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SEASONAL CHANGES ON EPIDIDYMAL HISTOLOGY AND TESTOSTERONE RECEPTORS IN IRAQI DOGS

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ABSTRACT

The present study was carried out on forty eight epididymis of sexually mature dogs to elucidate light microscope features and the presence of testosterone receptors inside epididymis in different seasons. Twenty four dogs aged 3 years were used during the periods from 1/2/2015-1/2/2016. The epididymis were carefully dissected from the testis after orchidectomy operations and fixed, dehydrated, clarified and embedded in paraffin. Sections were stained with hematoxylin and eosin and periodic acid schiff. As well as, paraffin embedded cytoimmunochemistry technique was used for detection of testosterone receptors. Muscular coats showed no significant differences during different seasons or among segments. Epithelial height, stereocilia length, total diameter and luminal diameters indicated presence of significant differences among segments and seasons. There were morphological changes in epithelial cells during seasons. The folding was noticed during winter only. Imunoreactivity of testosterone receptors were demonstrated during all seasons in all epididymal segments. The study concluded that there were seasonal variations in dogs' epididymis being highest reproductive activity during spring and lowest during summer.

Keywords: Dogs, Epididymis, Histology, Receptors, Testosterone.

INTRODUCTION

There was a long history of cohabitation between human and dogs but there was little information and many aspects of canine reproductive concerning the effect of breeding season (Gavrilovic *et al.,* 2008; Van den Berghe *et al.,* 2012). Seasonal changes in semen parameters were observed in undomesticated canids and the testosterone production reach peak during breeding season (Strzezek *et al.,* 2015). There were seasonal variations in reproductive activity in dogs inside Iraq (Zaid, 2015). Several functional and structural modifications take place in spermatozoa during its migration as well as its storing in epididymis (Varesi *et al.,* 2013). Testosterone was required for accomplishment of meiosis and differentiation of spermatids (De-Gier *et al.,* 2012). Cytoimmunochemistry staining was widely used in basic research to understand the distribution and localization of proteins in different parts of tissues (Bishop *et al*., 2013). This study was the first trial conducted in Iraq to evaluate the effect of season on epididymal histology and testosterone receptors presence in dogs.

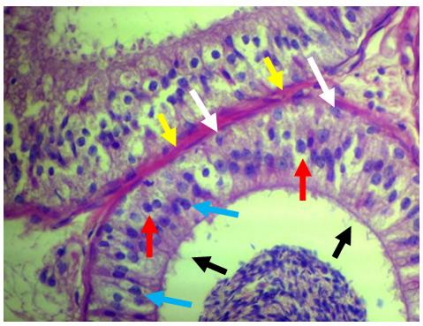
MATERIALS AND METHODS

A total of twenty four mature dogs, aged 3 years were used, these animals were caged at College of Veterinary Medicine, University of Baghdad during the periods from 1/2/2015 to

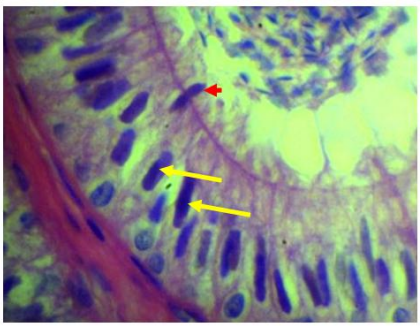
1/2/2016. Orchidectomy operations were done all over the year (six in each season). Then epididymis were carefully dissected from the testis and fixed in 10% formalin, dehydrated in graded ethanol, clarified in xylene, embedded in paraffin, sectioned at 4-5µm in glass slides and stained with Hematoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS). The cytoimmunochemistry technique for paraffin embedded sections was used for testosterone receptors detection. Sections were incubated with 10% normal goat serum, then incubated with primary antibody (1:1500) and secondary antibody. The primary antibody was determined using sequencing of genes according to NCBI (2015). The cytoimmunochemistry technique was done by using Santa Cruz kits. Examination using light microscope and measurements with oculometer was done; photographs (MDC2000 microscope camera) were taken. The measurements included the examination of two sections for each epididymis and twenty tubes from each section were selected and examined at magnification of 100× and 400× to determine cells appearance, types and diameters. Total diameter of epididymal ducts was measured for each tube at right angles to each other, epithelial height measures, stereocilia length and muscular coats measurement were done using the same forty tubes at four points at 0°, 90°, 180°, 270° and luminal diameter was measured by subtracting total diameter from epithelial height. All obtained data (mean ± standard error) were calculated from the forty tubes of each epididymis was listed and Analysis of Variance test and Least Significant differences was done according to (SPSS, 2001).

