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Light Microscopic Changes in The Epididymis of Different Age Groups of The African Greater Cane Rat (*Thryonomys swinderianus*, Temminck 1827)

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Abstract

The mammalian epididymis is an important tubular epithelial structure with key functions in spermatozoa maturation and storage. There is a dearth of information on age-related changes in the light microscopic details of the epididymis in cane rat. This study evaluated light microscopic changes in the epididymis of different age groups of the African greater cane rats (AGCR) using histological, histochemical, and histomorphometric techniques. Twenty (20) African greater cane rats were used for this study. The rats were randomly assigned into 4 groups of 5rats each as i. prepubertal (≤4 months), ii. pubertal (>4≤12 months), iii. adult (>12≤30 months) and iv. aged (>30 months). Sequel to intra-cardiac perfusion with fixative (10% buffered formalin), routine histology, and histomorphometry using GIMP2 Software was carried out on the processed epididymal tissues. Also, the glycogen and connective tissue content of the epididymis of each group was demonstrated using Periodic-Acid Schiff and Masson Trichrome stains, respectively. The epididymis was lined by simple cuboidal epithelium in prepubertal compared to the pseudostratified columnar cells observed in others. Both Periodic-Acid Schiff and Masson Trichrome intensities were significantly higher in pubertal and adult rats relative to others. Histomorphometric parameters displayed a progressive age-related increase across the groups of cane rats. These sets of data could probably be associated with reproductive quiescence in the prepubertal and remarkable vigor more particularly in the adult cane rats investigated. Consequent upon this, adult cane rats are recommended for optimal breeding.

Keywords: Age, light microscopy, epididymis, cane rat

1. Introduction

The mammalian epididymis is an important epithelial tube with prime functions of spermatozoa maturation and storage (Cornwall, 2009). Morphologically, epididymis was originally thought to be divided into three distinct regions (segments): the caput which is situated at the testicular cranial pole, the corpus found by the side of the testes and the cauda segment occupying the caudal pole of the testis (Hermo, 1995). The epididymis was later discovered to contain an additional segment, the initial segment, especially in the rat (Hermo et al., 1991; 1998).

The epididymal histoarchitecture has been observed to be divided into segments and zones by connective tissue stroma and typified by epithelial compositions with different cell types (principal, basal, apical, clear, and halo cells) of diverse functions (Robaire et al., 2006). In terms of cell population on the epididymal epithelium, the principal and basal cells constitute the major cells while the remaining cells on the epididymal epithelium are regarded as accessory cells (Agnes and Akbarsha, 2001). Studies on different mammalian species have identified several other slight histo-architectural differences such as additional zones and segments along the ductus epididymal length (Jones et al., 1979; Oke, 1989; Adebayo and Olurode, 2010).

The ductal diameter, epithelial height, parenchymal cell types and their distribution differ in the various segments of the mammalian epididymis (Akbarsha et al., 2015). The epididymal epithelial thickness has been established to vary along the tubule being thickest at the proximal caput and thinnest at the caudal region (Arrotéia et al., 2012). In most mammals, the proximal to the distal segment of the epididymis usually has a progressive increase in luminal diameter and periductal muscle coat thickness (Lasserre et al., 2001). Also, spermatozoa concentrations are usually scanty in the initial segment but are largely concentrated in the lumen of the cauda epididymal region (Yanagimachi et al., 1985; Cornwall, 2009). The epididymal external ductal diameter, epithelial height, periductal muscular wall width, and stereocilia height have been reported to vary in different age-category of hamster rats (Calvo et al., 1999).

African greater cane rat (AGCR) is a wild herbivorous rodent currently undergoing massive domestication to augment the acute shortage of animal protein in sub-Saharan Africa (Fayenuwo et al., 2003; Olude et al., 2014; Monadjem et al., 2015). Earlier investigations on the epididymal morphology of the cane rat paid attention to the adult-only (Olukole et al., 2009; Adebayo and Olurode,

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2010; Adebayo et al., 2016). There is a dearth of reports on the light microscopic changes in the epididymis of different age groups of the African greater cane rat (*Thryonomis swinderianus*, Temminck 1827). Therefore, this study was designed to evaluate light microscopic changes in the epididymis of different age-category of cane rat using histological, histochemical and histomorphometric approaches.

