Lectin histochemistry in the human epididymis

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A histochemical study using lectins to identify glycoconjugates present in the efferent ducts and ductus epididymidis of men without testicular or related disease was carried out. The lectins used and the oligosaccharide residues linked were: wheat germ agglutinin (WGA) for β -N-acetylglucosamine and sialic acid, concanavalin A (ConA) for α -mannose, *Ulex europaeus* agglutinin (UEA-I) for α -fucose, *Dolichos biflorus* agglutinin (DBA) for α -N-acetylgalactosamine, soy bean agglutinin (SBA) for β -N-acetylgalactosamine, and peanut agglutinin (PNA) for β -galactose. The lectin-binding pattern in the human epididymis presents similarities and differences to those observed in other mammals which also showed differences between species. The present results revealed that regional differences along the human ductus epididymidis were less pronounced than those reported in other mammals. The epithelial cells in the efferent ducts reacted positively to WGA. All along the length of the ductus epididymidis, the principal cells and the luminal content showed staining affinity for WGA and ConA. The epididymal principal cells and luminal content also reacted positively to DBA for α -N-acetylgalactosamine but only in the cauda epididymidis. A positive reaction to UEA-I was observed only in the luminal content of the cauda epididymidis. This finding suggests that changes in the oligosaccharide chains of secretions leading to a positive UEA-I reaction occur in the cauda epididymidis. The epididymal principal cells showed positive reactions to SBA and PNA over the apical microvilli but not in the cytoplasm. The reaction was observed in the caput and corpus epididymidis but not in the cauda. Positive reactions to SBA and PNA were also detected in the epididymal fluid and in the cytoplasm of mitochondria-rich cells (a minor population of epididymal epithelial cells). These cells also reacted to other lectins such as WGA, ConA and DBA, which were also detected in the principal cells.

Introduction

Mammalian spermatozoa are not mature when they leave the testis; their development continues during their passage through the epididymis (spermatozoon maturation) (Bedford, 1975). A contribution from the epididymis is necessary for this maturational process (Echeverría *et al.*, 1984) and, most importantly, spermatozoa acquire the ability to fertilize in the epididymis. Although the precise region of the epididymis where this acquisition occurs varies with species, it occurs generally in the corpus or proximal cauda epididymidis (Bedford, 1975).

The ductuli efferentes are a series of tubules that conduct spermatozoa from the rete testis to the epididymis (Ilio and Hess, 1994). These tubules leave the testis, initially as parallel straight tubules containing ciliated and non-ciliated cells in an epithelium of irregular height (Saito *et al.*, 1990; Yeung *et al.*,

1991). These ducts continue with the ductus epididymidis, a convoluted duct that extends from the caput to the cauda epididymidis. The epithelium lining the ductus epididymidis is columnar pseudostratified and comprises mainly principal cells and basal cells (Morita, 1966). Isolated mitochondria-rich cells (also called narrow cells, apical cells or flask cells according to their shape) may be seen in the caput and, more rarely, in the corpus epididymidis (Palacios *et al.*, 1991). Intraepithelial lymphocytes are evident in increasing numbers as one proceeds from the caput more distally (Ritchie *et al.*, 1984). Another epithelial cell type, designated as the clear cell, has been reported in mice and rats but not in humans (Toshimori *et al.*, 1988). The epididymal epithelium displays regional changes in features such as luminal diameter and smooth muscle investment (Vendrelly, 1981). The epididymis has been implicated in the secretion of a variety of substances ranging from small molecules to proteins (Hamilton, 1975; Turner, 1979). Biochemical analyses in various species have demonstrated the presence of specific epididymal proteins, which vary with the epididymal region (Lea *et al.*, 1978; Echeverría *et al.*,

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1982; Russell *et al.*, 1984; Olson and Hinton, 1985). Synthesis and secretion of proteins and glycoproteins by the epididymal epithelium have also been demonstrated using radioautography (Kopecny and Pech, 1977) or immunocytochemical procedures (Tezon *et al.*, 1985). Light microscope autoradiography studies have shown the greatest activity for protein or glycoprotein synthesis in the initial segment of the epididymis, with progressively lower protein incorporation in the middle and terminal segment (Kopecny and Pech, 1977). In the mouse epididymis, the synthesis and transport of proteins seem to be more rapid in the middle than in the initial segment (Flickinger, 1981).

