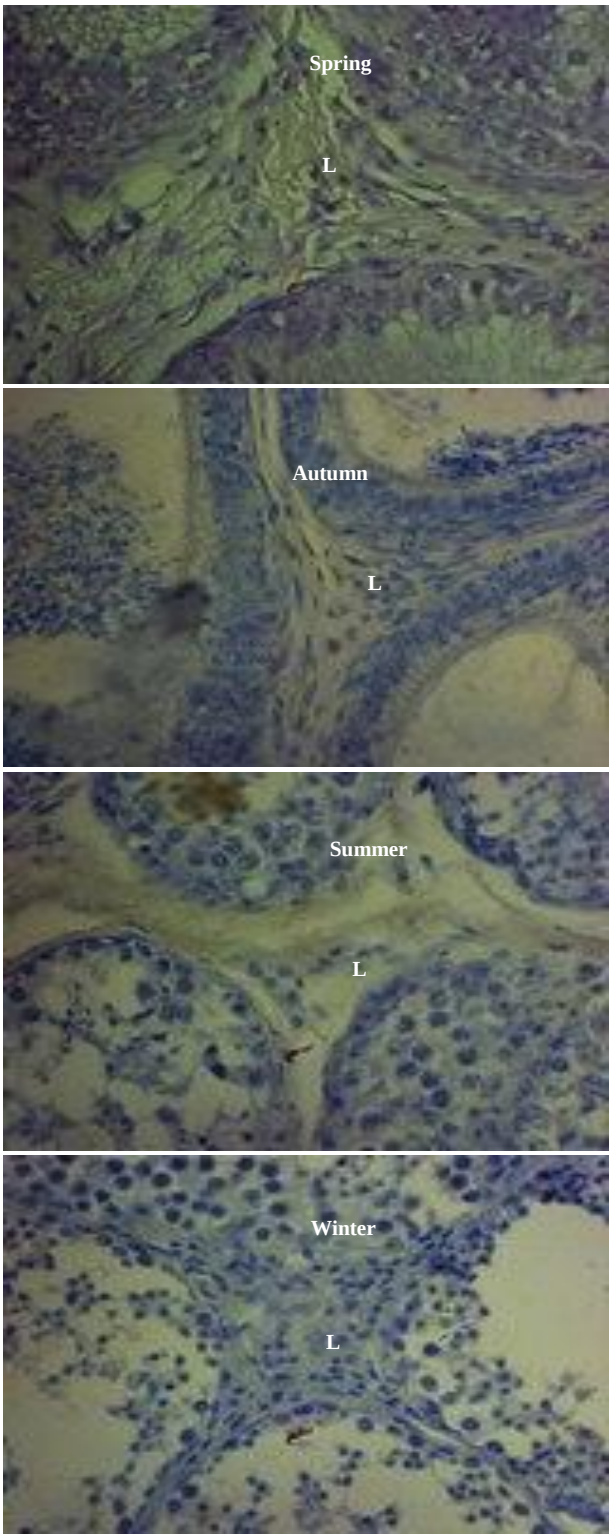
Figure, 3: Leydig cells (arrows) inside interstitial tissue during winter season. PAS X400.



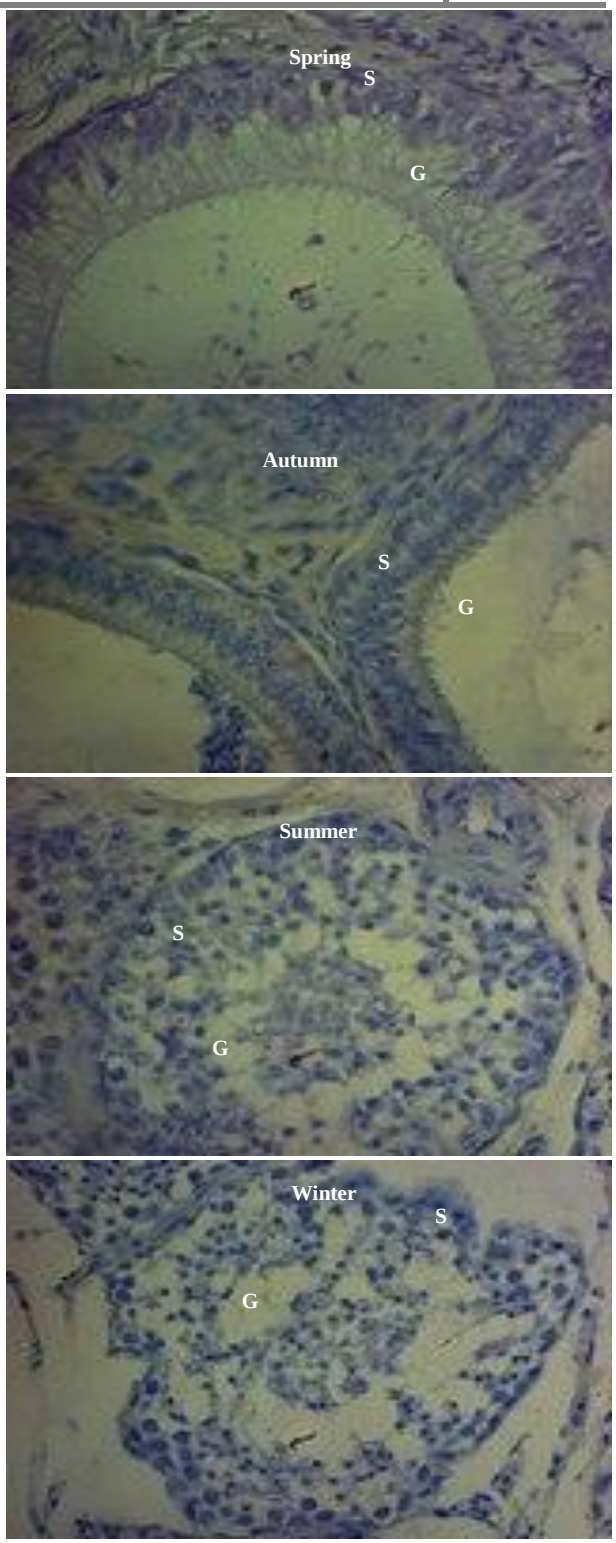
Figure, 4: Acidophilic nucleus cytoplasm (arrows) of Leydig cell during summer season. PAS X400.

