Some Immunohistochemical Studies on the Epididymal Duct in the Donkey (*Equus asinus*)

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Abstract

This work aimed to study the regional distribution pattern of some biologically active proteins of the epididymal duct of donkey through application of immune-histochemistry (IHC). Paraffin-embedded sections from different regions of the epididymal duct were used. Primary antibodies against S100, angiotensin converting enzyme (ACE), galactosyl-transferase (GalTase), vascular endothelial growth factor (VEGF), α-smooth muscle actin (α-SMA) and connexin 43 (Cx43) were used. The immunehistochemical findings exhibited a regional-specific, distribution pattern; different from other domestic mammals. Apical cells in the epididymal epithelium expressed a moderate to strong immune-staining with all of S100, ACE and Gal-Tase. Basal cells exhibited mode-rate to strong immunoreaction with both of GalTase and VEGF.

Keywords
Epididymis, Donkey, IHC

Introduction

Despite the economic value of donkeys, little is known about their reproduction biology. It is noteworthy that several investigations were concerned with histological and histochemical characteristics of the epididymis in the stallion (López et al., 1989; Arrighi et al., 1993;

Parillo et al., 1997; Retamal et al., 2000; Ha et al., 2003; Hejmej et al., 2007); however, few data are available about the donkey (Arrighi et al., 1991; 2004). Thus, the present work aimed to shed light on the regional differences of the epididymal duct in this species, and to outline some structural-functional relationships; in comparison to large ruminants. The diverse cellular components of the epididymal epithelium may account for its wide range of functional capacities. The epididymal epithelium consists of two main types of cells: principal (PCs) and basal (BCs); and two accessory cell types: apical cells (ACs) and intraepithelial leukocytes (IELs). ACs are the less frequent cell type in the different mammalian species (Sun and Flickinger, 1980; Goyal, 1985; Goyal and Williams, 1991; Palacios et al., 1991; Schön and Blottner, 2009). However, the term "apical cell" is confusing since it has been used to describe different cell types in the mammalian epididymal duct (Abou-Haila and Fain-Maurel, 1984; Martínez-Garcia et al., 1995). This confusion has hindered a better understanding of the morphological and functional characteristics of ACs (Martínez-Garcia et al., 1995). However, application of IHC might reveal some unexplained aspects of this type of cells and clarify their nature.

The unique functional competen-cy of the epididymis make it able to create regional-specific, complicated chrono-

logical changes in the composition of luminal fluid throughout its length. This helps the transformation of the imamture testicular sperms into mature sperms. The maturation process involves morphological and biochemical changes in the sperm plasma membrane in response to epididymal secretions and their proteins. Some of these proteins are responsible for induction of progressive motility and for acquisition of fertilizing capacity. (Retamal et al., 2000; Dacheux et al., 2003; Gatti et al., 2004; Axnér, 2006; Cornwall et al., 2007; Sostaric et al., 2008).

The proteins under study were chosen according to their relevance to certain functions; mainly secretion and absorption. This histochemical study of the epididymal duct of the donkey aim**s** to explain the interaction between the epithelial and the periductal components and their participation in providing an appropriate environment necessary for sperm maturation.

Materials and methods

Animals and tissues
Tissue specimens were obtained from 5 clinically healthy, adult donkeys (*Equus asinus*). Epididymidis were collected directly after castration**.** The epididymal duct was divided into three main parts: caput, corpus and cauda epididymidis. Specimens were taken from each part of the epididymal duct.

Chemicals and methods
Specimens were fixed in Bouin's fluid and a mixture of methanol-/glacial acetic acid (2:1). Bouin-fixed specimens were used for routine histological staining. For IHC, specimens were fixed in Bouin's fluid (ACE, S-100, α-SMA and Cx43) and in methanol/glacial acetic acid mixture (GalTase and VEGF). Tis-

sue specimens were dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraplast and sectioned at 5μm thickness. Tissue sections were mounted on positively charged, coated slides.

Routine histological staining:
Several conventional stains were carried out to investigate the general histological structure. These included Hematoxylin and Eosin, Masson and Goldner's trichrome stains, Alcian blue 8GX, Periodic acid-Schiff (PAS) reaction after McManus and Toluidine blue. All staining techniques were performed according to protocols in Romeis (1989).