Lectins have been used extensively as biochemical tools to isolate glycoproteins, glycolipids and polysaccharides and as probes to investigate the sugar residues of the cell surface. When conjugated to a marker, such as fluorescent dye, peroxidase or colloidal gold, lectins may be used as histochemical probes to identify and localize specific carbohydrate residues (Roth, 1978; Chang and Wong, 1992). Lectins are especially useful in studies of the quality, distribution, assembly and turnover of glycoconjugates in normal and pathological tissues, as well as during embryonic differentiation (Brown and Hunt, 1978; Lis and Sharon, 1986). Lectin histochemistry has been carried out in the epididymis of the mouse (*Mus musculus*), guinea-pig (*Cavia porcellus*), gerbil (*Jaculus jaculus*) and nutria (*Lutra lutra*) (Arya and Vanha-Perttula, 1986; Burkett *et al.*, 1987). The results of these studies revealed positive reactions for wheat germ agglutinin (WGA), peanut agglutinin (PNA) and concanavalin A (ConA), although the staining pattern varied among species and among regions of the epididymis. The aim of the present study was to examine the location and distribution of glycoconjugates in the human epididymis using a number of biotinylated lectin–peroxidase complexes.

Materials and Methods

Human epididymides from 17 adult men (aged from 25 to 55 years) were obtained at autopsy (6–10 h after death). These men had died either in traffic accidents (seven men) or from myocardial (five men) or cerebral (five men) infarction. The condition for selection was that the testes showed a normal histological pattern, including complete spermatogenesis. Samples were fixed in 10% (v/v) buffered formaldehyde for 24 h at room temperature. Tissue specimens were dehydrated through a graded ethanol series, cleared in xylene and embedded in paraffin wax (Paraplast: Panreac, Barcelona). Postmortem changes in the autopsy specimens were evaluated by immediately fixing and processing three epididymides obtained from testicular tumour surgery in the same way. Removal of tissues and the study of autopsy samples were approved by the Ethics Committee of the Hospital and made with the consent of the patients' relatives.

Thick sections, 5 µm, were cleared of paraffin with xylene and rehydrated through a series of decreasing ethanol concentrations followed by PBS. For glycoconjugate demonstration, in addition to histochemical staining with Alcian blue (pH 2.5)/periodic-acid–Schiff (AB/PAS) (Mowry, 1963), different horse-radish peroxidase-labelled lectins were used at concentrations of 10–20 µg ml⁻¹ in 0.1 mol PBS l⁻¹ (pH 7.2) for 60 min at

room temperature. Previously, the endogenous peroxidase was blocked by incubation in 3% H₂O₂. The lectins used were the following: wheat germ agglutinin (*Triticum vulgare*, WGA), concanavalin A (*Concanavalia ensiformis*, ConA), *Ulex europaeus* agglutinin-I (UEA-I), *Dolichos biflorus* agglutinin (DBA), soy bean agglutinin (*Glycine max*, SBA), and peanut agglutinin (*Arachis hypogaea*, PNA). The peroxidase activity was revealed by a diaminobenzidine–hydrogen peroxide system (DAB; Sigma, Barcelona). All lectins were supplied by Sigma.

For lectin negative controls, the following saccharides were added at a final concentration of 1 mg ml⁻¹ to the respective lectin solutions: *N*-acetyl-D-glucosamine for WGA, α-methyl-D-mannoside for ConA, L-fucose for UEA-I, *N*-acetyl-D-galactosamine for DBA and SBA, and β-galactose for PNA. Exposure to peroxidase and DAB system without lectins was performed as an additional negative control. The reactions of certain control sections were examined with DAB only to detect endogenous peroxidase activity in tissues. The sections were counterstained with Harris' haematoxylin, dehydrated, and mounted in DePex (Probus, Barcelona).

Mitochondrial-rich cells were identified in sections from the same specimens used here by means of enzymohistochemical and immunohistochemical techniques, using principally anti-AE1/AE3 cytokeratin antibodies, which immunoreact intensely against these cells. These techniques and the results obtained have been reported by Palacios *et al.* (1991).

Results

Comparison of the epididymides obtained during surgery with the autopsy specimens showed neither histological nor histochemical changes in the autopsy specimens.

