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RESEARCH ARTICLE

EFFECTS OF THE PUBERTY AND THE BREEDING SEASON ON SOME ENZYMES OF THE DROMEDARY CAMEL EPIDIDYMIS: HISTOCHEMICAL AND BIOCHEMICAL STUDIES

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ABSTRACT

The present study investigated the histochemical localization and activity of five different enzymes during breeding and non-breeding seasons and at pre- and post-pubertal ages in the camel epididymis. The plasma and epididymis samples were collected from a total of 40, one-humped, male camels (*Camelus dromedarius*) from the slaughterhouse. The samples were taken from pre-pubertal (1.0 – 3.5 years old) and post-pubertal camels (4.0 – 13.0 years old). The intensity of the studied enzymes i.e. alkaline phosphatase (ALP), acid phosphatase (ACP), glucose-6-phosphatase (G-6-Pase), adenosine triphosphatase (ATPase), and 5`-nucleotidase (5`-Nase) differed depending upon the region of the epididymis. The rutting season was associated with marked increases in the activity of ATPase in plasma and caput region, along with a significant rise in 5`-Nase activity in the caudal region; but the G-6-Pase activity did not differ significantly by season in both the plasma and epididymal tissues. The ALP and ACP activities showed slight reduction in the caudal region of the epididymis during the rutting season. With age advanced the caudal region showed significant increases in ATPase and ALP activities. The caput region showed a significant increase in 5`-Nase activity in the post-pubertal camels. The caudal region showed intensive reactivities of ATPase, ALP, and ACP more than the caput region in both seasons and ages. On the contrarily, caput region showed more activity of 5`-Nase than the caudal region. The current study concluded that the qualitative and quantitative enzymes activities of the epididymis were closely related with seasonal changes, stage of puberty, and with age advancing.

INTRODUCTION

The camel plays essential socio-economic roles and supports millions of people living in the dry and arid regions of Asia and Africa. Camels are the most suitable domestic animals during severe droughts, not only surviving these harsh conditions, but also continuing to produce and

reproduce[1]. Improving camel productivity, especially reproductive efficiency, is essential for addressing shortages in animal meat and milk. This requires effective management strategies based on a comprehensive understanding of camel reproductive biology[2].

The epididymis provides a microenvironment for sperm structural, functional changes, and maturation[3]. The regions of the epididymal duct were identified in prepubertal swamp buffalo based on location of ducts and epithelial height[4]. In camel, the epididymal duct was divided into initial, middle, and terminal segments[5]. The first two segments were areas of sperm maturation, while the last one would represent a storage region, as previously reported in the rabbit epididymis[6]. The epididymal epithelium plays a crucial role in sperm maturation, motility, and fertility through its absorptive and secretory activities, providing an optimal environment for these processes[3]. The epididymal duct is lined with a pseudostratified epithelium that includes principal cells, basal cells, apical cells, clear cells, lymphocytes, and macrophages (the latter two often referred to as halo cells). Principal cells make up about 80% of the epithelial cells in the epididymis[7]. These principal cells are tall columnar cells with stereocilia. The tubular epithelium is encased by a thin layer of lamina propria composed of loose connective tissue, followed by a smooth muscle coat. Circumtubular connective tissue covers the duct. The epididymis luminal diameter and sperm content increase progressively from the caput to the cauda[7].

The enzymes are the key molecules for cellular functions such as energy production, membrane transport, and metabolism. Insect enzyme activities were involved directly in maturation, concentration, and motility of spermatozoa[8]. Mammalian alkaline phosphatase (ALP) is a membrane-bound ectoenzyme attached to cells by phosphatidylinositol glycan anchors[9]. It responsible for dephosphorylation reactions that may be essential for normal male reproductive physiology and reflect germ cell function[10]. Acid phosphatase (ACP) is a marker enzyme for lysosomes, and it is concerned with the catabolic processes, which take place within the cells and regulate the annual male reproductive cycle in many species; e.g. lizards[11]. Also, it might be involved in the metabolism of phosphate compounds important to flagellar motility[8]. Glucose-6-phosphatase (G-6-Pase) is a multifunctional enzyme, displaying the ability to synthesize and hydrolyze glucose-6-phosphate to glucose for providing energy for different functions[12]. Its activity was localized in the endoplasmic reticulum, and at the outer membrane of the nuclear envelop[13]. Adenosine triphosphatase (ATPase) is an integral part of the cell membrane, plays important roles in intracellular functions and physiological activities[14]. Many basic and specialized cell functions have been attributed to Na, K-ATPase[14]. Inhibition of Na, K-ATPase lead to loss of sperm motility that causes infertility of sperm[15]. The 5`-nucleotidase (5`-Nase) is an enzyme, which has a glycosylphosphatidylinositol (GPI)-anchored structure, and produces adenosine from adenosine 5`-monophosphate[16]. Adenosine in the sperm microenvironment has been reported to play an important role in regulating sperm proper maturation, motility, and function[16]. Several mammalian 5`-Nases attached to membranes or present in the cytosol[17].

The histochemistry of the epididymis has been investigated in several laboratory and domestic species[18,19]. Although literature is available on the histoenzymic localization of phosphatases in bovine testis[20,21] and buffalo calves[22], relatively scare reports are available on qualitative and quantitative of enzymes in the camel epididymis in relation to age, pubertal stage, and seasonal variation of the male dromedary camel. Therefore, the present investigation carried out to study the effects of puberty stage and seasons of the year on localization and quantitative activities of phosphatases enzymes (ALP, ACP, G-6-pase, ATPase, and 5`-Nase) that play an essential role in sperm maturation

and fertility, as well as their potential as indicators of reproductive health and the functional state of the epididymis of the male dromedary camel.

MATERIAL AND METHODS

The present study was conducted in the Laboratory of Cell Biology and Histology, Department of Zoology, Faculty of Sciences, in cooperation with the Biotechnology Lab, Faculty of Agriculture, Mansoura University, Egypt. A total of 40 heparinized blood samples and epididymis of male, one-humped, camels (*Camelus dromedarius*) were collected from Bilbase slaughterhouse after slaughter in cool box during the breeding season (December-April) and non-breeding season (June-August). Blood and tissue samples were taken from camels at pre-pubertal (1.0-3.5 years old) and post-pubertal ages (4.0-11.0 years old). Five phosphatases' enzymes: ALP, ACP, G-6-Pase, ATPase, and 5`-Nase were histo-chemically and biochemically demonstrated.

Histochemical studies

Frozen cryostat sections (10 µm thickness) were used to demonstrate the localization and activities of the mentioned enzymes according to the following methods: the negative control sections were incubated in a substrate free medium.

Determination of ALP activity according to Anwer *et al.*[23]

Frozen sections of epididymis were fixed in a mixture of 95% methanol and 4% formalin for about 30 minutes and washed in distilled water. The media stock solution contains 150 mL of 0.2 mol tris-buffer + 25 mL of 0.05 mol P-glycerophosphate + 50 mL distilled water + 6 mL of 1.0% L-tartaric acid at pH 9.5. The sections were transferred to the following mixture for about 2 hours: 45 mL of the previous stock solution + 5 mL of 1.0% lead nitrate + 0.5 mL of 0.05 mol magnesium chloride.

Determination of ACP activity according to Tice and Barrnett[24]

Frozen sections of epididymis were fixed in a mixture of 25 mL methanol + 20 mL acetone + 5 mL formalin for about 2 hours, and then washed in distilled water. The specimens were incubated in the following mixture: 20 mL of 0.05 mol acetate buffer + 3 mL of 1.0% lead nitrate + 2 mL of 0.05 mol P-glycerophosphate for about 2 hours.

Determination of G-6-Pase activity according to Tice and Barrnett[24]

Frozen sections of epididymis were fixed in 2.5% glutaraldehyde (pH 7.4) for 3-4 minutes at 4°C, washed in distilled water, incubated in the following mixture for 30 minutes at 37°C: 20 mL of 0.1 mol acetate buffer at pH 6.4 + 3 mL of 1.0% lead nitrate + 2 mL of 0.05 mol glucose-6-phosphate disodium salt.

Determination of ATPase activity according to Wachstein and Meisel[25]

Frozen sections of epididymis were fixed in a mixture of 2.5% glutaraldehyde (pH 7.4) for 5-7 minutes at 4°C. They were washed with distilled water and incubated in the following mixture containing specific substrate: 2 mL of 0.2 mol tris-maleate buffer pH 7.4 + 8.5 mL distilled water + 3 mL of 1.0% lead nitrate + 1.0 mL of 0.1 mol magnesium chloride + 2.5 mL of 0.05 mol adenosine-5-tri-phosphate disodium salt for 1.0 hour at 37°C.