Recent studies (Table, 2-4) had conflicts with (9 and 16) who found an effect of the season on tubular diameters, germinal thickness and tubular area. The changes in tubular diameter had been used as an estimation of testicular activity in the dog (1). Whereas the finding of this study coincided with (9 and 12) of light microscope exam on testis tissue of the dog. The description of Leydig cells was similar to the finding of (9 and 12) who found that the Leydig cell of the dog was polyhedral and uninucleated. The changing of the histological parameters has clearly indicated that the uncontrolled season changes has an effect in testis due to the highly sensitivity of the external testis position which affected more than other internal part of the bodies. There were immunolocalization in Leydig cells during four seasons (Fig. 5). Seasonal changes in the immunolocaliztion in the Sertoli cells were noticed between spring, autumn and winter seasons from one side and summer season show no immunostaining of Sertoli cells with marked seasonal changes in immunolocalization were observed in germinal

epithelium (Fig. 6). The most extensive immunostaining was present in Leydig cells, Sertoli cells and germinal epithelium during spring and autumn seasons in dog in Iraq. While the immunoreactivity of winter season was present in Leydig and Sertoli cells only. Summer season have only the immunestaning of Leydig cells only.



Figure, 5: Immunolocalization of Leydig cells (L) during different seasons. ×400.



Figure, 6: Immunolocalization of Sertoli cells (S) and germinal epithelium (G) during different seasons. ×400.

The result of cytoimmunochemistry (Fig. 5 and 6) was nearly typical with the study (11 and 14) in raccoon dog. In Iraq the present study was the first of its kinds which conducted the effect of seasons on testosterone receptors inside dog's testes. Many of studies used this technique for investigation of several

problems in dog semen evaluation (23, 24 and 25) without referring to the effect of seasons. Seminal testosterone profile was peaks in October and reaches its rock bottom in April, in fact, the environmental changes appear to affect male dog gonadal physiology (20). In the dog, estrogens play a major role in negative feedback of the hypothalamic-pituitary-testicular axis (1). The enzymes which involved in producing testosterone in Leydig cells of male mammals were present in the smooth endoplasmic reticulum and mitochondria (20). During breeding season of the African hyrax, Leydig cell smooth endoplasmic reticulum proliferated and lipid droplets diminished, while plasma testosterone levels increased (1). After the administration of gonadotropins there will be changes in Leydig cell structure, when hCG given, Leydig cell smooth endoplasmic reticulum increase and elevation of plasma testosterone will happened (20). In conclusion the distribution of testosterone receptors during different seasons indicated that the best season of reproductive activity with best hormonal distribution were during spring and autumn and the lowest distribution was in summer.

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دور الشحمون الذكري في أثناء تغير المواسم في خصى الكلاب

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الخلاصة

أجريت هذه الدراسة في سنة 2015 لتقييم تأثير المواسم في مستوى الشحمون الذكري، فُحِصَت الخصى ومستقبلات الشحمون الذكري فحصاً نسجياً بواسطة استعمال التقنية الكيميائية المناعية النسجية. جُمعت عينات الدم وكلا الخصيتين من 24 كلب ذكر تراوحت أعمارهم ما بين 2-3 سنة وبوزن 18-22 كغم خلال 2015 والتي قسمت فيه على أربعة مواسم. لم تُسجيل أي فروق معنوية ما بين المواسم في مستوى الشحمون الذكري. تميز الفحص النسجي بوجود فرق معنوي ($P<0.01$) في المواسم في قطر النبيبات، سُمك الخلايا الجرثومية ومساحة النبيب. الفحوصات النسجية المناعية الكيمياوية أظهرت كذلك فروقات ما بين المواسم حيث انتشرت مستقبلات الشحمون الذكري في خلايا ليدك، خلايا سرتولي والظهارة الجرثومية في فصلي الربيع والخريف، في نفس الوقت لوحظ انتشار هذه المستقبلات في خلايا ليدك وخلايا سرتولي فقط في فصل الشتاء، في غضون ذلك تميز فصل الصيف بانتشار المستقبلات في خلايا ليدك فقط. من هذه النتائج نستنتج بأن هنالك تأثير للموسم في خصى الكلاب في العراق، وكان فصل الصيف الأقل في الفعالية التناسلية وعلى العكس تماماً ظهر فصلي الربيع والخريف كأعلى فصلين في الفعالية التناسلية للكلاب في العراق.

الكلمات المفتاحية: ذكر الكلب، المواسم، الشحمون الذكري، نسيج خصى الكلب، النسيج المناعي الكيميائي.