AB/PAS staining showed differential reactions in the epididymal epithelium. The luminal surfaces of the epithelial cells presented positive reaction to Alcian blue (acidic glycoproteins) in the efferent ducts and along the whole length of the ductus epididymidis. The apical cytoplasm of principal cells in the ductus epididymidis showed positive reaction to PAS (neutral glycoproteins). The luminal content of cauda epididymidis presented a strong positive reaction to Alcian blue and PAS.

The results of lectin histochemistry are summarized (Table 1). Reaction to WGA was positive in all epididymal regions. In the efferent ducts, the reactivity was localized in the supranuclear cytoplasm of the principal cells and ciliated cells (Fig. 1a, b). In the ductus epididymidis, the cytoplasm of all epithelial cell types stained diffusely with WGA (Fig. 1a, c). A positive reaction was also observed in the tubular lumen from the caput epididymidis onwards. The interstitial connective tissue and the smooth muscle tissue surrounding the tubules also stained all along the epididymis (Fig. 1b, c).

An intense reaction to ConA lectin was observed in the principal cells of the caput, corpus and cauda epididymidis and in the mitochondria-rich cells (present only in the caput and corpus epididymidis) (Fig. 1d). The luminal content of the ductus epididymidis also labelled for ConA in the corpus and cauda. No positive reaction to this lectin was found in the efferent ducts. The interstitial connective tissue and the smooth muscle tissue surrounding the tubules also stained all along the epididymis.

Table 1. Histochemistry of carbohydrates in the human epididymis

Lectins	Carbohydrate binding	Ductus epididymidis										
		Efferent ducts			Caput			Corpus			Cauda	
		PC	CC	L	PC	MRC	L	PC	MRC	L	PC	L
WGA	β -GlcNAc/sialic acid	+	+	+	+	+	+	+	+	+	+	+
ConA	α -Mannose	—	—	—	+	+	—	+	+	+	+	+
UEA-I	α -Fucose	—	—	—	—	—	—	—	—	—	—	+
DBA	α -GalNAc	—	—	—	—	+	—	—	+	—	+	+
SBA	β -GalNAc	—	—	—	+	+	+	+	+	+	—	+
PNA	β -Galactose	—	—	—	+	+	+	+	+	+	+	+

PC: principal cells; CC: ciliated cells; L: luminal fluid; MRC: mitochondria-rich cells; WGA: wheat germ agglutinin; ConA: concanavalinA; DBA: *Dolichos biflorus* agglutinin; SBA: soy bean agglutinin; PNA: peanut agglutinin; GlcNAc: N-acetylglucosamine; GalNAc: N-acetylgalactosamine; *Staining is visualized only in the adluminal surface. Basal cells were labelled with WGA and only occasionally with the other lectins.

UEA-I lectin labelling was only visualized in the luminal fluid of the cauda epididymidis (Fig. 1e, f). The epithelium of the efferent ducts, caput, corpus and cauda epididymidis showed a negative reaction to this lectin.

A strong reaction to DBA was found in the mitochondria-rich cells of the caput and corpus, the principal cells and luminal content of the cauda epididymidis and in fibroblast-like cells in the interstitial connective tissue throughout the epididymal length (Fig. 2a–c).

An intense reaction to SBA was observed in the luminal surface of the epithelial cells of the caput and corpus epididymidis as well as in their luminal content (Fig. 2d). The mitochondria-rich cells also presented a strong positive reaction to SBA (Fig. 2d). In the cauda, the epithelial cells showed no reaction to SBA, whereas the luminal content reacted positively to this lectin (Fig. 2e). All along the length of the ductus epididymidis, small round cells that appeared isolated in the epididymal epithelium and, more rarely, in the interstitial connective tissue (probably lymphocytes), also reacted to SBA (Fig. 2d, e).

Reaction to PNA was localized in the luminal surface of the principal cells and luminal content in all the epididymal regions except for the efferent ducts (Fig. 2f, g). A strong label was also observed in the cytoplasm of mitochondria-rich cells (Fig. 2g).

Discussion

The lectin-binding pattern of the human epididymis obtained in the present study presents similarities and differences with regard to that observed in other mammals, such as gerbils, guinea-pigs, nutria, and mice (Arya and Vanha-Perttula, 1986; Burkett *et al.*, 1987) which, in turn, showed differences among species. In addition, the present results reveal that regional differences along the human ductus epididymidis were less pronounced than those reported in other mammals.