RESULTS AND DISCUSSION

The epididymal duct is divided into three main segments (caput, corpus and cauda). The widest diameter was observed in the cauda segment. The entire epididymis duct exhibits a pseudostratified epithelial lining, with or without stereocilia, wrapped by a lamina propria and circular smooth muscle layer coat. The whole height of epithelium was decreased significantly during summer ($P<0.01$) in caput and corpus as compared with other seasons, meanwhile, the cauda during summer show significant increase than caput (Tab. 1). The epithelium consists of four cell populations: principal, apical, basal (Pl.1). This study revealed the presence of apoptosis cells (Pl. 2 and 3).



Plate(1): Histological section stained showing the corpus segment during winter. Notice the stereocilia (black arrows) principal cells (red arrows), apical cells (blue arrows), basal cells (white arrows) and muscular coat (yellow arrows). H&E 100×.



Plate(2): Histological section stained showing the corpus segment during winter. Notice the nuclei of principal cell (yellow arrows) and apoptotic cell (red arrow head). H&E 400×.

Table (1): Epithelial height (µm) in different segments of the dog's epididymis in different seasons.

Seasons	Segments		
	Caput	Corpus	Cauda
Spring (March, April and May)	36±5 ab	38±6 a	38±4 a
Summer (June, July and August)	26±6 c	29±4 bc	34±7 ab
Autumn (September, October and November)	35±5 ab	37±6 a	36±4 ab
Winter (December, January and February)	34±9 ab	38±8 a	41±4 a
Least Significant Differences	7.6		

- Numbers represent mean ± standard error.
- The similar small letters represent no significant differences between means.
- The different small letters represent significant differences between means at level of $P<0.01$.

The thickness of mucosal coat showed no significant differences between epididymis segments in different seasons (Tab. 2).

Table (2): Muscular coat thickness (µm) in different segments of the dog's epididymis in different seasons.

Seasons	Segments		
	Caput	Corpus	Cauda
Spring (March, April and May)	17±11 a	13±1 a	19±10 a
Summer (June, July and August)	16±5 a	22±2 a	7±1 a
Autumn (September, October and November)	16±11 a	12±1 a	18±9 a
Winter (December, January and February)	25±4 a	7±3 a	30±3 a

- Numbers represent mean ± standard error.
- The similar small letters represent no significant differences between means.

The length of stereocilia also exhibits significant difference ($P<0.01$) between caput and corpus during winter, as well as, summer season was decrease significantly ($P<0.01$) than other seasons in dogs epididymis (Tab. 3).

Table (3): Length of stereocilia (µm) in different segments of the dog's epididymis in different seasons.

Seasons	Segments		
	Caput	Corpus	Cauda
Spring (March, April and May)	10±1 ab	10±2 ab	9±1 ab
Summer (June, July and August)	0±0 c	0±0 c	0±0 c
Autumn (September, October and November)	10±1 ab	10±2 ab	8±1 ab
Winter (December, January and February)	6±1 b	12±5 a	8±1 ab
Least Significant Differences	4.5		

- Numbers represent mean ± standard error.
- The similar small letters represent no significant differences between means.
- The different small letters represent significant differences between means at level of $P<0.01$.

The total diameter of epididymal ducts showed seasonal differences among segments. The caput during winter season recorded the largest diameters (675±255 µm) comparing to other seasons. The cauda during summer (280±90 µm) was the lowest than other seasons. The caput in spring and winter had a significant greater than other segments, while the cauda during summer showed a significant lesser than other segments (Tab. 4).

Table (4): Total diameters (µm) in different segments of the dog's epididymis in different seasons.

Seasons	Segments		
	Caput	Corpus	Cauda
Spring (March, April and May)	500±289 b	365±168 cd	465±120 bc
Summer (June, July and August)	553±275 ab	380±106 cd	280±90 d
Autumn (September, October and November)	490±280 bc	360±160 cd	460±120 bc
Winter (December, January and February)	675±255 a	380±154 cd	495±125 bc
Least Significant Differences	172		

- Numbers represent mean ± standard error.
- The similar small letters represent no significant differences between means.
- The different small letters represent significant differences between means at level of $P<0.01$.