Determination of 5'-Nase activity according to Wachstein and Meisel[25]

Frozen sections of epididymis were fixed in a mixture of 2.5% glutaraldehyde (pH 7.4) for 5-7 minutes at 4°C. The specimens were washed in distilled water and incubated in the following mixture for 1.0 hour at 37°C: 12 mL of 0.2 mol of tris-maleate buffer, pH 7.4 + 8.5 mL distilled water + 3.5 mL of 1.0% lead nitrate + 1.0 mL of 0.1 magnesium chloride + 2.5 mL of 0.05M adenosine-5'-monophosphate disodium salt. All the sections of the different specimens treated for demonstrating the different enzymes were washed in distilled water and stained in 1.0% ammonium sulfide for 2-3 minutes. After a further washing in distilled water the specimens were mounted in neutral glycerin

jelly and examined by using bright field microscopy.

Biochemical assays

The caput and caudal regions (2 g) of pre-pubertal and post-pubertal ages in two seasons were washed with saline (0.9% NaCl), blotted on a piece of filter paper, weighed and homogenized in ice cold distilled water to yield (10% weight/volume) using a Potter-Elvehjen glass homogenizer fitted with a Teflon pestle. The freshly prepared homogenate was used for biochemical assays. The homogenates were centrifuged at 10000 ×*g* for 10 minutes at 4°C using a HERMLE Z306 centrifuge (HERMLE Labortechnik GmbH, Wehingen, Germany), and the supernatants were aspirated for biochemical assays. The ALP, ACP, G-6-Pase, ATPase, and 5`-Nase enzyme activities in tissue homogenate and plasma were measured according to methods of Belfield and Goldberg[26], Wootton and King[27], Swanson[28], LeBel *et al*.[29] and El-Aaser and El-Merzabani[30], respectively.

Statistical analysis

The obtained data were analyzed statistically by a two-way ANOVA using SAS System for Windows, version 6.12 (SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test was used to determine the least significant difference among the means.

RESULTS

Effects of puberty and breading season on the histochemical localization and activity of ALP in the camel epididymis

The activity of ALP was demonstrated in the epithelium cells, sub epithelium, stereocilia and luminal contents in the epididymis. The intensity of ALP staining varied across different regions; also, its localization changed depending on the region. In pre-pubertal camels during non-breeding season ALP was intensive along the circumtubular smooth muscle cells and the basal membrane in caput region (Figure 1A), and some cells wedged between principal cells showed activity. It was not possible to identify these cells with any degree of certainty. No activity of ALP was found in the lumen of caput. However, in caudal region, ALP activity was intensive along the basal lamina, and apical region of the epithelium, while the middle epithelial cells and stereocilia showed weak to moderate reaction. The lumen showed positive reaction (Figure 1B). In post-pubertal camels during non-breeding season, ALP was intensive along the circumtubular smooth muscle cells and the basal membrane in caput region. The principle epithelial cells showed weak reaction, but the stereocilia showed intensive reaction to ALP. However, the lumen of caput showed negative reaction (Figure 1C). In the caudal region, the circumtubular smooth muscle cells showed moderate reaction, and the apical region of the epithelium showed very intensive reaction, while the luminal contents showed almost intensive reaction (Figure 1D). The endothelial cells of intertubular blood vessels had a moderate to strong reaction in both regions (Figures 1C, D).

In the caput region of pre-pubertal camels during breeding season, the activity of ALP was intensive in the basement membrane, circumtubular smooth muscle cells, and the endothelial cells of intertubular blood vessels (Figure 1E). However, in the caudal region, the luminal contents showed very intensive reactivity to ALP, while the circumtubular smooth muscle cells, endothelial cells of intertubular blood vessels, and basal epithelial cells showed moderate reactivity (Figure 1F). In post-pubertal camel during breeding season, the caput region showed intensive ALP activity in the basal epithelium, stereocilia, and circumtubular smooth muscle layer. Also, intensive reaction was observed in some parts of the lumen of the tubules, while the principal cells of the epithelial layers showed no reactivity (Figure 1G). However, in the caudal region, the basal and apical epithelium showed intensive reactivity, while the principal cells, stereocilia, and circumtubular smooth muscle layer showed moderate reaction. The luminal

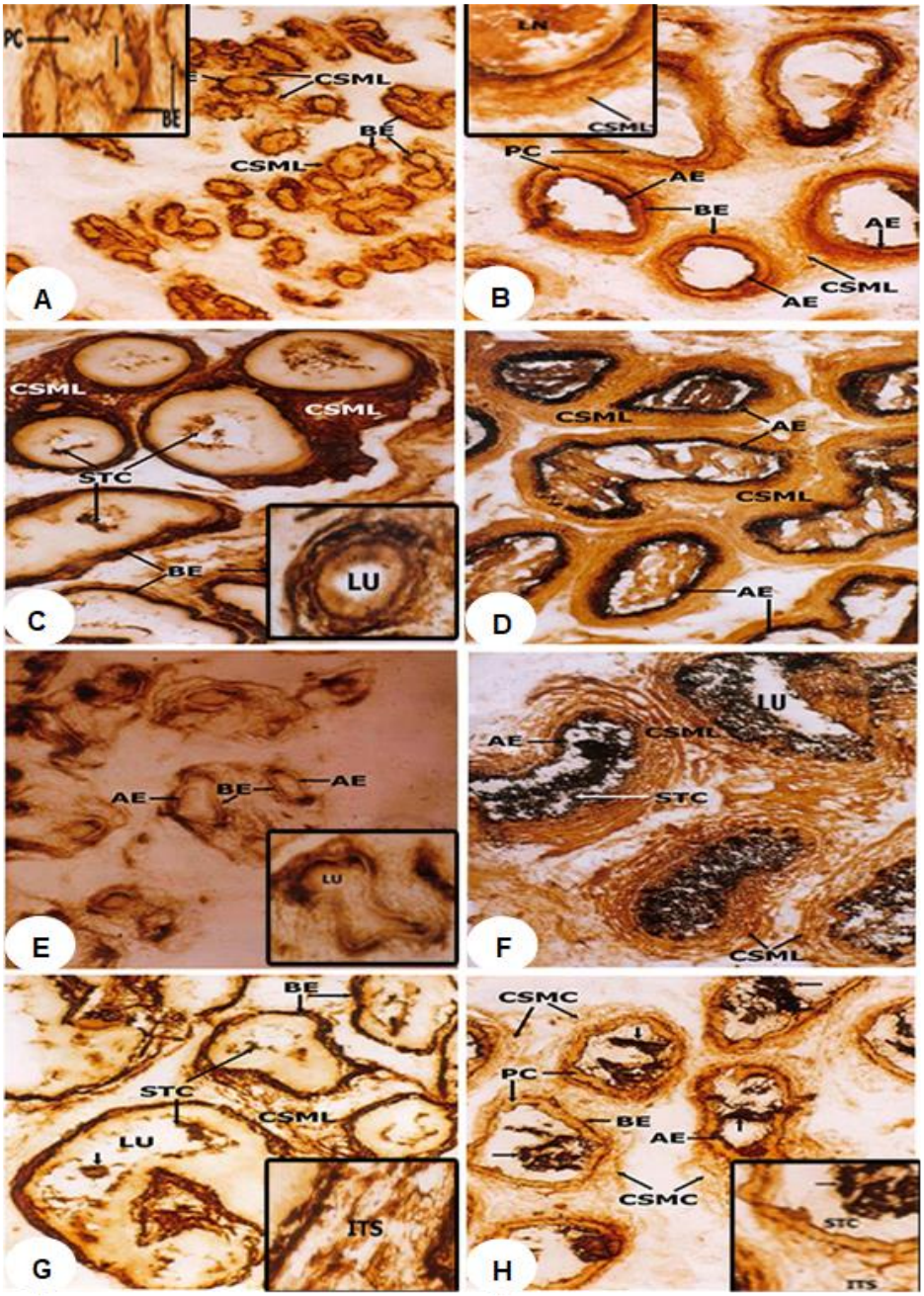

Figure 1: Photomicrographs of frozen cryostat sections for demonstrating alkaline phosphatase (ALP) activity in caput and caudal regions, respectively, of epididymis of pre-pubertal camel in non-breeding season **(A** and **B)**, post-pubertal camel in non-breeding season **(C** and **D)**, pre-pubertal camel in breeding season **(E** and **F)**, and post-pubertal camel in breeding season **(G** and **H)**. **(A)** Showing intensive ALP activity in the circumtubular smooth muscle layer (CSML) and basal epithelium border (BE; ×40); higher magnification showing intensive ALP in the basal epithelium border (BE; ×100). PC: principal cells. **(B)** Showing intensive ALP activity in BE and the apical epithelium borders (AE), moderate ALP activity in CSML and PC (×40); higher magnification showing moderate activity of ALP in the lumen (LU) of caudal region and CSML (×100). **(C)** Showing intensive ALP activity in CSML, BE, and stereocilia (STC; ×40); higher magnification showing no reactivity of ALP in LU of caput (×100). **(D)** Showing moderate ALP activity in CSML, and intensive reaction in AE and LU of caudal region (×40). **(E)** Showing intensive reactivity of ALP in BE and AE (×40); higher magnification showing no ALP reactivity in LU of the caput (×100). **(F)** Showing moderate ALP activity in CSML and intensive reaction in STC, AE, and LU of caudal region (×40). **(G)** Showing intensive reactivity of ALP in CSML, BE, STC, and some parts of LU (arrow; ×40); higher magnification showing intensive ALP activity in the intertubular tissues (ITS) and in some parts of LU of some tubules (×100). **(H)** Showing intensive ALP activity in BE and AE, weak reaction in PC and CSML, and very intensive reaction in LU of tubules (arrow; ×40); higher magnification showing weak reaction in STC and ITS, while very intensive reaction was observed in LU of tubules (arrows; ×100).

contents showed a very intensive reaction (Figure 1H). In general, the activity of ALP was higher in caudal than in caput region in pre- and post-pubertal camels in both seasons.

