Histological and Lectin Histochemical Characterization of the Epididymal Duct in the Dromedary (*Camelus dromedarius*)

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Abstract

Study of the regional microscopic anatomy of the epididymal duct of the dromedary camel revealed that the initial segment was lined with high pseudostratified columnar epithelium bearing stereocilia; varying height levels from place to place give the duct a “festooned” appearance. The nuclei of both principal and basal epithelial cells were located in the basal one-third. The duct was surrounded by a layer of circularly oriented smooth muscle cells; this peritubular smooth muscle thickened toward the termination of the epididymal duct. The middle and terminal segments of the epididymal duct were lined with similar epithelium, but with no noticeable variations in height. The nuclei in these regions were located in the basal one-half of the cells and spermatozoa were more evident in the lumen of the middle and terminal segments.

Epithelial cells were stained with six lectins: Con-A, DBA, MAA, SBA, SNA-1, and WGA. Binding sites for Con-A, WGA, and SNA-1 were seen in the initial segment. No binding sites for SBA, DBA, or MAA were detected in the initial segment. Sugar moieties for all lectin studied were detected in the middle and terminal segments, except for DBA and MAA in the middle segment, or WGA and MAA in the terminal segment. These lectins showed selective regional binding to different portions of the epididymal duct and varying degrees of binding to different cellular

compartments. These regional variations in carbohydrate composition imply the probability of functional compartmentalization in the epididymal duct.

Key Words

Epididymis, dromedary camels, lectin histochemistry, principal cells.

Introduction

The excurrent duct system of the testis consists of the ductuli efferentes, the ductus epididymidis, and the ductus deferens (Roberts, 2010) *"If anyone asks what the epididymis is, we shall answer that is a vessel constituting by various twists a body affixed to the back of the testicle"* de Graaf, 1668; see Jocelyn & Setchell, 1972; Hinton, 2010). The ductus epididymidis proper is a single highly convoluted tubule connected to the testis by the efferent ductules (Wrobel and Bergmann, 2006). The function of the epididymis is to bring testicular spermatozoa (which are nonmotile and incapable of fertilization) to functional as well as morphological maturity. The epididymis also protects the spermatozoa as they become mature and provides an environment for storage following the maturation process. The pseudostratified columnar epithelium of the epididymis is comprised of several cell types including principal, basal, apical, halo, clear and narrow cells, each of which vary in number and size along the epididymal duct. Much needs to be learned about the function of each cell type. So far, principal cells are known for active secretion and endocytotic activities. Clear and narrow cells play a significant role in the acidification of the luminal fluid. Basal cells may have a role in protection of spermatozoa from oxidative stress and xenobiotics. Halo cells are lymphocytes and may have a role in immune protection. The function of apical cells is unclear, though they show endocytic activities (Hinton, 2010).

Ultrastructural investigation of the epithelium of the epididymal duct of the dromedary camels revealed that the epithelium consists of principal cells, basal cells, halo cells, apical cells and dark cells (Tingari, 1989). The principal cells are by far the most abundant and probably the most recognizable at the light microscopic level. The different segments of the epididymal duct exhibit species-specific morphological features. To the best of our knowledge, Tingari and Moniem (1979) have produced the only report of the histological characteristics of the epididymal duct in the dromedary camel.

Several authors have used lectin histochemistry to study the glycolconjugates in the epididymal duct in

other species including the mouse (Lee and Damjanov, 1984; Burkett et al., 1987), hamster (Calvo et al., 1995), human (Arenas et al., 1996), boar (Calvo et al., 2000), horse (Ha et., 2003), and alpaca (Parillo et al., 2009), but none of these studies considered the dromedary camel. Considering the widespread use and distribution of the dromedary, and its importance to many regions of the world including the Middle East, northern India, and Africa, this is a deficit in the knowledge for this organ that need to be corrected.

Lectins are sugar-binding proteins that are highly specific for their sugar moieties (Alroy et al., 1984; Brooks and Hall, 2001). This makes lectin histochemistry a powerful method for mapping the presence of glycans in tissues; doing so allows accurate detection not only of structurally integral cellular components such as glycoconjugates, but also functionally significant molecules because glycoconjugates can act as cell receptors, tumor markers, or immunomodulators. Histochemistry bridges the gap and clarifies the relationship between morphological description and the functional significance of this variation. Understanding the morphology of the camel reproductive organs is imperative for improving its reproductive performance. The purpose of this report was to study the histological features of each segment of the epididymal duct in the dromedary camel and to map the cellular differences in glycoconjugates demonstrated by lectin histochemistry.