Immunohistochemistry:
Immunohistochemical studies were performed using the Avidin biotin complex method (ABC) according to Hsu et al., (1981). Dewaxed and rehydrated sections were subjected to inactivation of endogenous peroxidases by incubation in 1% H_2O_2 for 15 minutes. Then after the sections were placed in 0.01 mol/L citrate buffer (pH 6) and heated in microwave (700 watt) for 10 minutes for antigen retrieval. The sections were blocked by PBS containing 5% bovine serum albumin (BSA) for an hour, and then each section was incubated with its corresponding primary antibody (types, sources and dilutions of antibodies, and the duration of incubation are shown in Table 1). The sections were washed by PBS for 5 minutes 3 times and incubated with biotinylated secondry antibodies (types, sources and dilutions are shown in Table 1), for 30 minutes at room temperature. The sections were washed by PBS for 10 minutes. Then the secondary antibody was detected with Vectastain ABC kit (Vector Laboratories Inc.) firstly each section is covered with 100 x dilution of A & B reagent in PBS (1 μl reagent A+ 1 μl reagent B + 98 μl PBS), then washed

by PBS for 10 minutes 3 times and the color was developed using DAB reagent (Sigma-Aldrich, St. Louis, MO, USA). Sections were counterstained with haematoxyline for 30 seconds, washed in water, dehydrated through graded ethanol, cleared in xylene and mounted with DPX permanent mounting media (Sigma-Aldrich, St. Louis, MO, USA).and photographed by light microscopy .

Positive and negative controls

Immunohistochemical negative controls, where each primary or secondary antiserum or the ABC reagent was omitted, gave no positive staining. Positive controls were used according to the instructions provided by the manufacturers of the primary antibodies. For assessment of the immunolabelling a semi-quantitative subjective scoring was performed by three indepen-dent observers.

Results

Light microscopic observations:

The epididymal duct of donkey was lined by a pseudostratified columnar epithelium with stereo-cilia on the apical surface (Fig. 1, 2, 3 and 4). The epididymal epithelium consisted of PCs, BCs, ACs and IEL (Fig. 1, 2 and 3). ACs looked like cones with their dome-shaped bases facing outward and pear-shaped nuclei found in the apical portion of the cells. Their cytoplasm was lighter than that of the surrounding PCs and their luminal surfaces were not provided with stereocilia but with shorter microvilli (Fig. 2). The epithelium in the cauda showed many epithelial folds, some of which was crossly cut (Fig. 4). The peritubular structures included thin highly vascularized lamina propria of loose connective tissue followed by muscular coat of circularly oriented

SMCs. This coat was the thickest in the cauda. The interstitial connective tissue that binds the coils of the duct together housed several types of cells especially mono-nuclear leukocytes.

Immunohistochemical observations:

The most important immunehistochemical findings were summarized in table 2.

S100: immunolocalization of S100 in the caput epididymidis displayed a negative reaction (in the cytoplasm) and a strong reaction (in the nuclei) of PCs. The epididymal epithelium in the corpus showed moderately positive apical cytoplasm and strongly positive nuclei. In the cauda the epithelium possessed a strongly positive PCs alternating with negative ones. The positively reacting cells, especially of the epithelial folds (Fig. 6), displayed an intense staining pattern in the apical cytoplasm and in the nuclei. Whereas ACs presented weak to strong reaction (Fig. 5), BCs reacted negatively throughout the whole length of the epididymal duct. On the other hand nerves, nerve fibres (Fig. 5 and 6), interstitial cells and pericytes of some capillaries expressed a strong reaction along the entire length of the epididymis.

ACE: stereocilia and luminal surfaces of PCs showed moderate (in the caput) to strong (in the cauda) reactivity (Fig. 7 and 9). However, in the caput and the corpus regions, the reaction varied from tubule to another, ranged from negative to moderate reaction (Fig. 7) ACs in the epididymal caput region displayed a strong immune-reaction for ACE, giving the patchy appearance of the apical epithelial surface. ACs of the corpus exhibited variable signal ranged from absolutely negative to moderate reaction. The Golgi zone of PCs, only in the corpus (Fig. 8) expressed a strong re-

action. Whereas BCs displayed a negative reaction, the vascular endothelium expressed strong to very strong reaction (Fig. 8) along the length of the epididymal duct.

GalTase: immunolocalization of Gal-Tase in the caput region was represented by a well-distinct reaction in the apical surfaces of some PCs, giving a patchy staining pattern. ACs expressed staining pattern varied from negative to moderate reaction (Fig. 10). The reaction in the corpus was restricted to moderately stained supranuclear cytoplasm in PCs and variable staining pattern in ACs. BCs showed positively reacting juxtanuclear cytoplasm both in the caput and the corpus regions (Fig. 11). .In the cauda the epithelium was entirely positive; showing moderate (apical portion) to strong (basal portion) intensity of the reaction .The peritubular structures reacted negatively, however, the vascular endothelium and the peritubular muscular coat showed a moderate and weak reactivity reaction respectively.

VEGF: the BCs in the caput region expressed moderate VEGF-binding sites. These always enclose intra-epithelial vacuolar macrophages (Fig. 13).In the corpus the reaction was similar but much weaker than that in the caput. The epithelium in the cauda displayed a moderate to strong reactivity, which was mainly expressed by the basal portions of the epithelium. With the exception of some interstitial mononuclear leukocytes, displaying a strong reaction (Fig. 12), all the peritubular structures showed no binding site. These cells are speculated (on the basis of morphological characteristics) to be plasma cells; however, further work required to elucidate their nature. The vascular endothelium in the interstitium of caput and corpus showed negative to weak reac-

tion, however; that of the cauda expressed a weak to moderate reactivity.