On the other hand, the luminal diameters did not exhibit differences among seasons except in summer cauda being lesser than other seasons. Generally, the widest summer caput

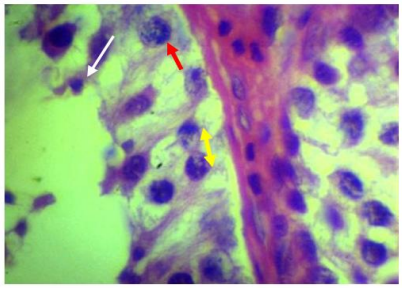
increased significantly (P<0.01) than other segments, the same in winter caput which increased more than corpus (Tab. 5).

Table (5): Luminal diameters (µm) in different segments of the dog's epididymis in different seasons.

Seasons	Segments		
	Caput	Corpus	Cauda
Spring (March, April and May)	412±265 abc	303±156 bcd	390±170 abc
Summer (June, July and August)	438±280 ab	262±76 cd	168±90 d
Autumn (September, October and November)	410±260 abc	300±150 bcd	400±170 abc
Winter (December, January and February)	475±251 a	250±147 cd	405±172 abc
Least Significant Differences	162		

- Numbers represent mean ± standard error.
- The similar small letters represent no significant differences between means.
- The different small letters represent significant differences between means at level of P<0.01.

The difference in surface cellular modification indicated the presence of seasonal variations. In all seasons, the principal cells display important morphological changes from one season to another. The oval nuclei of the principal cells during spring, autumn and winter were deep blue with heterochromatin mainly in all segments state of apoptosis to regenerate the cells (Pl. 2 and 3). The cytoplasmic vacuoles in all epididymal segments demonstrated in all seasons (Pl. 4). The basal cells showed no changes among segments during different seasons. In general, the apoptotic cells increased during summer seasons than others (Pl. 2 and 3). The sperms demonstrated inside epididymal lumen in all seasons (Pl. 1 and 2), except summer caput and corpus (Pl. 5). Folding phenomenon was demonstrated during winter season only (Pl. 6).



Plate(3): Histological section stained showing the caput segment during summer. Notice the nuclei of principal cells (red arrow), cell division (yellow double arrow) and apoptotic cell (white arrow). H&E 400×.

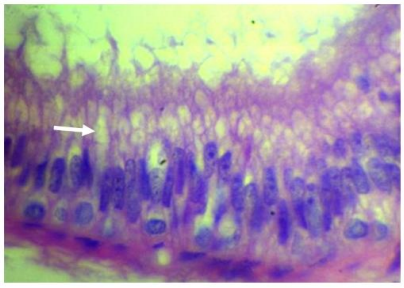
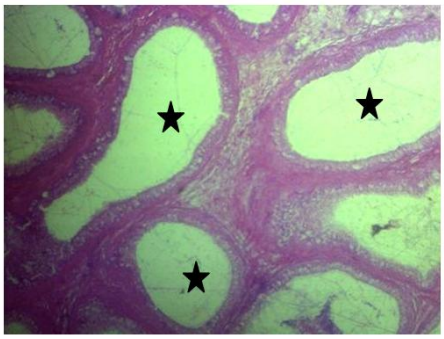


Plate (4): Histological section stained showing the cauda segment during autumn. Notice the cytoplasmic vacuoles (white arrow). H&E 400×.



Plate(5): Histological section stained showing the caput segment during summer. Notice the absence of sperms inside epididymis lumen (black stars). H&E 100×.

