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**SEASONAL HISTOMORPHOMETRIC STUDIES ON THE EPIDIDYMAL DUCT
OF THE ONE-HUMPED CAMEL (CAMELUS DROMEDARIUS)**

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ABSTRACT

This investigation was carried on right and left epididymis of 76 sexually mature (6-7 years) apparently healthy, one humped camel (Sudanese breed), which collected directly after slaughtering from Kerdasa slaughter house, Giza , Egypt all over the year from December 2011 till November 2012. The epididymis was composed of the efferent ductules and the epididymal duct. The epididymal duct was divided into initial, middle and terminal segments. The middle segment was subdivided into proximal, intermediate and distal parts. The terminal segment was further subdivided into proximal and distal parts. Samples from different regions were processed histologically. Paraffin sections 5-6 μ m thick were stained with H&E, Van Gieson's, Masson's trichrome and PAS stains were used for histomorphometrical study. The epididymal duct showed significant seasonal changes in the total diameter, epithelial height, length of stereocilia, luminal diameter, thickness of peritubular muscular coat and the cellular distribution. The values of these elements were increase during spring season in comparison with other seasons. The results were analyzed statistically. The epididymal duct was lined by a pseudostratified columnar epithelium. The epididymal epithelium was composed of five cell types: principal, basal, apical, dark and halo cells. Abundant apical like protrusions, numerous cytoplasmic vacuoles and PAS positive granules characterizing the principal cells in spring season. In conclusion the spring season is the breeding season in camel in Egypt.

Keywords: Seasonal changes, epididymis, camel, histomorphometry

INTRODUCTION

Camel is described as a seasonal breeder (Al Eknah, 2000 and Maraiet *al.*, 2009) or it may maintain their reproductive capacity throughout the year (Zayed *et al.*, 1995). The seasonal breeding behavior of the male camel is referred to as rut (Khanet *al.*, 2003). The onset of breeding season of male camel is markedly divergent in Egypt. The rutting season has been reported to occur at December uptill March (Ebada, 1994), while according to Abd El-maksoud (2010) the rutting season occurs during spring. The excurrent duct system of the testis includes the efferent ductules, the ductus epididymidis and the ductus deferens (Roberts, 2010). The epididymis consists of highly convoluted duct that links the efferent ductules to the vas deferens (Turner *et al.*, 2003). Like other economically valuable animals, studying the morphology of the camel reproductive organs is essential to improving and exploiting their reproductive activity. Detailed description of the structure of the epididymal duct and their morphometry in the dromedary during breeding and non-breeding seasons has been quite few (Abd El-maksoud, 2010). In addition, data on the stage of seasonal changes in camel are quite divergent. In view of this, the aim of this study is to elucidate more light and some details on the histomorphometrical

features of the epididymal duct of the one humped camel during different seasons.

MATERIAL AND METHODS

Right and left epididymis of 76 sexually mature (6-7 years) apparently healthy, one humped camel (Sudanese breed) were collected directly after slaughtering from Kerdasa slaughter house, Giza, Egypt all over the year from December 2011 till November 2012.

Anatomically, the epididymal duct was consisted of three regions; head (caput), body (corpus) and tail (cauda). Histologically and histomorphometrically, the epididymis in the present investigation was subdivided into 7 parts. The head (caput) was subdivided into two parts, ascending first part of the head and initial part of epididymal duct. The middle segment (corpus) was further subdivided into proximal, intermediate and distal parts and finally the terminal segment (cauda) was composed of proximal and distal parts (Fig.1).

The present study included the following lines:

1- General histological study:

The obtained segments were fixed in buffered 10 % formalin of (pH 7.0) and Bouin's fluid for 24 hours. Fixed specimens were dehydrated, cleared and embedded in paraffin wax. Step serial sections of 5-6 μ m

thick were obtained and stained with: Harris hematoxyline and eosin (H&E), Van Gieson's stain, Masson's trichrome stain, Periodic acid Schiff technique (PAS). The aforementioned methods were used as outlined by Drury and Wallington (1980).