Data presented in Tables "1 and 3" indicated that the activity of ALP in blood plasma in pre-pubertal camels were significantly ($P \leq 0.05$) higher than those in post-pubertal camels. However, the activity of ALP of caudal epididymal tissues in post-pubertal camels were significantly ($P \leq 0.05$) higher than those in pre-pubertal camels (Tables 1 and 4). During breeding season there was insignificant change in the ALP activity of plasma and epididymal tissues, except caput region. The results obtained showed also higher activity in the caudal region compared with the caput region in both seasons and at both ages.

Table 1: Effects of season, stage, and their interaction on alkaline phosphatase (ALP), acid phosphatase (ACP), and glucose-6-phosphatase (G-6-Pase) enzymes activities in plasma and tissues of caput and caudal regions (means ± their standard error).

		ALP			ACP			G-6-Pase		
		Plasma (U/L)	Caput (U/mg tissue)	Caudal (U/mg tissue)	Plasma (U/L)	Caput (U/mg tissue)	Caudal (U/mg tissue)	Plasma (U/L)	Caput (U/g tissue)	Caudal (U/g tissue)
Season	(A)	74.6± 5.9	130.0± 16.0	422.0± 129.1	4.6± 0.7	87.3± 58.3	177.0± 55.3	376.0± 84.2	0.69± 0.07	0.27± 0.04
	(B)	65.9± 8.1	220.0± 51.9	387.3± 54.22	5.7± 0.6	83.1± 38.4	152.7± 12.7	650.0± 120.7	0.52± 0.05	0.45± 0.14
Stage	(C)	81.4± 2.5[a]	171.7± 56.2	275.5± 54.2[b]	4.8± 1.0	16.8± 1.5	118.6± 22.2	654.9± 130.9[a]	0.68± 0.08	0.25± 0.05
	(D)	61.0± 7.5[b]	178.3± 24.4	534.6± 100.5[a]	5.4± 0.4	153.6± 54.8	211.1± 43.9	371.7± 69.5[b]	0.53± 0.03	0.47± 0.13
Interaction	A×C	82.1± 4.4	108.7± 21.9	164.8± 46.8	3.4± 1.1	16.4± 3.3	98.0± 45.1	530.8± 127.6	0.80± 0.11	0.22± 0.06
	A×D	67.1± 10.2	151.2± 18.8	680.7± 121.0	5.8± 0.6	158.2± 109.5	256.0± 83.6	221.2± 29.1	0.57± 0.02	0.32± 0.04
	B×C	80.9± 2.0	234.7± 106.5	386.2± 15.1	6.9± 0.9	17.2± 0.2	139.1± 1.6	820.5± 251.7	0.55± 0.09	0.27± 0.08
	B×D	54.8± 11.6	205.3± 43.5	388.5± 120.3	5.0± 0.6	149.1± 54.8	166.2± 25.0	522.2± 81.4	0.48± 0.06	0.62± 0.24

A: Non breeding season, B: Breeding season, C: Pre-pubertal, D: Post-pubertal. Means in the same column with different letters (e.g., 'a' and 'b') differ significantly at $P \leq 0.05$.

Effects of puberty and breading season on the histochemical localization and activity of ACP in the camel epididymis

In pre-pubertal camels during non-breeding season, the activity of ACP was strong in the apical and basal borders of the epithelium cells of caput, while the basal epithelium of cauda was weakly reacted. The apical epithelium and luminal contents showed moderate to intensive reaction. The circumtubular smooth muscle cells and inter tubular tissue showed weak to moderate reactivity to ACP (Figure 2A, B). In post-pubertal camels during the non-breeding season, the granules in the supranuclear and apical zones of the epithelial cells (principal cells) in the caput exhibited the strongest ACP activity and appeared larger than those scattered throughout the rest of the cytoplasm (Figure 2C). The peritubular smooth muscles and intertubular tissue showed no ACP activity in both the caput and cauda regions. A strong positive reaction was observed in the luminal content of the cauda, while the tubules in the caput were negative. The stereocilia in the cauda exhibited weak to moderate ACP reactivity (Figure 2C, D).

