Immunohistochemical Studies of Epididymal Duct in Buffalo

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Abstract

Paraffin-embedded sections from different regions of the epididym duct from clinically healthy, adult buffalo-bulls were studied usin conventional histological and immunohistochemical techniques. Prima antibodies against angiotensin converting enzyme (ACE galactosyltransferase (GT), S-100, alpha smooth muscle actin (α-SMA connexin-43 (Cx43) and vascular endothelial growth factor (VEGF) wer applied. Immunohistochemistry aimed to underline morphofunctiona correlations in different epididymal segments. The results revealed tha in addition to the well-known principal and basal cells, the epididym epithelium possessed apical cells and intraepithelial leukocytes Immunohistochemistry showed that, with the exception of VEGF whic reacted negatively, all of the others were immunolocalized in th corresponding specific epididymal structures. Apical cells displayed strong reactivity with ACE along the entire length of the epididymal duc Principal cells in the caput epididymidis expressed a stron immunoreactivity against S-100 (cytoplasm and nuclei) and GT (Gol zone). The peritubular muscle coat manifested a marke immunoreactivity against (α-SMA) and (Cx43). In conclusion thes findings might reflect absorptive, secretory and other epididym activities involved in creating an appropriate microenvironment essentia for sperm maturation.

Keywords: *Immunohistochemistry, Epididymal, Buffalo.*

Introduction

Testicular sperms lack both the ability to move and to fertilize an ovum. Howe acquire these properties during their passage through the epididymis (Bedfor Metamorphosis of an immature and immotile sperm into a mature sperm ca progressive motility and fertility may be a result of highly regulated and sequential events in the epididymis (Cornwall and Hann, 1995). Furth spermatozoa of scrotal mammals possess limited biosynthetic activities (Da al., 1989) and display a crucial dependence on the activities of the ep epithelium for their maturation and survival (Moore and Bedford, 1979, Hir Palladino, 1995).

The biochemical composition of the epididymal fluid microenvironment regional variation along the length of the epididymal duct. This variation res the differential absorptive and secretory activities of the epididymal epithelium and Marsh, 1971; Hinton and Palladino, 1995).

The present work aimed to shed lights on the morphofunctional correlations application of immunohistochemistry.

Materials and Methods

Epididymal specimens were obtained from 7 adult clinically healthy buff slaughtered at the central abattoir of Cairo. All the specimens were taken dire slaughter. The epididymal duct was divided into the three main parts: head, b tail (Dyce et al., 1996). Specimens were fixed in different fixatives for histological staining and immunohisto-chemistry. They were then dehydrat graded series of alcohol, cleared in xylene and embedded in paraplast and s at 5μm. Immunohistochemical studies were performed using the avidin-biotin- Method according to Hsu et al. (1981). Sections were deparaffinized in rehydrated in a descending grade of alcohol until distilled water. Sections w washed three times in a phosphate buffer saline (PBS), pH 7.4 for 5 minut Endogenous peroxidase was inhibited with 1% H2O2 for 10 minutes foll intense washing under current tap water for 10 minutes. Sections were washe (three times, 5 minutes each) and then covered with serum-free, protein block for 10 minutes at room temperature. They were then incubated with the primary antibody (Table 1) for 12 hours at 4ºC (for an hour at room temperatur of S-100 and α-SMA). For detection of connexin it was necessary to unr antigen through heating the sections in citrate buffer (pH 6.00) three times (1 each) in microwave before adding the primary antibody. In case of negative primary antibodies were replaced by PBS. Incubation with secondary antibodi 1) for 30 minutes at room temperature. Washing the sections in PBS (thre minutes each). Incubation of sections with streptavidin-biotin horseradish p complex at room temperature for 30 minutes. Washing in PBS (three times, each). Developing of the reaction by using diaminobenzidin (DAB) soluti minutes at room temperature. Counter-staining of the nuclei with Haematoxy seconds. Sections were dehydrated through ascending grades of ethanol, xylene, and covered with DPX.

