

Morphological and histochemical changes in the dromedary camel epididymis in relation to reproductive activity

Zarroug Hassan Ibrahim^{1,2}, Khalid Ali Al-Kheraije¹ and Shio Kumar Singh³

¹Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia,

²Permanent Address: College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum-North, Sudan and

³Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India

Summary. Environmental conditions such as temperature, light and food availability are known to influence the physiological status of animals. The male dromedary camel (*Camelus dromedarius*) is considered as a seasonal breeder with maximal sexual activity during certain period of the year followed by a decrease in activity during the remaining period. On the other hand, the male camel is also shown as an atypical seasonal breeder because this does not undergo sexual quiescence with complete cessation of spermatogenesis. This animal, however, shows remarkable physiological and behavioral changes during its maximal sexual activity. The annual breeding (rutting) period also influences the epididymis. In this review, an attempt has been made to present the available literature pertaining to gross anatomical, histological, histochemical, immunohisto-chemical and molecular changes in camel epididymis during breeding and nonbreeding periods, and the changes are believed to be correlated with male sexual behavior and libido. This review may also exhibit the dromedary camel breeding period, which is still unresolved, and thus may prove helpful in determining the exact time of mating, which is important for the success of assisted reproductive outcomes. Further, the review may contribute to a better understanding of the epididymal physiology in camel and may also prove useful in improving reproductive efficiency and population of this animal.

Key words: Dromedary camel, Histochemistry, Epididymis, Morphology, Seasonal reproductive activity

Introduction

The dromedary or one-humped camel (*Camelus dromedarius*) is one of the two species within the genus *Camelus* and the other being the Bactrian or two-humped camel (*Camelus bactrianus*). The *Camelus dromedarius* is a very useful animal to humans. Several studies targeting better performance of dromedary camel population have been conducted, including reproductive efficiency (Taha and Abdalla, 1980; Tingari and George, 1984; Taha et al., 1994; Sumar, 2000; Hegazy et al., 2004; Sckidmore, 2005; Ghazi, 2007). The reproductive performance in dromedary camels is relatively lower than in other domestic animals (Novoa, 1970) because of many complex genetic and natural constraints (El-Hassanein et al., 2004). The short breeding season and limited male libido are considered to be the main obstacles in the growth of dromedary camel livestock (El-Hassanein, 2003). However, the idea that the *Camelus dromedarius* breeding is seasonal is also disputed. Male camels have been reported as seasonal breeders with a marked sexual activity during certain months of the year (rutting season) and remain sexually quiescent during the remaining months (Musa and Abusineina, 1978; Tingari et al., 1984a; Sumar, 2000; Hegazy et al., 2004; Ghazi, 2007). Recently, Al-Bulushi et al. (2019) have shown that there is clearly a defined reproductive seasonality in Omani male camels ranging from December to March, with a peak reproductive efficiency from December to January. However, Ibrahim and Singh (2014) have reported that there is no cessation of epididymis and testis activity in male dromedary camels throughout the year, though their activity is reduced during non-breeding months. Similarly, Tingari et al. (1984a) have reported dromedary camel as an atypical seasonal breeder and that there is no complete cessation of spermatogenesis.

Corresponding Author: Prof. Shio Kumar Singh, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi-221005, India.

e-mail: shioks@rediffmail.com or shioks53@gmail.com

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The seasonal breeding activity in male dromedary camels leads to variations in blood androgen level (Babiker et al., 2011; Al-Bulushi et al. 2019), and so sexual desire and behavior are affected. These behavioral and functional changes are believed to result in remarkable morphological and biochemical changes in the male reproductive organs, which are evident from gross anatomy of testis (Singh and Bharadwaj, 1978a; Tingari et al., 1984a; Masood, 2007; Zayed et al., 2012; Pasha et al., 2013) and epididymis (Singh and Bharadwaj, 1978a; Zayed et al., 2012), histology of testis (Singh and Bharadwaj, 1978b; Tingari et al., 1984a., Zayed et al., 1995) and epididymis (Alkafafy et al., 2011; Hafez et al., 2011; Abd-Elaziz, et al., 2012; Zayed et al., 2012; Ibrahim and Singh, 2014), ultrasonography of testis (Hussain, 2010; Pasha et al., 2011a), and from immunohistochemistry of epididymis (Alkafafy et al 2016; Ibrahim et al., 2017), efferent ducts (Ibrahim, 2015) and poll glands (Tingari et al., 1984b; Ibrahim et al., 2020; Ibrahim and Al-Kheraije, 2020).

Because of its important role in sperm maturation, and sperm storage and resorption, epididymis has received more attention than other parts of the testicular excurrent duct system (Nicander and Malmqvist, 1977; Abe et al., 1983). Morphological and histochemical studies on male camel reproductive organs could be of great importance for a better understanding of its physiological adaptive activity as it is thought to be responsible for the reproductive performance in this species (Osman and el-Azab, 1974). Recently, Ibrahim and Abdel-Maksoud (2019) have studied the effect of reproductive activity on epididymal structure and function; the findings showed that the reproductive activity significantly affected the immunohistochemistry and ultrastructure of the camel epididymis. Seasonal morphological and histochemical changes in the reproductive organs may also lead to identification of a clearly defined reproductive period with maximal reproductive functions (Tingari et al., 1984a). During

this period, a successful breeding program including mating and semen collection could be performed and so improve dromedary camel conception and development of assisted reproductive techniques (Al-Bulushi et al., 2019). The present review therefore describes the morphological and histochemical changes in the dromedary camel epididymis during breeding and nonbreeding periods.

Structure and function

The dromedary camel epididymis is located at the cranial border of the testis and extends from the lower end to just above the upper border. According to Zayed et al. (2012), the camel epididymis is situated on the dorso-cranial testicular border, and the head is curved on the testicular cranial pole. The epididymis, which is composed of the efferent ductules proximally and a long epididymal duct distally, is tightly packed in a thick layer of tunica albuginea which is also covered from outside by the visceral layer of tunica vaginalis (Zayed et al., 2013). Epididymis is in the form of highly convoluted and small tubules with a diameter of 2 mm (Hafez and Hafez, 2001). The gross anatomical description of this organ as having distinct regions like head (caput), body (corpus) and tail (cauda) is considered unsatisfactory (Glover and Nicander, 1971). These regions could be subdivided into segments, either depending on epithelial height (Reid and Cleland, 1957) or cytological characteristics and epithelial height (Nicander, 1958). The segments exhibit different morphological features and vary in size, number and in type of cells in different animal species (Tingari and Moniem, 1979). On these bases, Tingari and Moniem (1979) identified and designed distinct histological regions in camel epididymis as initial segment, middle segment and terminal segment; further, the middle segment was subdivided into proximal part, intermediate part and distal part. The segment-based design has also been adopted by Zayed et al. (2012) and Ibrahim and Singh (2014) in dromedary camel epididymis (Fig. 1).

It is well established that spermatozoa acquire fertilizing capacity during their passage through the proximal part of the epididymis before being stored in the distal region (Abe et al., 1983). Therefore, the functional maturation of spermatozoa is believed to be mediated through the surrounding luminal fluid of the epididymal duct. Further, Nicander and Malmqvist (1977) have reported that the epididymal fluid has regional chemical variations which are thought to be associated with functional activity of the epithelial lining in different segments.

The histological structure of the epididymis in all mammalian species including dromedary camels is in the form of a pseudostratified columnar epithelium, surrounded by a thin layer of loose connective tissue, and supported by a smooth muscle coat (Fig. 2); the high stereo-ciliated epithelium in the proximal epididymis decreases gradually towards the terminal segment



Fig. 1. Diagram of camel epididymis showing three regions: caput (a), corpus (b) and cauda (c). (Ibrahim and Singh, 2014). (Reproduced with permission).

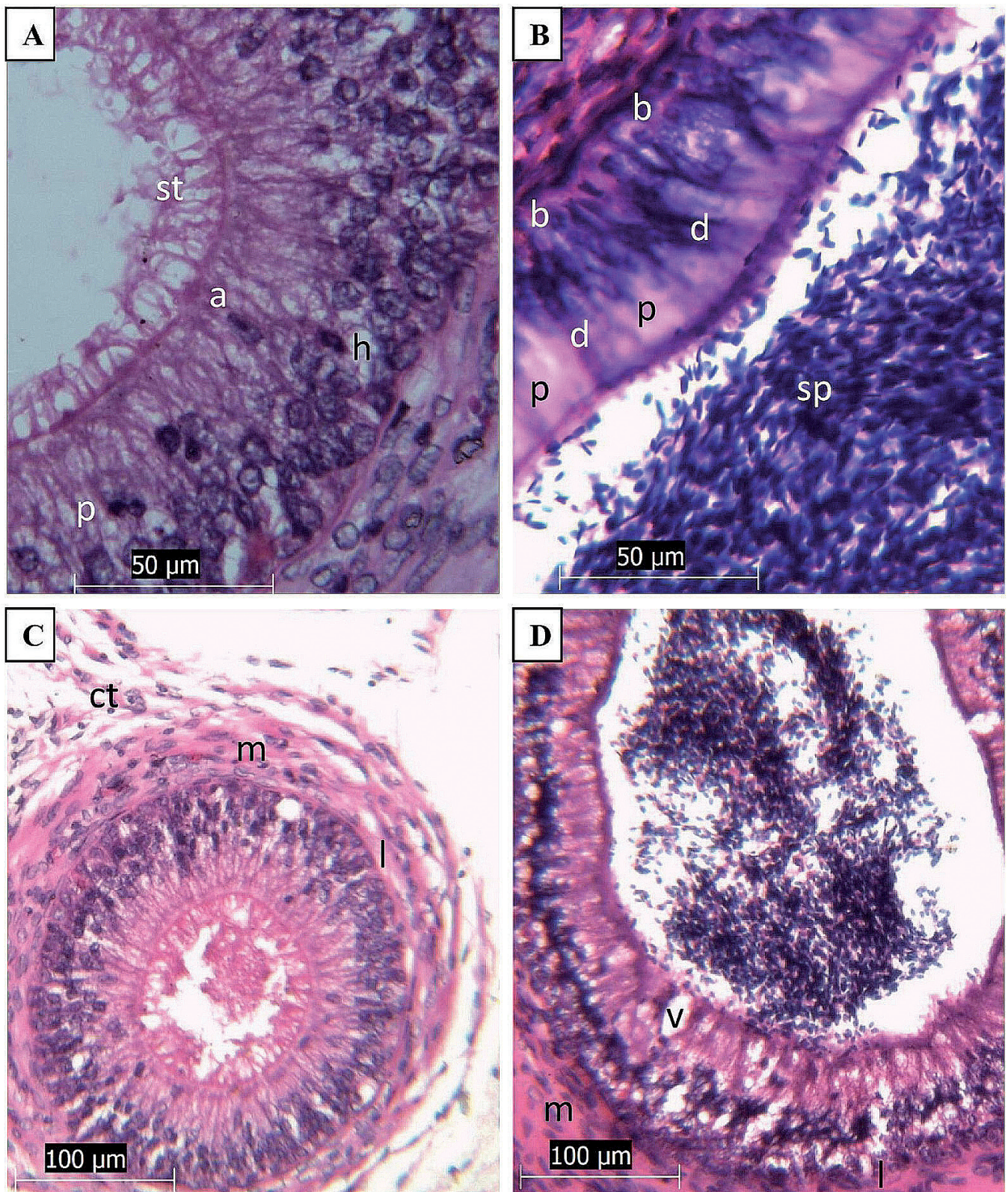


Fig. 2. Photomicrographs of camel epididymis stained with hematoxylin and eosin (H&E). **A.** Caput region showing tall pseudostratified columnar epithelium with long stereocilia (st), principal cells (p), apical cells (a) and halo cells (h). **B.** Cauda region showing low epithelium and stereocilia with basal cells (b), dark cells (d) between the principal cells (p) and wide lumina filled with spermatozoa (sp). **C.** Corpus region showing high epithelium and lamina propria (l) surrounded by a thin smooth muscle coat (m) and peritubular connective tissue (ct). **D.** Cauda region showing low epithelium with abundant vacuoles (V) and lamina propria (l) surrounded by a thick smooth muscle coat (m). (Ibrahim and Singh, 2014). (Reproduced with permission).