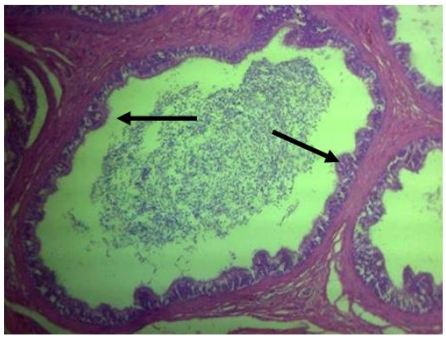
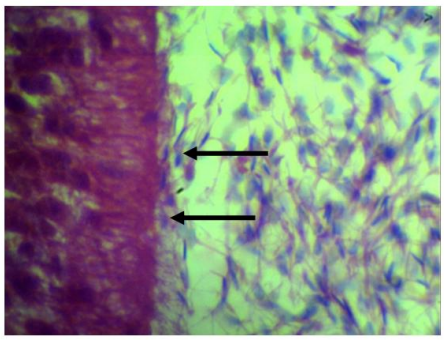
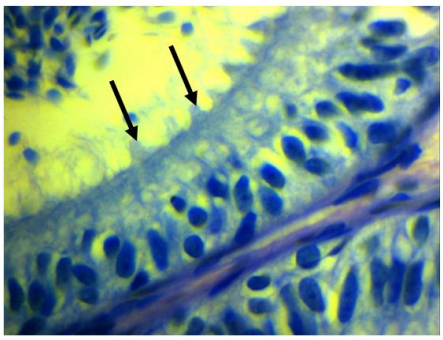


Plate (6): Histological section stained showing the caput segment during winter. Notice the folding of epididymis lumen (black arrows). H&E 100×.

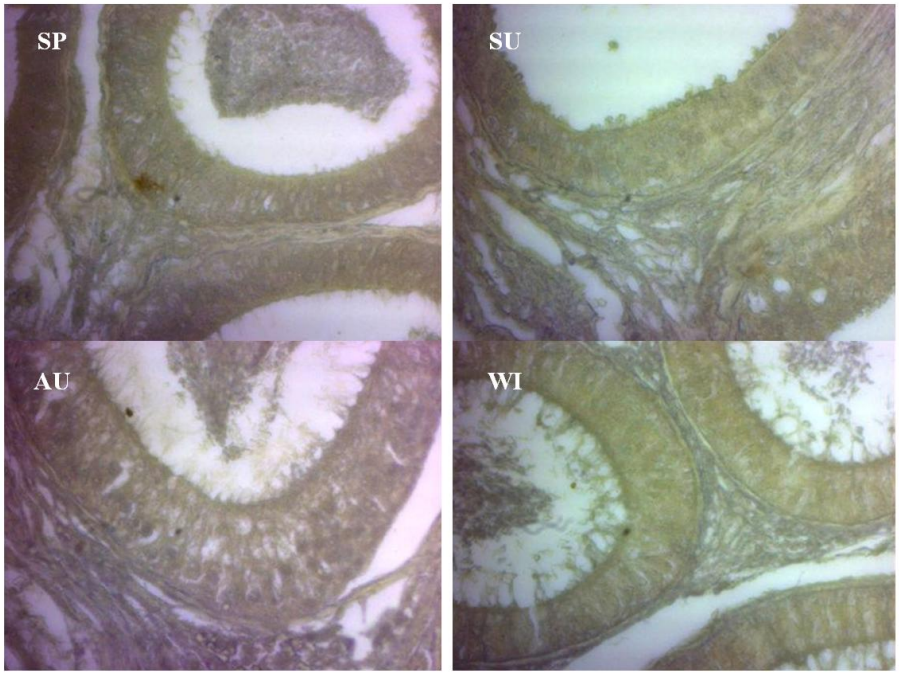


Plate(7): Histological section stained showing the corpus segment during spring. Notice the attachment of sperms with stereocilia (black arrows). H&E 400×.



Plate(8): Histological section stained showing the cauda segment during winter. Notice the apical projection in epididymis lumen (black arrows). PAS 400×.

The cytoimmunochemistry study clearly indicated that the testosterone receptors present in all seasons of segments parts inside dog epididymis (Pl. 9).



Plate(9): Immunohistochemistry staining of formalin–fixed, paraffin-embedded for testosterone receptors. Notice the distribution of brown colour (testosterone receptors) inside all the epididymis segments during spring (SP), Summer (SU), Autumn (AU) and winter (WI). 40×

Seasonal effect on epididymal histology and testosterone receptors distribution in dogs has not been reported before; the epididymis plays an important role in maturation and storage of male spermatozoa (Zayed *et al.*, 2012). The epididymis, exhibits variable structures and functions along its length (Schimming *et al.*, 1997). Concerning the epithelial height, muscular coat, stereocilia height, total diameter and luminal diameters, observations currently were similar to previously mentioned by Schimming *et al.* (1997) and Schimming and Vicentini (2001) in dogs epididymis. The epithelium represents the absorbable power of the epididymis segments (Zayed *et al.*, 2012). In this consideration, previous reported that 90% of fluid entering the epididymis will be absorbed again (Whelly *et al*., 2012). The law height of the caput and corpus may be due to decrease secretary activity during summer season. The muscular coat helps in strength the movement of sperms inside epididymis and in ejaculation (Junqueira and Carneiro, 2009).