Materials and Methods

Testicles and the attached epididymis of six apparently healthy adult male dromedary camels were collected immediately after slaughter at the central abattoir in Cairo, Egypt. Samples of the initial, middle, and terminal segments of the epididymis were obtained, fixed in Carnoy's solution for about 4 hours, and paraffin-embedded. Sections 6.0 µm thick were prepared. For histological evaluation, sections were stained with Hematoxylin and Eosin or with Masson's Trichrome stain using Gurr's (1956) modification as given in Humason (1979). Weigert's iron Hematoxylin was replaced by Verhoeff's stain. This stain combination allows the differentiation of collagen fibers (blue), muscle (red) and elastic fibers (black) and renders cell nuclei deep mauve; cytoplasmic elements stain in varying shades of red and mauve.

Lectin histochemistry was performed using biotinylated lectins following the protocol of Brooks and Hall (2001). Embedded sections were deparaffinized in xylene and rehydrated in increasingly dilute etha-

nol/distilled water baths. For antigen retrieval, slides were trypsinized for 10 minutes at 37°C (Trypsin-EDTA, Media Tech Inc., Manassas, VA, USA). Slides were incubated in 0.3% hydrogen peroxide in methanol for 30 minutes to block endogenous peroxidase, then washed and incubated with Carbo-Free Blocking solution (Vector Laboratories, Burlingame, CA, USA) for 30 min at room temperature followed by incubation with the biotinylated lectin for 60 min in a humid chamber at room temperature.

Table 1 lists the lectins (E.Y. Laboratories Inc., San Mateo, CA, USA) used in this study, their source, their abbreviations, the lectin concentration used, their major sugar specificities, and their inhibitory sugars. After many preliminary trials, concentrations were selected that gave minimal background staining yet were sufficient to detect relatively low levels of specific sugar residues. A final incubation, after washing, with ABC (avidin: biotinylated horseradish peroxidase complex) (Vector Laboratories) lasted 30 minutes under the same conditions. Immunoreactivity was visualized with 3, 3 - diaminobenzidine (DAB substrate kit, Vector Laboratories) in a dark place as outlined in the manufacturer's protocol. Slides were counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted.

Some sections were treated with neuroaminidase (0.1 units/ml of type VI from *Clostridium perfringens*, Sigma, USA) (Jones et al., 1995). Controls for non-specific staining included incubation in which the biotinylated lectins were replaced with unconjugated lectins, biotinylated bovine serum albumin (E.Y. Laboratories) or buffer, preincubation of a lectin, except MAA and SNA-1 with its competing sugar (Vector Laboratories), or sections to be stained with MAA or SNA-1-were preincubated with neuroaminidase (Sigma, USA).

Results

The epididymal duct was lined by a high pseudostratified columnar epithelium with stereocilia. The initial segment showed varying height levels from place to place giving the duct a "festooned" appearance. The nuclei of the principal and basal cells were located in the basal one-third of the epithelial cells. The duct was surrounded by a layer of circularly oriented smooth muscle cells; this peritubular smooth muscle thickened toward the termination of the epididymal duct. The nuclei were located in the basal one-half of the cells and spermatozoa were more evident in the lumen of the middle and terminal segments as shown in Fig. (1).

Binding sites for Con-A, WGA, and SNA-1 were seen in the initial segment. No binding sites for SBA, DBA, or MAA were detected in the initial segment. Sugar moieties for all lectins studied were detected in the middle and terminal segments except for DBA and MAA in the middle segment, or WGA and MAA in the terminal segment. Lectin histochemistry demonstrated variations in the content and distribution of glycosidic residues of glycoconjugates in different epididymal regions; these variations are shown in table (2).

Binding sites for Con-A were detected throughout the epididymal duct in the stereocilia, glycocalyx, the apical and the basal cytoplasm, and Golgi zone with variations in binding intensity as shown in Fig. (2). The basal cells in the middle segment showed unstained Golgi zone.

Fig. (3) shows the binding sites for SBA in the three segments of the epididymal duct. SBA binding sites were readily detected only in the stereocilia, the Golgi zone. Binding was less intense in the basal and the apical cytoplasm in the middle segment and the luminal content of the terminal segment. The glycocalyx of the cells of the middle segment did not show binding sites for SBA. A sub population of cells did not show binding for SBA in the middle segment.