(Tingari and Moniem, 1979; Hermo and Robaire, 2002; Ibrahim and Singh, 2014) of the organ.

The gradual decrease in the epididymal epithelial height is suggested to mechanically facilitate spermatozoa passage towards the terminal segment (Abd El-maksoud, 2010). Further, the high epithelial cells in the initial segment may indicate more absorptive and secretory activities in this segment. In line with this, Crabo (1965) reported that over 90% of the fluid reaching the epididymis is absorbed in the caput. A gradual increase is also evident in the thickness of the smooth muscle layer coat of the epididymal duct that reaches to its maximum in the terminal segment (Tingari and Moniem, 1979; Parillo et al., 2009; Hafez et al., 2011; Ibrahim and Singh, 2014). The regional histological variations in the epididymis are suggested to correlate with different functional activities throughout the duct necessary for sperm maturation; the absorptive and secretory activities of the epididymal epithelial cells are necessary for the spermatozoa to acquire fertilizing capacity (Zhou et al., 2018). Further, spermatozoa forward motility and ability to efficiently encounter the ovum and its vestments are acquired in different epididymal segments (Sullivan and Mieusset, 2016). According to Tingari and Moniem (1979), the regional histological appearance of the camel epididymal duct fits well with the terminology proposed by Glover and Nicander (1971). On this basis, Tingari and Moniem (1979) have reported that the initial and middle segments of the epididymis were concerned with sperm maturation, while the terminal segment served for storage of sperm and the initial segment with its stereociliated epithelium showed evidence of micropinocytosis. There are two main types of epithelial cells in the mammalian epididymis, namely principal cells and basal cells (Glover and Nicander, 1971; Tingari, 1989; Sostaric et al., 2008). Other cell types including apical cells and clear cells are also present; halo cells and narrow cells have also been identified in dromedary camels (Tingari, 1989; Ibrahim and Singh, 2014) and stallions (Sostaric et al., 2008). Ibrahim and Abdel-Maksoud (2019) identified five types of epithelial cells in the epididymis of dromedary camel, and these are principal cells, basal cells, apical cells, dark cells and halo cells. Macrophages and lymphocytes are also found scattered along the epididymal wall and lumen (El-Sakhawy et al., 2016). It has been reported that the

macrophages and lymphocytes might induce immune tolerance, and hence prevent the animal body from immune reaction against spermatozoa (Marchlewicz, 2001). The presence of macrophages is suggestive of their participation in the process of sperm resorption.

Intraepithelial macrophages may also occur in the epididymal epithelium (Schon and Blottner, 2009). According to Tingari (1989) and Pasha et al. (2011a), principal cells have been described as having ultrastructural characteristics of absorption especially in the proximal segment and intermediate part of the middle segment, as indicated by abundant endocytotic apparatus with endosomes, multi-vesicular bodies, lysosomes and vesicles, and secretory function, especially in the terminal segment as shown by well-developed Golgi apparatus, numerous smooth and rough endoplasmic reticulum and scattered mitochondria; basal cells, which are comparatively simple in structure exhibit general paucity of organelles; halo cells consist of scanty cytoplasm and large nuclei (Reid and Cleland, 1957) and morphological evidence relates them to intraepithelial cells and circulating lymphocytes (Hoffer et al., 1973); apical cells contain scanty mitochondria and small Golgi apparatus; and dark cells are tall and they have many electron dense bodies. Dark cells have been described as dying principal cells (Tingari, 1989).

It is well established that spermatozoa acquire fertilizing ability and motility during their transit through the epididymis (Cooper and Yeung, 2006). Some other functions are also ascribed to the epididymis, including sperm transport, sperm concentration, male gamete immune-protection, and storage of spermatozoa (Cornwall, 2009; Belleannée et al., 2012).

Seasonal gross anatomical variations

Zayed et al. (2012) have shown in camel an increase in epididymal weight and volume during breeding season compared to nonbreeding season (Tables 1, 2), and this may be attributed to the fact that the reproductive activity in this animal increases only during certain months during which there is an increase in the number of spermatozoa in the epididymis leading to an increase in organ weight and volume compared to that in the nonbreeding season. Further, the breeding season also causes an increase in volume and exerts a prominent

Table 1. Weight (g) of testis and epididymis in camel in different seasons of the year

Season	Testis	Epididymis	%Epididymis/Testis + Epididymis
Spring	99.93±22.47	26.83±2.21	21.16
Summer	77.80±25.70	18.50±5.63	19.21
Autumn	88.50±22.13	18.95±1.08	17.63
Winter	68.68±14.96	18.82±1.15	22.60

Reproduced with permission from Zayed et al., 2012.

Table 2. Volume (cm³) of testis and epididymis in camel in different seasons of the year.

Season	Testis	Epididymis	%Epididymis /Testis + Epididymis
Spring	97.83±21.78	25.66±4.96	20.77
Summer	77.33±24.01	18.33±5.03	19.17
Autumn	85.75±20.46	21.25±2.50	19.80
Winter	71.80±16.17	19.80±0.40	22.90

Reproduced with permission from Zayed et al., 2012.

effect on the echotexture picture of camel testis and epididymis (Pasha et al., 2011a).

Seasonal general histological variations

The dromedary camel epididymis exhibited seasonal changes in epithelial cells shape and height, stereo-cilia length, luminal diameter, peritubular muscular coat and in cell distribution (Singh and Bharadwaj 1980; Tingari et al., 1984a; Alkafafy et al., 2011; Zayed et al., 2012; Ibrahim and Singh, 2014; El-Sakhawy et al., 2016; Alkafafy et al. 2016; Ibrahim and Abdel-Maksoud, 2019).

Principal cells showed lightly stained oval nuclei during breeding season which indicated increased activity of these cells compared to darkly stained nuclei in nonbreeding season (Abd El-maksoud, 2010; Zayed et al. (2012). Zayed et al. (2012) reported that the epididymal basal cells were relatively increased during breeding season which might indicate increased activity, noting that these cells are considered to be stem cells for the principal cells. These authors also found that the surrounding lamina propria in the epididymal duct is very thick in breeding season and thin in other seasons, which may be related to the increased total diameter of the epididymal duct in the breeding season.

According to Ibrahim and Singh (2014), there is an increase in apical cytoplasmic folding and intraepithelial vacuoles accompanied by distended lumina with increased spermatozoal content in the epididymis during the breeding season compared to the nonbreeding season (Fig. 3). Zayed et al. (2012) also reported inward folding in the epithelium of camel epididymis as well as increased number of principal cells during breeding season. Ibrahim and Abdel-Maksoud (2019) reported that the principal cells in the caput region are significantly decreased in number during nonbreeding season with respect to breeding season; this may indicate that most of these cells start to undergo regression during the inactive period. In the same manner, the abundant apical protrusions and numerous cytoplasmic vacuoles in the principal cells in breeding season are suggestive of increased absorptive and secretory functions compared to that in other seasons (Zayed et al., 2012).

Moderate seasonal variations have also been described in the efferent ductules in terms of total

diameter, epithelial height, cilia length, muscular coat thickness and cellular distribution (Zayed et al., 2013). The maximal epithelial lining in dromedary camel epididymis is demonstrated during breeding season (Singh and Bharadwaj, 1980; Abd El-maksoud, 2010; Alkafafy et al. 2011; Ibrahim and Singh., 2014; El-Sakhawy et al. 2016) (Table 3). During nonbreeding season, the epididymal epithelial cells are distorted and their heights become one third to one half that recorded during breeding season (Singh and Bharadwaj 1980). This may be related to the decreased sperm production during this season. Though increased epithelial height with narrow luminal diameter is recorded during breeding season, decreased epithelial height with wide luminal diameter is seen during nonbreeding season (Alkafafy et al. 2011; Ibrahim and Singh, 2014). According to Ibrahim and Singh (2014) and Ibrahim and Abdel-Maksoud (2019), the luminal content of spermatozoa was increased during the breeding season. It has been suggested that the decreased epithelial thickness and wide lumina could facilitate easy spermatozoa passage in the camel epididymal duct (Alkafafy et al., 2011; Ibrahim and Singh, 2014) and efferent ductules (Zayed et al., 2013). Aguilera-Merlo et al. (2005) have reported similar seasonal epididymal histologic changes in a typical seasonal breeder (viscacha) attributing the changes to be testosterone-dependent. On the other hand, however, contradictory findings have also been reported in camel efferent ductules (Zayed et al., 2013) and epididymis (Abd El-maksoud, 2010) showing low epithelial thickness during breeding season and high epithelial thickness during nonbreeding season. It has been suggested that the low epithelium thickness during the breeding season is an indication of high activity and the high epithelium thickness during nonbreeding season is an indication of low activity accompanied by accumulated secretion within the cells leading to increased cell size (Abd Elmaksoud, 2010; Zayed et al., 2013).

A remarkable increase is reported in the stereocilia length of the camel epididymal epithelium in the cauda region in rutting Sudanese camels (Ibrahim and Singh, 2014), while prominent variations in length and interdigitating stereocilia, including that in the caput region are observed in rutting Egyptian camels (Zayed et al., 2012) (Table 3).

Table 3. Seasonal morphometric parameters of different epididymal regions in camel (Ibrahim and Singh, 2014).

Epididymis region	Season	Epithelial height	Stereocilia length	Luminal diameter	Muscular thickness
Caput	Winter	78.97±3.79	21.90±1.80	154.31±7.75	19.62±4.20
	Summer	74.22±3.27	18.70±2.07	162.22±3.27	26.10±4.50
Corpus	Winter	51.71±4.49	16.04±3.09	167.94±18.09	28.71±5.02
	Summer	48.92±2.04	12.12±2.00	195.38±18.43	32.92±3.99
Cauda	Winter	48.78±9.34 ^a	12.70±1.81 ^a	273.55±14.10	31.21±6.30
	Summer	32.53±8.88	6.95±1.99	331.23±28.69 ^a	48.06±1.99 ^a

All values (mean ± SD, n=24) are in micrometers (µm). ^a: Values are significantly different in cauda region during winter and summer seasons. Reproduced with permission from Ibrahim and Singh, 2014.