Alpha-SMA: immunoreactivity for α-SMA was strongly evident both in the peritubular and vascular SMCs along the whole length of the duct. The reaction was further more intense in the vascular SMCs. (Fig. 14 and 15).

Cx43: binding sites for Cx43 could not be seen either in epididymal epithelium or in peritubular structures. However, isolated intraepithelial and interstitial cells (Fig. 16) displayed intense diffuse cytoplasmic reaction.

Discussion

The results of the current investigation revealed that the epididymal epithelium in the donkey, similar to the other species, consists of the well-known PCs, BCs, ACs and intraepithelial leukocytes (Goyal, 1985; Goyal and Williams, 1991; Palacios et al., 1991; Alkafafy, 2005 and; Schön and Blottner, 2009). Although a general name is given for the most numerous cell populations in the epididymal epithelium, PCs may perform either secretory or absorptive activities or both (Moore and Bedford, 1979). BCs comprise the second consistent cell populations of the epididymal epithelium. The function of BCs is to great extent unknown. (Amann, 1989) Though they are assumed to be reserve cells (Bidwai and Bawa, 1981) for epididymal epithelium renewal, this assumption was disproved (Arrighi et al., 1993). Despite the several studies on ACs, the ambiguity of their description has delayed a better understanding of its morphofunctional characterristics (Martínez-Garcia et al., 1995). However, the present study showed that ACs expressed obvious immune-staining to S100, ACE and GalTase.

This will be discussed later, explaining the plausible function of this type of cells.

The peritubular connective tissue showed numerous mononuclear cells. It is noteworthy that a variety of angiogenic and chemotactic factors regulate the vascular permeability (Lissbrant et al., 2003) and help in the migration of mono-nuclear leukocytes from blood into the interstitial and consequently into the epididymal epithelium (Clauss et al., 1990). Existence of interstitial and intraepithelial leukocytes may participate in the induction of immune tolerance preventing any immune reaction against sperms (Marchlewicz, 2001).

The variable regional S100-reactivity of PCs goes in line with previous studies on the human (Haimoto et al., 1987) ox (Alkafafy, 2005) and buffalo bull (Alkafafy et al, 2009) epididymis. Unlike the case in the ox, ACs exhibited obvious immune-reaction to S100 both in the caput and in the corpus epididymidis. Similar findings were recorded in the buffalo bull. As a multifunctional subfamily of $Ca2^+$-binding proteins, S100 has wide variety of diverse functions. These range from calcium-buffering through intracellular activities like energy metabolism and motility; to extracellular functions like secretion, chemotaxis, and neurite extension (Heizmann et. al., 2002). It is noteworthy that nerve fibers in the epididymal interstitium expressed a strong reactivity with S100. This goes in line with Slater et al., (2000) who reported that S100 can be used as an axonal marker. Despite these suggested functions, the exact biological role of S100 in the epididymis is not yet known. However, Amselgruber et al., (1994) and Cruzana et al., (2003) assumed that S100 proteins are involved in the absorptive and secretory functions in the intratesticular

excurrent duct system. Similarly, it may promote comparable tasks in the extra-testicular excurrent duct system. Additionally the vascular endothelium expressed a moderate to strong immune-reactivity for S100 proteins. Similar findings were reported in bison (Czykier et al., 1999); bovine (Amselgruber et al., 1994, Alkafafy, 2005) and murine (Czykier et al., 2000) epididymis. It was, therefore, assumed that it may participate in the processes of transcytosis.

The current findings showed that ACE-binding sites in the caput and the corpus regions were evidently localized in some tubules and absolutely absent in the other some. This variation may reflect subregional functional difference. Similar results were recorded in the ox (Alkafafy, 2005).The stereocilia and luminal surfaces of the PCs showed remarkable reactivity for ACE especially in the caput and cauda regions. Similar results were reported in the human (Vivet et al., 1987) and in the ox (Alkafafy, 2005), but not in buffalo bull (Alkafafy et al., 2009). It is evident that ACE converts angiotensin I, locally produced by epididymal epithelium, into angiotensin II. The latter is concerned with regulation of electrolyte and fluid transport in the epididymis (O'Mahony et al., 2000).