Results

Light microscopical studies revealed that the epididymal duct is l pseudostratified columnar epithelium with stereocilia. The cellular eleme epithelium included principal cells (PCs), basal cells (BCs), apical cells intraepithelial leukocytes (Fig 1 a, b and c). The peritubular elements inclu highly vascularized lamina propria (Fig 1c) of loose connective tissue and coat of circularly orinted smooth muscle cells. The interstitial connective ti the coils of the duct together. It houses mast cells (Fig 1d) and several especially macrophages and lymphocytes.

Immunostaining for ACE in the epididymal head region displaye reaction in apical cells and in subepithelial blood vessel (Fig 2a). However, surfaces of principal cells and some basal cells showed a weak to modera Although similar reaction was detected in the body (Fig 2 b) and tail (Fig 2 basal cells exhibited moderate reactivity (body) or reacted negatively (t luminal surfaces of principal cells showed no reaction either in body or

Moreover, intraepithelial leukocytes expressed negative reaction along the length of the epididymal duct.

Immunolocalization of galactosyltransferase in the epididymal head regi represented by a well-distinct reaction in Golgi zone and apical surfaces o principal cells (Fig 2 d). No binding sites could be localized either in the body o tail regions.

Immunolocalization of S-100 in the epididymal head region displayed a reaction in cytoplasm and nuclei of PCs (Fig 3 a). The epididymal epithelium body region was almost negative. However, nuclei and apical cytoplasm of son showed positive reaction in alternation with negatively reacting large areas epithelium. In tail region the epithelium was entirely negative. Whereas apic presented negative to weak reaction, basal cells and intraepithelial leucocytes r negatively throughout the whole length of epididymal duct. On the other hanc fibres (Fig 3 b) and vascular endothelium expressed a moderate to strong r along its entire length.

A strong immunoreactivity for α-SMA was evident both in the peritubul vascular smooth muscle elements (Fig 3 c) along the whole length of the duct.

Binding sites for connexin 43 were restricted to the smooth muscle cells peritubular muscle coat (Fig 3 d).

No binding sites for VEGF could be found either in the epididymal epithe in the peritubular components along the entire length of the duct.

Discussion

Angiotensin converting enzyme (ACE) is a membrane-bound glycoprotein is detectable in all tissues and body fluids of mammals (Soffer, 1976). ACE ca the formation active angiotensin II from the inert angiotensin I in many organs in the male reproductive tracts (Wong and Uchendu, 1990). This lends a support hypothesis that renin-angiotensin system can control the reproductive functions et al., 1998).

In the present work the vascular endothelium particularly of subepithelia vessels expressed a strong immunoreaction for ACE along the length of the epid duct. Similar findings were reported in the epididymis of rabbit (Berg et al., human (Vivet et al., 1987) and cow-bulls (Alkafafy, 2005). Furthermore, bindin for ACE were observed on the luminal surface and stereocilia of the epid epithelium in human (Vivet et al., 1987) and in cow-bulls (Alkafafy, 2005). My fi showed that ACE-binding sites were evident in the head region rather than ir regions. This was in accord with those found in rat (Strittmatter et al., 1985) cow-bulls (Alkafafy, 2005).

In the present study there was a moderate reaction for ACE in the l epithelial surface but not in stereocilia. Moreover, apical cells displayed a reaction throughout the entire length of the duct. This contradicts the case in co (Alkafafy, 2005) that failed to develop immunoreaction against ACE in apical cell difference in immunoreactivity may reflect a divergent physiological role in di species. Although the exact functional significance of the apical cells is not yet (Martínez-Garcia et al., 1995), they could be involved in resorption (Jensen 1999) and acidification (Au and Wong, 1980; Jensen et al., 1999) of the epid fluid. It is noteworthy that ACE converts angiotensin I (locally produced by epid epithelial cells) into angiotensin II. The latter may mediate its action by pa and/or autocrine routes (Leung et al., 1999) via specific receptors (Leung et al.,

on both apical and basal surfaces of epididymal epithelium, playing its role regulating electrolyte and fluid transport in the epididymis (Leung et al. O'Mahony et al., 2000). It is noteworthy that, the epididymal fluid is hyperosr blood plasma (Levin and Marsh, 1971). This hyperosmolar milieu may be a f prolonging the survival of spermatozoa during their transit along the epididyn (Hinton and Palladino, 1995). However, my findings are in disagreement wit reported by Adamali and Hermo (1996) and Alkafafy (2005) that assume tha cells play no significant role in regulation of fluid and electrolyte movemen epididymis.