2. Materials and Methods

2.1. Animals

Twenty (20) healthy male African greater cane rats used for this study were procured from a commercial farm (Pavemgo cane rat, Lagos State, Nigeria) with a record of birth. The rats were stabilized for one week in the Experimental Animal Unit of the Faculty of Veterinary Medicine. They were fed daily on dry corn feed and water provided *ad libitum*. The protocol for this experiment was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) and issued an ethical clearance (***UI-ACUREC/18/*0120).**

2.2. Experimental Design

Modified age-classification earlier reported by Soro et al. (2014) was adopted. The AGCR were randomly sorted into four groups of five (n=5) rats per group as highlighted below:

(A). Prepubertal (Pre); ≤4 months, (B). Pubertal (Pub); >4≤12 months, (C). Adult; >12≤30 months and (D). Aged (AG); >30 months). At the end of stabilization on day 8, the rats were sedated with combined sedatives; xylazine and ketamine (20:80 mg/kg body weight respectively) injected intramuscularly. Thereafter, primary perfusion fluid (0.9% sodium chloride (Aventra, Fidson, Nigeria) plus 25,000 IU of heparin (2IU\ML) (Heparinum; Polfa) and fixative proper (10% buffered formalin) were intra-cardially perfused.

2.3. Tissue processing for histology, histochemistry and histomorphometry

For histology, epididymal tissues were processed using the protocol of Adebayo and Olurode (2010). The stained tissues were then examined under a light microscope (Olympus BX3-CBH, USA) for variation in histo-architecture concerning age. Photomicrographs of the slides of the epididymides of different age groups of AGCR were evaluated for histomorphometric variations in epididymal (ductal diameter, ductal height, luminal diameter, stereocilia height and peritubular muscle coat thickness) parameters using GIMP2 Software. Following routine histology, the glycogen and connective tissue content of the epididymis of each group were demonstrated using Periodic-Acid Schiff (PAS) and Masson Trichrome (MT) stains, respectively. Also, the images of PAS and MT stained slides were quantified using ImageJ software.

2.4. Statistical analysis

Data obtained from histomorphometry and image J quantification of PAS and Masson trichrome staining intensities were statistically analyzed using GraphPad Prism Version 4.00 for Windows, GraphPad Software (San Diego, CA, USA). The results were expressed as group mean ± standard error of the mean (SEM), with a level of significance at $p<0.05$. The differences across the four groups of AGCR were compared using a one-way analysis of variance (ANOVA), and Tukey was used for multiple comparisons post hoc.

3. Results

3.1. Histological changes in the epididymis of different age groups of the African greater cane rat

The epididymis of the AGRC comprised six distinct zones: Zones I, II and III (initial segment), Zone IV (caput), Zone V (corpus) and Zone VI (cauda) (Fig 1 A-C). The first (initial) segment unlike others is further partitioned by connective tissue septae into three (3) histologically distinct sub-segments or regions or zones, namely: proximal (zone I), middle (zone II) and caudal (zone III) initial segments (Fig. 1 A and B). The shape of the lumen of all the epididymal segments in the pre-pubertal rat was round (Fig. 2). However, in the pubertal and aged AGCR, the proximal to the distal sub-segments of the initial epididymal segment had stellate-shaped lumen (Figs. 2-4), while their caput, corpus and cauda segments bear characteristic round luminal shape (Figs. 5-7). Concerning age-related differences in the nature of the epithelial lining of the epididymal segments, pre-pubertal epididymal duct was lined by predominantly simple cuboidal to columnar cells (Figs. 2-7), while the epithelial lining of the epididymal duct in pubertal and adult rats were the typical pseudostratified ciliated columnar epithelium (Figs. 2-7).

3.2. Age-related changes in the content of glycogen and collagen fibres in the epididymis of the African greater cane rat

Positive Masson's Trichrome stained areas within the segments of the epididymal duct of all AGCR groups were observed as bluish collagen substances in the ductal interstices and in the peritubular muscle coats (Figs. 2-7). Concerning PAS staining of the epididymis, positive areas appeared as magenta colour in the epididymal interstitium, lamina propria, peritubular muscle coat, perinuclear region of epididymal epithelial cells, ductal stereocilia and luminal content especially in the caput, corpus and caudal segments (Figs. 2-7). On the intensity of MT and PAS (Figs. 2-7) expressions in the segments of epididymal duct, significant age-related increase ($p<0.05$) in values were consistently observed for both stains. Strong PAS and MT intensities were displayed in most of the segments of pubertal and adult epididymis compared to other AGCR groups.