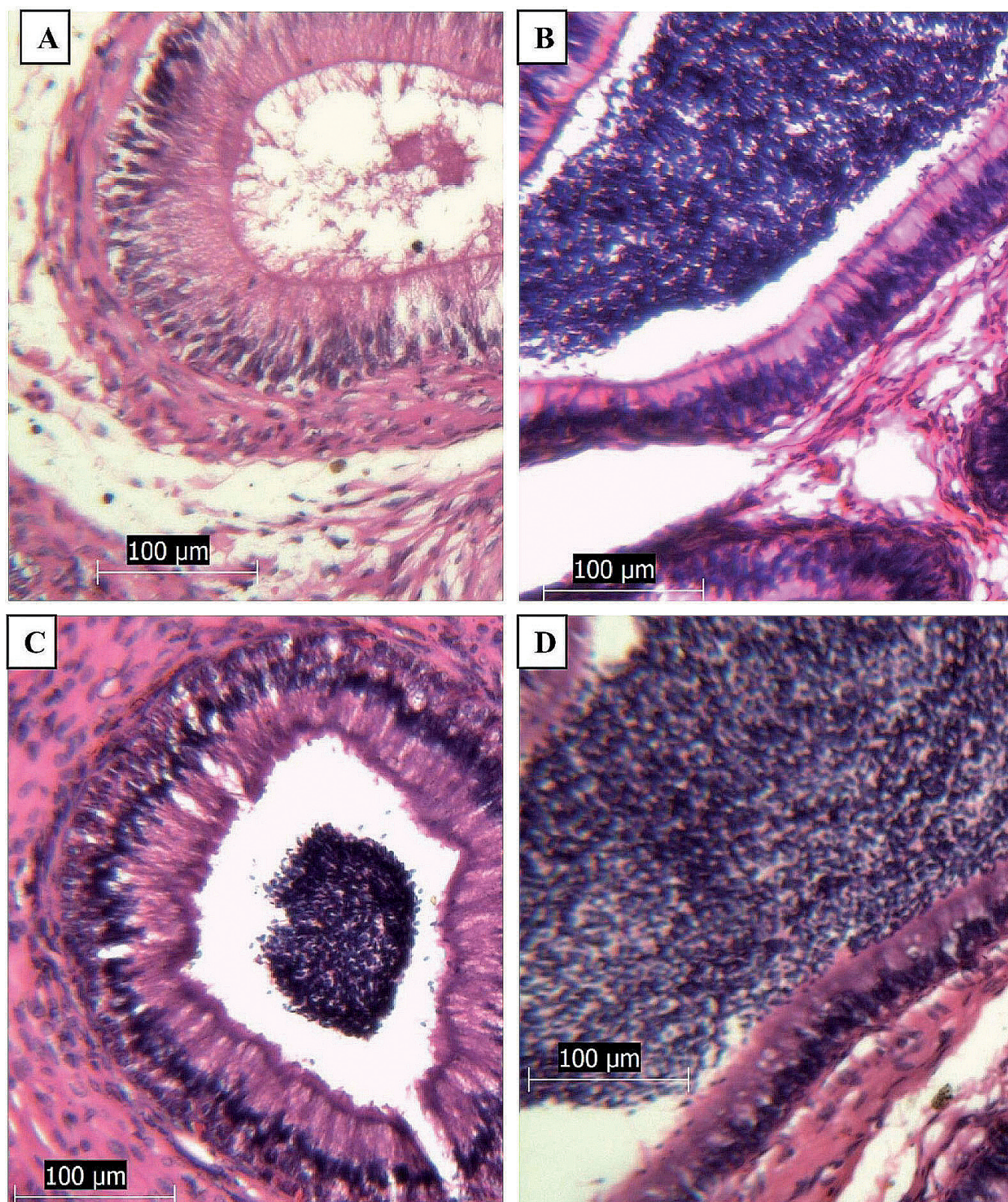


Fig. 3. Photomicrographs of camel epididymis stained with H&E: Caput region (A) during winter showing increased epithelial and stereocilia height, decreased luminal diameter and decreased luminal spermatozoa from that seen in caput region (B) during summer. Cauda region (C) during winter showing increased epithelial and stereocilia heights decreased luminal diameter and decreased luminal spermatozoa from that observed in cauda (D) during summer. (Ibrahim and Singh, 2014). (Reproduced with permission).

As reported by EL-Sakhawy et al. (2016), the diameter of dromedary camel epididymis decreases gradually from the initial segment to the intermediate portion of the middle segment and then increases gradually in the distal portion of the middle segment with maximal diameter in the distal portion of the terminal segment; the total epididymal diameter is significantly changed among different segments in different seasons. Zayed et al. (2012) and Ibrahim and Singh (2014) have noted that the camel epididymal diameter (μm) varies from season to season in Egyptian and Sudanese camels and the maximal diameter is seen during the rutting season (Zayed et al., 2012) (Table 3). According to Abd El-maksoud (2010) and El-Sakhawy et al. (2016), there is a marked seasonal change in the epididymal duct total diameter, epithelial thickness, stereocilial length, luminal diameter, peritubular muscular coat thickness and in cellular distribution.

Intraepithelial vacuoles in the camel epididymis are often seen during breeding season (Zayed et al., 2012; Ibrahim and Singh, 2014) (Fig. 4). Such vacuoles are suggested to be absorbed fluids (Wrobel, 1972), which condense in the cytoplasm and get ready to be released into the lumen through the apical cytoplasmic membrane. Abd El-Maksoud (2010) considered the large intraepithelial vacuoles of camel epididymis as unique structures, which are called the intraepithelial glands of the epididymal body. The lumen of these intraepithelial glands is filled with intensely positive bromophenol blue secretory granules in the breeding season (Abd El-Maksoud et al., 2019) which may indicate protein

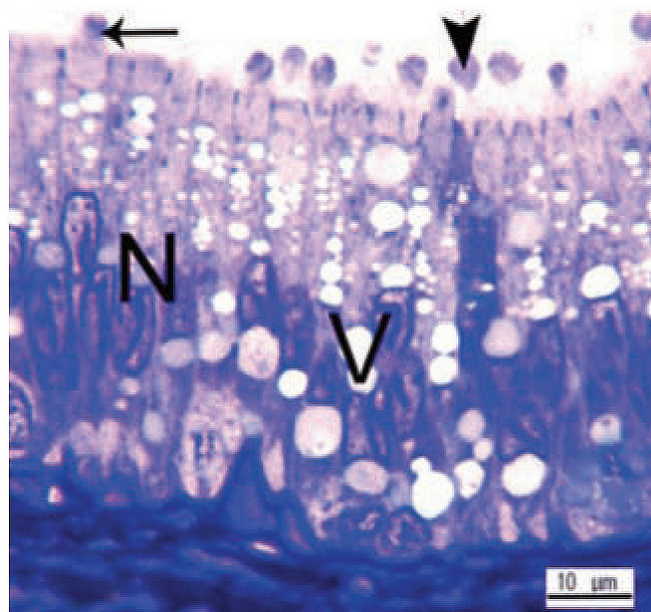


Fig. 4. Semithin sections stained with toluidine blue showing the terminal segments of the camel epididymal duct in spring. The nuclei (N) show very deep indentations. The cytoplasmic vacuoles (V) are demonstrated and also show apical protrusions (arrow) and vesicles (arrow head). (Zayed et al, 2012). (Reproduced with permission).

secretion (Pearse, 1968).

The circumtubular muscular coat thicknesses increased during breeding season in dromedary camel epididymis compared to nonbreeding season (Ibrahim and Singh, 2014) (Table 3), and Zayed et al. (2013) have also made similar observations in the efferent ductules of one-humped camel. This feature is suggested to be because of the increased muscular peristaltic movement needed for easy spermatozoal flow through the epididymal tubules. As the muscular coat thickness is maximal in the cauda epididymidis (Tingari and Moniem, 1979; Alkafafy, 2005; Alkafafy et al., 2011; Ibrahim and Singh, 2014), it is suggested to play a role in sperm ejaculation. Similarly, Zayed et al. (2013) have reported that the thicker muscular coat in the terminal segment of the epididymis during breeding season might help in powerful ejaculation.

Generally, spermatozoa are crowded in the intermediate portion of the middle segment of the epididymis and are packed in the terminal segment (Tingari and Moniem, 1979). During breeding season, a compact spermatozoal mass occupies the entire lumen of the intermediate portion of the middle epididymal segment, while during nonbreeding season, spermatozoal content is variable in the distal portion of the middle segment, large in the distal part of the middle segment and remarkable in the terminal segment (Tingari et al., 1984a). According to Ibrahim and Singh (2014), spermatozoa are detected in the epididymal lumen during both breeding and nonbreeding seasons, though they are less in number during nonbreeding season. It could be stated that spermatozoal content in the epididymis is related to their release from the testis; increased sperm content in the epididymal segments during breeding season and decreased content during nonbreeding season reflect the functional state of the testis and the spermatogenic activity in camels; the observations of increased spermatozoa during breeding season and decreased spermatozoa during nonbreeding season suggest that the spermatogenic activity keeps going, though at a reduced pace during nonbreeding season compared to the breeding season. Thus, Tingari et al. (1984a) and Ibrahim and Singh (2014) consider the dromedary camel as an atypical seasonal breeder because it does not undergo sexual quiescence in which spermatogenesis is completely suppressed.

Seasonal ultrastructural variations

The study of Ibrahim and Abdel-Maksoud (2019), to the best of our knowledge, appears to be the first study that describes the seasonal ultrastructural changes in epididymis of dromedary camels. These authors have reported that the seasonal reproductive activity in dromedary camels causes considerable ultrastructural changes in the epididymis with consequent functional effect; the changes are mainly observed in the stereocilia and in the different epithelial cells of the duct, namely, the principal, dark, basal, and apical cells (Fig. 5). The

principal cells showed extremely long stereocilia and multiple tight junctions in the reproductive season; a few desmosomes could be seen between the principal cells and between the principal and dark cells (Fig. 6). Some intracytoplasmic vesicles with or without vacuoles are also evident in the apical part of the cytoplasm in dark and principal cells. During nonbreeding season, the stereocilia are much shorter and fewer in number. Abundant multivesicular inclusion bodies and large cytoplasmic vacuoles are the most common features in the principal cells during nonbreeding season (Ibrahim and Abdel-Maksoud, 2019). The vacuoles are mainly found in the apical and basal part of cytoplasm. On the other hand, in the nonbreeding season principal cells show smaller nuclear diameter, less developed Golgi apparatus, a few rough endoplasmic reticulum (rER) and mitochondria. Also in the nonbreeding season, the dark cells are much smaller than that in breeding season, especially in the caput region, and their nuclei are basal and exceptionally lobulated (Fig. 7).