Moreover, ACs exhibited a variable reaction throughout the caput and the corpus regions. This reaction was the strongest in the caput and variable (negative to moderate) in the corpus regions. These findings go in line with the results reported in buffalo bull, but contradict the case in ox that exhibited no reactivity to ACE in ACs. In the cauda ACs, negative or positive, could not be found; however the stereocilia and luminal surfaces of the PCs exhibited strong ACE-binding. Taken together it seems that PCs (represented

by stereocilia) in the cauda may compensate the absence of ACs, in expressing the activity of ACE and in turn in performing its functions, at least in the donkey. Moreover, prevalence of the epithelial folds in the cauda might suggest an active absorptive competency of this segment of the epididymis. Immunolocalization of ACE in Golgi zone of PCs in the corpus epididymidis might reflect synthesis and secretion (Berg et al., 1986) of ACE in the PCs in this region. It is noteworthy that remodeling of sperm surface molecules may be a consequence of interactions between spermatozoa and the epididymal secretions, and may reflect adsorption or addition of new molecules to the sperm membrane (Retamal et al., 2000). Accordingly, ACE activity could be determined in sperm membrane from ejaculated and epididymal spermatozoa in horses (Ball et al., 2003). Furthermore the vascular endothelium, mainly of subepithelial blood vessels, expressed a strong reactivity to ACE. Similar findings were reported in the epididymis of human (Vivet et al., 1987), ox (Alkafafy, 2005) and buffalo bull (Alkafafy et al., 2009). It is noteworthy that the endothelial ACE may participate in regulating the vascular tone and in turn control the blood flow (Franke et al., 2003) through the epididymal tissues.

PCs of the epididymal epithelium in the caput and corpus regions of ruminants (Alkafafy, 2005; Alkafafy et al., 2009), expressed a strong to moderate intensity of GalTase-immunostaining both in the supranuclear Golgi zone, apical cytoplasm and stereocilia. Unlike the case in ruminants, PCs in donkey's epididymis displayed a different distribution pattern represented by a well-distinct reaction in the apical surfaces of only some PCs, but not in the Golgi zone or stereocilia. In the current work and dis-

similar to ruminants, the epithelium lining the cauda was entirely positive exhibiting a moderate to strong GalTase-immunoreaction. Similarly, both of ACs and BCs expressed moderate to strong reaction to GalTase. These findings disagree with those found in ruminants and confirm distinct species differences. On contrary to ox and buffalo bull (Alkafafy, 2005; Alkafafy et al., 2009), it seems that the PCs of the caput epididymidis are not the main secretory cells; and that the PCs of the cauda might be suggested to co-play such role. This notion may agree with Pavlović et al., (1981), who reported, in their immunohistochemical studies, that the epithelium of the bovine cauda epididymidis possesses greater secretory activity than that of the caput. The present findings may propose secretory activities for both ACs and BCs. It is noteworthy, that ACs populations are more numerous in donkey than those in ruminants. Moreover, ACs displayed a strong GalTase-immunoreaction in donkey, but not in ruminants. Taken together, abundance of ACs (both numerically and distinct GalTase-immunoreactivity) may compensate the deficient GalTase-reactivity of the Golgi zone in PCs, per-forming an alternative secretory pathway at least in the donkey. It is noteworthy that GalTase belongs to a functional family of enzymes that are responsible for the biosynthesis of carbohydrate moieties of GPs (Hennet, 2002). Alterations of sperm membranes may result from the incorporation of GPs which are of epididymal origin (Retamal et al., 2000; Cornwall et al., 2007; Sostaric et al., 2008).

The exact functional relevance of ACs is not yet known (Martinez-Garcia et al., 1995); however they may be involved in reabsorption and acidification (Jensen et al., 1999) of the epididymal fluid. However, the remarkable reactivity to

both S100 and ACE expressed by ACs in the present work showed deviation from the findings reported by Adamali and Hermo (1996) and Alkafafy (2005) in the epididymis of the rat and the ox respectively. Taken together, the expression of both of ACE, S-100 and GalTase in the ACs in the caput and corpus epididymidis, in the current work might point to the significance of this type of cells both in the absorptive and the secretory activities in the epididymal duct of the donkey.

VEGF-reactivity could be seen in the basal portion of the epididymal epithelium and in some interstitial mononuclear leukocytes. In agreement with this result, VEGF was expressed in several cell types in the human male genital system including BCs and certain peritubular cells of the epididymis (Ergun et al., 1998), in the rat testis and epididymis (Ai et al., 2008) and in mast cells in the bovine epididymal interstitium (Alkafafy, 2005). On the other hand, no VEGF-binding sites could be found, neither in the epithelium nor in the interstitium, in the epididymal duct of the buffalo bull (Alkafafy et al, 2009). Dissimilar to human, vascular endothelium exhibited a weak to moderate reactivity, especially in the cauda. This variation may be attributed to species differences. It is known that VEGF is angiogenic protein implicated in both physiological and pathological conditions (Ekerbicer et al., 2008). This may be attributed to its ability to increase the microvascular permeability (Ergun et al., 1998). The functional significance of VEGF-positively reacting cells may elucidate the role of this factor as a paracrine effector in influencing the permeability of lymphatic and blood vessels via its diverse receptors. It is distinguished that, the in vitro VEGF-treatment of epididymal tissue induced endothelial fenestrations and opening of

interendothelial junctions. Additionally, it was concluded that these gaps might be of importance not only for molecular transport but also for migration of mononuclear cells from blood into interstitium and consequently into the epididymal epithelium. Occurrence of leukocytes both in the interstitium and within the epididymal epithelium may participate in the induction of immune tolerance in the male excurrent duct system (Marchlewicz, 2001), preventing the initiation of immune reaction against sperms.