In the human epididymis, a diffuse and uniformly distributed WGA labelling (β -N-acetylglucosamine and sialic acid detection) was localized in all the epithelial cells in all the epididymal

segments. A positive staining to this lectin was also found in gerbils, guinea-pigs, nutria, and mice (Arya and Vanha-Perttula, 1986; Burkett *et al.*, 1987), although only epididymal regions I and II were stained in mice, and only regions II and III were stained in nutria. The wide reaction to this lectin may be related to structural oligosaccharides. In the mouse epididymis, Burkett *et al.* (1987) observed a strong staining for WGA in the cilia and the apical surfaces of ciliated cells in the efferent ducts of mice. As the ciliated cells are not known to secrete macromolecules, it is more probable that newly synthesized glycoconjugates are incorporated directly into the cell plasma-membrane as integral membrane glycoproteins than that they are exocytosed.

Nevertheless, WGA staining also appears in the epididymal lumen. Although the presence of stained material in the lumen and epithelial cells does not necessarily imply that the protein is secreted (it could be taken up from the lumen), it is well known that, in contrast to the absorptive function of the efferent duct epithelial cells (Ilio and Hess, 1994), the ductus epididymidis epithelium is principally involved in glycoprotein secretion (Yeung *et al.*, 1991), and thus, the present findings suggest that some WGA-labelled glycoprotein is also secreted in the human epididymis. In the boar epididymis, Dacheaux and Dacheaux (1988) immunolabelled antagglutinin in all the principal cells of the caput and corpus epididymidis as well as in the lumen. These authors also found that antagglutinin is a sialoprotein that binds WGA and ConA in the corpus and cauda epididymidis of this species. In addition, Bedford *et al.* (1973), in humans, and Toshimori *et al.* (1988), in mice, detected sialic acid residues in spermatozoa and assumed that these residues are from the sperm maturation antigen (SMA). Toshimori *et al.* (1988) concluded that mouse SMA is secreted by the epididymal clear cells – and not by the principal cells – because only the clear cells of the corpus epididymidis showed a positive reaction to sialic acid. In the study reported here, where WGA lectin labelled the SMA, this would not have been synthesized by the clear cells, which are absent in most mammals including humans, but by other epithelial cells. In mice, the clear cells are present only as isolated cells in the corpus epididymidis. It may be that, in humans and in some

Fig. 1. (a–c) Wheat germ agglutinin staining for the demonstration of the presence of β -N-acetylglucosamine/sialic acid in the human epididymis. (a) Control section of the caput epididymidis from a 41-year-old man. Positive staining (due to peroxidase activity) is observed only in lysosomal granules (arrow) of the efferent duct epithelium (EF). DE: ductus epididymidis. (b) Efferent ducts from a 26-year-old man. In addition to the lysosomal reaction, the supranuclear cytoplasm of the epithelial cells (arrow) and the interstitial connective tissue (star) were weakly stained. (c) Caput epididymidis from a 30-year-old man. The epithelium of the ductus epididymidis (DE), luminal content (asterisk), and the connective tissue (star) are diffusely stained. (d) Concanavalin A staining for the demonstration of the presence of mannose in the corpus epididymidis from a 33-year-old man. The epithelial cells reacted intensely to this lectin (DE). A weaker reaction was observed in the connective tissue (star). (e, f) *Ulex europaeus* agglutinin-I staining for fucose. (e) Control section of the corpus (asterisk) and cauda (star) epididymidis from a 42-year-old man. No positive reaction is observed. (f) Corpus (asterisk) and cauda (star) epididymidis from a 39-year-old man. A positive reaction is observed only in the luminal content but not in the epithelium (E) of the cauda. All sections were counterstained with Harris' haematoxylin. Scale bar: 1 μ m for all micrographs.