It has been earlier reported that rat epididymal principal cells show markedly well-developed organelles including Golgi apparatus and rER, suggesting increased secretory and absorptive activities (Hoffer et al., 1973). Similar findings have recently been reported by Ibrahim and Abdel-Maksoud (2019) in dromedary camel epididymis. According to the latter authors, the absorptive activity is amplified during the nonbreeding

season, which is evident from infiltration of pinocytotic vesicles in the principal cells apical cytoplasm and numerous multivesicular inclusion bodies and cytoplasmic vacuoles. The blood-epididymis-barrier (BEB) guarantees a proper microenvironment for sperm protection from the immune system (Akbarsha et al., 2015). In this respect, Ibrahim and Abdel-Maksoud (2019) described the BEB in dromedary camel as more functional during the breeding seasons and the tight junction is found to be more obvious in the rutting season than that in nonrutting season. Moreover, it has been noted that the harmful effects of some stressors might adversely affect the cellular tightness resulting in alterations in sperm fertility and function (Hermo et al., 2007). Similarly, Ibrahim and Abdel-Maksoud (2019) noted that during nonbreeding season heat stress might alter the BEB. Intraepithelial leukocytes may participate in holding epididymal sperm antigens away from the immune system, i.e. prevent a specific immune response against sperm antigens. According to Zayed et al. (2012), principal cells display accumulation of dense bodies and abundant lysosome-like dense bodies in rutting camel epididymis, which may be related to active sperm digestion and epithelial cell remnants with typical signs of involuting organ.

Compared to nonbreeding season, the camel epididymal basal cells show many structural changes, including numerous cytoplasmic extensions, plasma

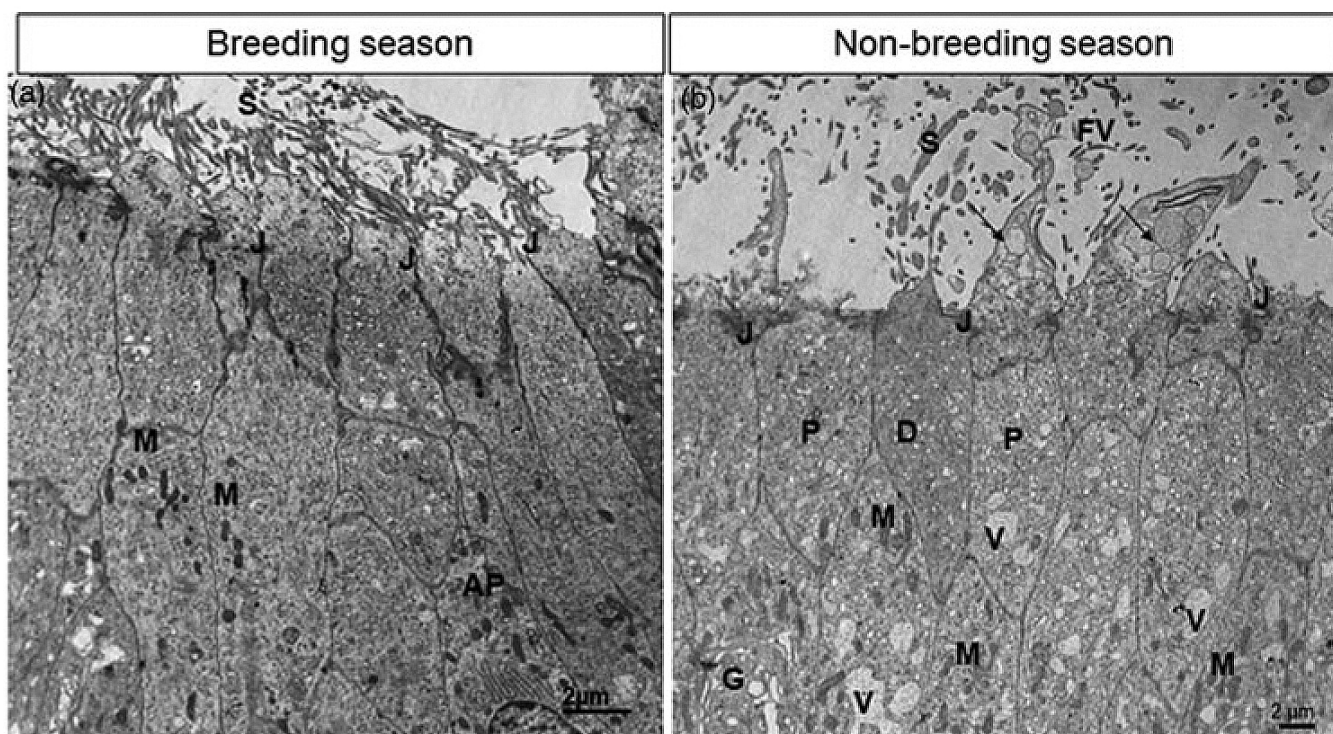


Fig. 5. Stereocilia covering the free surface of the head region of camel epididymis in the breeding (a) and nonbreeding (b) seasons. Arrows indicate pinocytotic vesicles in the apical cytoplasm of the principal cells. AP, apical cell; D, dark cell; FV, free vesicles; G, Golgi complex; J, junctional complexes; M, mitochondria; P, principal cell; S, stereocilia; and V, vacuoles. (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

membrane folding, large euchromatic nuclei, and abundant mitochondria and rER during breeding season (Ibrahim and Abdel-Maksoud, 2019) (Fig. 8). Increased number of mitochondria is believed to be essential for reproductive activity and regulation of reproductive function (Ramalho-Santos et al., 2009). These findings could also indicate that the function of basal cells is not confined to only their stem-like cells. It has been suggested that during the breeding season the reduction in tubular smooth endoplasmic reticulum with proliferation in the condensed endoplasmic reticulum is because of the relatively high level of testosterone production (Friedlander et al., 1984; Pasha et al., 2011b).

Apical cells are mitochondria-rich cells which play many important functional roles in the epididymis, such as holocrine secretion, reabsorption of testicular fluid and acidification of epididymal luminal fluid (Martinez-Garcia et al., 1995). In reproductive season, the apical cells appear wedge-shaped with broad basal ends and small basal nuclei and they are rich in mitochondria and many cytoplasmic processes (microvilli) (Fig. 9); during nonbreeding season, the cells show large central nuclei, small luminal bleb-like microvilli, ill-developed Golgi apparatus and a few mitochondria and rER (Ibrahim and Abdel-Maksoud, 2019).

The halo cells have been shown in the lining epithelium of many mammalian epididymal ducts (Hoffer et al., 1973; Wang and Holstein, 1983). They are also present in camel epididymis (Ibrahim and Abdel-Maksoud, 2019). The latter authors have shown that the

intraepithelial leukocytes such as macrophages and lymphocytes are markedly increased during nonbreeding season (Fig. 10). The presence of these intraepithelial leukocytes together with multivesicular inclusion bodies suggests an immune function as these elements serve as a barrier between sperm antigens and the general blood circulation (Wang and Holstein, 1983).

Histochemical variations

Studies of Tingari and Moniem (1979) show that the PAS-positive material, which is probably glycogen, is absent in the camel epididymal cells, while it is present in epididymal stereocilia, sub-epithelial tissue and blood vessels. On the other hand, PAS-positive material (glycogen) has been detected in the epididymis of rabbit (Linnetz and Amann, 1968), horse, ram and bull (Nicander, 1958). According to Tingari and Moniem (1979), it is difficult to offer an explanation for the absence of glycogen in the epididymis of camel. In contrast to the findings of Tingari and Moniem (1979), PAS-positive granules have been demonstrated in dromedary camel epididymis (Zayed et al., 2012) and in efferent ductules (Zayed et al., 2013) in different reproductive seasons with relative intensity in the breeding season. PAS-positive reaction gives an indication for the presence of glycoprotein (Abou-Häila and Fain-maurel, 1984) which may be an indication of increased secretory and absorptive activities. Further, principal cells of dromedary camel epididymis are

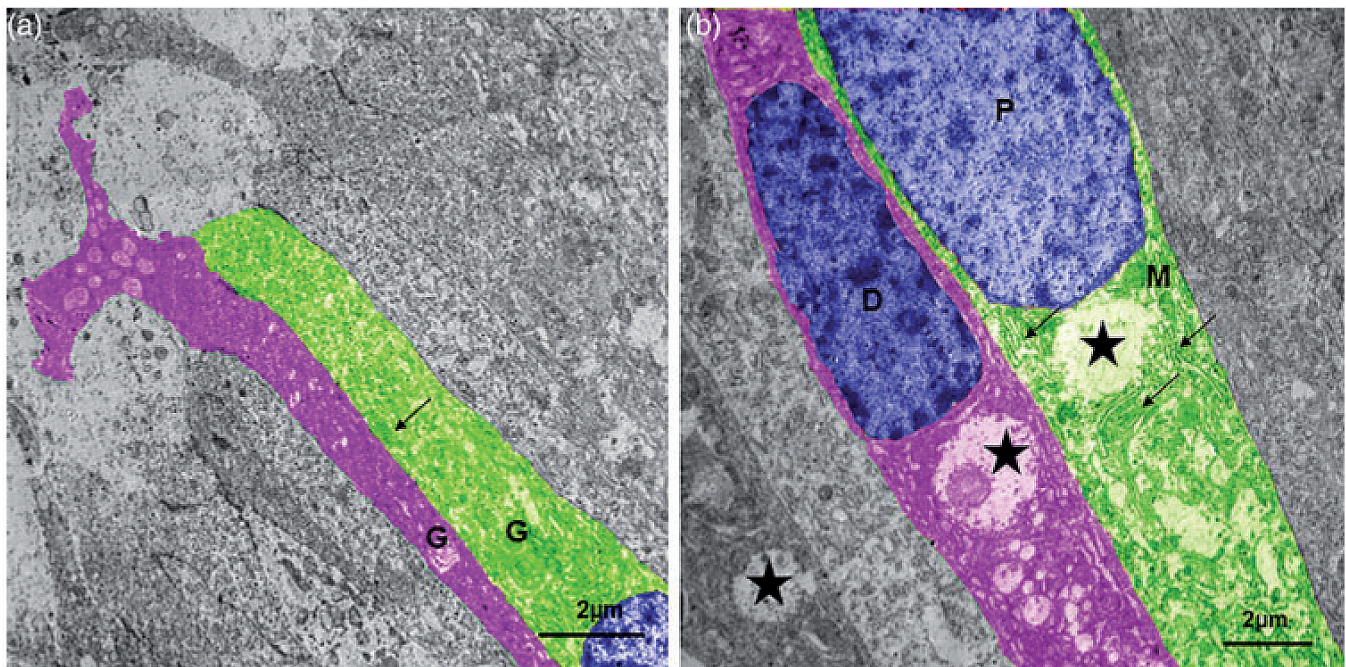


Fig. 6. Ultrastructure of the principal (P) and dark (D) cells in the reproductive season of camel epididymis. **a.** The apical portion of the apical and dark cells shows well-developed Golgi complexes (G) and rough endoplasmic reticulæ (arrows). **b.** The basal portion of the apical and dark cells shows an electron opaque spherical cellular inclusion closely attached or may fuse to the basal border of the nucleus (asterisks). M, mitochondria. (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

shown to be PAS-positive in Egypt during spring season, and this is considered as an indication of increased secretory and absorptive activities in spring than in other seasons (El-Sakhawy et al., 2016). It should be remembered that the epididymal epithelial cells synthesize and secrete glycoproteins as part of luminal fluid and these glycoproteins are a carrier medium for sperm during their passage along the duct in addition to their role in sperm maturation and fertilization (López et al., 1989). In the efferent ductules, the nonciliated cells secretory activity is denoted by detection of PAS-positive granules and globules in their infra- and supra-nuclear cytoplasm (Ahmed et al., 2013). The acid mucopolysaccharides are found mainly in the apical part of the epithelial cells in efferent ductules of camels.