2– Histomorphometrical and statistical analysis:

Sections stained with H&E, Van Gieson's, Masson's trichrome and PAS stains were used for histomorphometrical study. Approximately 10 tubular cross sections from each epididymal parts were measured by high power lens (X 40). A computerized microscopic image analyzer attached for full HD microscopic camera (Leica Microsystems, Germany) was used to

determine the histomorphometric parametes. The data were tested firstly for normal distribution using Normality test. Normal distributed data were compared by ANOVA (One-Way Analysis of Variance). Significant differences were determined by Duncan's Multiple Range Test. The data sets, that had not fulfilled the assumption of normal distribution, were analyzed using Kruskal- Wallis One Way Analysis of Variance on Ranks. Significant differences were determined by Dunn's Test. All statistical analyses were performed by SigmaStat (Jandel scientific software V3.1).

Table 1: Total diameter (Mean±SE) of different segments of the camel epididymal duct in different seasons

Segments		Total diameter (µm)			
		Winter (January)	Spring (may)	Summer (July)	Autumn (October)
Initial segment (IS)		156.851±4.102 a,b	163.498±4.649 a	139.31±4.183 b	150.219±5.054 a,b
Middle segment (MS)	Proximal part (P)	97.075±6.639 a	152.66±5.062 b	121.859±6.966 c	135.39±4.02 b,c
	Intermediate part (I)	115.19±9.238 a	133.078±6.387 a	131.654±1.147 a	125.669±5.959 a
	Distal part (D)	111.383±3.601 a	169.428±5.981 b	153.516±5.772 b,c	143.443±4.335 c
Terminal segment (TS)	Proximal part (P)	168.33±3.263 a,c	208.316±9.284 a	187.385±18.487 c	163.104±7.396 a
	Distal part (D)	182.421±8.001 a	246.311±13.871 b	217.951±5.759 c	232.325±6.216 b,c

a-c Values with a different superscript within rows were significantly different (P<0.05)

Table 2: Epithelial height (Mean±SE) of different segments of the camel epididymal duct in different seasons

Segments		Epithelial height (µm)			
		Winter (January)	Spring (may)	Summer (July)	Autumn (October)
Initial segment (IS)		32.582±0.942 b	42.634±1.558 a	34.569±1.663 b	32.094±1.206 b
Middle segment (M)	Proximal part (P)	12.569±1.201 b	27.208±1.526 a	25.743±3.16 a	24.427±2.043 a
	Intermediate part (I)	20.047±2.502 b	29.267±0.637 a	22.374±0.841 b	21.056±1.872 b
	Distal part (D)	17.673±1.502 b	26.809±1.068 a	18.143±0.411 b	19.149±1.861 b
Terminal segment (TS)	Proximal part (P)	11.286±0.897 c	31.11±1.306 a	24.501±1.17 b	22.218±1.659 b
	Distal part (D)	11.268±0.457 b	19.115±1.045 a	17.143±0.322 a,c	13.418±0.947 b,c

a-c Values with a different superscript within rows were significantly different (P<0.05)

Table 3: Length of stereocilia (Mean±SE) of different segments of the camel epididymal duct

Segments		Length of stereocilia (µm)			
		Winter (January)	Spring (may)	Summer (July)	Autumn (October)
Initial segment	(IS)	3.329±0.334 A	4.16±0.503 A	3.346±0.598 a	3.972±0.532 a
Middle segment (MS)	Proximal Part (P)	2.437±0.687 A	6.591±0.189 B	3.609±0.289 c	5.149±0.453 d
	Intermediate part (I)	2.51±0.124 b	3.774±0.585 a	3.767±0.319 a	1.609±0.168 b
	Distal part (D)	1.579±0.216 a	2.14±0.0374 a	2.049±0.285 a	1.151±0.0236 a
Terminal segment (TS)	Proximal part (P)	1.803±0.141 a	1.915±0.129 a	1.242±0.232 a	1.888±0.174 a
	Distal part (D)	1.325±0.0851 a	1.964±0.248 a	1.406±0.178 a	0.923±0.0494 a

a-d Values with a different superscript within rows were significantly different (P<0.05).