WGA binding sites were detected only in the stereocilia, glycocalyx, Golgi zone, and the basal and the apical cytoplasm in the initial segment as shown in Fig. (4). SNA-1 binding sites were detected in the glycocalyx, and the apical and the basal cytoplasm in the middle and terminal segments. Golgi zone remained unstained for SNA-1 in all segments (Fig. 5). Binding sites for DBA showed weak staining only in the terminal segment at all levels, except that Golgi zone remained unstained in a subpopulation of principal cells (Fig. 6).

Peritubular smooth muscle layer and the intertubular connective tissue showed binding sites for Con-A, WGA, and SNA-1, but not for SBA, DBA, or MAA in the initial segment; however, in the middle segment no binding sites were detected for any of the lectins used in this study except for WGA and SNA-1. Binding sites for Con-A, SNA-1, and DBA were detected in the peritubular smooth muscle layer and intertubular connective tissue in the terminal segment, Figures (2-6).

Discussion

This study demonstrates the regional morphological and histochemical

features of the epithelial lining of the epididymal duct segments in the dromedary, which correspond to regional variations in function needed for functional maturity of spermatozoa. Morphological and histochemical features of the terminal segment seem to provide optimum environment for storage of spermatozoa after maturation.

Variations in the regional morphological and histochemical features reported in this study in the dromedary camel have been reported in other mammalian species (Lee and Damjanov, 1984; Burkett et al., 1987; Calvo et al., 1995; Calvo et al., 2000; Ha et., 2003; and Parillo et al., 2009). Those variations were less pronounced in human (Arenas et al., 1996).

Of the six lectins studied, Con-A was the most widely distributed throughout the epididymal duct This may be due to its widespread distribution in the structural oligosaccharides; Con-A binds tightly to mannose-rich precursor oligosaccharide chains, complex mannose-type oligosaccharides found in membrane glycoproteins, and lysosomal enzymes (Arenas et al., 1996).
The glycoproteins we have demonstrated to be present in the principal cells correspond to and support the findings of others, chiefly Srivastav et al., (2004). The involvement of the principal cells in synthesis and secretion of glycoproteins needed for sperm maturation is well documented in other species, and there is no reason to think that their role is different in the dromedary. Further investigation of the regional histology of the epididymis may reveal some structural or physiological oddities related to the species' desert habitat.

Confinement of the binding sites for DBA to the terminal segment has been previously reported in the human epididymis (Arenas et al., 1996). This binding pattern was different in other species.

The presence of most of the lectins studied in the luminal content of the epididymal duct is in agreement with what is known about the secretory activities of the epididymal duct. It is known that the epididymis is involved in glycoprotein secretion (Yeung et al, 1991).

The presence of a subpopulation of cells with different binding properties (e.g., as we have shown, SBA in the middle segment and DBA in the terminal segment) suggests the existence of different types of cells other than the principal cells. This has, in fact, been documented at the ultrastructural level (Tingari, 1989).

A cell type that is worth noting is the "mitochondria rich cell." These cells form a minor population among the epididymal epithelial cells in most mammals. They are usually present only in the initial segment of the epididymis (Martí-nez-García et al, 1995, Arenas et al., 1996). The morphological pattern of the mitochondria-rich cells varies from slender to round apical cells, but features they have in common include short microvilli and an electron-dense cytoplasm with abundant mitochondria. More than one cell type is known to exist in other species supports our contention that this is also the case in the dromedary, but what the exact functional significance may be is still as yet unclear.

We have reported in this study that the glycoconjugates composition of the glycocalyx of the epithelial cells differs along the epididymal length. This reflects the functional complexity of the glycocalyx. 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 (Arenas et al., 1996).

Regional variations in the thickness of peritubular smooth muscles are presumably related to the movement of spermatozoa along the epididymal duct. It is aided by the contraction of the smooth muscle fibers (Hinton, 2010). This contraction is probably most needed in the terminal segment to move the spermatozoa to the ductus deferens after storage.