According to Robaire et al. (2006) and Samuelson (2007), the principal cells secrete proteins and enzymes including phosphatases and glycosidases, which are

essential for fertilization. Further, Mandal et al. (2006) in buffalo have shown a protein factor called forward motility stimulating protein (FMSF-1), which is a glycoprotein associated with activation of sperm motility. In their study on camel testis, Shalaby et al. (2012) reported that there was a marked increase in the activity of 5'-nucleotidase (5'-Nase), adenosine

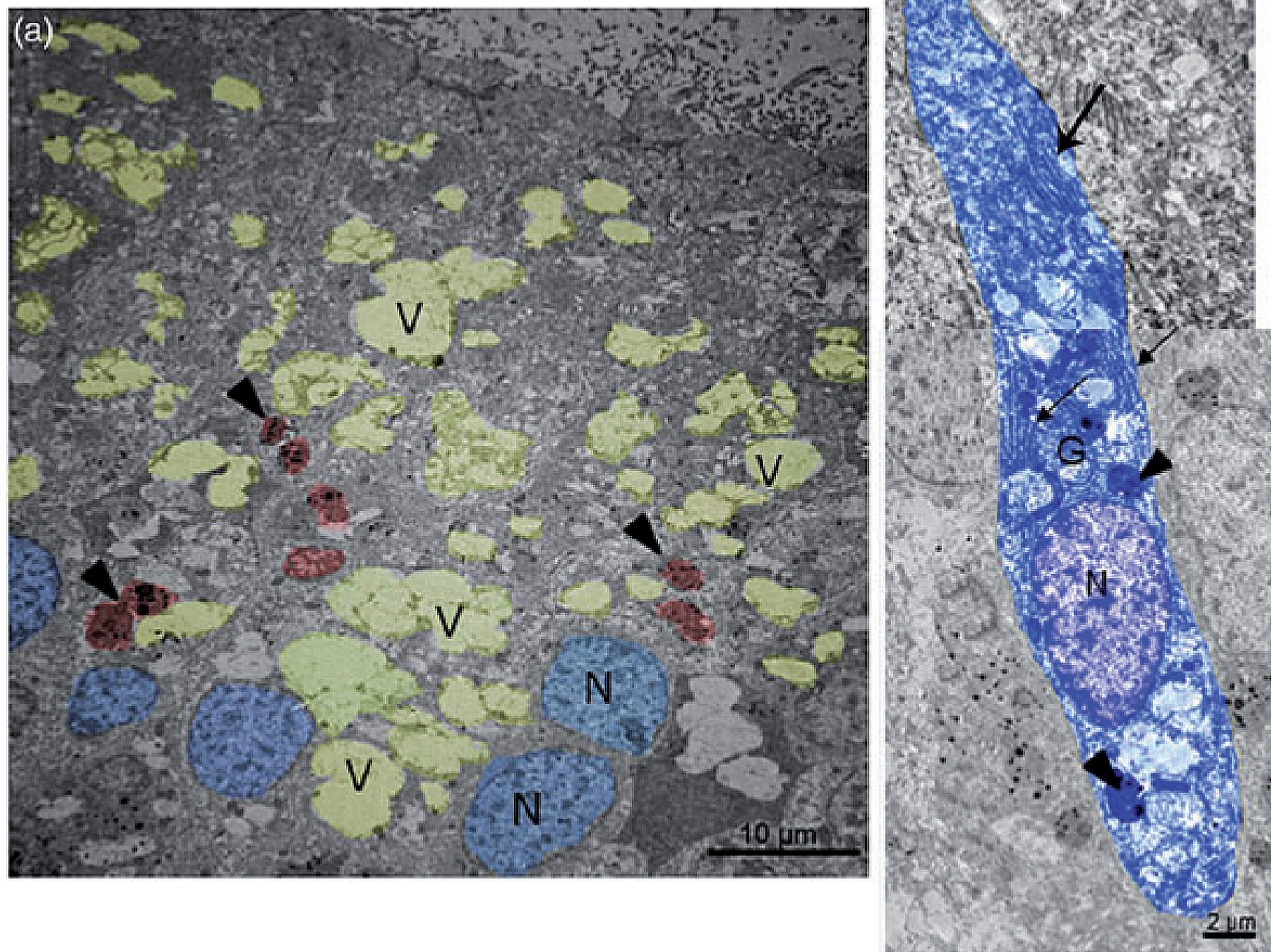


Fig. 7. Fine structure of the principal cells in the nonbreeding season. **a.** The epididymal epithelium from the body region of the camel epididymis shows an extensive predominance of cytoplasmic vacuolation (V) and multivesicular inclusions (arrowheads). Higher magnification of principal cell (**b**): rough endoplasmic reticulum (arrows). G, Golgi complex; M, mitochondria; and N, nucleus. (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

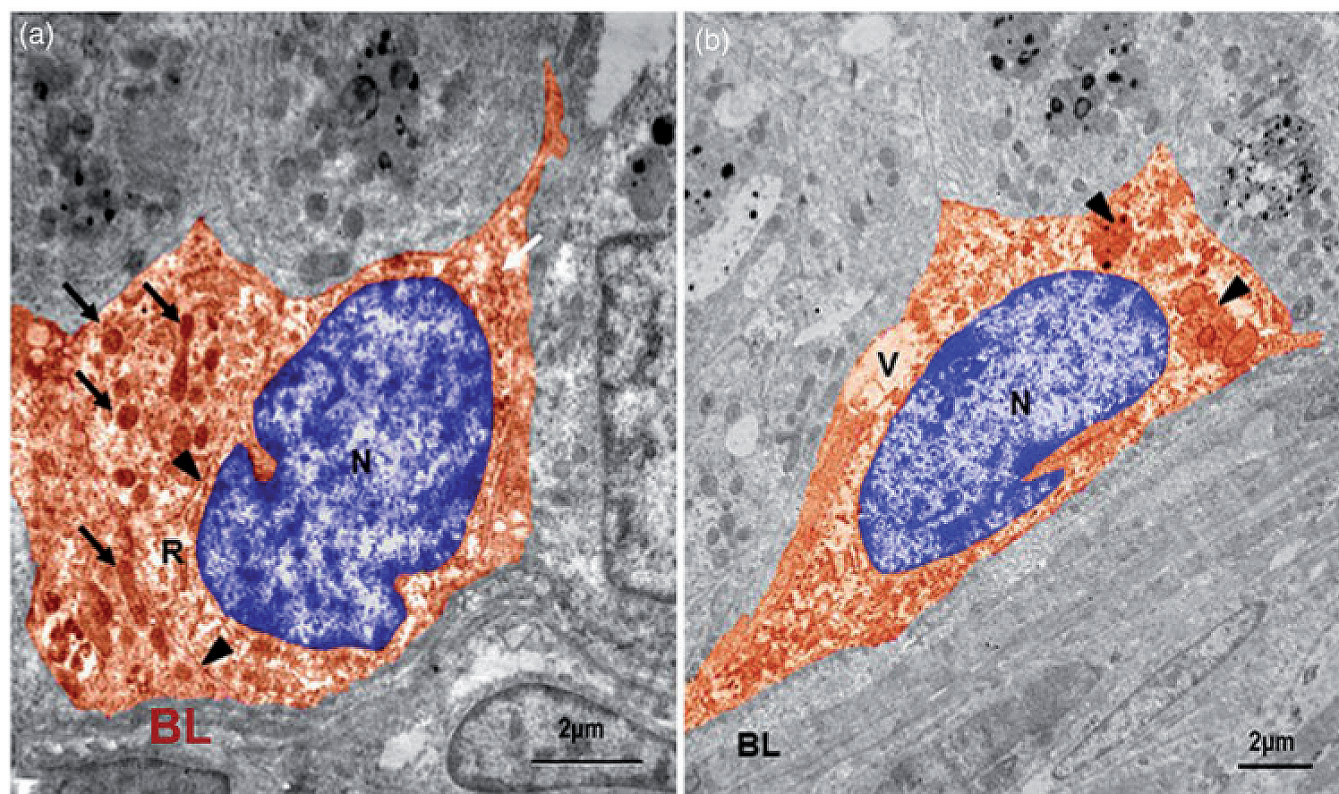


Fig. 8. Ultrastructural features of the basal cells in the body of camel epididymis in the breeding (a) and nonbreeding (b) seasons. Black arrows indicate elongated or round mitochondria, while white arrows indicate centrioles. Arrowheads in a indicate microfilament bundles, while in b indicate multivesicular inclusion bodies. BL, basal lamina; N, nucleus of the basal cell; R, rough endoplasmic reticulum; and V, a cytoplasmic vacuole. (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

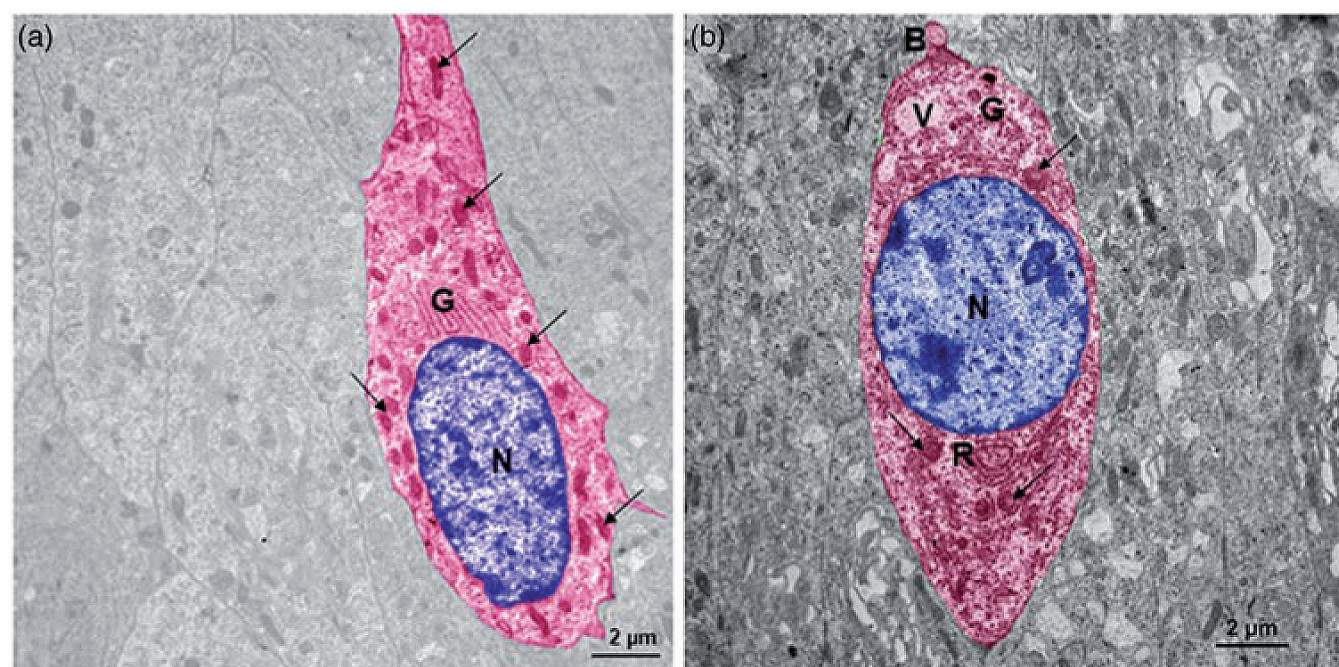


Fig. 9. Ultrastructural features of apical cells of the camel epididymis in the breeding (a) and nonbreeding (b) seasons. Arrows indicate mitochondria. A bleblike structure (B) is seen in the apical cell membrane of the apical cell in the nonbreeding season. G, Golgi complex; N, nucleus of the apical cells; R, rough endoplasmic reticulum; and V, intracytoplasmic vacuoles. (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