3.3. Histomorphometric parameters of the epididymis of different age-groups of the African greater cane rat

3.3.1. Ductal diameter (DD)

The DD was the smallest in all the segments of the epididymis in the prepubertal AGCR relative to others. The various segments of the epididymal duct showed a significant increase in DD from prepubertal to aged AGCR. The comparison along the rest of the epididymal duct of each AGCR group showed a significant ($p<0.05$) craniocaudal increase in the DD with the highest in the caudal segments (Table 1).

3.3.2. Ductal luminal diameter (DLD)

The ductal luminal diameter was significantly low ($p<0.05$) in the prepubertal relative to other AGCR groups. Also, the DLD of the entire epididymal segment was significantly higher ($p<0.05$) in the aged AGCR compared to others. The DLD of the epididymal duct in all age groups showed a significant increase ($p<0.05$) with advancing age. On the difference along the epididymal duct within each group, a significant craniocaudal increase ($p<0.05$) in luminal diameter was observed (Table 1).

3.3.3. Ductal epithelial height (DEH)

The ductal epithelial height of the prepubertal AGCR was significantly low ($p<0.05$) when compared to other groups. The DEH in nearly all the epididymal segments (initial, middle and distal segments) were not significantly different ($p>0.05$) from pubertal to aged groups, though a markedly reduced DEH was noticed in the caudal segment of the aged AGCR. In the comparison along the epididymal duct, a progressive significant craniocaudal decrease in DEH was seen in this study (Table 1).

3.3.4. Ductal stereocilia height (DSH)

The ductal stereocilia height of the prepubertal epididymis was significantly low ($p<0.05$) relative to other groups. The DSH values from the pubertal to aged AGCR were not significantly different ($p>0.05$), though an insignificant decrease in DSH was exclusively found in the epididymal segments of aged AGCR. Concerning variation along the segments of the epididymal duct of each AGCR group, a craniocaudal decrease in the trend of DSH values was noted. Also, the initial segments of each group bear significantly higher ($p<0.05$) stereocilia (Table 1).

3.3.5. Periductal muscle coat thickness (PMCT)

The periductal muscle coat thickness was significantly higher ($p<0.05$) in the adult AGCR compared to other groups. The PMCT appeared to increase significantly ($p<0.05$) with age. A fairly progressive increase in PMCT was noticed with consistently significant ($p<0.05$) values obtained in the caudal epididymal segment of all AGCR groups (Table 1).

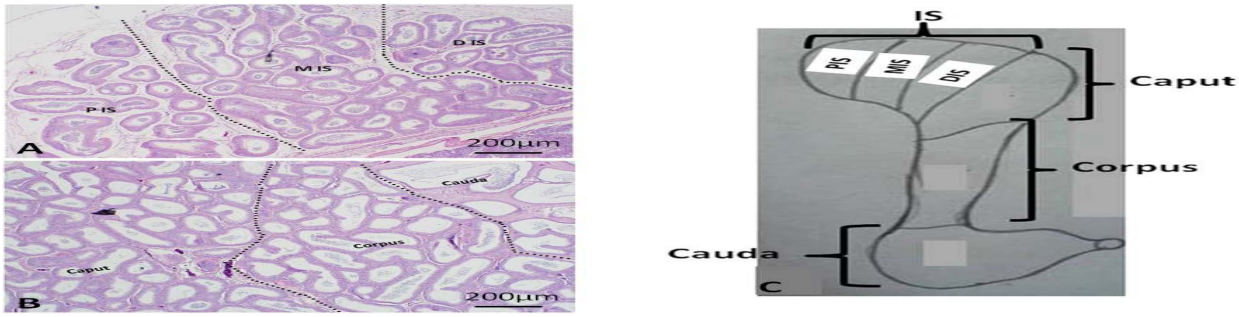

Figure 1 A-C. Photomicrographs and schematic diagram of the epididymal segments of the African greater cane rat. Note the four main segments of the epididymis; Initial segment (IS), caput, corpus and cauda. The initial segment (IS) is partitioned by connective tissue septae (dashed lines) into the proximal initial segment (PIS), middle initial segment (MIS) and distal initial segment (DIS). Scale bar: 200µm.