Fig. 2. (a–c) *Dolichos biflorus* agglutinin staining of α -galactosamine. (a) Control section of the corpus epididymidis from a 52-year-old man showing a negative reaction to this lectin. (b) Corpus epididymidis from a 39-year-old man. Only an isolated, apically placed, mitochondria-rich cell (arrow) appears stained in the epithelium. Some fibroblast-like cells stained in the connective tissue (arrowheads). (c) Transition from the corpus (asterisk) to the cauda (star) epididymidis from a 41-year-old man. A positive reaction was observed in the epithelial lining (E) and the luminal content of the cauda epididymidis. Fibroblast-like cells are also stained in the connective tissue (arrowheads). (d, e) Soy bean agglutinin staining for β -galactosamine. (d) Corpus epididymidis from a 37-year-old man showing an intense reaction in the luminal surface of the epithelial cells (E) and luminal content (L). Mitochondria-rich cells displaying apical locations (arrows) are also stained. The dark spots (arrowhead) in the epithelium are probably intraepithelial lymphocytes. (e) Cauda epididymidis from a 33-year-old man. Only the luminal content appears positively stained (L). The dark spots (arrowheads) in the epithelium (E) may be intraepithelial lymphocytes. (f, g) Peanut agglutinin (galactose) staining for galactose in the epididymis from a 42-year-old man. (f) No reaction is observed in the efferent ducts. The dark granules (arrow) are lysosomes which also appeared stained in negative control sections. (g) Corpus epididymidis showing intense staining in the luminal surface of the principal cells (E) and luminal content (L). Several narrow mitochondria-rich cells (numerous in this section) are also stained (arrow). All sections were counterstained with Harris' haematoxylin. Scale bar: 1 μ m for all micrographs.

other mammals, an ill-defined subtype of principal cells or other cell type of the epididymal epithelium present in very small numbers, such as the mitochondria-rich cell, may be responsible for this secretion.

ConA binds tightly to mannose-rich precursor oligosaccharide chains, complex mannose-type oligosaccharides found in membrane glycoproteins, and lysosomal enzymes (Shibuya *et al.*, 1988). ConA does not bind to complex chains with three or more *N*-acetylglucosamine branches (Baenziger and Fiete, 1979). Several researchers have reported ConA-binding sites in the cisternae of the rough endoplasmic reticulum (Chang and Wong, 1992), and within the cisternae of the Golgi apparatus (Roth, 1983; Pavelka and Ellinger, 1985). We observed an intense staining affinity for ConA in the cytoplasm of principal cells in the caput, corpus and cauda epididymidis. A positive reaction to this lectin was also found in the luminal content but only in the last two regions. These observations differ from the lectin histochemistry study of the epididymis of gerbils, guinea-pigs, nutria, and mice by Arya and Vanha-Perttula (1986). These researchers only observed a cytoplasmic positive reaction to ConA in gerbils and only in region II of the epididymis. If antagglutinin – which may be detected with WGA and ConA in boars (Dacheaux and Dacheaux, 1988) – or other similar glycoproteins are also present in other mammals including humans, it is possible that the epididymal principal cells that are positive to these two lectins are responsible for the synthesis and secretion of these proteins in men and gerbils, whereas in other mammals, such as guinea-pigs, nutria, and mice, the cells involved in these secretions are only WGA positive. We can speculate that, in humans, one of these proteins may be HE2, a secretory epididymal glycoprotein that associates with the equatorial region of the sperm head (Osterhoff *et al.*, 1994).

The oligosaccharide residues detected by DBA (α -*N*-acetylgalactosamine) could be contained in residual proteins in the lysosomes or in mucin-type-*O*-linked glycoproteins from mucous secretions (Madrid *et al.*, 1994). Positive reactions to DBA have been reported in the cytoplasm of epididymal epithelial cells in most mammals studied. However, whereas in guinea-pigs this reaction was positive all along the length of the ductus epididymidis, in mice, gerbils and nutria, the reaction was limited to the initial epididymal regions (I and II) (Arya and Vanha-Perttula, 1986). Lectin histochemical studies on the mouse epididymis by Burkett *et al.* (1987) found a positive reaction to DBA all along the epididymis, but only in basal cells. In the present study, a reaction to DBA was observed only in the epithelial cells and luminal content of the cauda epididymidis. In mice, a reaction to DBA may be related to the secretion of a glycoprotein (CP27) which is the major glycoprotein secreted in the cauda epididymidis of this species that is involved in maturation of spermatozoa (Flickinger *et al.*, 1988). However, there is no information about a similar protein in humans.