triphosphatase (ATPase) and glucose-6-phosphatase (G6-Pase) during the breeding season, while there was a marked decrease in the activities of acid phosphatase (ACPase) and alkaline phosphatase (ALPase). These authors suggested that the qualitative enzyme activity of ACPase, ALPase, 5'-Nase, ATPase and G-6-Pase is closely associated with the stage of puberty and seasonal changes. The activity of acid phosphatase has mainly been detected in the basal cytoplasm of the epididymal cells in the middle segment and in supra-nuclear region in the initial and terminal segments of camel epididymis (Tingari and Moniem, 1979). Supra-nuclear localization of acid phosphatase has also been observed in the proximal part of middle segment in rabbit (Linnetz and Amann, 1968). Alkaline phosphatase has only been observed in the epididymal stereocilia, subepithelial connective tissue and blood vessels. The activity of acid phosphatase is shown to be high in the camel efferent ductules (Ahmed et al., 2013). Further, this activity is suggested to be associated with the mammalian epididymal lysosomes (López et al., 1989; Delhon and von Lawzewitsch, 1994).

The alkaline phosphatase activity has been detected in the apical part of principal cells in rabbit epididymis (Linnetz and Amann, 1968). In accordance with the findings of Tingari and Moniem (1979) in camel epididymis, positive alkaline phosphatase reaction has only been seen in the efferent ductules stereocilia, subepithelial connective tissue and blood vessels (Ahmed et al., 2013). Positive alkaline phosphatase activity has also been observed in the stereocilia of

camel (Al-Shaikly et al., 1981) and horse (López et al., 1989). The activity of alkaline phosphatase is believed to be associated with cellular active transport (López et al., 1989) and with metabolites secretion that participate in sperm maturation (Al-Shaikhly et al., 1981; Beu et al., 2007). Alkaline phosphatase, AMPase and ATPase are reported to be localized in the camel epididymal sub-epithelial tissues, stereocilia, blood vessels and luminal contents (Tingari and Moniem, 1979). According to Tingari and Moniem (1979), the detection of the three enzymes in the stereocilia and subepithelial connective tissue is consistent with a suggested function of active transport between the epithelial cells and the endovascular structures on one hand, and between luminal contents and epithelial cells on the other hand. These authors have also suggested the considerable importance of the glycolytic pathway and the high enzymatic activity of LDH, indicating a high metabolic activity of the epididymal cells.

Immunohistochemical variations

The immunohistochemical characterization of the epididymis has been reported in some mammalian species including human (Marchlewicz, 2001), ox (Alkafafy, 2005), buffalo (Cruzana et al., 2003), donkey (Alkafafy, 2009) and European bison (Czykier et al., 2010). However, similar studies on the dromedary camel epididymis were lacking in the literature until 2011 when Alkafafy et al. (2011) published the first report on the immunohistochemical characteristics of camel

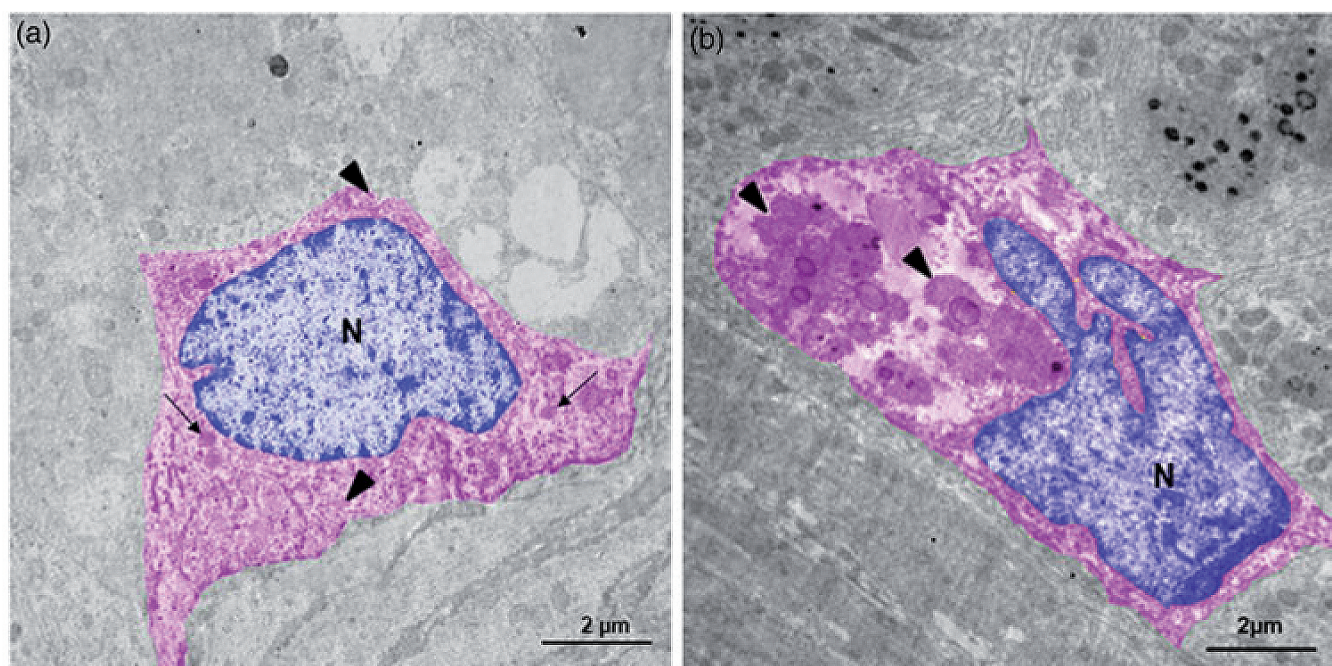


Fig. 10. Ultrastructural features of halo cell of the camel epididymis in the breeding (a) and nonbreeding (b) seasons. Rough endoplasmic reticulum is indicated by arrowheads and lysosomes indicated by arrows in a. In b, arrowheads indicate multivesicular inclusion bodies. N, nuclei of the halo cells. (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

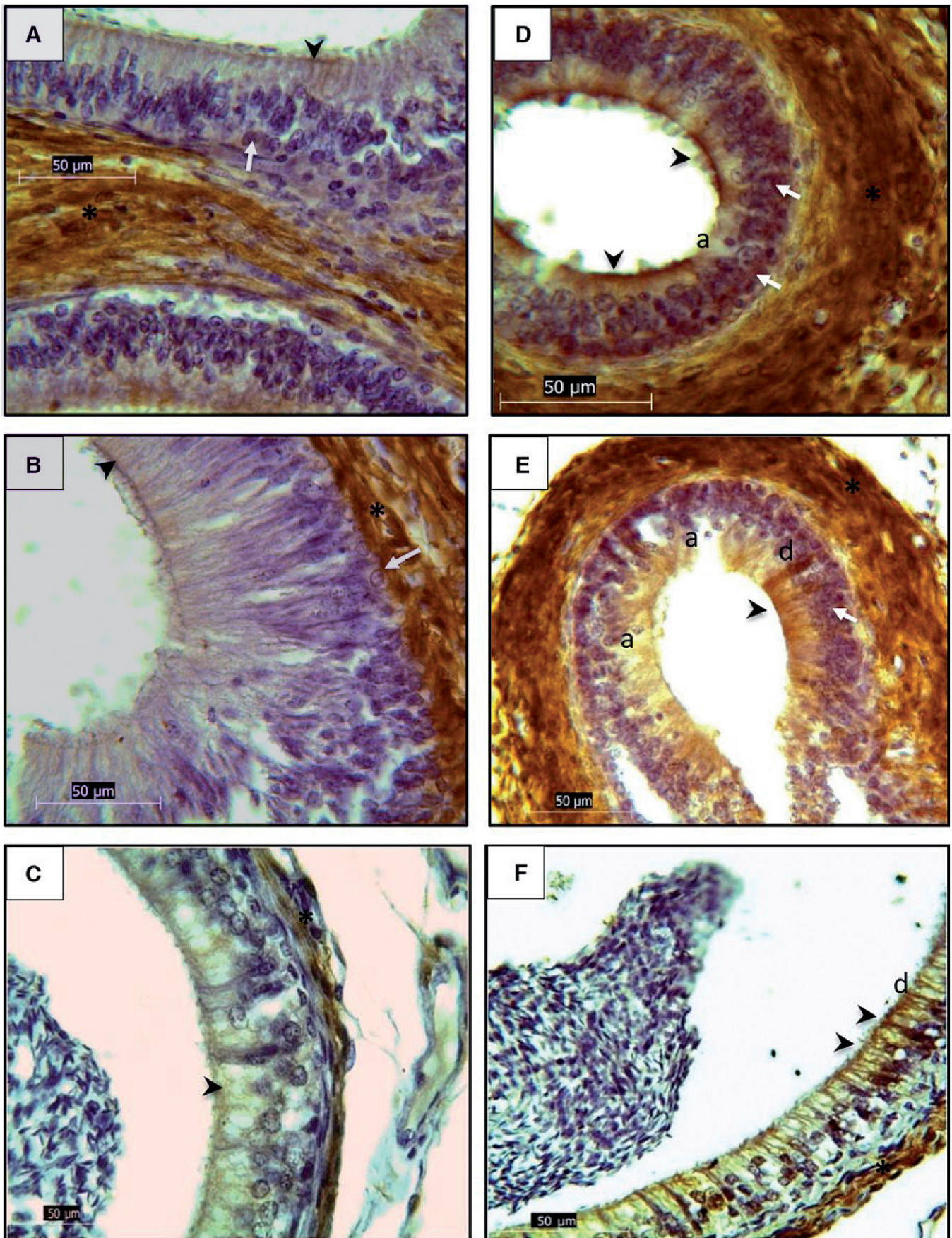


Fig. 11. Photomicrographs showing seasonal S-100 immunoreactions in the camel epididymis. **A** (caput), **B** (corpus) and **C** (cauda) during nonbreeding season showing mostly S-100- negative epithelial cells with some positive reactions in the apical parts of principal cells (arrowheads) and nuclei of basal cells (arrows). **D** (caput), **E** (corpus) and **F** (cauda) during breeding season with relatively strong reaction in many epithelial cells, especially in apical parts of principal cells (arrowheads), dark cells (d) and basal cells (arrows). Apical cells (a) show negative reaction. Positive reaction is seen in the surrounding connective tissue (stars), which becomes strong during breeding season with the gradual decrease from caput to cauda. Stereocilia and spermatozoa exhibit negative or weak reactions in both seasons. (Ibrahim et al., 2017). (Reproduced with permission).