The cilia support the movement of molecules in and out of the cells (Junqueira and Carneiro, 2009); these findings indicated that the summer exhibited a low absorptive function of the epididymis. In all seasons, except summer season, there were increases in total and luminal diameters in caput and slightly narrowing in corpus segment then rewinding in cauda.

In our opinion, the decrease of cauda diameter during summer season may be related to decrease the capacity storage of spermatozoa due to low testicular production.

The presences of sperms were demonstrated in all seasons except summer, this may due to the decrease of testicular activity in dogs during summer (Zaid, 2015). Cellular structures of epididymis epithelium display seasonal changes showing decrease activity in summer and increase activity in other seasons; the most affected cells are the principal cells, these cells contain oval nuclei with light stained that indicate increase metabolic activity, while the dark stained nuclei signs decrease activity in summer, this fact was in agreement with Zayed *et al.* (2012) in his seasonal study in camel. Moreover, the presence of apoptosis cells, which is dying cells represents decrease activity during summer; these cells firstly, reported in this study and varied with Zayed *et al.* (2012) who regarded these cells as dark cells. The study hypothesized that any dead cell eliminated through the tube lumen. The increasing basal cells, which create principal cells as a stem forming cells during winter coincide with increasing total and luminal diameters. This is due to increasing building up requirement during next highly reproductive spring season. This is in agreement with (Schimming *et al.*, 1997. This is attendant with inwards folding which is a mark for increasing numbers of principal lining cells as a preparation steps for next spring season. The accumulation of dense lysosomal bodies inside principal cells exhibits active digestion of sperms and epithelial cells, this finding agreed with Zayed *et al.* (2012). Vacuoles may be representing the lyses of secretory product during routinely dehydrating, clarifying process; the apical projection and sperm attachment were firstly noticed presently (Pl. 7 and 8). Whereas, the intraepithelial gland which describe by Zayed *et al.* (2012) in camel epididymis was not seen in this study.

There are immunelocalization of testosterone receptors in all segment parts in all seasons of the years; there is marked immunostaining in epithelial, muscular coat, stereocilia of epididymis of the dogs, this indicated that the epididymis affected by testosterone all over the year. This result agreed with the findings of Ibrahim and Zaid (2015) and Zaid (2015) who found that the testosterone show no seasonal changes in Iraqi dogs, so that the immunoreactivity of testosterone receptors inside epidiymis is ready for reproduction in all seasons.

Conclusion: The dog epididymis showed seasonal reproductive variations in Iraq with highest activity during the spring and adverse lowest activation during summer and the testosterone hormone control epididymis activity in all seasons.

LITERATURE CITED

De-Gier, J., Buijtels, J.J., Albers-Wolthers, C.H., Oei, C.H., Kooistra, H.S. and Okkens, A.C. 2012. Effects of gonadotropin-releasing hormone administration on the pituitary-gonadal axis in male and female dogs before and after gonadectomy. *Theriogenology*, 77 (5): 967-978.

Bishop, M.L., Fody, E.P. and Schoeff, L.E. 2013. Clinical Chemistry: Principles, Techniques, and Correlations. 7th ed. Lippincott Williams & Wilkins, North American, 784pp.

Gavrilovic, B.B., Andersson, K. and Forsberg, C.L. 2008. Reproductive patterns in the domestic dog-A retrospective study of the Drever breed. *Theriogenology*, 70: 783-794.

Ibrahim, N.S. and Zaid, N.W. 2015. Effect of the season on male dog testosterone, SSH, LH level in Iraq. *Indian Journal of Research*, 4(8): 332-333.

Junqueira, L.C. and Carneiro, J. 2009. Basic Histology: Text and Atlas. 11th ed. McGraw-Hill Medical, New York.

NCBI. 2015. National Center for Biotechnology Information. (Avaliable at: http://www.ncbi.nlm.nih.gov/).