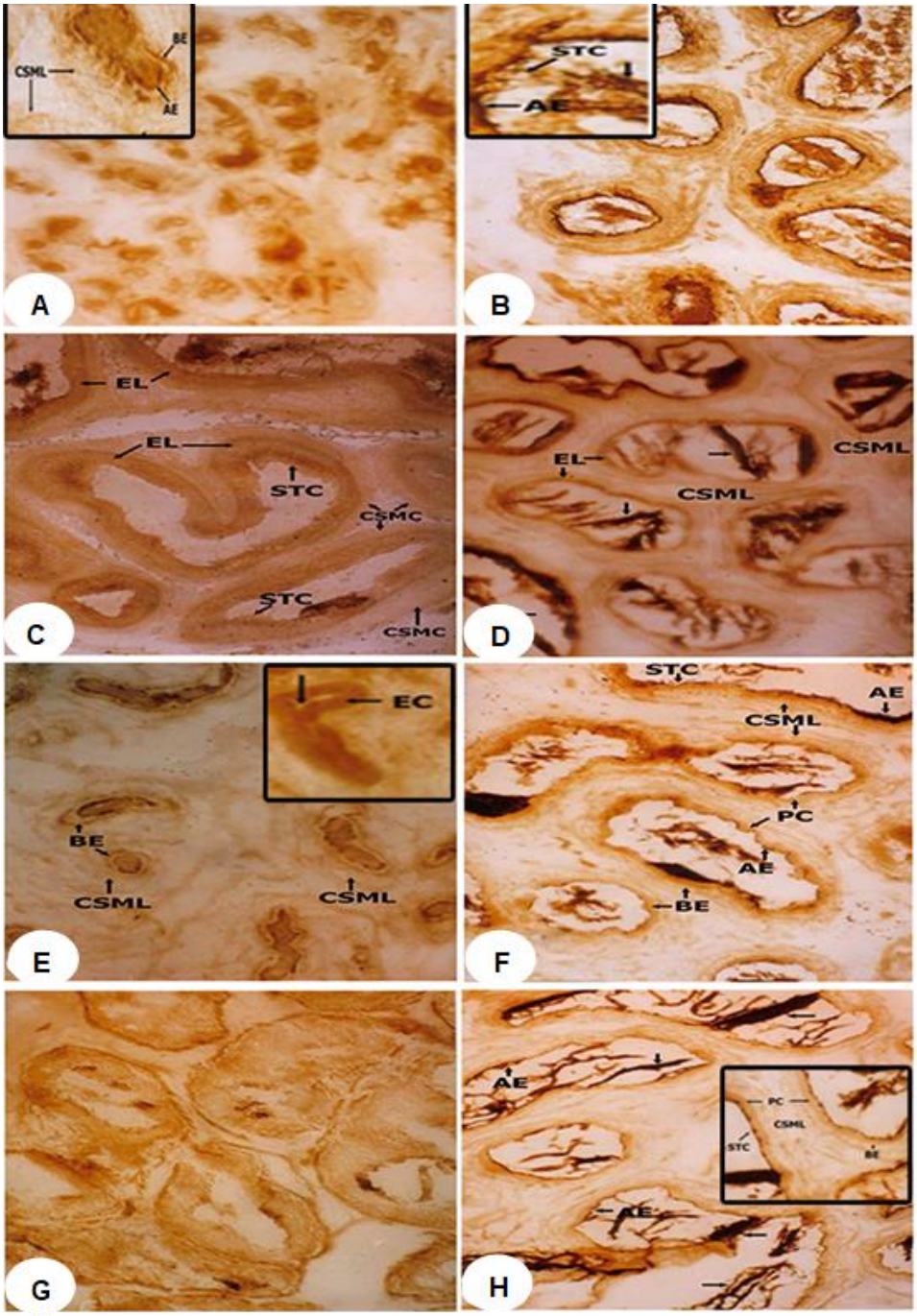

Figure 2: Photomicrographs of frozen cryostat sections for demonstrating acid phosphatase (ACP) activity in caput and caudal regions, respectively, of epididymis of pre-pubertal camel in non-breeding season **(A** and **B)**, post-pubertal camel in non-breeding season **(C** and **D)**, pre-pubertal camel in breeding season **(E** and **F)**, and post-pubertal camel in breeding season **(G** and **H)**. **(A)** ×40; Higher magnification showing intensive reaction of the basal (BE) and apical (AE) epithelial borders and weak to moderate reaction of the circumtubular smooth muscle layer (CSML) to ACP (×100). **(B)** ×40; Higher magnification showing intensive reaction of AE and lumen (arrow), and weak reaction of CSML, BE, principal epithelium (PC), and stereocilia cells (STC) to ACP (×100). **(C)** Showing weak ACP activity in CSML and STC, while epithelium layer (EL) had moderate reactivity. The lumen of some tubules showed extrusion of enzyme, and intensive ACP activity was found in the lumen of some tubules (×40). **(D)** Showing no reaction in CSML, weak reaction in EL, and intensive ACP activity in the lumen of the tubules (arrows; ×40). **(E)** Showing weak reaction in CSML, and positive reaction in BE (×40); higher magnification showing no reaction in the lumen of the tubules (arrow), but the epithelial cells (EC) showed positive reaction to ACP (×100). **(F)** Showing intensive reaction to ACP in AE and the lumen (arrows), and weak reaction in CSML, BE, and STC (×40). **(G)** Showing moderate reaction to ACP activity (×40). **(H)** Showing intensive reaction AE and the lumen (arrows; ×40); higher magnification showing weak reaction to ACP in CSML, BE, PC, and STC (×100).

In pre-pubertal camels during breeding season the activity of ACP was intensive in epithelial cells and weak in the circumtubular smooth muscles cells. The lumen of the caput was free of the enzyme activity (Figure 2E). In caudal region, intensive reaction was noticed in apical epithelium and in the lumen, while weak reaction was found in the circumtubular smooth muscles cells and principal cells (Figure 2F). However, in post-pubertal camels during breeding season the basal membrane and stereocilia of the caput showed moderate reaction to ACP and the luminal content was free of the enzyme (Figure 2G). The apical epithelium and luminal content of the caudal region had strong reaction to ACP (Figure 2H). The circumtubular smooth muscle, intertubular tissue, and endothelial cells of intertubular blood vessels had negative reaction in both caput and caudal regions (Figure 2G, H).

The activity of ACP enzyme was shown in Table "1", the activity of plasma enzyme was insignificantly changed in breeding and non-breeding seasons. In addition, the caput and caudal tissues showed insignificant changes in enzyme activity during breeding and non-breeding seasons. The enzyme activity increased in caudal compared with caput in both pre- and post-pubertal ages, and in breeding and non-breeding season. Generally, the activity of ACP showed an insignificant change as affected by seasons and stage of puberty (Tables 1, 3, and 4).

Effects of puberty and breading season on the histochemical localization and activity of G-6-Pase in the camel epididymis

During the non-breeding season, the localization of G-6-Pase activity in the epididymis of both pre- and post-pubertal camels was similar. The luminal border of epithelial cells showed strong positive activity, the basement membrane was also reactive, and the circumtubular smooth muscle cells had a weak to moderate reaction (Figure 3A, B). The G-6-Pase activity in the caput was higher in pre-pubertal camels than in post-pubertal ones, while in the cauda, the activity was greater in post-pubertal camels. The enzyme distribution in the epithelial and smooth muscle cells of the cauda is like that of the caput. However, the lumens of tubules in pre-pubertal camels were non-reactive, whereas those in post-pubertal camels showed moderate reactivity due to the presence of spermatozoa (Figure 3). During the rutting season, G-6-Pase activity in the cauda of post-pubertal camels was higher than in pre-pubertal ones. Additionally, the enzyme activity in the tubule lumens of the caudal region was greater during the breeding season compared to the non-breeding season (Figure 3).

Data in Table "1" showed that the activity of G-6-Pase in plasma was significantly ($P \leq 0.05$) increased in pre-pubertal than post-pubertal camels and was insignificantly changed in the breeding and non-breeding seasons. However, the activity of G-6-Pase in epididymal tissues did not differ significantly (Tables 1, 3, and 4).

Effects of puberty and breading season on the histochemical localization and activity of ATPase in the camel epididymis

In pre- and post-pubertal camels, the epithelium of the epididymal tubules, the interstitial, and lumen were positively reacted to ATPase, as well as spermatozoa in the lumen were reacted. The activity of ATPase in the epididymal epithelium was located apically (Figure 4A-D). In non-breeding season, the intensity of ATPase activity in caput of pre-pubertal camels was slightly more intensive than in post-pubertal camels, while ATPase activity in caudal region of post-pubertal camels increased as compared to that in pre-pubertal camels, especially in the luminal epithelial cells and interstitial cells (Figure 4). In breeding season, the ATPase activity in caput of pre-pubertal was higher than in post-pubertal camels. However, reactivity of ATPase in caudal region of post-pubertal camels' epididymis was higher than in the pre-pubertal camels. Rutting season was associated with increased ATPase activity in studied epididymal segments (Figure 4E-H).