The cytoplasm of the periductal SMCs showed a strong positive reactivity to α-SMA. This goes in line with the findings reported in the epididymal duct of the domestic fowl (Maretta and Marettová, 2004), the ox (Alkafafy, 2005) and the buffalo bull (Alkafafy et al, 2009). Also, the SMCs in the walls of epididymal blood vessels expressed a distinct α-SMA-reactivity. Similar findings were reported by Marettová and Maretta (2002) in the sheep ovarian blood vessels. It is noticeable that α-SMA is most significant in studying differentiation of SMCs in normal and pathological conditions (Skalli et al., 1989).

Binding sites for Cx43 could not be found either within the epididymal epithelium or in the interstitium. Similar results were reported in the epididymis of the ox (Alkafafy, 2005). Despite the similarity to the case in the ox, some deviation was reported in the donkey. This was represented by the positively reacting scattered intraepithelial and interstitial mononuclear cells. These cells are speculated to be macrophages; however, their nature might be elucidated by further specific immune-histochemical studies. Moreover, the current findings disagree with those reported in the rat (Dufresne et al., 2003), the stallion (Hejmej et al., 2007),

and the buffalo bull (Alkafafy et al, 2009), which expressed a positive reaction in the epithelium. This may be another aspect of species variations in the function of the epididymis. The difference in the distribution pattern of Cx43 in stallion and donkey agrees with a pervious comparative study (Arrighi et al., 2004) showed that the different immunophenotypes exist even in species with a very close zoological relationship. Existence of gap junctions (represented by Cx-binding sites) in the epididymis might reflect the co-ordination (Segretain and Falk, 2004), between various epididymal components, necessary for regulation of different epididymal functions. For instance, the storage and passage of sperm through the epididymal duct is affected by the active contractions of the epididymis. Thus the PMC, in a coordination (via intercellular channels represented by Cx-binding sites) with the epithelium; may control periods spent by sperms within the different epididymal regions, required for their modifications and ultimately for their ripening.

In conclusion, unlike the case in male ruminants, ACs in the epididymal epithelium expressed a moderate to strong immunestaining with all of S100, ACE and GalTase. Thus, ACs are assumed to perform both secretory and absorptive active-ties. BCs exhibited moderate to strong immunoreaction with both of GalTase and VEGF. This may signify the role of this type of cells in chemotaxis and induction of immune tolerance in the epididymis. Immuno-localization of α-SMA and Cx43 in the epididymal duct might explain the interaction between the epithelial and the periductal components for regulation of diverse epididymal functions and providing an appropriate microenvironment necessary for sperm maturation.

Acknowledgements

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are upregulated in the aging rat prostate. Acta Histochem. 102(4): 427-438.

Sostaric, E.; Aalberts, M.; Gadella, BM. and Stout, TA. (2008): The roles of the epididymis and prostasomes in the attainment of fertilizing capacity by stallion sperm. Anim. Reprod Sci., 107(3-4):237-248.

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Table 1: Identity, sources, and working dilutions of primary and secondary antibodies.

Primary antibodies					Secondary antibodies		
Against	Origin	Source	Dilution	Incubation time	Type	Source	Dilution
S100	Rabbit	Dako, Hamburg	1:400	½ hr at room temperature	Biotinylated pig anti-rabbit IgG	Dako, Hamburg	1:300
ACE	Chicken	Institute of Vet. Anat. II, LMU Munich	1:500	Overnight at 4°C	Biotinylated rabbit antichicken IgG	Rockland, USA	1:400
GalTase	Chicken	Institute of Vet. Anat. II, LMU Munich	1:500	Overnight at 4°C	Biotinylated rabbit antichicken IgG	Rockland, USA	1:400
VEGF	Rabbit	Dako, Hamburg	1:800	Overnight at 4°C	Biotinylated pig anti-rabbit IgG	Dako, Hamburg	1:300
αSMA	Mouse	Dako, Hamburg	1:200	1 hr at room temperature	Biotinylated rabbit antimouse IgG	Dako, Hamburg	1:300
Cx 43	Mouse	BD Bioscience, Heidelberg	1:200	Overnight at 4°C	Biotinylated rabbit antimouse IgG	Dako, Hamburg	1:300

Table 2: Immunolocalization of different proteins in the epididymal duct of
         donkey