Intraepithelial leukocytes failed to develop reaction with ACE along th length of the duct either in the present work or in cow-bulls (Alkafafy, 2005).

Galactosyltransferase (GT) is a member of a functional family of enzyr work co-ordinately in the biosynthesis of carbohydrate moieties of glycoprote glycolipids (Ramakrishnan et al., 2001). Immunoreaction for GT appeared to b localized in the Golgi zone and apical cytoplasm of the PC of the epitheliu epididymal head region. My results indicate that the caput epididymidis is the r of synthesis and secretion of GT. This supports the findings of previous (Nicander and Malmqvist, 1977; Kohane, et al., 1980; Liu et al., 1991; Arrig 1993; Cornwall and Hann, 1995; Alkafafy, 2005), which indicated that the P caput epididymidis are the main site of glycoprotein synthesis in different ma species. Some of the secretory glycoproteins in the epididymal fluid posses established role in the induction of forward motility (Acott and Hoskins, 19 acquisition of fertilizing capacity (Orgebin-Crist and Jahad, 1978). M alterations of sperm membranes result from the incorporation of proteins, su lipids which are of epididymal origin (Moore, 1998).

S-100 is a protein appears in a subcellular fraction first isolated fror brain. It was called S-100 due to its Solubility in a 100 % saturated sc ammonium sulphate at neutral pH (Moore, 1965). Immunoreactivity for S-100 was detected in the PC and weakly in the AC and BC. Similar results were re human (Haimoto et al., 1987) and bovine (Alkafafy, 2005) epididymis. Sin been associated with several activities including regulation of the transm diffusion of monovalent cations (Zimmer et al., 1995), S-100 may be involv processes regulating pH, electrolyte and water content of the kidney tub (Molin et al., 1985). Thus, S-100 may promote a similar task in the excur system of the male genital tract (Alkafafy, 2005).

In the present work, the vascular endothelium expressed a moderate immunoreaction for S-100 proteins. Similar findings were reported in the e cells of arteries, veins, and capillaries of bison (Czykier et al., 1999); bovine 2005) and murine (Czykier et al., 2000) epididymis. It is noteworthy th immunoreactivity in vascular endothelial cells was regularly detected in (Amselgruber et al., 1994). It was therefore assumed that it may particip processes of transcytosis and cell contractility (Czykier et al., 2000).

Alpha SMA is mainly found in cells having contractile functions (Sk 1986). It is an isoform typical of smooth muscle cells (SMC) and is prese

amounts in vascular SMC (Skalli et al., 1989). A monoclonal antibody (Skall 1986) has been used for recognizing exclusively α-SMA and it appears t powerful probe in the study of SMC differentiation in normal and path conditions (Skalli et al., 1986; 1989).

In the present work the cytoplasm both of periductal and vascular SMC a strong positive α-SMA-immunoreaction. Thus our findings agree with those r by Francavilla et al. (1983) and Alkafafy (2005) who found similar findings epididymal ducts of the rat and cow-bull respectively. Since the passage o through the epididymal duct is affected by the active contractions of the epi (Hamilton, 1975), the PMC play an important role in the epididymal function.