Table 4: Luminal diameter (Mean±SE) of different segments of the camel epididymal duct in different seasons

Segments		Luminal diameter (µm)			
		Winter (January)	Spring (may)	Summer (July)	Autumn (October)
Initial segment	(IS)	76.528±6.347 b	98.832±4.949 a	83.774±3.657 a,b	80.629±9.321 a,b
Middle segment (MS)	Proximal part (P)	71.251±8.015 b,c	98.466±4.92 a	71.026±2.984 b,c	84.668±4.145 a,c
	Intermediate part (I)	76.481±4.729 a	88.82±5.369 a	75.273±1.558 a	84.79±9.212 a
	Distal part (D)	75.83±3.922 a	120.507±5.936 b	117.568±6.552 b	104.939±1.7 b
Terminal segment (TS)	Proximal part (P)	145.91±3.035 a,c	164.503±10.372 a	139.586±20.754 b,c	100.901±6.057 b
	Distal part (D)	162.501±7.978 a	206.266±7.312 b	184.759±6.255 a,b	201.229±8.58 b

a-c Values with a different superscript within rows were significantly different (P<0.05)

Table 5: Thickness of muscular coat (Mean±SE) of different segments of the camel epididymal duct in different seasons

Segments		Thickness of muscular coat (µm)			
		Winter (January)	Spring (may)	Summer (July)	Autumn (October)
Initial segment	(IS)	8.616±1.151 a	12.174±1.315 b	10.826±0.524 a,b	10.303±0.754 a,b
Middle segment (MS)	Proximal part (P)	7.087±1.131 b,c	10.581±1.245 a	7.853±0.602 b,c	8.386±0.954 a,c
	Intermediate part (I)	7.168±0.508 b	10.668±0.764 a	7.268±0.523 b	8.846±0.301 b
	Distal part (D)	6.788±0.653 b,c	9.747±0.378 a	6.682±0.784 b,c	8.167±0.259 a,c
Terminal segment (TS)	Proximal part (P)	15.162±0.921 b	21.638±0.732 a	14.336±1.381 b	20.285±0.734 a
	Distal part (D)	16.446±0.809 b	24.19±1.897 a	22.244±0.234 a	18.403±0.367 b

a-c Values with a different superscript within rows were significantly different (P<0.05).

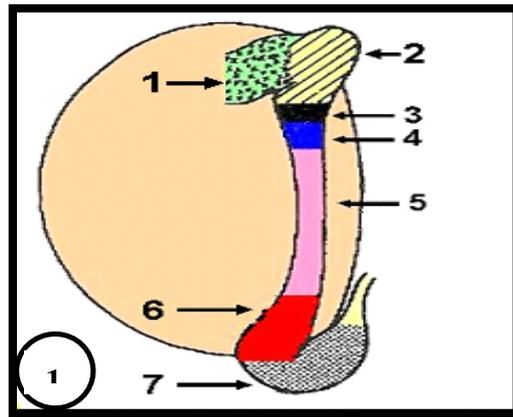


Fig. (1). Schematic drawing of the right testis and epididymis of camel. The head, body and tail had been dissected from the upper pole of the testis and stretched to show the different segment of the epididymis from which the specimens were obtained. 1- Ascending first region of the head, 2- Initial segment (IS), 3-Proximal part of the middle segment (MS-P) ,4-Intermediate part of the middle segment (MS-I) ,5-Distal part of the middle segment (MS-D), 6-Proximal part of the terminal segment (TS-P), 7-Distal part of the terminal segment (TS-D).

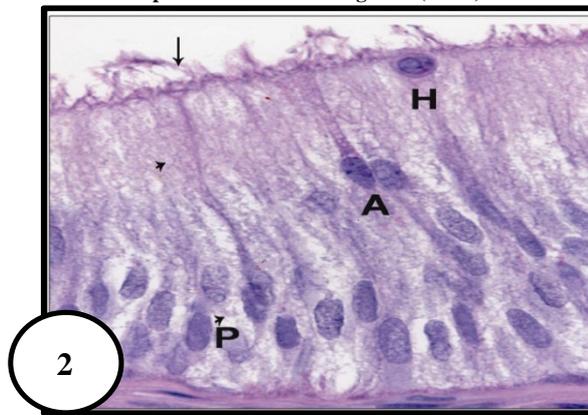


Fig. (2). A photomicrograph of a section at the level of the initial segment of camel epididymal duct during spring showing: Principal cell (P) and its PAS positive supra and infranuclear cytoplasmic granules (arrow heads), Halo cell (H), apical cell (A) and PAS positive stereocilia (arrow) PAS X 1000