Schimming, B.C. and Vicentini, C.A. 2001. Ultrastructural features in the epididymis of the dog (*Canis familiaris*, L). *Anatomia, Histologia and Embryologi*, 30 (6): 327-323.

Shimming, B.C., Vicentini, C.A., Orsi, A.M., Franceschini-Vicentini, I.B. and Abreu-Rays M.A. 1997. Regional histology of the ductus epididymis in the dog (*Canis familiaris*, L.). *Revista Chilena de Anatomia*, 15(1): 5- 12.

SPSS. 2001. Software for windows. IBM SPSS statistics version 22.

Strzezek, R., Szemplinska, K., Filipowicz, K. and Kordan, W. 2015. Semen characteristics and selected biochemical markers of canine seminal plasma in various seasons of the year. *Polish Journal of Veterinary Science*, 18 (1): 13-18.

Van den Berghe, F., Paris, D.P.P.B., Van Soom, A., Rijesselaere, T., Van der Weyde, L., Bertschinger, H. J. and Paris, M.C.J. 2012. Reproduction in the endangered African wild dog: Basic physiology, reproductive suppression and possible benefits of artificial insemination. *Animal Reproduction Science*, 133: 1-9.

Varesi, S., Vernocchi, V., Faustini, M. and Luvoni, G.C. 2013. Quality of canine spermatozoa retieved by percutaneous epididymal sperm aspiration. *Journal of Small Animal Practice*, 54: 87-91.

Whelly, S., Johnson, S., Powell, J., Borchardt, C., Hastert, M.C. and Cornwall, G.A. 2012. Nonpathological extracellular amyloid is present during normal epididymal sperm maturation. *PLOS ONE*, 7 (5): 1-8.

Zaid, N. W. 2015. Effect of seasons on some testicular parameters in male Iraqi dogs. Ph.D. Thesis in College of Veterinary Medicine, University of Baghdad.

Zayed, A.E.Z., Aly, K., Ibrahim, I.A.A. and Abd El-Maksoud, F.M. 2012. Morphological studies on the seasonal changes in the epididymal duct of the one-humped camel (*Camelus dromedarius*). *Veterinary Science Development*, 2(3): 7-14.

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تأثير التغيرات الموسمية في نسيج البربخ ومستقبلات الشحمون الخصوي في الكلاب العراقية

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تاريخ الاستلام: ٢٠١٣/٠٣/١٢ تاريخ القبول: ٢٠١٧/٠٧/١٠

الخلاصة

أجريت الدراسة الحالية باستخدام ثمانية وأربعون بربخ لكلاب بالغة جنسياً لغرض تقييم التغيرات النسيجية ووجود مستقبلات الشحمون الخصوي داخل البربخ بواسطة المجهر الضوئي خلال المواسم المختلفة. تم استخدام أربعة وعشرون كلباً بعمر ٣ سنوات خلال المدة الممتدة من ٢٠١٥/٢/١-٢٠١٦/٢/١.

فصل البربخ بعناية عن الخصية بعد أجراء عملية الأخصاء الجراحي و ثبت البربخ ومن ثم أجري الأنكاز والترويق وطمره في شمع البرافين، صبغت المقاطع النسيجية بصبغة الهيماتوكسلين والأيوسين وكاشف شف الدوري؛ وكذلك استخدمت تقنية التحري المناعي النسيجي الكيميائي عن مستقبلات هرمون الشحمون الخصوي. أظهرت النتائج أن الغطاء العضلي لم يظهر فرقاً معنوياً خلال المواسم المختلفة وما بين القطع المختلفة. أشارت الدراسة الحالية إلى ارتفاع الظهارة، ارتفاع الأهداب، القطر الكلي وقطر الأنبوب إلى وجود فرقاً معنوياً بين القطع والمواسم. وجدت كذلك تغيرات شكليائية في الخلايا الظهارية خلال المواسم المختلفة. وتم ملاحظة ظاهرة الانطواء خلال موسم الشتاء فقط. لوحظ وجود فعالية مناعية لمستقبلات الشحمون الخصوي خلال المواسم الأربعة في كل قطع البربخ المدروسة.
استنتجت هذه الدراسة وجود اختلاف تناسلي موسمي في بربخ الكلب وسجل أعلى ارتفاع في الفعالية التناسلية للبربخ خلال موسم الربيع والأقل أثناء موسم الصيف.