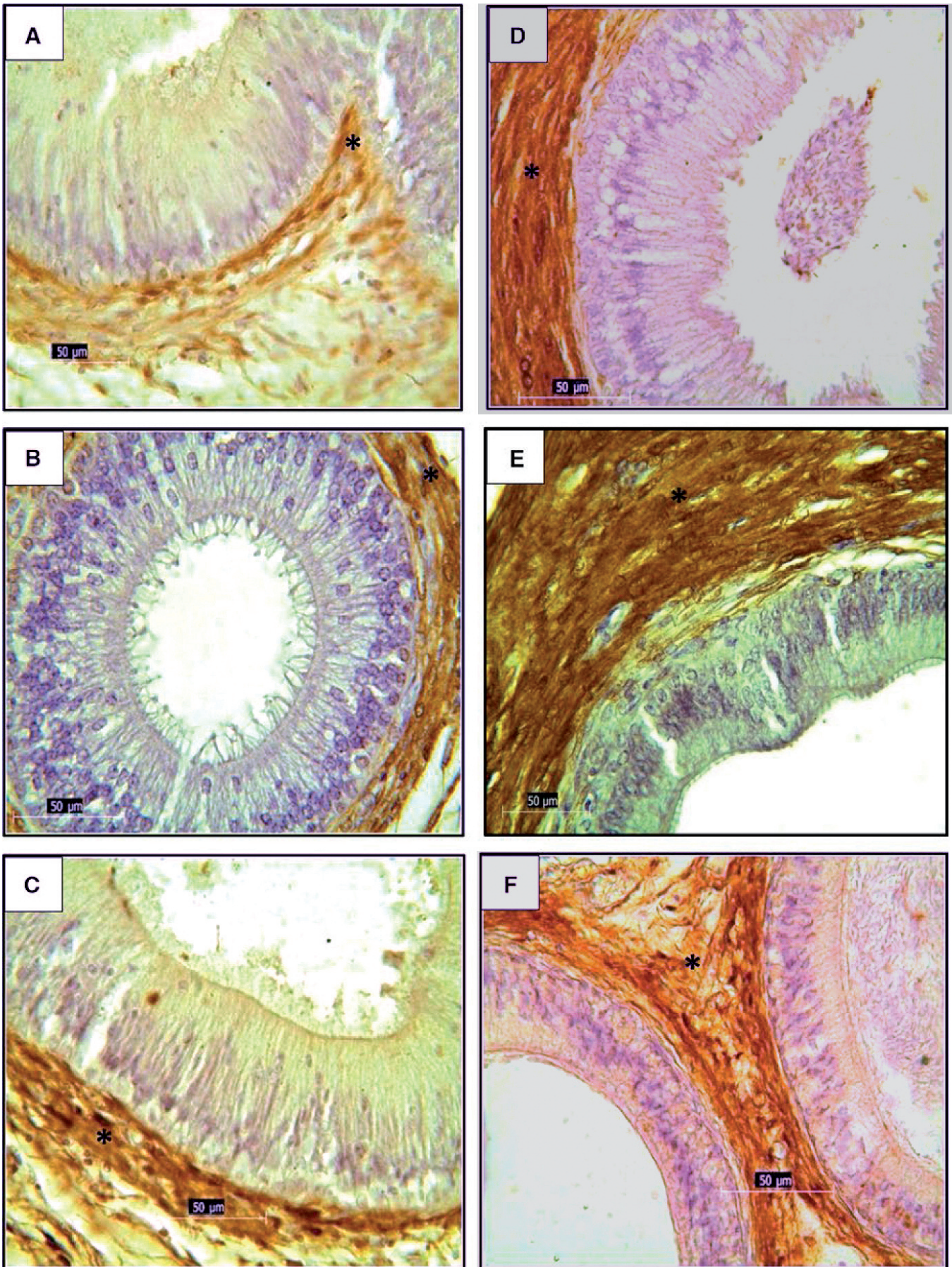


Fig. 12. Photomicrographs showing epithelium of caput (A), corpus (B) and cauda (C) regions of camel epididymis during nonbreeding season, and epithelium of caput (D), corpus (E) and cauda (F) during breeding season with negative immunoreactivities to alpha smooth muscle actin (α -SMA). Less intense α -SMA- immunopositive signals are seen in the muscular coat (stars) of the epididymis during nonbreeding season than that seen during the breeding season. (Ibrahim et al., 2017). (Reproduced with permission).

epididymis. In recent years, a number of authors have made immunohistochemical observations on dromedary camel reproductive organs including epididymis (Ibrahim et al., 2017; Ibrahim and Abdel-Maksoud, 2019), efferent ductules (Ibrahim, 2015) and testis (Alkafafy et al., 2016). However, limited published work is available on immunohistochemistry of the epididymis or any other reproductive organ in relation to reproductive activity. Immunohistochemistry has been used to show the spatial labeling of some important biologically active proteins in different segments of the camel epididymis, which may also reveal the potential structural-functional relationship (Alkafafy et al., 2011). According to these authors, angiotensin converting enzyme (ACE), galactosyltransferase (GalTase), S100, alpha smooth muscle actin (α -SMA), vascular endothelial growth factor (VEGF) and connexin-43 (Cx43) have functional relevance including secretion, absorption and contractility. S-100 and α -SMA

immunopositive signals have been observed in the different regions of dromedary camel epididymis (Alkafafy et al., 2011; Ibrahim et al., 2017). S-100 immunopositivity has been observed in both the epithelial cells and the inter-tubular connective tissue, whereas α -SMA reactivity has only been shown in the inter-tubular connective tissue, especially in the smooth muscle coat and blood vessels. Positive S-100 labelling has also been reported in the epididymis of ox (Alkafafy, 2005), buffalo (Alkafafy et al., 2011) and donkey (Alkafafy, 2009). Ibrahim et al. (2017) showed that the intensity of S-100 and α -SMA immunoreactivity is increased during the breeding season in different regions of dromedary camel epididymis as compared to that during the nonbreeding season (Figs. 11, 12). These authors stated that the camel epididymal morphology and physiology could be affected by these proteins. S-100 is known to be associated with many cellular activities such as diffusion and modulation of physical

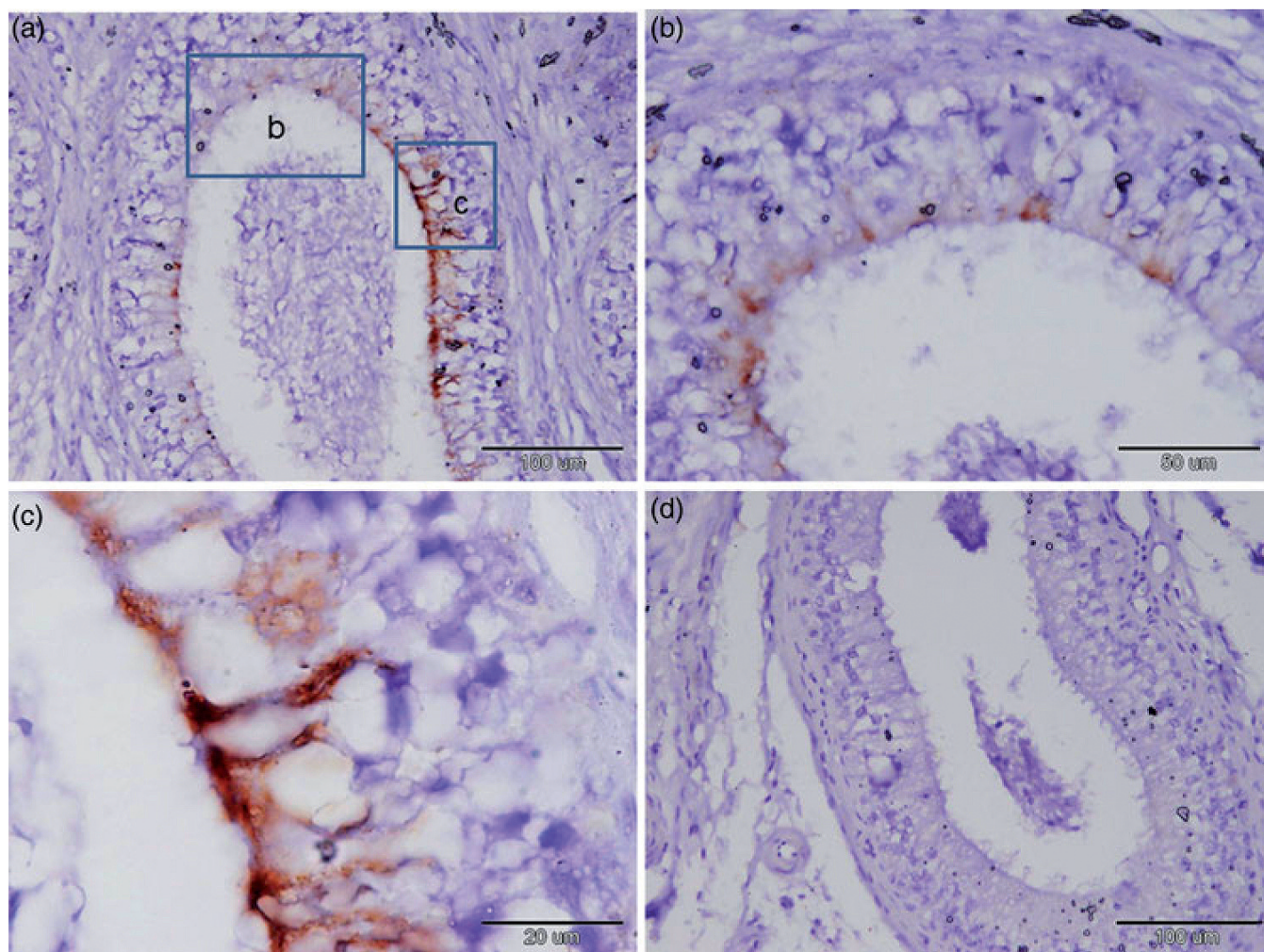


Fig. 13. Paraffin sections of the body of the camel epididymis showing a strong immunoreactivity for CX-43 in the epididymal epithelium during the rutting season (**a-c**), but an absence of immunoreaction during the nonrutting season (**d**). **b** and **c** are magnifications of squared fields in **a**. (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

condition of membranes (Zimmer et al., 1995); it may also play a role in cell contraction and in the process of transcytosis (Czykier et al., 2000) and contribute to the establishment of blood-testis-barrier (Cruzana et al., 2003). Further, S-100 is associated with the absorptive and secretory activities in the intra-testicular excurrent ducts (Cruzana et al., 2003), sperm signaling and regulation of migration of phagocytes via the modulation of tubulin polymerization (Martinez-Heredia et al., 2008). According to Ibrahim et al. (2017), there is an increase in S-100 immunoreaction in the camel epididymal epithelium and smooth muscle coat during the breeding season compared to that during the nonbreeding season. These authors report that during the breeding season the secretory and absorptive activities of camel epididymis are increased. Further, increased S-100 immunoreactivity has been observed in efferent ductules (Ibrahim, 2015) and testis (Alkafafy et al. 2016) during breeding season. Stronger S-100 expression in the vascular smooth muscles of the epididymis has also been reported in adult European bison than in young animals (Czykier et al. 2010). It has been concluded that the expression of S-100 is increased during breeding season in the camel epididymis and that this protein may play a role in sperm maturation in this species (Ibrahim et al., 2017).