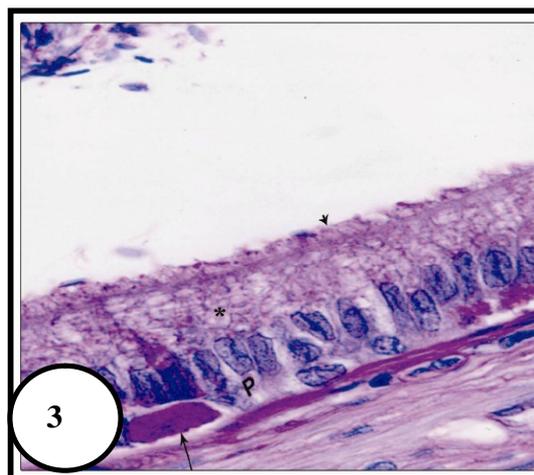


Fig. (3). A photomicrograph of a section at the level of the distal part of terminal segment of camel epididymal duct during spring showing: Principal cell (P) with its oval nuclei in the lower third, numerous vacuoles and PAS positive granules (star) in the apical part, basal cell with its large PAS positive globule (arrow) and intense PAS positive stereocilia (arrow head). PAS X 1000

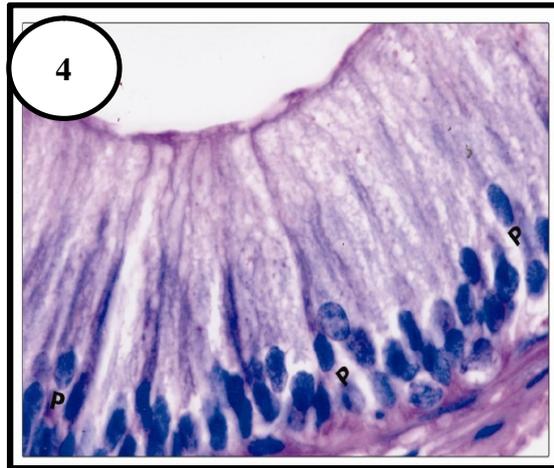


Fig. (4). A photomicrograph of a section at the level of the initial segment of camel epididymal duct during summer season showing: Principal cells (P) with their condensed irregularly oval heterochromatic nuclei. PAS X 1000

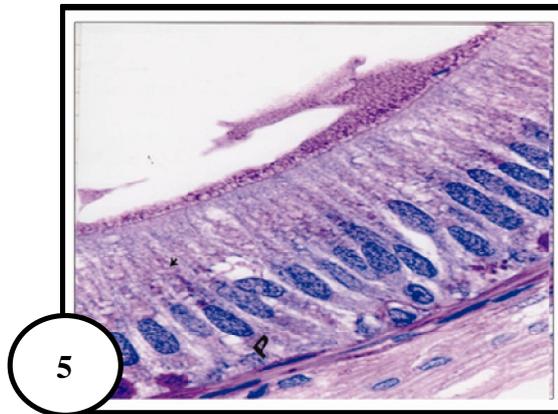


Fig. (5). A photomicrograph of a section at the level of the distal part of middle segment of camel epididymal duct during spring showing: Principal cells (P) with their PAS positive granules (arrow head) and apical like protrusions. Basal cell with its PAS positive globule (double arrows); PAS X 1000

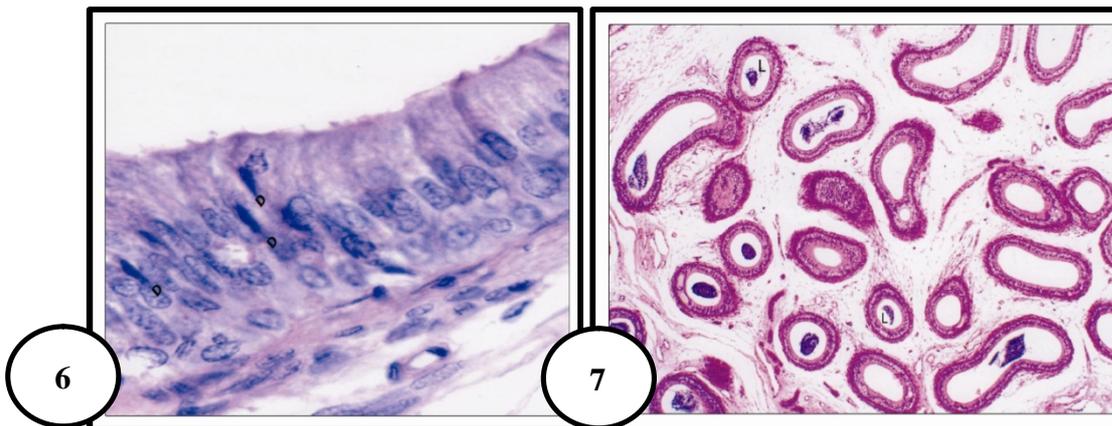


Fig. (6). A photomicrograph of a section at the level of the distal part of middle segment of camel epididymal duct during autumn season showing: Numerous dark cells (D); PAS X 1000