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Table 1: lectins used for identifying carbohydrate residues in the epididymal duct of the dromedary

Source of lectin	Common name	Acronym	Concentration (ug/ml)	Carbohydrate specificity	Inhibitory sugar
Canavalia ensiformis	Jack bean	CON A	10	α-D-mannose/α-D-glucose	α-D-methylmannose
Dolichos biflorus	Horse gram	DBA	50	α-D-*N*-acetylgalactosamine.	α-D-*N*-acetylgalactosamine
Maackia amurensis	Maackia seed	MAA	50	*N*-acetylneuraminic acid	NA
Glycine max	Soybean	SBA	50	α- and β- D-*N*-acetylgalactosamine.	α- and β- D- *N*-acetylgalactosamine
Sambucus nigra	Elderberry	SNA-1	50	*N*-acetylneuraminic acid/ α-*N*-acetylgalactosamine	NA
Triticum vulgaris	Wheat germ	WGA	50	*N*-acetylglucosamine/ sialic acid	*N*-acetylglucosamine

NA: not applicable

Table 2: lectin binding profiles in the epididymal duct of the dromedary

	Initial segment	Middle segment	Terminal segment
CON-A			
Stereocilia	+++	+++	++
Glycocalyx	+++	+++	+
Apical cytoplasm	+++	+++	+
Golgi zone	+++	+++ (-)	+
Basal cytoplasm	+++	+++	+
Peritubular sm	+	-	+
Intertubular CT	+	-	+
Luminal contents	+++	+++	+
SBA			
Stereocilia	-	+++	-
Glycocalyx	-	-	-
Apical cytoplasm	-	+	-
Golgi zone	-	+++ (-)	-
Basal cytoplasm	-	+	-
Peritubular sm	-	-	-
Intertubular CT	-	-	-
Luminal contents	-	+++	++
WGA			
Stereocilia	+++	-	-
Glycocalyx	+++	-	-
Apical cytoplasm	++	-	-
Golgi zone	+++	-	-
Basal cytoplasm	++	-	-
Peritubular sm	+	+	-
Intertubular CT	+	+	-
Luminal contents	+++	++	-
SNA-1			
Stereocilia	-	+	++
Glycocalyx	-	+	++
Apical cytoplasm	-	+	++
Golgi zone	-	-	-
Basal cytoplasm	-	+	++
Peritubular sm	+++	+	++
Intertubular CT	+++	+	++
Luminal contents	-	+	+
DBA			
Stereocilia	-	-	+
Glycocalyx	-	-	++
Apical cytoplasm	-	-	+
Golgi zone	-	-	+ (-)
Basal cytoplasm	-	-	+
Peritubular sm	-	-	+
Intertubular CT	-	-	+
Luminal contents	-	-	+

Incubation of sections with neuroaminidase eliminated any detected staining with SNA-1. All control sections failed to disclose appreciable reactivity at any of the above site.

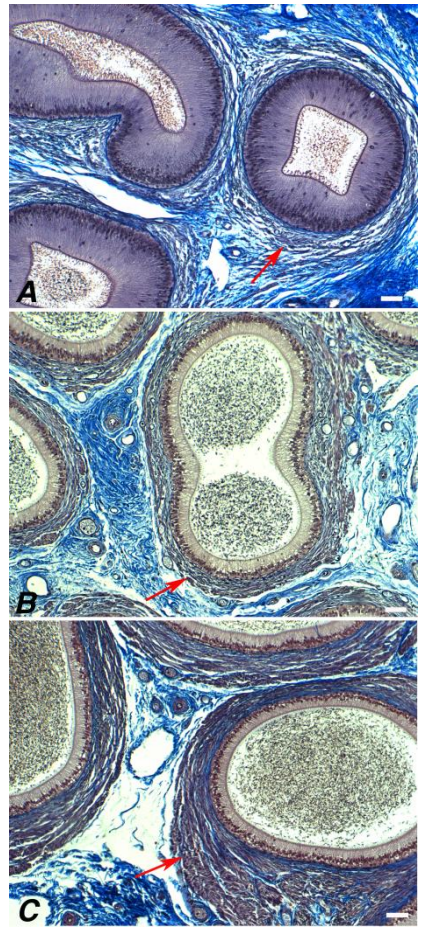

Fig (1): Sections of the epididymal duct of *Camelus dromedarius* stained with Masson's Trichrome and Verhoeff's stains showing that the epithelial lining is a high pseudostratified columnar with stereocilia. A is showing the initial segment of the epididymal duct with the characteristic varying height levels from place to place giving the duct a "festooned" appearance. The nuclei of principal and basal cells were located in the basal third of the epithelial cells. The nuclei were located in the basal one-half of the cells and spermatozoa were more evident in the lumen of the middle (B) and terminal (C) segments. Arrows in A, B, and C indicate the peritubular smooth muscle (reddish mauve color), which thickens toward the termination of the epididymal duct. A loose connective tissue (blue) surrounded the duct. Bar = 500 µm.