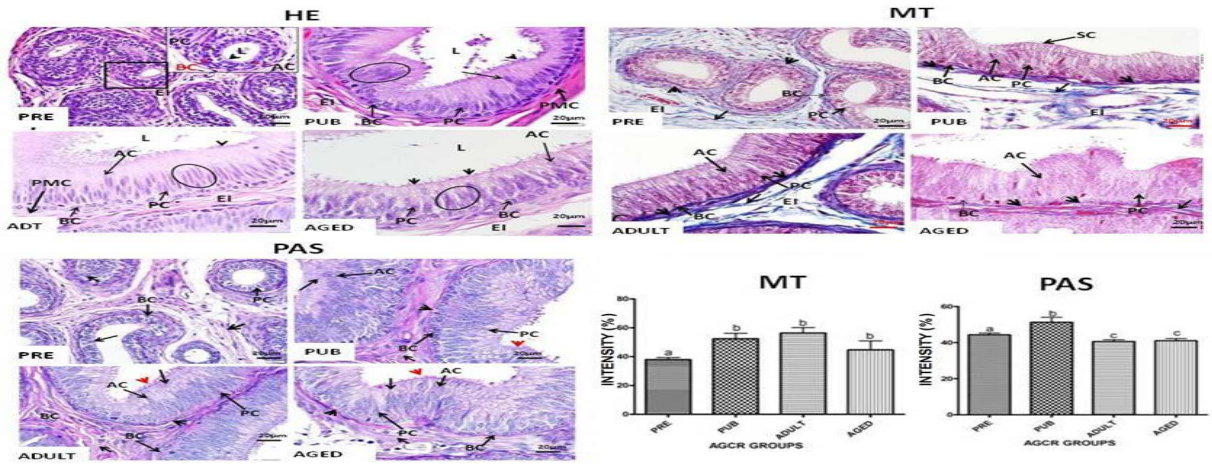

Figure 2. Photomicrographs of the proximal initial segment of the epididymis in different AGCR groups. In HE sections, the conspicuously reduced but almost absent stereocilia height (arrowhead), a round ductal lumen (L) lined by simple columnar epithelial cells and more cellular epididymal interstitium (EI) in prepubertal (Pre) AGCR. While in others, note the display of stellate-shaped ductal lumen prominently lined by pseudostratified columnar epithelium (oval) with prominent stereocilia (arrowhead) as well as component cell types; basal cells (BC), Principal cells (PC) and apical (AC). For the Masson trichrome (MT) sections, Note the blue staining collagen fibres (arrow) in the epididymal ductal interstices (EI) together with pink- staining smooth muscles (arrowheads) surrounding the epididymal ducts. Concerning the PAS section, note the PAS-positive areas in the interstitium (short arrow), lamina propria (arrowhead), ductal stereocilia (red arrowhead), and supranuclear region (long arrow) of the epididymal epithelium. Scale bar: 20µm. Bars with different superscripts (a, b, c) are significantly different.