Fig. (7). A photomicrograph of a section at the level of the intermediate part of the middle segment of camel epididymal duct during summer season showing:
- Lumina (L) contained few spermatozoa H & E X 40

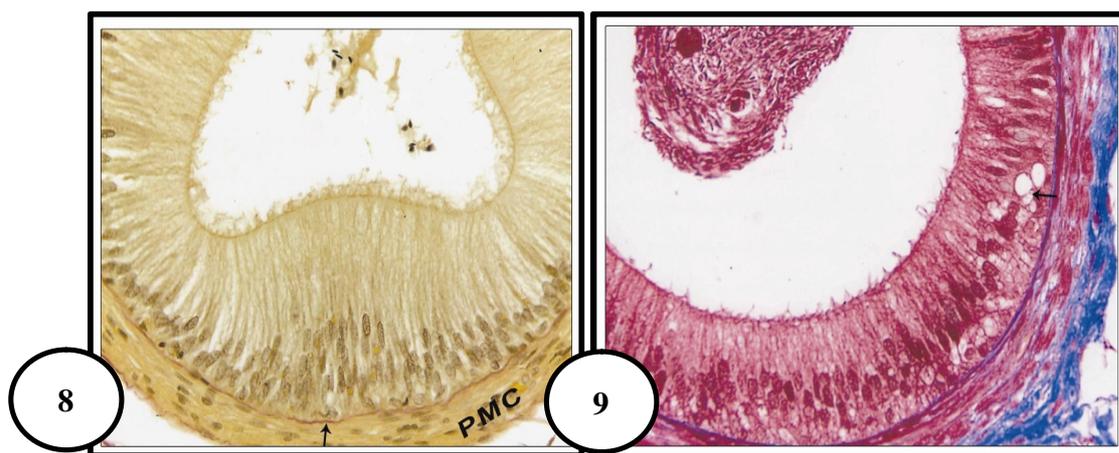


Fig. (8). A photomicrograph of a section at the level of the initial segment of camel epididymal duct during spring showing: Peritubular internal and external collagen fibers (arrows) and peritubular muscular coat (PMC) Van Gieson X 400
 Fig. (9). A photomicrograph of a section at the level of the distal part of middle segment of camel epididymal duct during spring showing: Increase in thickness of the peritubular muscular coat and aggregation of vacuolated basal cells (arrow) Masson's trichrome X 400

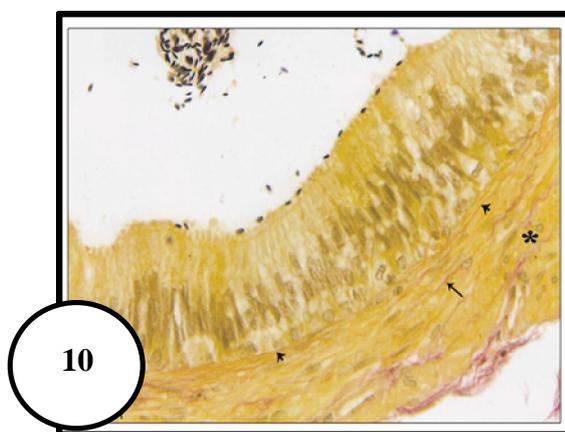


Fig. (10). A photomicrograph of a section at the level of the proximal part of terminal segment of camel epididymal duct during spring showing: Peritubular fine collagen fibers (arrow head) and distribution of collagen fibers (arrow) among the thick peritubular smooth muscle fibers (stars) Van Gieson X 400

RESULTS AND DISCUSSION

The traditional macroscopic segmentation of the epididymis into head, body and tail was insufficient to reflect the structural-functional relationship of this organ (Glover and Nicander, 1971). Based on histological, Histochemical and ultrastructural characteristics, the epididymis of most mammalian species could be subdivided into several segments. The number and distribution of these segments was species-specific