A positive reaction to UEA-I lectin, which preferentially labels terminal fucose residues located in the outer region of oligosaccharide chains (Sugii and Kabat, 1982), has been reported in the epididymal principal cells of gerbils (regions II and III), guinea-pigs (region III) and mice (regions I and II) but not in nutria (Arya and Vanha-Perttula, 1986). We observed that the luminal content of the cauda epididymidis

presents a positive reaction to this lectin. However, the reaction to this lectin was not detected in the epithelial cells in any epididymal region. This suggests that, in men and nutria, the UEA-labelled secretion is not synthesized and secreted in its final form and that the changes leading to a positive UEA reaction in the oligosaccharide chains of secretions occur in the cauda epididymidis. These changes may be explained by two mechanisms: either a fucose residue is added to the oligosaccharide chain or this residue is eliminated by enzymatic digestion. The last hypothesis is in agreement with the high content of α -glucosidase found in the human cauda epididymidis (Yeung *et al.*, 1990; Purvis and Egdetveit, 1993).

In the results presented here, the principal cells of the epididymis showed positive reactions only to SBA (β -*N*-acetylgalactosamine) or PNA (β -galactose) over the apical microvilli. SBA staining was localized in the caput and corpus epididymidis, whereas PNA staining was observed all along the epididymis, except for the efferent ducts. Arya and Vanha-Perttula (1986) observed that in the epididymis of gerbils, guinea-pigs, mice and nutria, the apical surface and microvilli are strongly reactive to PNA in all regions, while SBA displayed weak staining. These findings suggest that the glycoconjugates forming the glycocalyx of the epithelial cells differ along the epididymal length. It has been reported that the residues labelled by these lectins could also be part of the glycoproteins secreted by epithelial cells as cementing or barrier substances (Meyer, 1986). In addition, other saccharide residues that were also detected in the cytoplasm, such as sialic acid and α -mannose, also appear in the glycocalyx of the epithelial cells in some epididymal regions. Lectins have been used to characterize the glycoconjugates in the glycocalyx of various epithelial cells bearing microvilli (Roth, 1983; Gonnella and Neutra, 1985; Tanimura *et al.*, 1987) and, it has been shown that, along the length of the renal tubules, the glycocalyx exhibits marked variations that have been related to their specialized functions (Spicer *et al.*, 1981; Roth and Taatjes, 1985).

In the present study, positive reactions to SBA and PNA were also detected in the epididymal fluid and in the cytoplasm of mitochondria-rich cells. Although staining of the intraluminal content might be attributed to the glycocalyx of sloughed principal cells, this staining might also be the result of secretion by mitochondria-rich cells. These cells also reacted to WGA and ConA, and they were the only epididymal cells positive to DBA (α -*N*-acetylgalactosamine), which was also detected in the intraluminal content. These cells form a minor population of epididymal epithelial cells in most mammals studied and are usually present only in the initial epididymal segments (Martínez-García *et al.*, 1995). The morphological pattern of these cells varies, even in the same epithelium, from slender cells (narrow cells) to round apical cells without apparent connection with the epithelial basal lamina (apical cells); but they all contain short microvilli and an electron-dense cytoplasm with abundant mitochondria (mitochondria-rich cells). Histochemical and biochemical studies have also shown differences between these cells and the principal cells in the epididymis with respect to enzymatic activities and intermediate filament types (Palacios *et al.*, 1991). Although the function of the apical mitochondria-rich cells is unknown, the

following possible functions have been suggested: holocrine secretion (Martan and Risley, 1963), cooperation with the principal cells in reabsorption of testicular fluid (Sun and Flickinger, 1980), and acidification of epididymal fluid (Kierszenbaum *et al.*, 1981). The present results suggest that human mitochondria-rich cells are also secretory cells responsible for a secretion that reacts to SBA and PNA and, perhaps, to WGA, ConA and DBA. These secretions probably vary from one species to another since lectin studies in mice, gerbils, nutria and guinea-pigs indicated that the mitochondria-rich cells are WGA- and ConA-positive in the four species: SBA is positive in mice, gerbils and nutria; PNA is positive in mice, gerbils and guinea-pigs; DBA is positive in gerbils and guinea-pigs and UEA is positive in mice (Arya and Vanha-Perttula, 1986).

The small intraepithelial and interstitial cells that react with SBA are probably lymphocytes. These cells, present in small numbers, are a normal constituent of epididymal epithelium (Ritchie *et al.*, 1984). In the samples used in this study, we did not observe inflammatory infiltrates into the epithelium or interstitium.

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