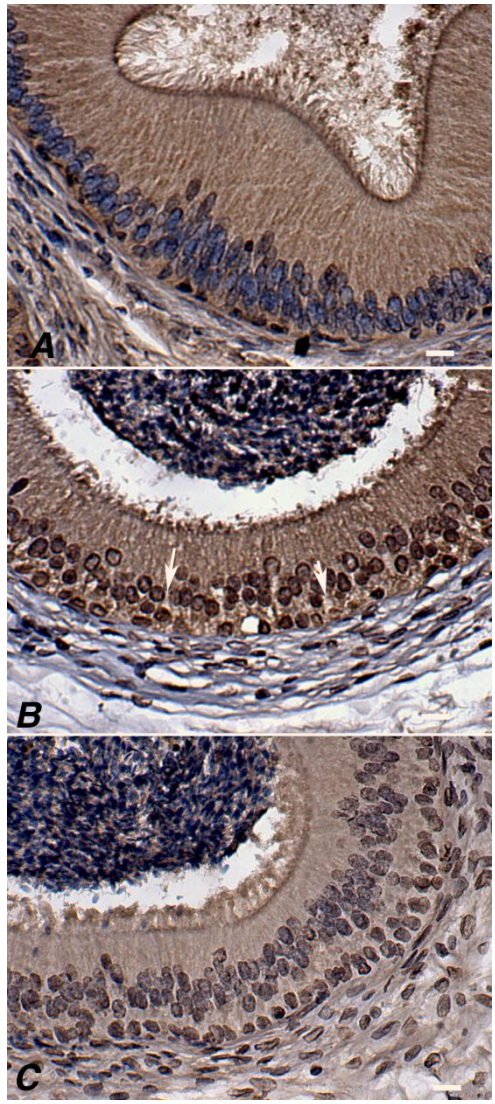

Fig (2): Con-A binding sites in the initial (A), middle (B), and terminal (C) segments of the epididymal duct. Binding sites for Con-A were detected in all segments at all levels with less intense binding at the terminal segment. Arrows indicate unstained Golgi zone of basal cells in the middle segment. Peritubular smooth muscle layer and the surrounding connective tissue remained unstained for Con-A in the middle segment. Bar = 125 µm.