Connexins (Cx) are members of a large family of integral membrane (Thomas et al., 2002). They are the subunit proteins which oligomerize intercellular channels known as gap junctions through which ions and met diffuse directly from cell to another (Roscoe et al., 2001). They facilitate ordination of individual cells in an organ (Evans and Martin 2002) and pr mechanism for regulating the biological function of a whole tissue or organ (Th al., 2002). In the present work binding sites for connexin 43 could be found at t of the epithelium and between the peritubular SMC. These findings agree wit of Cyr et al. (1996) who reported that connexin 43 was immunolocalized epididymis of rat (Cyr et al., 1996). Existence of gap junctions in the epididym reflect the co-ordination between the epididymal epithelium and the peritubular coat necessary for regulation of different epididymal functions. However, the fin the current work disagree with those mentioned by Alkafafy (2005) who could binding sites either in the epithelial lining or between the peritubular SMC in epi duct of cow-bulls.

VEGF is a heparin-binding growth factor specific for vascular endothel (Leung et al., 1989). It is a potent angiogenic inducer that has been impli physiological and physiopathological conditions (Armesilla et al., 1999). This attributed to its ability to increase the microvascular permeability (Connoll 1989). In disagreement with the current study, VEGF was expressed in sev types in the human male genital system including certain epithelial and peritubu of the epididymis (Ergün et al., 1998). In the present work, VEGF-reactivity c be seen either in epididymal epithelium or in the interstitium. Though mast ce visualized by Alcian blue staining, they showed no immunostaining react VEGF. This is contradictory to the case in cow-bulls (Alkafafy, 2005). This attributed to species differences. It is noteworthy that MC release a va angiogenic factors (Crivellato et al., 2004), and may be associated with regu vascular permeability (Lissbrant et al., 2003). Furthermore, they may have m chemotactic effect (Clauss et al., 1990). This may help migration of mononucl from blood into interstitium and consequently into the epididymal epithelium. E of macrophages both in the interstitium and within the epididymal epithelium al T-lymphocytes may participate in the induction of immune tolerance in the testi excurrent ducts (Marchlewicz, 2001).

***In conclusion**, immunohistochemistry might reflect functional correlate epididymal duct. Those include secretory (GT) and absorptive (S100, ACE) characterizing the epithelium of caput epididymidis. Moreover, selective al*

potential could be indicated by localization of ACE in AC along the leng Localization of Cx43 in the epididymal duct might explain the inter-play b epithelial and periductal structures necessary for co-ordination and re diverse epididymal functions necessary for providing an appropriate microe essential for sperm maturation.

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Table1: Antibodies used in immunohistochemistry.

Primary antibody (Ab) against	Dilution of Primary Ab.	Origin of Primary Ab.	Secondary Antibody	Dilution Second Ab.
ACE	1: 500	Chicken	Antichicken, IgG Biotin from Rabbit	1: 400
GT	1: 500	Chicken	Antichicken, IgG Biotin from Rabbit	1: 400
S100	1: 400	Rabbit	Antirabbit, IgG Biotin from pigs	1: 300
α-SMA	1: 200	Mouse	Antimouse, IgG Biotin from Rabbit	1: 300
Cx43	1: 400	Mouse	Antimouse, IgG Biotin from Rabbit	1: 300
VEGF	1: 800	Rabbit	Antirabbit, IgG Biotin from pigs	1: 300

Figure legends

Fig 1:

a) H&E-stained epididymal tubule of head region displaying apical cell (A stereocilia (st) and peritubular muscle coat (PMC). Scale bar = 25μ

b) H&E-stained epididymal tubule of body region displaying apical cell (A basal cell (BC), principal cell (PC), stereocilia (st), peritubular muscle coa intraepithelial leukocytes (arrow head). Scale bar = 25μ

c) Masson trichrom-stained epididymal tubule of tail region displaying pr cells (PC), basal cells (arrow), stereocilia (st), peritubular muscle coat (P intraepithelial leukocyte (asterisk). Scale bar = 25μ

d) Alcian blue-stained part of epididymal head region showing a positive mast cell (MC) in the interstitium, Ep. E= epididymal epithelium, Lu = lum sperm.