Protein	Region	Epithelium			Interstitium			
		AC	BC	PC	PMC	BV	N	I C
S100	Caput	+/+++	-	++/+++	-	+/++	+++	+++
	Corpus	+/+++	-	+/++	-	+/++	+++	+++
	Cauda	NF	-	++/+++	+/++	+/++	+++	+++
ACE	Caput	+++	-	-/++	-	+++	-	-
	Corpus	-/++	-	-/++^{GZ}	-	+++	-	-
	Cauda	NF	-	++/+++	-	+++	-	-
GalTase	Caput	-/+++	+	-/+++	+	+/++	-	-
	Corpus	-/++	+	-/++	+	+/++	-	-
	Cauda	NF	+	+++	+	++	-	-
VEGF	Caput	-	++	-	-	-/+	-	+++
	Corpus	-	+	-	-	-/+	-	+++
	Cauda	NF	+++	+/++	-	+/++	-	+++
α-SMA	Caput	-	-	-	+++	++/+++	-	-
	Corpus	-	-	-	+++	++/+++	-	-
	Cauda	NF	-	-	+++	++/+++	-	-
Cx43	Caput	-	-	-	-	-	-	+++
	Corpus	-	-	-	-	-	-	+++
	Cauda	NF	-	-	-	-	-	+++

Apical (AC), basal (BC), principal (PC) and interstitial (IC) cells; peritubular muscle coat (PMC);
blood vessel (BV) and nerve (N); negative (-), weak (+), moderate (++), strong (+++),
negative to weak (-/+), negative to moderate (-/++), negative to strong (-/+++), weak to moderate (+/++),
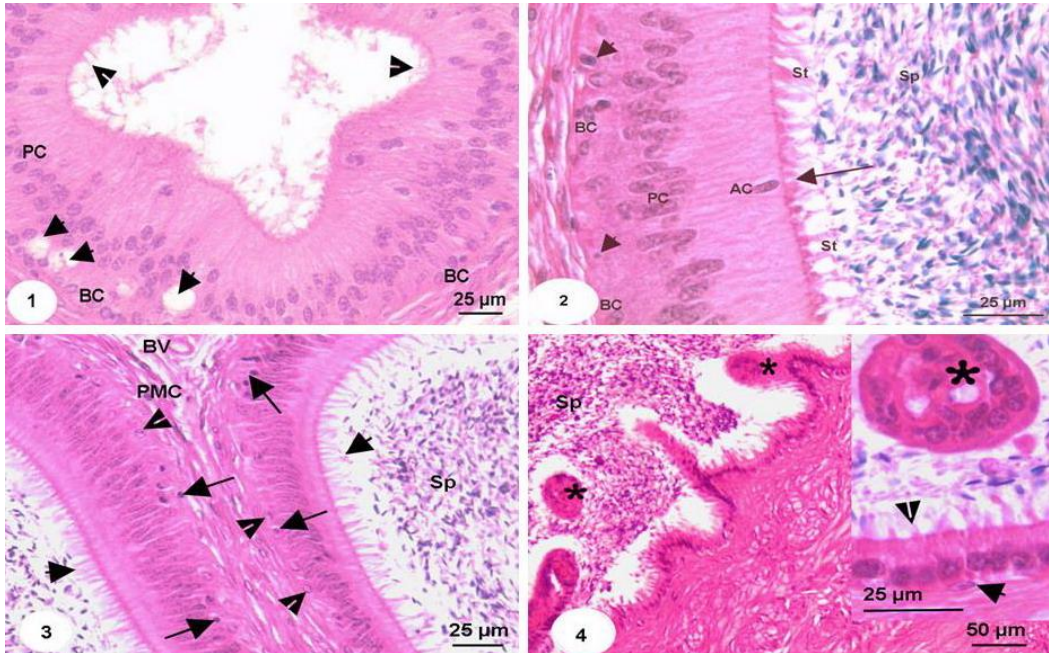moderate to strong (++/+++), not found (NF), Golgi zone (GZ).

Fig (1): H&E-stained caput epididymidis displaying principal cell (PC), basal cell (BC) and foamy intraepithelial macrophages (arrowheads).

Fig (2): H&E-stained caput epididymidis showing apical cell (AC), basal cell (BC), principal cell (PC), stereocilia (st), sperm (Sp), intraepithelial lymphocytes (arrowheads).

Fig (3): H&E-stained corpus epididymidis displaying BCs (notched arrowheads), intraepithelial lymphocytes (arrows), stereocilia (st) and peritubular muscle coat (PMC).