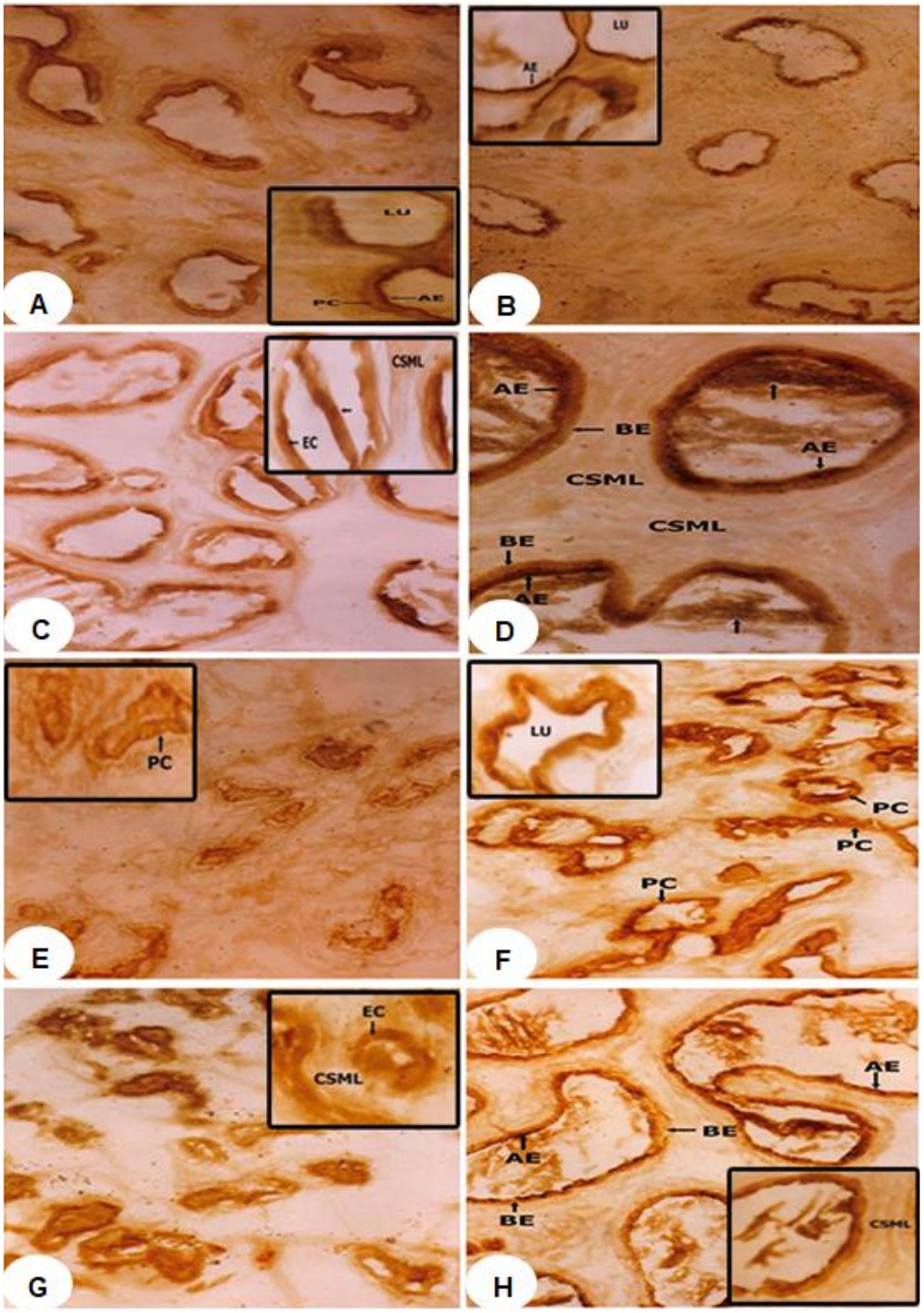

Figure 3: Photomicrographs of frozen cryostat sections for demonstrating glucose-6-phosphatase (G-6-Pase) activity in caput and caudal regions, respectively, of epididymis of pre-pubertal camel in non-breeding season **(A** and **B)**, post-pubertal camel in non-breeding season **(C** and **D)**, pre-pubertal camel in breeding season **(E** and **F)**, and post-pubertal camel in breeding season **(G** and **H)**. **(A)** ×40; Higher magnification showing intensive reaction of G-6-Pase activity in apical epithelium border (AE), principal cells (PC), and weak to moderate reaction in the surrounding muscles. The lumen (LU) was free of the enzyme activity (×100). **(B)** ×40; Higher magnification showing intensive reaction of G-6-Pase activity in AE and weak reaction in the circumtubular smooth muscle layer (CSML). LU was free of the enzyme reactivity (×100). **(C)** ×40; Higher magnification showing intensive activity of G-6-Pase in the epithelial cells (EC) and the luminal contents (arrow), and weak reaction in CSML (×100). **(D)** Showing high activity of G-6-Pase in AE and the luminal contents (arrows), moderate reaction in the basal epithelium (BE) and weak reaction in CSML (×100). **(E)** ×40; Higher magnification showing intensive reaction of G-6-Pase in PC (×100). **(F)** Showing G-6-Pase activity in PC (×40); higher magnification showing no reactivity of G-6-Pase in LU and weak reaction in CSML (×100). **(G)** ×40; Higher magnification showing moderate reactivity to G-6-Pase in EC, weak reaction in CSML, and no reactivity was found in LU (×100). **(H)** Showing very intensive reaction in AE and moderate reaction in BE (×40); higher magnification showing weak reactivity for G-6-Pase in CSML (×100).

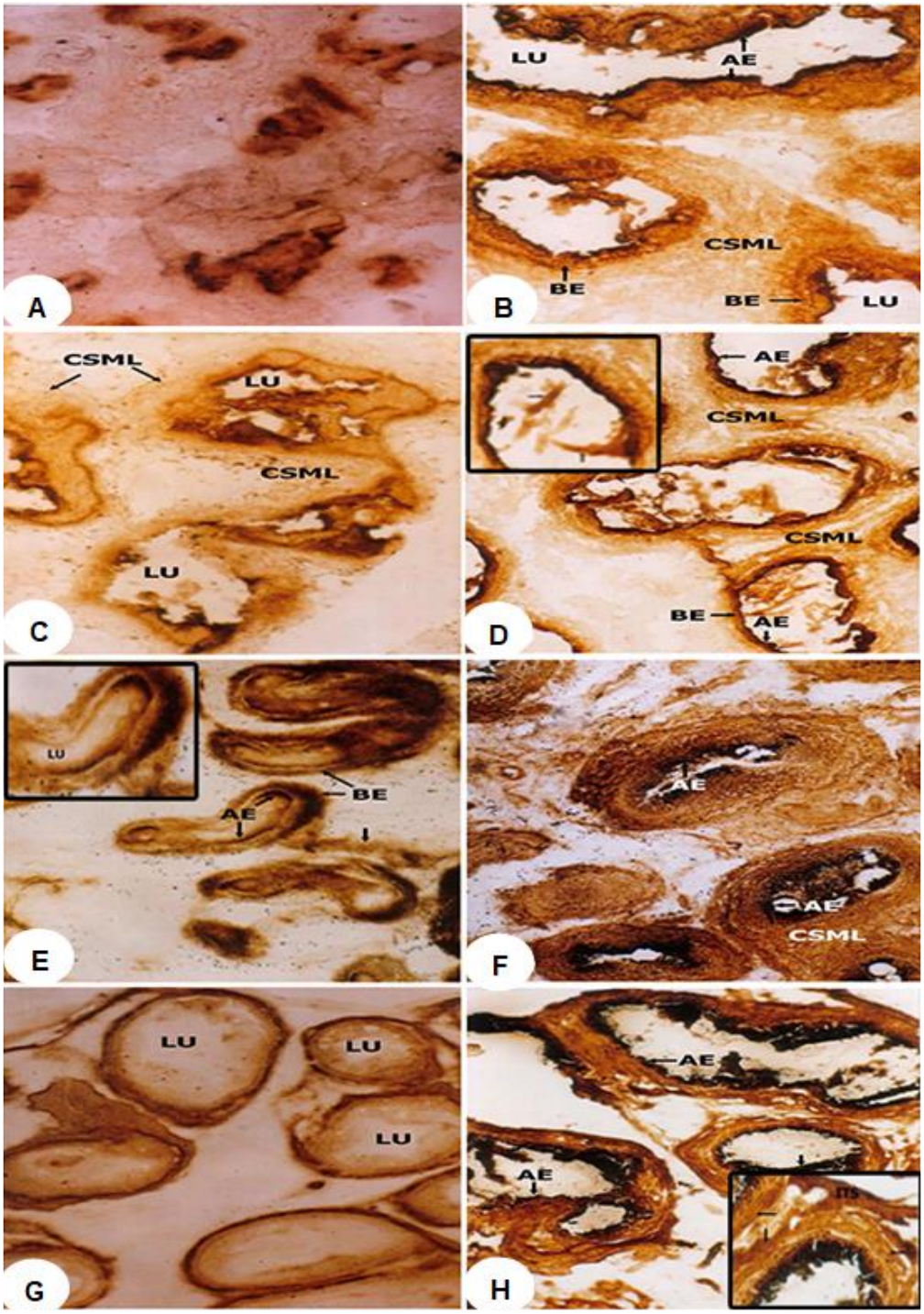

Figure 4: Photomicrographs of frozen cryostat sections for demonstrating adenosine triphosphatase (ATPase) activity in caput and caudal regions, respectively, of epididymis of pre-pubertal camel in non-breeding season **(A** and **B)**, post-pubertal camel in non-breeding season **(C** and **D)**, pre-pubertal camel in breeding season **(E** and **F)**, and post-pubertal camel in breeding season **(G** and **H)**. **(A)** Showing intensive reaction for ATPase in the tubules and weak reaction in inter-tubular tissues (×100). **(B)** Showing intensive reaction to ATPase in the apical epithelium border (AE), moderate reaction in the basal epithelium border (BE), weak to moderate in the circumtubular smooth muscle layer (CSML), and no reaction in the lumen (LU; ×100). **(C)** Showing intensive reaction in apical epithelium, no reactivity of ATPase in LU, and weak reaction in CSML (×100). **(D)** Showing intensive reaction in AE and moderate reaction in BE and CSML to ATPase (×40); higher magnification showing weak to moderate reaction in some LU (×100). **(E)** Showing intensive reaction in AE and BE and some parts of CSML (arrow; ×40); higher magnification showing no activity in LU (×100). **(F)** Showing very intensive reaction in AE and CSML (×40). **(G)** Showing no reaction in LU (×40). **(H)** Showing very intensive reaction in AE and some parts of LU (arrow; ×40); higher magnification showing intensive reaction in AE, high activity in CSML (arrows) and intertubular tissues (ITS; ×100).

The activity of ATPase in plasma and epididymal regions was higher during the breeding season compared to the non-breeding season, with a significant difference ($P \leq 0.05$) observed only in the caput (Tables 2-4). Additionally, the caudal region showed a significant increase ($P \leq 0.05$) in post-pubertal camels, while the caput region exhibited the opposite trend (Tables 2-4).

Table 2: Effects of season, stage, and their interaction on adenosine triphosphatase (ATPase) and 5`-nucleotidase (5`-Nase) enzymes activities in plasma and tissues of caput and caudal regions (means ± their standard error).

		ATPase			5`-Nase		
		Plasma (U/L)	Caput (U/g tissue)	Caudal (U/g tissue)	Plasma (U/L)	Caput (U/g tissue)	Caudal (U/g tissue)
Season	(A)	231.2± 52.2	0.33± 0.05[b]	0.89± 0.13	334.2± 92.0	0.87± 0.16	0.20± 0.04[b]
	(B)	252.8± 16.5	0.48± 0.10[a]	0.90± 0.17	432.5± 89.5	0.69± 0.13	0.45± 0.11[a]
Stage	(C)	231.5± 45.1	0.47± 0.11[a]	0.63± 0.09[b]	335.3± 94.0	0.54± 0.09[b]	0.39± 0.13
	(D)	249.9± 37.6	0.34± 0.05[b]	1.17± 0.09[a]	419.3± 89.5	1.02± 0.12[a]	0.26± 0.03
Interaction	A×C	209.5± 81.8	0.26± 0.04	0.60± 0.09	218.1± 52.6	0.60± 0.07	0.17± 0.04
	A×D	252.9± 75.5	0.25± 0.07	1.12± 0.03	450.4± 166.4	1.14± 0.21	0.23± 0.04
	B×C	260.7± 9.1	0.69± 0.02	0.66± 0.18	491.6± 187.0	0.48± 0.18	0.61± 0.19
	B×D	246.9± 29.7	0.42± 0.05	1.21± 0.20	388.1± 94.9	0.90± 0.10	0.29± 0.03

A: Non breeding season, B: Breeding season, C: Pre-pubertal, D: Post-pubertal. Means in the same column with different letters (e.g., 'a' and 'b') differ significantly at $P \leq 0.05$.