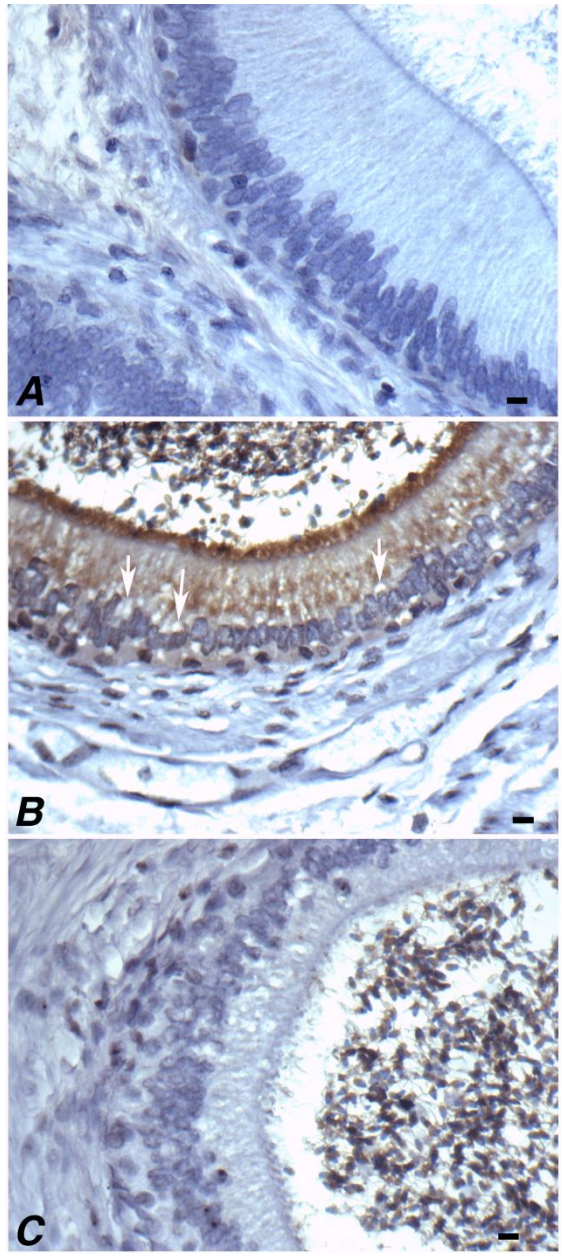

Fig (3): SBA binding sites were detected only in the epithelium of the middle segment (B) particularly in the stereocilia and the Golgi zone and to a less extent in the basal and the apical cytoplasm. Arrows indicate unstained Golgi zone in a subpopulation of principal cells. A: initial segment and C: terminal segment. Bar = 125 µm.

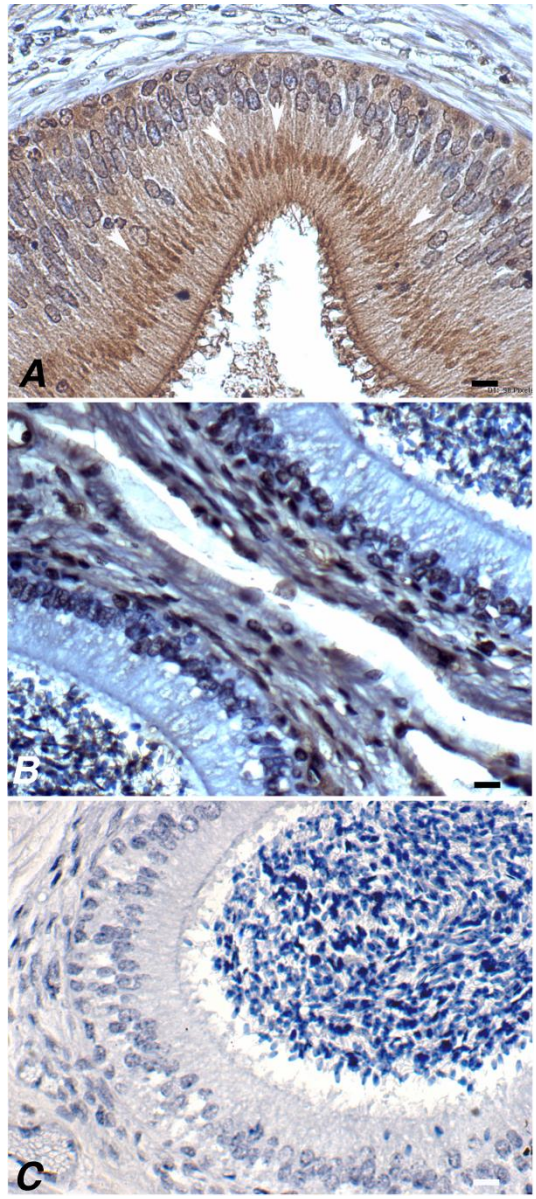

Fig (4): WGA binding sites were detected only in the stereocilia, glycocalyx, Golgi zone, and the basal and the apical cytoplasm in the initial segment (A). The peritubular smooth muscle layer and the intertubular connective tissue are showing weak binding in the middle segment. No binding sites are detected in the terminal segment (C). Bar = 125 µm.