Scale bar = 25μ

Fig 2:

a) Immunolocalization of ACE in the epididymal head region. A strong re an apical cell (arrow) and in a blood vessel (arrow head), Lu = lumen, PM peritubular muscle coat. Scale bar = 25μ

b) Immunolocalization of ACE in the epididymal body region. The reactio moderate in basal cells (arrow heads) and strong in vascular endotheliu or negative in intraepithelial leukocytes (arrows), Lu = lumen. Scale bar

c) Immunolocalization of ACE in the epididymal tail region. A strong rea an apical cell (arrow) and in vascular endothelium (BV). Scale bar = 25μ

d) Immunolocalization of galactosyltransferase in the epididymal head r The reaction was evident in Golgi zone (GZ) and apical surfaces (arrow principal cells, BV = blood vessels, st = stereocilia. Scale bar = 25μ

Fig 3:

a) Immunolocalization of S-100 in the epididymal head region displayin reaction in cytoplasm and nuclei of principal cells (PC) and negative (ar head) to weak (asterisk) reaction in apical cells. The basal cells (arrows negative, Sp = sperm, st = stereocilia. Scale bar = 25μ

b) Immunolocalization of S-100 in epididymal tail region showing a stro reaction in nerve fibre (N) and vascular endothelium (BV). Scale bar = 2

c) Immunolocalization of α-smooth muscle actin in peritubular (PMC) a vascular (arrow head) muscle cells in the epididymal tail region. Scale 100μ

d) Immunolocalization of connexin 43 in the epididymal tail region. Dist binding sites (arrow heads) were found between smooth muscle cells a epididymal epithelium, BC = basal cell, PC = principal cell, PMC = perit muscle coat. Scale bar = 25μ

Fig 1

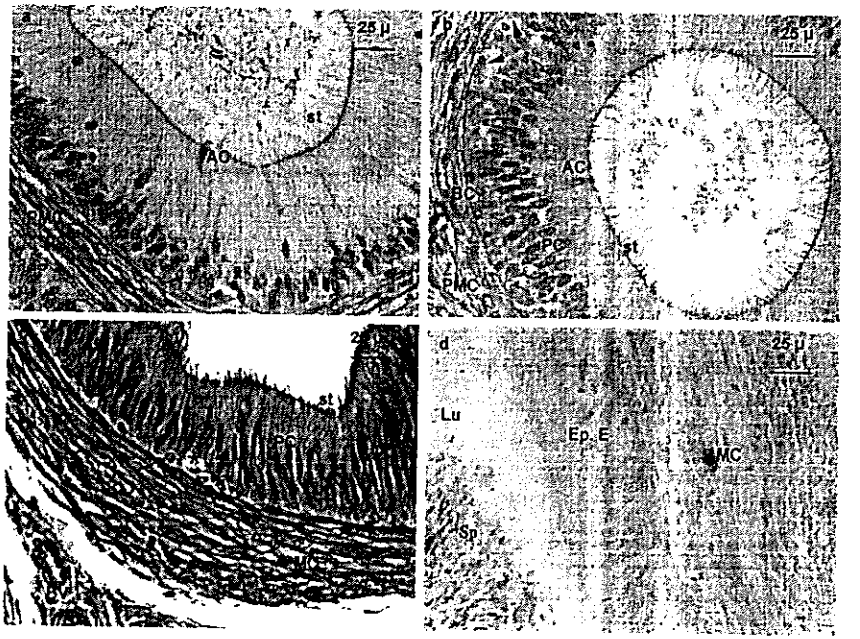
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Fig 2