Table 3: Analysis of variance of effects of season, stage, and their interaction on enzymes activity in plasma.

	Mean square				
	ALP	ACP	G-6-Pase	ATPase	5`-Nase
Season	282.1	5.0	280437.6	1750.7	36011.8
Stage	1565.3*	1.3	299525.9*	1269.9	26331.3
Interaction	21.2	14.4	44707.8	2823.7	100007.5
Error	283.0	2.6	60468.8	14536.1	62132.4

ALP: alkaline phosphatase, ACP: acid phosphatase, G-6-Pase: glucose-6-phosphatase, ATPase: adenosine triphosphatase, 5`-Nase: 5`-nucleotidase, *: significant ($P \leq 0.05$).

Table 4: Analysis of variance of effects of season, stage, and their interaction on enzymes activities in epididymal regions.

	Mean square									
	Caput					Caudal				
	ALP	ACD	G-6-Pase	ATPase	5`-Nase	ALP	ACD	G-6-Pase	ATPase	5`-Nase
Season	24304.5	51.5	0.08	0.06*	0.10	3770.5	1777.8	0.09	0.0	0.19*
Stage	129.8	56166.0	0.07	0.05*	0.69*	201369.9*	25692.8	0.15	0.9**	0.05
Interaction	3881.2	72.0	0.02	0.3**	0.01	197887.5*	12868.1	0.05	0.02	0.11
Error	10548.1	11249.3	0.02	0.01	0.07	23645.5	7233.9	0.05	0.01	0.03

ALP: alkaline phosphatase, ACP: acid phosphatase, G-6-Pase: glucose-6-phosphatase, ATPase: adenosine triphosphatase, 5`-Nase: 5`-nucleotidase, *, **: significant ($P \leq 0.05$ and $P \leq 0.01$, respectively).

Effects of puberty and breading season on the histochemical localization and activity of 5`-Nase in the camel epididymis

In non-breeding season, the epithelial cells and the lumen content of caput in the young camels showed intensive reaction to 5`-Nase, while the muscular layer showed weak reaction (Figure 5A). Caput of adult camels showed more intensive reaction to 5`-Nase than in young camels. The reaction in the stereocilia, apical epithelium and the lumen content, the basement, and middle epithelium showed intensive reactivity. However, the circular muscles fiber showed weak reactivity (Figure 5C). The activity of 5`-Nase in caudal region in pre-pubertal camels was strongest in the apical area of the epithelial cells and basal membrane, while the circumtubular smooth muscles showed moderate reaction (Figure 5B). The same localization of 5`-Nase activity was found in caudal region of adult camels. In addition, the lumen content showed moderate reactivity to 5`-Nase (Figure 5D).

In breeding season, the 5`-Nase of caput in young camels showed intensive reactivity in the lumen border and the apical epithelial cell layer. The basal epithelium and basal membrane showed moderate reaction, while circumtubular smooth muscles showed weak to moderate reactivity (Figure 5E). The same distribution of 5`-Nase activity was found in caput of the adult camels, but the intensity was more in adult than young camels (Figure 5E, G). In the caudal region, the activity of 5`-Nase was intensive in the basal epithelial cells. The circumtubular smooth muscles showed weak to moderate reactivity, while the lumen content showed negative reactivity to 5`-Nase. The same distribution was found in the caudal of post-pubertal camels, but the intensity was lower in post-pubertal camels than that in the pre-pubertal camels (Figure 5F, H).

The activity of 5`-Nase is shown in Tables (2-4), revealing that the activity in plasma insignificantly changed by seasons and stage of puberty. The activity of 5`-Nase in caput significantly ($P \leq 0.05$) increased in post-pubertal camels than pre-pubertal camels. However, in the caudal region, the enzyme activity was significantly ($P \leq 0.05$) higher in breeding than non-breeding season.

DISCUSSION

The epididymis is a vital part of the male reproductive system, responsible for the storage, maturation, and survival of sperm until ejaculation, as well as maintaining the blood-epididymal barrier[31]. The epididymis environment results from intraepithelial ion exchange and secretory and absorptive activities of the epithelium that is regulated by androgenic hormones[32]. Studying seasonal histochemical changes in the epididymis can help identify a distinct reproductive period with optimal reproductive functions. During this peak period, a successful breeding program, including mating and semen collection, can be executed to enhance dromedary camel conception rates and the development of assisted reproductive techniques[33].

In the present study the activity of ALP was demonstrated throughout the basal and apical borders of epithelium cells with stereocilia, subepithelial connective tissue, and luminal contents of the epididymis. This runs parallel with Goyal and Vig[18] and Ariyaratna *et al*.[4] in bulls and buffalo who demonstrated heavy ALP activity along the basal and apical region of epithelium and with stereocilia. Tingari and Moniem[34] demonstrated ALP activity in the subepithelial connective tissues, blood vessels, stereocilia, and luminal contents in the epididymis of camel. Early study demonstrated ALP activity on the external or internal side of the lipid bilayer[35]. ALP is predominantly a plasma membrane enzyme, localized in the absorptive or secretory surfaces of cells[18]. The epithelium lining the caput region of the camel epididymis that shows strong ALP positivity at the apical and basal borders, likely plays a significant role in transporting testicular fluid from the lumen to the intertubular tissue[36].

The present investigation clarifies that strongly ALP reactivity in the basement membrane in all regions of epididymis. The