Fig (4): H&E-stained cauda epididymidis showing epithelial folds (asterisks), sperm (Sp). The inset displays a crossly cut epithelial fold (asterisk), flat basal cell (arrowhead) and stereocilia (notched arrowhead).
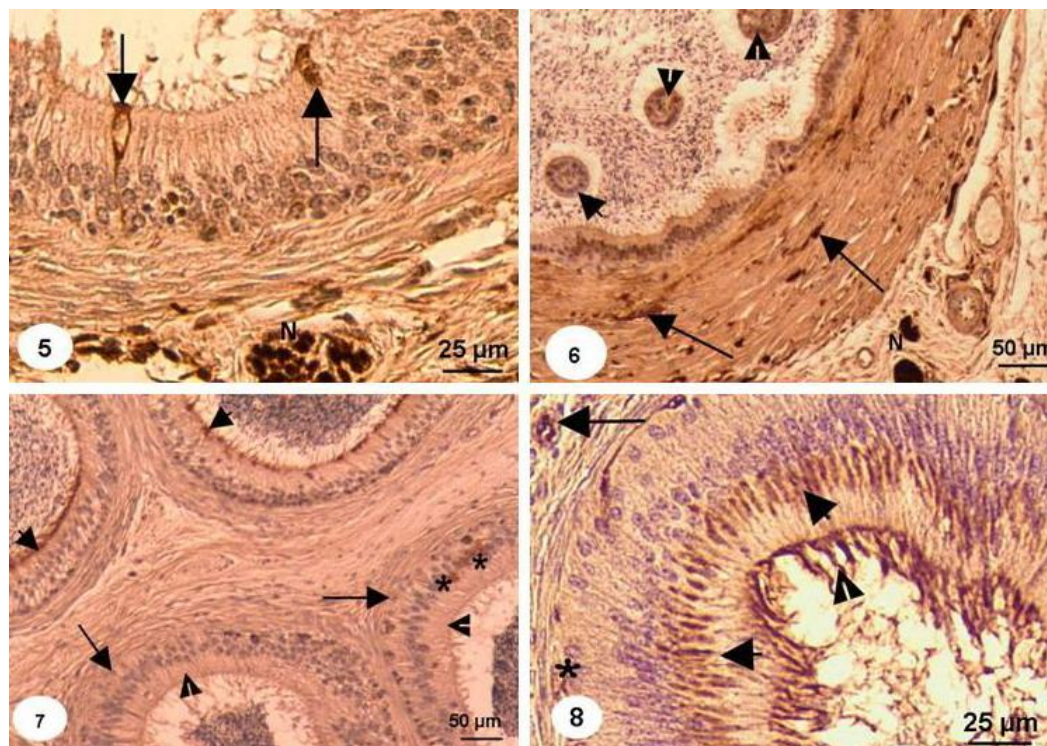
Fig (5): Immunolocalization of S100 in the caput epididymidis. The reaction was evident in apical cells (arrows) and in a nerve (N).

Fig (6): Distribution of S100-binding sites in cauda epididymidis showing some crossly cut epithelial folds with positive nuclei (notched arrowheads) and the other some were negative (arrowhead); a strong reaction in a nerve (N) and in nerve fibres (arrows).

Fig (7): ACE-binding sites in caput epididymidis displaying a strong reaction in apical cytoplasm and stereocilia (arrowheads) of PCs of some tubules and negative reaction (notched arrowheads) in the other some. Some PCs possessed strongly positive supranuclear areas (asterisks). The BCs (arrows) were negative.

Fig (8): Distribution of ACE-binding sites in corpus epididymidis showing PCs with strongly positive supranuclear Golgi areas (arrowheads) and stereocilia (notched arrowheads); strongly reacting vascular endothelium (arrows) and negatively reacting BCs (asterisk).
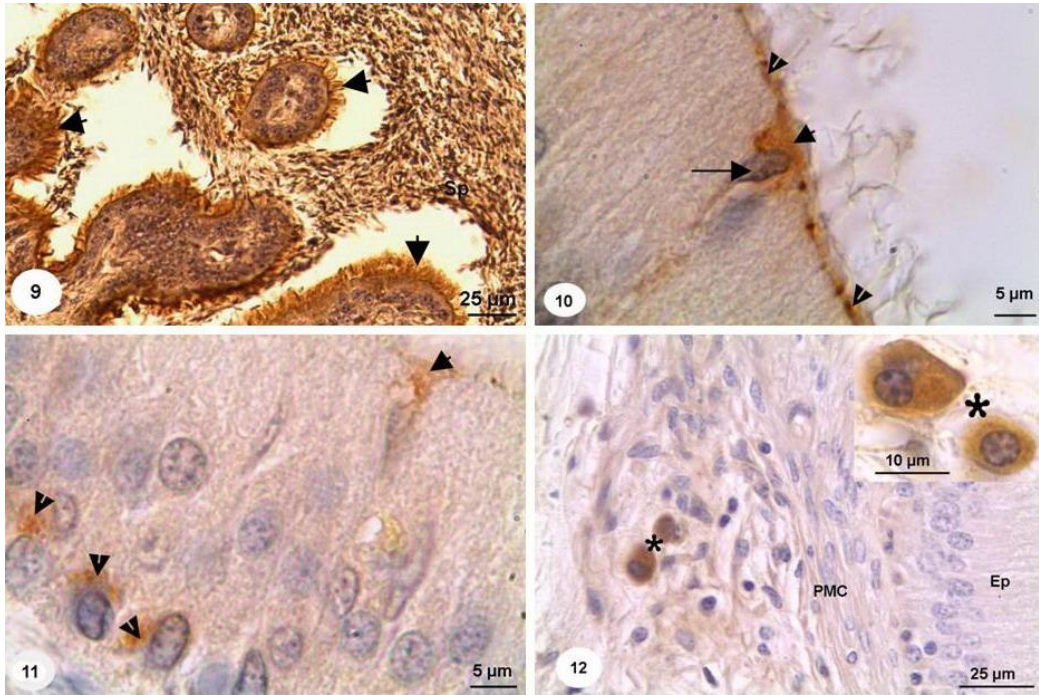
Fig (9): Immunolocalization of ACE in cauda epididymidis showing distinct binding sites on stereocilia (arrowheads) and apical surface of the epididymal epithelium.