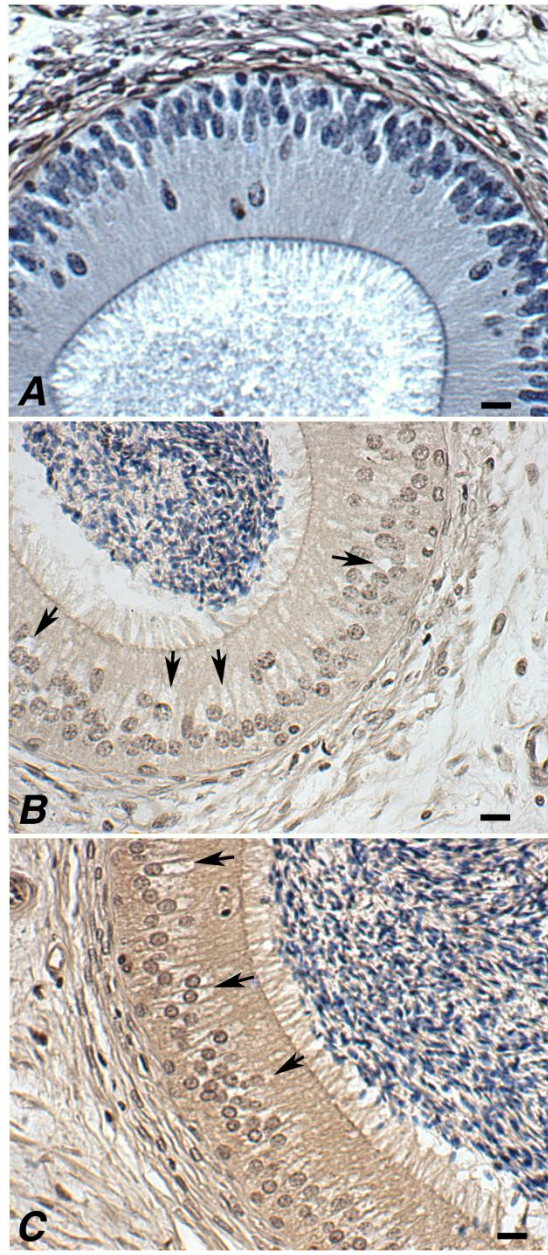

Fig (5): SNA-1 binding sites were detected in the glycocalyx, and the apical and the basal cytoplasm in the middle (B) and terminal (C) segments. Golgi zone (arrows) remained unstained for SNA-1. The epithelium in the initial segment (A) did not show any binding sites for SNA-1. Bar = 125 µm.

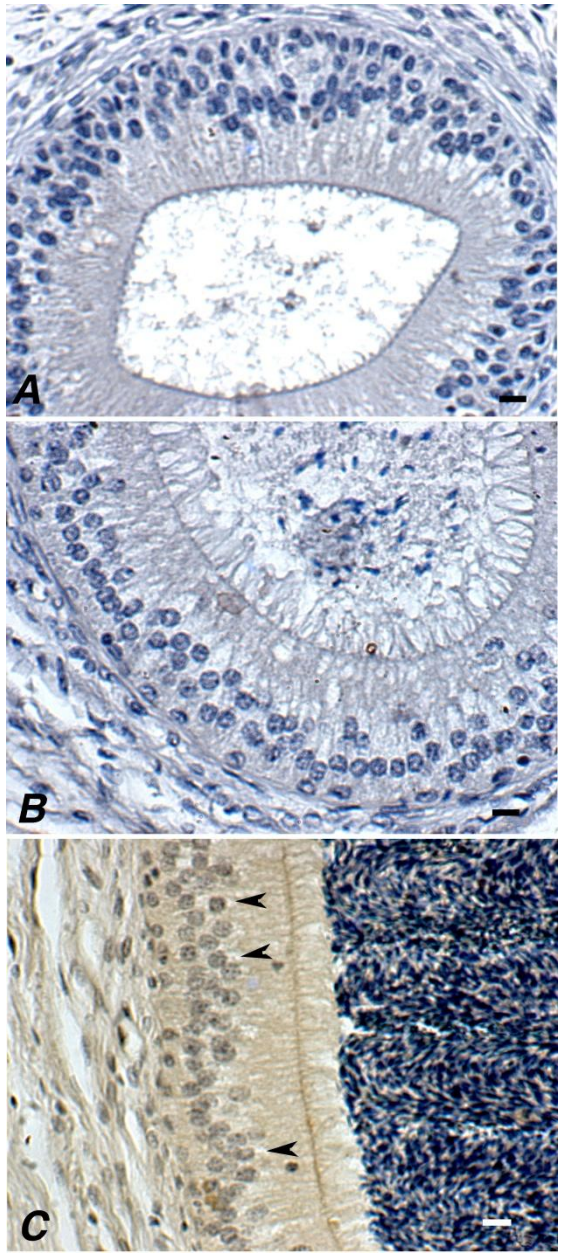

Fig (6): DBA staining in initial (A), middle (B), and terminal (C) segments. Only the terminal segment showed staining, a weak positive binding for DBA at all levels. Golgi zone remained unstained in a subpopulation of principal cells (arrow heads). Bar = 125 µm.