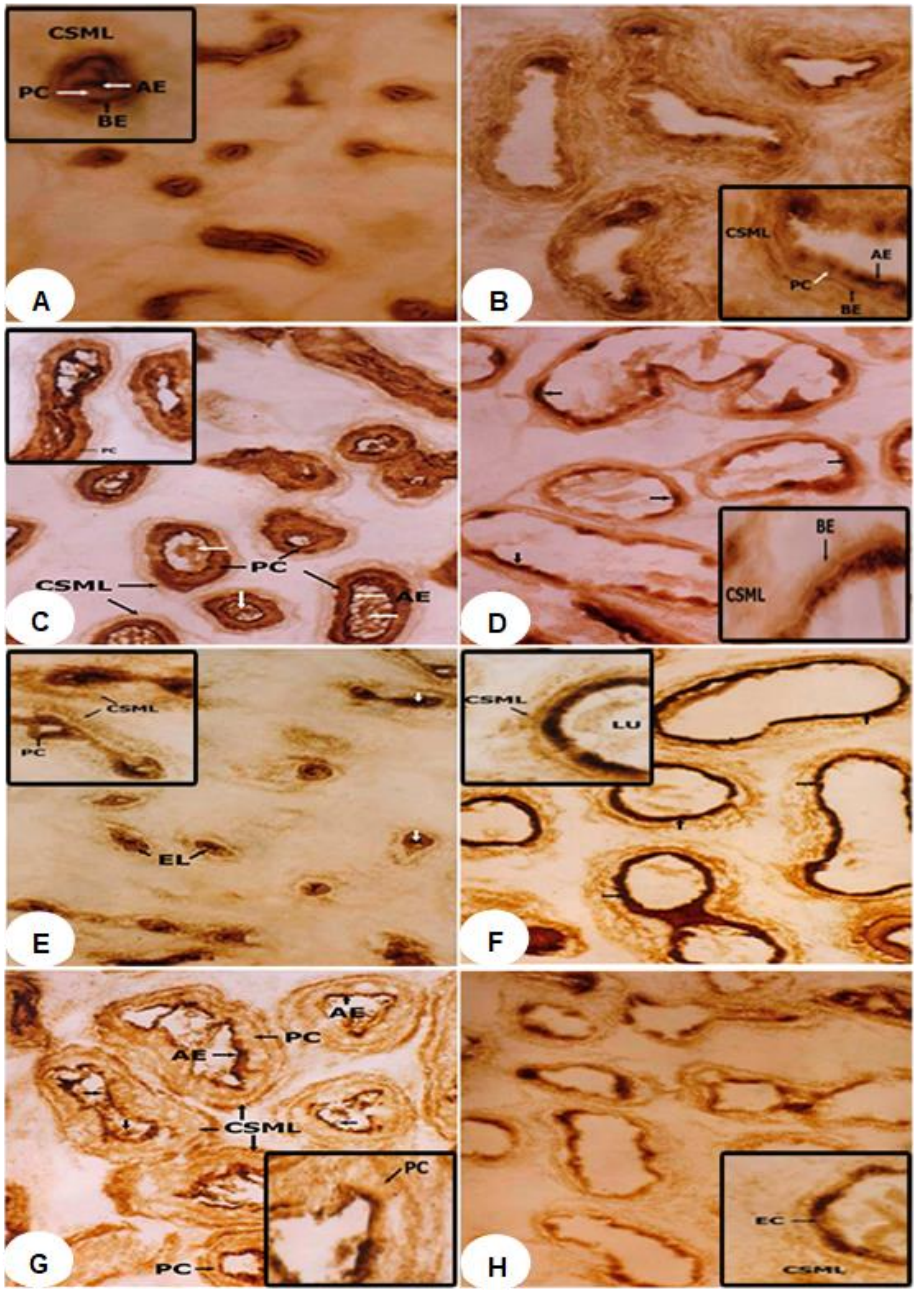

Figure 5: Photomicrographs of frozen cryostat sections for demonstrating 5`-nucleotidase (5`-Nase) activity in caput and caudal regions, respectively, of epididymis of pre-pubertal camel in non-breeding season **(A** and **B)**, post-pubertal camel in non-breeding season **(C** and **D)**, pre-pubertal camel in breeding season **(E** and **F)**, and post-pubertal camel in breeding season **(G** and **H)**. **(A)** Showing intensive reaction to 5'-Nase (×40); higher magnification showing intensive reaction to 5'-Nase in the basal (BE) and apical (AE) epithelium borders, moderate in the principal cells (PC), and weak reaction in the circumtubular smooth muscle layer (CSML; ×100). **(B)** ×40; higher magnification showing high reactivity of 5`-Nase in BE and AE, and moderate reactivity in the PC and CSML (×100). **(C)** Showing very intensive reaction in AE, intensive reaction in PC and luminal contents (arrows), and weak reactivity in CSML (×40); higher magnification showing intensive reaction to 5'-Nase in PC, and very intensive reaction in AE and some lumen contents (×100). **(D)** Showing intensive reaction to 5'-Nase in apical epithelium (arrows; ×40); higher magnification showing weak reaction to 5`-Nase in BE and CSML (×100). **(E)** Showing intensive reaction in the center of tubules (arrows) and intensive reaction in the epithelial layer (EL; ×40); higher magnification showing intensive reaction to 5'-Nase in PC, and moderate reaction in CSML (×100). **(F)** Showing very intensive reaction to 5'-Nase in the epithelial cells (EC; arrows; ×40); higher magnification showing moderate reactivity to 5`-Nase in CSML and no activity was found in LU (×100). **(G)** Showing intensive reaction to 5'-Nase in AE and stereocilia (arrows), and moderate reaction in CSML and PC (×40); higher magnification showing moderate reactivity to 5'-Nase in PC (×100). **(H)** ×40; Higher magnification showing intensive reaction to 5'-Nase in EC and weak reaction in CSML (×100).

ALP activity in luminal contents of caput gave almost negative, except the caput of adult camel in breeding season, while in caudal was intensive in almost. This may be due to that ALP could be involved directly in some steps of maturation of spermatozoa and positively correlated with sperm concentration[37]. In addition, the intensive activity of caput in rutting season may probably be because maturation of spermatozoa takes place in the caput and corpus regions and hence the increased absorptive activity and increased localization[38]. The higher ALP activity in the epithelial cells of the caudal region of the epididymis compared to the caput region indicates a parallel between increased enzyme activity and the emission/storage of epididymal spermatozoa in golden hamster[39]. The intensive ALP activity of endothelial cells in caput and luminal contents in caudal region was in breeding season. Therefore, the high activity of ALP during the reproductive season may be under hormonal control and may have some role in fertilization.

The mean of plasma ALP activity of prepubertal camels showed a significant increase compared with the postpubertal camels. Similar findings were reported in camel testis[40,41]. The high ALP activity in the pre-pubertal stage indicates a higher metabolic rate during development, as seen in young animals compared to adults due to bone development[42]. The ALP activity was increased in caudal region than caput region in both seasons and both ages. Also, Raj *et al*.[43] showed high ALP activity in caudal segment of epididymis compared to corpus epididymis, because there is a good correlation between ALP and sperm motility. The present data showed insignificant changes in the ALP activity of plasma and caudal tissues of epididymis in non-breeding and breeding seasons. On the contrary, Kataria and Bhatia[40] observed a significant increase in ALP and ACP activities in serum of dromedary camels during non-breeding season compared with the breeding season. An increase in ALP activity in caudal region of post pubertal camel during non-rutting season maintain sperm viability and functional integrity during prolonged storage, possibly by providing energy or maintaining ionic balance[39]. Conversely, during the rutting season, camels increased reproductive activity with frequent ejaculation and sperm turnover. Consequently, the demand for long-term sperm storage in the caudal region decreases, leading to reduced ALP activity as the focus shifts to producing and transporting fresh, mature sperm. The caput region of post pubertal camel during rutting season showed an insignificant change in ALP activity. However, during the non-rutting season, sperm production and maturation slow down, reducing metabolic demands in the caput region. As a result, ALP activity decreases due to the diminished need for the enzyme in initial sperm maturation processes[44]. Generally, the results of the biochemical analyses confirmed the results of histochemical analyses in the current investigation.

ACP is a key lysosomal enzyme vital for energy-intensive biological processes such as development, growth, maturation, and histolysis[45]. If ACP is located in proacrosomal vesicles, it will be significant for flagellar motility[46]. In the present study, the ACP activity was present in the borders of epithelial cells of all regions and was negative in the circumtubular smooth muscle and intertubular tissue. These results agree with that obtained by Goyal and Vig[18] and Ariyaratna *et al.*[4] who found that ACP was present in the luminal border of the epithelium of the epididymis and the most pronounced activity in the apical region epithelium and stereocilia in bulls and buffalo. The luminal content of caudal region in both pre- and post-pubertal camels in breeding and non-breeding seasons showed intensive reaction that is make ACP activity in caudal higher than caput region. These results run parallel with the results of Adams[47] who revealed that ACP activity in the regions of the epididymis of the guinea pig was increased gradually from caput to

caudal. The present data revealed that the intensity of ACP activity was more in caudal region of post-pubertal than pre-pubertal camel. The ACP activity was increased in epididymis with goat maturity[48]. The present result indicated also that ACP activity was increased in caudal when compared with caput either pre- or post-pubertal ages in both breeding and non-breeding season**.** These results run parallel with the results of Adams[47] in guinea pig. The higher ACP activity in caudal region than in the caput region regardless the age or season, indicating that the caudal region plays a more significant role of ACP in sperm maturation, preparation for fertilization or storage. A decrease in ACP activity might indicate a reduction need for the breakdown of phosphate compounds, suggesting that spermatozoa have already matured and are in storage[45]. Additionally, lower ACP activity could signify a shift in the metabolic demands of the epididymal tissues, conserving energy for other reproductive processes that are more critical during the breeding season. ACP also plays a role in regulating the reproductive cycle, and a decrease in its activity might be part of the physiological changes that prepare the reproductive system for optimal functioning during the breeding season, such as maintaining the integrity and function of the epididymal epithelium[11].

ACP activity in plasma, caput and caudal regions of post-pubertal animals was higher than pre-pubertal animals. This coincides with Kishore *et al*.[49] who reported that the ACP activity increased with the onset of puberty especially in the epididymal epithelium of Madras Red rams. On the contrary, Olaho-Mukami *et al*.[50], found that calves between 3 months and a year of age had higher ACP activity than calves in the age group of 5 years and above. The higher ACP activity in post pubertal animals compared to prepubertal animals suggests that ACP activity increases with sexual maturity. This increase may be associated with the development and functioning of reproductive organs, as ACP is often linked to processes such as sperm maturation and storage.

The localization of the G-6-Pase in the present study was observed in the principal cells of the epithelia of the epididymis; the same finding was reported in mice[51]. The increased G-6-Pase activity of the principal cell may be attributed to the secretary and absorptive functions of these cells[52]. The lumen of the caput and caudal of pre-pubertal camels have no reaction, while lumen of the tubules in caput and caudal of the post-pubertal camels had moderate reactivity to G-6-Pase. Increased G-6-Pase activity in the lumen of post pubertal caput might be linked to the need for more glucose production and release to support the energy requirements of sperm cells during these early development stages. Glucose is necessary for both glycolysis and the synthesis of metabolites important for sperm function[53]. The G-6-Pase activity in the cauda ensures adequate glucose levels in the epididymal fluid, helping to maintain sperm viability, and prepare them for activation during ejaculation. When sperm are mobilized, they rely on stored glucose for energy to power their motility, and increased G-6-Pase activity ensures that free glucose is available when needed[53].