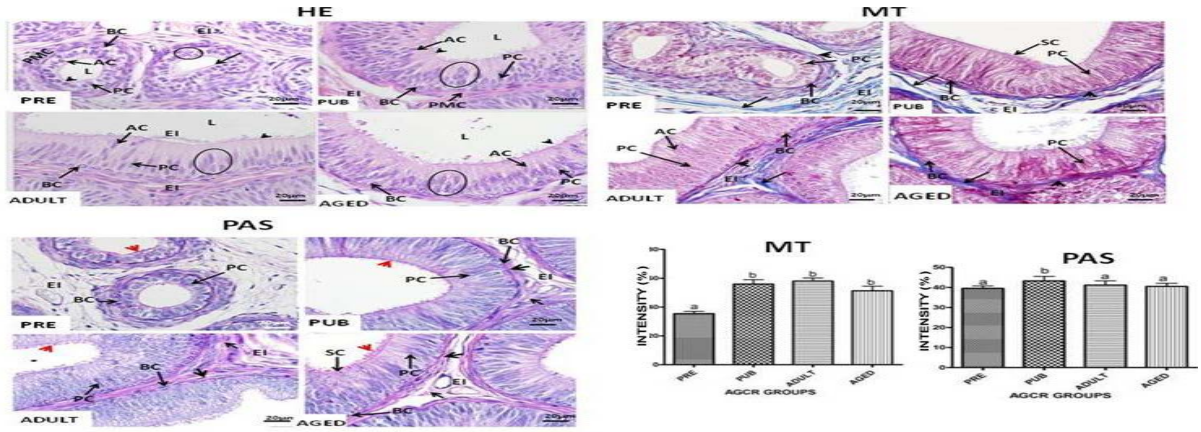

Figure 3. Photomicrographs of the middle initial segment of the epididymis in different AGCR groups. In the HE sections, note the markedly reduced stereocilia height (arrowhead), the round ductal lumen (L) lined by simple columnar epithelium and more cellular epididymal interstitium (EI) in pre-pubertal rat compared to the stellate-like lumen, duct lined by pseudostratified columnar epithelium (oval) with prominent stereocilia (arrowhead) as well as the presence of basal cells (BC), Principal cells (PC) and a moderate increase in apical cells (AC) seen in other AGCR. Also, observe in Masson trichrome (MT) sections the blue staining collagen fibers (arrow) in the epididymal ductal interstices (EI) together with pink-staining smooth muscles (arrowheads) surrounding the epididymal ducts. Note the PAS-positive areas in the interstitium (short arrow), lamina propria (arrowhead) and supranuclear region (long arrow) of the epididymal epithelium in the PAS sections. Scale bar: 20µm. Bars with different superscripts (a, b) are significantly different.

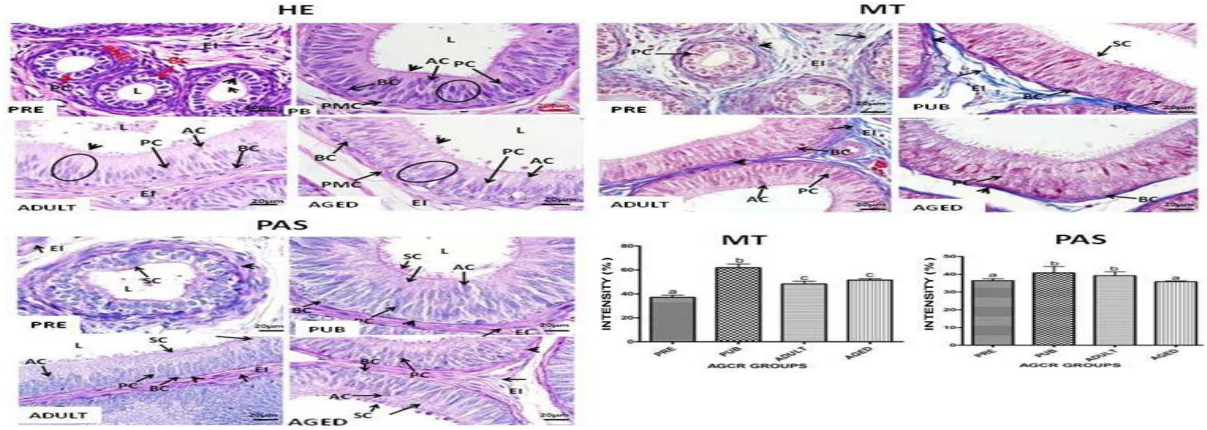

Figure 4. Photomicrographs of the distal initial segment of the epididymis in different AGCR groups. Note in the HE sections the reduced stereocilia height (arrowhead), the round ductal lumen (L) lined by simple columnar epithelium and more cellular interstitium in the prepubertal rat compared to the stellate-like luminal shape, ducts lined by pseudostratified columnar epithelium (oval) with prominent stereocilia (arrowhead) as well as the presence of basal cells (BC), Principal cells (PC) and markedly increase in apical cells (AC) in other groups. Note also the blue staining collagen fibres (arrow) in the epididymal ductal interstices (EI) together with pink-staining smooth muscles (arrowheads) surrounding the epididymal ducts in all the MT sections. Observe the PAS-positive areas in the interstitium (short arrow), lamina propria (arrowhead), and supranuclear region (long arrow) of the epididymal epithelium of all AGCR. Scale bar: 20µm. Bars with different superscripts (a, b, c) are significantly different.