(Wrobel, 1998). Furthermore, Glover and Nicander (1971) stated that the number of epididymal segments might vary within the same species depending upon the criteria of classification. The number of epididymal segments was eight in rabbit (Jones *et al.*, 1979) and buffalo (Abdouet *et al.*, 1985), seven in guinea pig (Hoffer and Greenberg, 1978) and buffalo (Ebada, 2000), six in buffalo (Goyal and Dhingra, 1975), horse (López *et al.*, 1989) and cat (Axner *et al.*, 1999), five in bull (Alkafafy, 2005) and

goat (Goyal and Williams,1991), and four in cat (Sanchez et al., 1998).

In camel and in consistence with Tingari and Moniem (1979), Ebada(1994) and Abd El-maksoud (2010) whom stated that, the epididymis was subdivided into initial, middle and terminal segments, of which the middle segment was further subdivided into proximal, intermediate and distal parts. The current study did not support the previous findings in camel, since the epididymis was divided into initial, middle and terminal segments. The middle segment was further subdivided into proximal, intermediate and distal parts and the terminal segment was also subdivided into proximal and distal parts. For these reasons, the epididymis of camel in the present work was subdivided into seven segments (with addition of efferent ductules) (Fig. 1).

The epididymal duct was lined by a pseudostratified columnar epithelium. The epididymal epithelium was composed of five cell types: principal, basal, apical, dark and halo cells. These findings simulated with former observations in the camel (Tingari, 1989; Ebada, 1994 and Abd El-maksoud, 2010).

The principal and basal cells were the most frequent cell types throughout the whole length of the epididymal duct. The occurrence of the apical, dark and halo cells was less numerous in comparisons with

those of principal and basal cells. Macrophages and lymphocytes were found scattered throughout the epididymal duct and also present within the lumen. It was possible that the macrophages along with the lymphocytes might participate in the induction of immune tolerance and thus prevent the body from mounting immune reaction against spermatozoa (Marchlewicz, 2001). Also, the presence of macrophages might be of some importance in the process of sperm resorption.

Similar to findings of Abd El-maksoud (2010) in camel, the present study showed significant seasonal differences of the epididymal duct expressed by total diameter, epithelial height, length of stereocilia, luminal diameter, thickness of peritubular muscular coat and the cellular distribution.

The total diameter showed significant changes among different segments of the epididymal duct in different seasons. It decreased gradually from initial segment to the intermediate part of the middle segment then increased gradually in the distal part of the middle segment until reaching the maximum diameter in the distal part of the terminal segment (Table 1). The above was observed in all seasons.

On the other hand, the height of the epithelium decreased toward the distal part of the middle segment, and then the

epithelial height increased in the proximal part of the terminal segment followed by a marked decrease in the distal part of such segment (Table 2 and Figs. 2, 3). This gradual decrease in the epithelial height distal wards the epididymal duct might mechanically facilitate passage of the sperms toward the terminal segment (Abd El-maksoud, 2010). Our observation revealed that, the high epithelium in the initial segment might however, indicated a more absorptive power and secretory activity of the epithelium in this segment. In this respect, Crabo (1965) in bull had been reported that, over 90% of the fluids entering the epididymal duct was absorbed in the head of the epididymis.

In the current study, the highest epididymal epithelium was demonstrated in spring season (Fig. 2) followed by summer (Fig. 4), autumn and winter. Singh and Bharadwaj (1980) had been revealed that, the epididymal epithelium increased in height at December to March and Abd El-maksoud (2010) recorded that, the slightly low lining epithelium was identified in spring season. The latter author illustrated that the decreasing epithelial height in spring season to higher activity, whereas the high epithelium in other seasons was an indication to low activity. This was due to accumulation of secretion within the cells of high epithelium.

The present investigation revealed that the distribution and morphology of various cells constituting the epididymal epithelium showed significant seasonal changes. In all seasons, the principle cells formed the predominant cells throughout the whole epididymal duct and displayed important morphological changes from season to another. In spring season, the nuclei of the principal cells were vesicular oval with few chromatin condensations mainly adherent to the inner surface of the nuclear membrane. These nuclei were displaced at different levels in the cells (Fig. 2). The nuclei of the principal cells were elongated oval in winter season and condensed irregular oval in summer (Fig. 4) and autumn. The nuclei in the latter seasons possessed numerous heterochromatin patches and they were mostly arranged in the basal parts of the cells. Similar to findings of Abd El-maksoud (2010) in the camel, the lightly stained oval nuclei in the spring season indicating increased activity of the principal cells comparing to the darkly stained nuclei in other seasons; a sign of the decreased activity.