α -SMA immunoreaction has been detected in the muscle and connective tissue of the epididymis in some mammalian species including donkey (Alkafafy, 2009) and European bison (Czykier et al., 2010). α -SMA, which has only been detected in cells having contractile

function (Osborn and Weber, 1983), could also be expressed in the peritubular muscles of the epididymis of donkey (Alkafafy, 2009), ox (Alkafafy, 2005) and camel (Alkafafy et al., 2011; Ibrahim et al., 2017). Though epithelial cells are α -SMA negative, positive α -SMA immunoreaction is seen in the peritubular muscle coat and in the connective tissue of camel epididymis; the reaction becomes relatively intense especially in the terminal segment during the breeding season (Ibrahim et al., 2017). Increased α -SMA reaction in the breeding season compared to nonbreeding season suggests increased contraction of epididymal muscles which facilitates the sperm movement through the duct. According to Ibrahim et al. (2017), the epididymal structure might be affected by S-100 and α -SMA proteins and that the increase in the intensity of immunoreaction in these proteins during breeding season could enhance the epididymal function in camels, and hence, the reproductive efficiency.

Recently, Ibrahim and Abdel-Maksoud (2019) evaluated the effect of reproductive activity on functional characteristics of dromedary camel epididymal epithelium using the immunoreactivity of Cx-43, VEGF and AR (Figs. 13-15). They reported that the principal cells are amplified during the breeding season and their endocytotic functions are more active during nonbreeding season, which is evident from the strong immunoreactivity of Cx-43, especially in the apical cytoplasm. On the other hand, principal cells showed granular VEGF immunoreactivity in the apical and basal parts of the cytoplasm; a strong

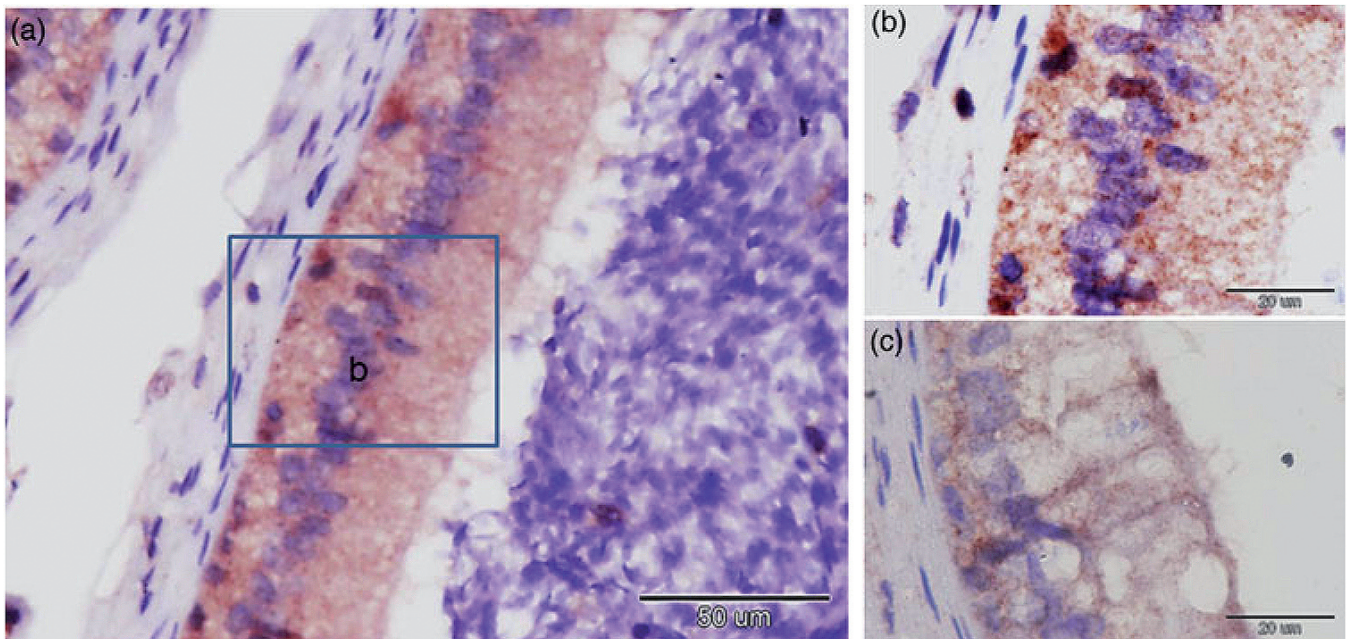


Fig. 14. Paraffin sections of the body of the camel epididymis showing a strong immunoreactivity for vascular endothelial growth factor (VEGF) in the epididymal epithelium during the nonrutting season (a, b), but a weak immunoreaction in the rutting season (c). (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

immunoreactivity for VEGF could also be seen in the cytoplasm of apical and some basal cells. These authors concluded that the reproductive activity significantly affects the immunohistochemical and ultrastructural appearance of the epithelial lining of the dromedary camel epididymis.

Seasonal molecular characterization

Very limited molecular studies, represented by immunohistochemistry (Saleh et al., 2002) and lectin-histochemistry (Abd-Elmaksoud et al., 2008), have been done on reproductive organs of dromedary camel. To the best of our knowledge, Alkafafy et al. (2016) have published the only report on the use of molecular procedures for analyzing the expression of S-100 genes to confirm the spatial distribution of S-100 in different parts of the camel testis during rutting and non-rutting seasons. These authors reported that the expression of S-

100 mRNA is significantly higher in adult animals in breeding season than in other seasons. Further, they also postulated that the seasonal changes in the gene expression in relation to histological structure and sexual activity are suggestive of the role of S-100 in regulation of exocrine and endocrine testicular functions in dromedaries.

Conclusion

The information presented in this review shows considerable structural and histochemical changes in camel epididymis in relation to reproductive activity and these changes are suggested to be androgen-dependent. The camel epididymis may also serve as a potential source of many biologically active proteins in this animal with increased activity during breeding season compared to the nonbreeding season. Further, these proteins may contribute to the improvement in camel

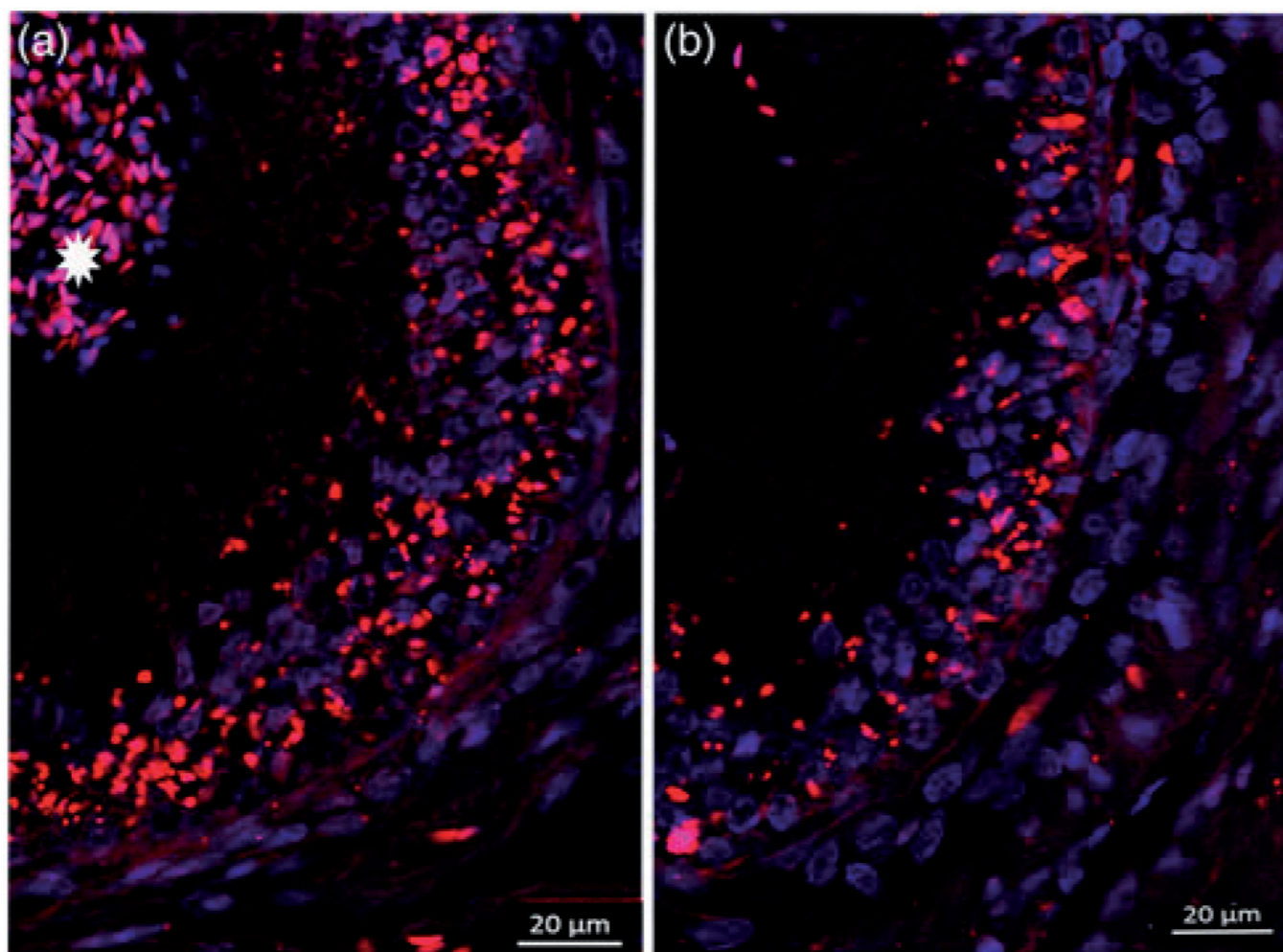


Fig. 15. Immunofluorescence labeling for androgen receptor (AR) (red) with DAPI (blue) counterstain for nuclei of the epididymal epithelium showing a stronger immunofluorescence for AR in the rutting season (a) than that in the nonrutting season (b). Note: sperm in the lumen (asterisk). (Ibrahim and Abdel-Maksoud, 2019). (Reproduced with permission).

fertility and reproductive efficiency. This review may also add to our understanding of the dromedary camel breeding period and help determine the exact mating time, which is important for the success of assisted reproductive outcomes and so the reproductive efficiency. The reviewed literature may also help us to reanalyze what we know now about epididymal physiology in mammalian species living in harsh environmental conditions with limited food and water supply and that this knowledge may prove beneficial towards improving the reproductive efficiency and herd population of camels owned by poor families in many underdeveloped countries in Africa and Asia.

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