Fig (10): Distribution of GalTase-binding sites in caput epididymidis displaying a strong reaction (arrowhead) in an AC (arrow) and in the apical cytoplasm (notched arrowheads) of PCs.

Fig (11): Immunolocalization of GalTase in corpus epididymidis showing a moderate reaction in an AC (arrowhead) and in juxtanuclear cytoplasm (notched arrowheads) of BCs.

Fig (12): VEGF-binding sites in corpus epididymidis displaying a strong reaction in two interstitial plasma cells (asterisk). Both of the epithelium (Ep) and the peritubular muscular coat (PMC) were negative. The inset showed higher magnification of the two plasma cells (asterisk).
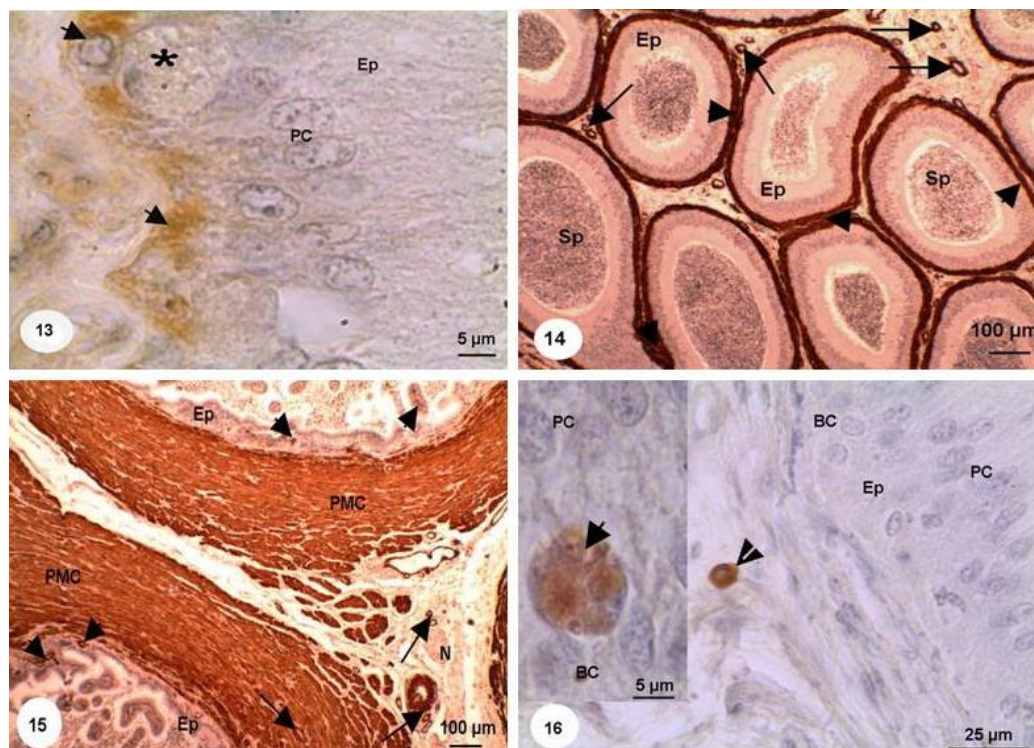
Fig (13): VEGF-binding sites in caput epididymidis showing a moderate reaction in cytoplasm of BCs (arrowheads) surrounding a negatively reacting foamy intraepithelial macrophage (asterisk). Epithelium (Ep) and principal cells (PC).

Fig (14): Distribution of α-SMA-binding sites in caput epididymidis displaying a strong reaction in the peritubular (arrowheads) and the vascular (arrow) SMCs.

Fig (15): Immunolocalization of α-SMA in cauda epididymidis showing a strong reaction in the peritubular (PMC) and the vascular SMCs of subepithelial (arrowheads) and interstitial (arrows) blood vessels. The epithelium (Ep) and nerve (N) were negative.

Fig (16): Cx43-binding sites in caput epididymidis displaying a strong reaction in an interstitial (notched arrowhead) and intraepithelial (arrowhead, in the inset) macrophages. The epithelium (Ep), including the principal (PC) and the basal (BC) cells, was negative.