In the same manner, our observations showed that the abundant apical like protrusions, the numerous cytoplasmic vacuoles and PAS positive granules characterizing the principal cells in spring season (Fig. 5) were indication to increase

absorptive and secretory activities comparing with other seasons. In this respect, López *et al.* (1989) had mentioned that the epididymal epithelium synthesized and secreted glycoproteins into the luminal fluid, which was the carrier medium for spermatozoa during the epididymal passage and played a role in sperm maturation, capacitation and fertilization activities. Furthermore, Mandalet *al.* (2006) had found protein factor termed as forward motility stimulating protein (FMSF-1). This factor was glycoprotein in nature and possessed a physiological activator of sperm motility. The principal cells function was secretion and absorption. In addition to glycoproteins, the principal cells secreted proteins and enzymes such as phosphatases and glycosidases which were essential in the process of fertilization (Robaire *et al.*, 2006 and Samuelson, 2007).

In the present work, the basal cells did not exhibited significant changes among the different seasons. The dark cells in the current study were never found in the distal part of the terminal segment in spring season, whereas in the other season particularly in autumn, they were increased in number distalwards of the epididymal duct (Fig. 6). Abd El-maksoud (2010) in camel mentioned that, the dark cells increased in summer indicating decreased activity after an active period in spring.

The longest stereocilia were noticed in the proximal part of the middle segment, and then decreased gradually distalwards till they reached the lowest length in the terminal segment (Table 3). These findings were in accordance with the preceding studies in camel (Tingari and Moniem, 1979) and contradict other studies in the same animal (Ebada, 1994 and Abd El-maksoud, 2010). The principal cells of the equine caput and corpus epididymidis were equipped with long stereocilia and displayed morphological hallmarks of absorption and digestive activities (López *et al.*, 1989). The stereocilia increased the cell surface area facilitating the movement of molecules into and out of the cell (Junqueira and Carneiro, 2005).

In spring, the luminal diameter decreased gradually toward the intermediate part of the middle segment, then increased distalwards greatly. In spring, the maximum luminal diameter was noticed in the distal part of the terminal segment. Also, maximum luminal diameter was reached in all parts in spring season followed by autumn, summer and winter (Table 4). In spring, the lumina of the middle and terminal segments were densely crowded with sperms.

In summer, the middle segment of the epididymal duct revealed few sperms in their lumen (Fig. 7), while the lumen of

the terminal segment showed no sperms. During winter and autumn the epididymal duct showed dispersed few amount of sperms within their lumen.

In the present study, the distal part of the terminal segment was characterized by the widest lumen and lowest epithelial height comparing with other parts of the epididymal duct. This made a place for storage of sperms. Pineda (1989) and Samuelson (2007) recorded that the cauda epididymidis served as a reservoir for storage of viable spermatozoa.

The highest thickness of the peritubular muscular coat was apparent during spring season in all parts of the epididymal duct (Table 5). Our results pointed that the highest thickness of the peritubular muscular coat was apparent during spring season in all parts of the epididymal duct comparing with other season. Furthermore, there was an increase in the thickness of the peritubular muscular coat till the distal part of the middle segment (Figs. 8, 9), followed by dramatic increase in the thickness of this muscle in both parts of the terminal segment (Fig. 10). In agreement with findings of Abd El-maksoud (2010) in spring season, the thickest muscular coat observed in the terminal segment which might be helpful for powerful ejaculation (AlKafafy, 2005). In the present work, the muscular coat consisted of circularly

arranged smooth muscle cells in the initial and middle segments, whereas in the terminal segment formed of abundant circularly and obliquely arranged smooth muscle fibers. According to Abd El-maksoud (2010) in the camel, the obliquely smooth muscle fibers in the terminal segment increased in proportion and tended to be longitudinally oriented. Furthermore, the latter author mentioned that, the head and body of the epididymis demonstrated spontaneous rhythmic peristaltic contractions that served to convey the sperms along the duct. Fewer such contractions were observed in the tail region.

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