In breeding season, the plasma and post-pubertal caudal region showed higher G-6-Pase activity than non-breeding season. These results also coincide with results obtained by Shalaby *et al*.[41] in camel testis. This suggests elevated metabolic activity to meet the higher energy, and glucose demands associated with reproductive functions. Kanai *et al.*[51] noticed a marked regressive change occurred in the endoplasmic reticulum of principal cells in non-breeding season. Therefore, the reduction of G-6-Pase activity in principal cells in non-breeding season may be related to the regressive changes in the endoplasmic reticulum[51,52]. The biochemical results for G-6-Pase activity of the epididymis regions showed that the activity of enzyme was significantly increased in plasma of pre-pubertal than post-pubertal camels; this

might reflect a higher demand for glucose to support rapid growth and development.

Sodium-potassium ATPase is a widely present plasma membrane enzyme that hydrolyzes ATP to regulate cellular sodium and potassium levels, as well as fluid volume[54]. In the current study, ATPase activity in the epididymis exhibited an intense reaction in the epithelium of the caput and the caudal regions. A more intense reaction was observed in the apical epithelium of the caudal region, and a moderate reaction was seen in the muscular layer. These results align with the findings of Byers and Graham[54], who reported similar localization of ATPase activity in the rat epididymis. This distribution is consistent with the fluid resorptive function of these epithelia, as described by Diamond and Bossert[55]. In the caudal segment, ATPase is in the apical part of the epithelium near fluid accumulation sites is crucial for regulating absorptive and secretory activities, creating an environment conducive to sperm motility and fertility as they pass through the epididymal duct[56,57].

In the present study, ATPase activity was higher in the caudal region compared to the caput, in both seasons particularly in post-pubertal animals. This is because the caudal epididymis stores spermatozoa. ATPase is vital for normal sperm function, as it produces ATP necessary for cellular activities such as motility[58]. Moreover, research by Koçak-Toker *et al*.[59] showed that inhibiting Na, K-ATPase results in the loss of sperm motility, indicating that decreased Na, K-ATPase activity in the sperm plasma membrane may be linked to infertility. The current findings indicate that ATPase activity in the epididymis fluctuates based on the breeding season and camel age. Notably, enzyme activity in the plasma and caudal region shows a slight increase, while the caput tissue exhibits a significant rise during the breeding season. This suggests that the breeding season boosts enzyme activity, likely to support reproductive functions. Additionally, post-pubertal camels display higher enzyme activity in the caudal region compared to pre-pubertal camels, suggesting that maturity also affects enzyme levels. Swain *et al*.[60] observed that goat sperm motility is highest during the breeding season, particularly in sperm from the caudal region. This suggests a link between increased enzyme activity and enhanced sperm motility, highlighting its importance for optimal sperm function and fertility during this period.

The 5`-Nase is a GPI-anchored protein that has a role in protecting spermatozoa from immune attack during their maturation, storage, and fertilization[61]. The current data indicates a strong reaction for 5'-Nase activity in the epithelial layer lining the epididymal duct. This finding may be due to the epididymis being the site for reabsorbing testicular fluid and performing secretory functions that aid in sperm maturation. This process requires energy and ATP, which are generated from the breakdown of 5'-Nase molecules, thus regulating maturation[58,62].

In the current study, the breeding season was associated with increased 5'-Nase activity in both plasma and the caudal region. Additionally, the caput region of post-pubertal camels exhibited significantly higher 5'-Nase activity compared to pre-pubertal camels, confirming the histochemical findings. The elevated intensity of 5'-Nase in the caudal region of post-pubertal camels during the breeding season, compared to the non-breeding season, is likely due to the caudal region's role in storing spermatozoa. Increased 5'-Nase activity may enhance and regulate sperm motility, while also protecting sperm from immune attacks as they traverse the male genital tract[63]. Furthermore, 5'-Nase is essential for both normal sperm physiology and the pathophysiology of male infertility. The strong reaction of 5'-Nase in the caput region of both pre-mature and post-mature animals across both seasons suggests that it plays a vital role in protecting and maturing spermatozoa, as the caput is the epididymal segment responsible for the complete maturation of sperm[64].

In conclusion, the intensity of the studied

enzymes of the camel epididymis varied depending on the specific epididymal region. In addition, the enzyme activities in the epididymal segments were closely related to seasonal changes, stages of puberty, and advancing age. The breeding season was associated with a marked increase in plasma and caput ATPase, as well as plasma and caudal 5'-Nase and G-6-Pase. The breeding season also linked to an insignificant change in plasma and caudal ALP, as well as caput and caudal ACP enzyme activities. The caudal region exhibited higher activity of ATPase, ALP, and ACP compared to the caput region across both seasons and ages. In contrast, the caput region displayed a more intense reaction in 5'-Nase and G-6-Pase during both seasons and ages. In post-pubertal camels, there was a notable increase in caudal ATPase and caput 5'-Nase, alongside a significant decrease in plasma G-6-Pase. ALP activity was higher in the plasma of pre-pubertal camels compared to post-pubertal camels, reflecting a higher metabolic rate during the pre-pubertal stage. In contrast, the caudal region of post-pubertal camels exhibited significantly increased ALP levels compared to the caudal region of pre-pubertal camels. This indicates that enzyme reactivity in epididymal tissue differs from that in plasma based on specific functions. ACP activity was weak in pre-pubertal camels, but moderate in post-pubertal ones. The elevated ACP activity in post-pubertal animals compared to pre-pubertal ones indicates that ACP activity rises with sexual maturity, likely due to its role in the development and functioning of reproductive organs, including sperm maturation, and storage.

ETHICAL APPROVAL

The animals blood and tissues used in this study were obtained from a licensed slaughterhouse after the animals were sacrificed as part of routine food production, with the permission of the slaughterhouse manager. Prior permission for animal use, an approval of the protocol was also obtained from the Institutional Animal Care and Use Committee of Mansoura University, code number: MU-ACUC (AGR.R.24.11. 10).

CONFLICT OF INTEREST

There are no conflicts of interest.

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تأثيرات مرحلة البلوغ وموسم التكاثر على بعض إنزيمات البربخ للجمل العربي: دراسات كيميائية نسيجية وكيميائية حيوية

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بحثت الدراسة الحالية التوطين الكيميائي النسيجي ونشاط خمسة إنزيمات مختلفة خلال موسم التكاثر وخارج موسم التكاثر وفي الأعمار قبل وبعد البلوغ في بربخ الإبل. تم جمع عينات بلازما الدم والبربخ من إجمالي 40 جملًا ذكرًا ذو سنام واحد (*Camelus dromedarius*) من المسلخ. تم أخذ العينات من الإبل قبل البلوغ (1.0 – 3.5 سنة) وبعد البلوغ (4.0 – 13.0 سنة). اختلفت شدة الإنزيمات المدروسة: الفوسفاتيز القلوي، والفوسفاتيز الحمضي، والجلوكوز-6-فوسفاتيز، وأدينوسين ثلاثي فوسفاتيز، و5`-نوكليوتيداز باختلاف منطقة البربخ. ارتبط موسم التكاثر بزيادة ملحوظة في نشاط أدينوسين ثلاثي فوسفاتيز في كل من بلازما الدم ومنطقة والرأس، إلى جانب ارتفاع ملحوظ إحصائيًا في نشاط 5`-نوكليوتيداز في المنطقة الذيلية، لكن نشاط الجلوكوز-6-فوسفاتيز لم يتغير بشكل ملحوظ إحصائيًا حسب الموسم في كل من بلازما الدم وأنسجة البربخ. وأظهر نشاط كل من الفوسفاتيز القلوي والفوسفاتيز الحمضي انخفاضًا ملحوظًا في المنطقة الذيلية من البربخ خلال موسم التكاثر. ومع تقدم العمر، أظهرت المنطقة الذيلية زيادة كبيرة في نشاط كل من أدينوسين ثلاثي فوسفاتيز والفوسفاتيز القلوي. وأظهرت منطقة الرأس زيادة كبيرة في نشاط 5`-نوكليوتيداز في الإبل بعد البلوغ. وأظهرت المنطقة الذيلية تفاعلًا مكثفًا لإنزيمات أدينوسين ثلاثي فوسفاتيز والفوسفاتيز القلوي والفوسفاتيز الحمضي أكثر من منطقة الرأس في كل من المواسم والأعمار. وعلى العكس من ذلك، أظهرت منطقة الرأس تفاعلية أكبر لإنزيم 5`-نوكليوتيداز مقارنة بالمنطقة الذيلية. وخلصت الدراسة الحالية إلى أن أنشطة الإنزيمات النوعية والكمية للبربخ كانت مرتبطة ارتباطًا وثيقًا بالتغيرات الموسمية، ومرحلة البلوغ، ومع تقدم العمر.