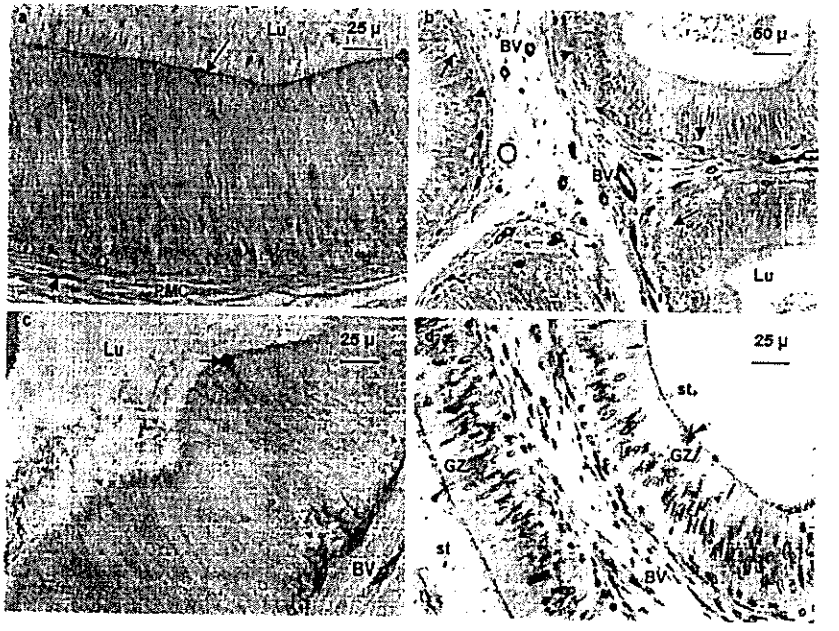
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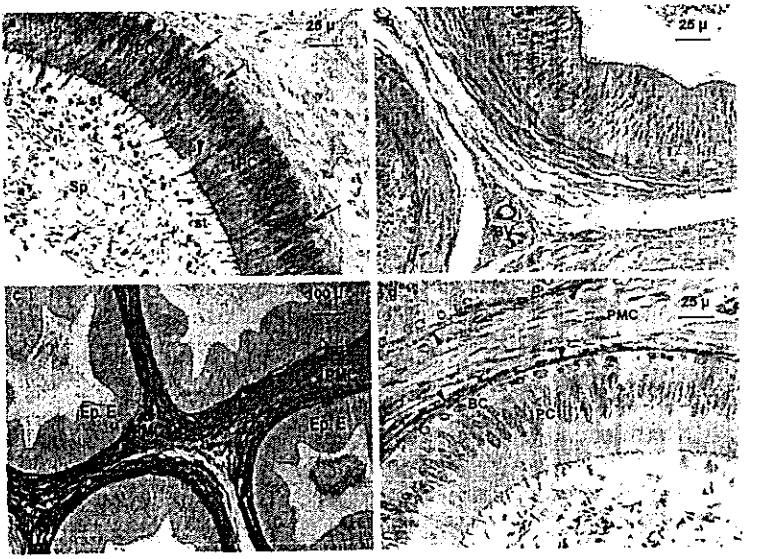
Fig 3



لعربى

ستوكيميائية مناعية علي قناة البربخ في فحول الجاموس

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راسة قناة البربخ في فحول الجاموس اليافعة باستخدام الطرق الهستولوجية التقليدية و المناعية. استخدمت الدراسة أجساما مضادة للبروتينات VEGF , connexin-43, α-SMA, S-100, لتحصيرها مناعيا ؛ مستهدفة إبراز الارتباط الوظيفي بالتراكيب في مناطق قناة البربخ المختلفة. بينت الدراسة أن النسيج الطلائي المبطن لقناة البربخ يا أساسية، قاعدية و قمية. كما أظهرت النتائج أنه باستثناء VEGF و الذي تفاعل سلبيا كل البروتينات الأخرى. و عليه فقد تم تحصير ACE بوضوح في الخلايا القمية في لبربخ في حين أن الخلايا الأساسية بمنطقة رأس البربخ قد أظهرت تفاعلا قويا مع كل من وبلازم و الأنوية) و GT (أجسام جو لجي). كما أبدت الطبقة العضلية المحيطة بقناة جليا مع كل من α-SMA و connexin-43.

الدراسة إلي أن تقنية الهستوكيمياء المناعية قد تعكس تضافر القدرات الفسيولوجية بربخ و خصوصا القدرات الامتصاصية (بتحصير ACE, S-100) و الإفرازية) و تبرز دورها في توفير البيئة الملائمة لإنضاج الحيوانات المنوية.