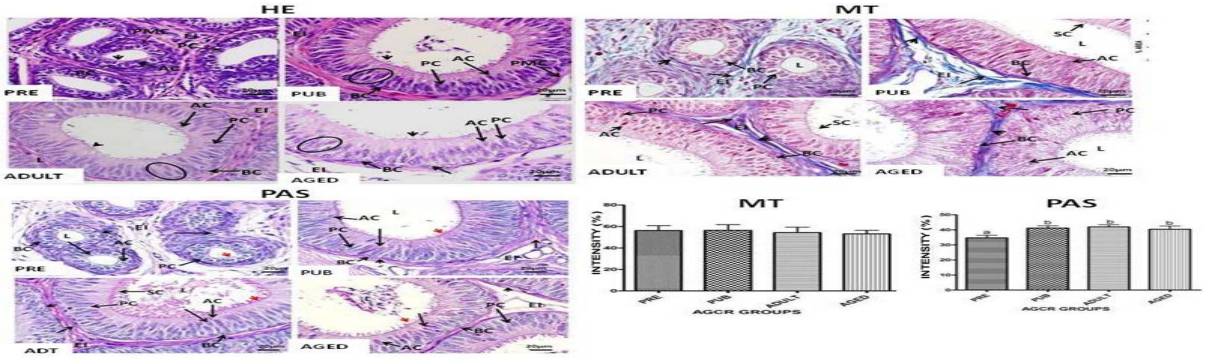

Figure 5. Photomicrographs of the caput segment of the epididymis in different AGCR groups. Observe in prepubertal (Pre) the display of markedly reduced stereocilia height (arrowhead), roundish ductal luminal (L) shape lined by simple cuboidal to columnar epithelial lining relative to the stellate to roundish like luminal shape, ducts lined by pseudostratified columnar epithelium (oval) with prominent stereocilia (arrowhead) as well as the presence of basal cells (BC), principal cells (PC) and more apical cells (AC) in other groups. Take note of the bluish staining collagen fibers (arrow) in the epididymal ductal interstices (EI) together with pink-staining smooth muscles (arrowheads) surrounding the epididymal ducts in the MT sections of all groups. Note the PAS-positive areas in the interstitium (short arrow), lamina propria (arrowhead), ductal stereocilia (red arrowhead), epithelial perinuclear region (long arrow) and luminal spermatozoa especially from pubertal to aged AGCR. Scale bar: 20µm. Bars with different superscripts (a, b) are significantly different.

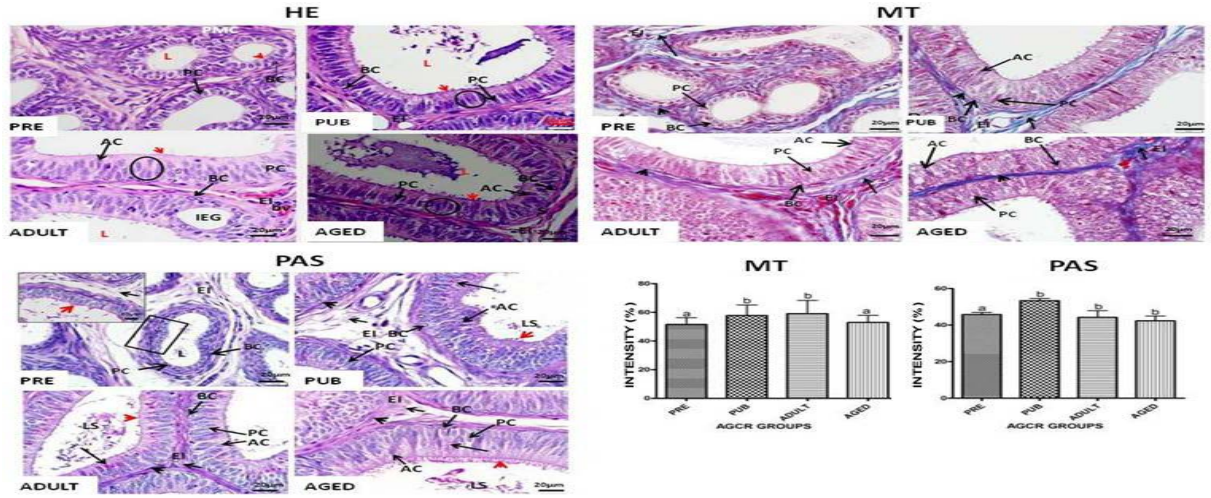

Figure 6. Photomicrographs of the corpus segment of the epididymis in different AGCR groups. In the HE sections, note the reduced stereocilia height (arrowhead) and roundish ductal luminal (L) shape lined by simple cuboidal to columnar epithelial cells in the prepubertal rat compared to the numerous intraepithelial glands, round ductal lumen shape containing spermatozoa, ducts lined by pseudostratified columnar epithelium (oval) with prominent stereocilia (arrowhead) as well as the presence of basal cells (BC), Principal cells (PC) and reduced apical cells (AC) seen in others. Also, note in MT stained sections the bluish staining collagen fibers (arrow) in the epididymal ductal interstices (EI) together with pink-staining smooth muscles (arrowheads) surrounding the epididymal ducts. Bv- blood vessel. Scale bar: 20µm. Bars with different superscripts (a, b) are significantly different.

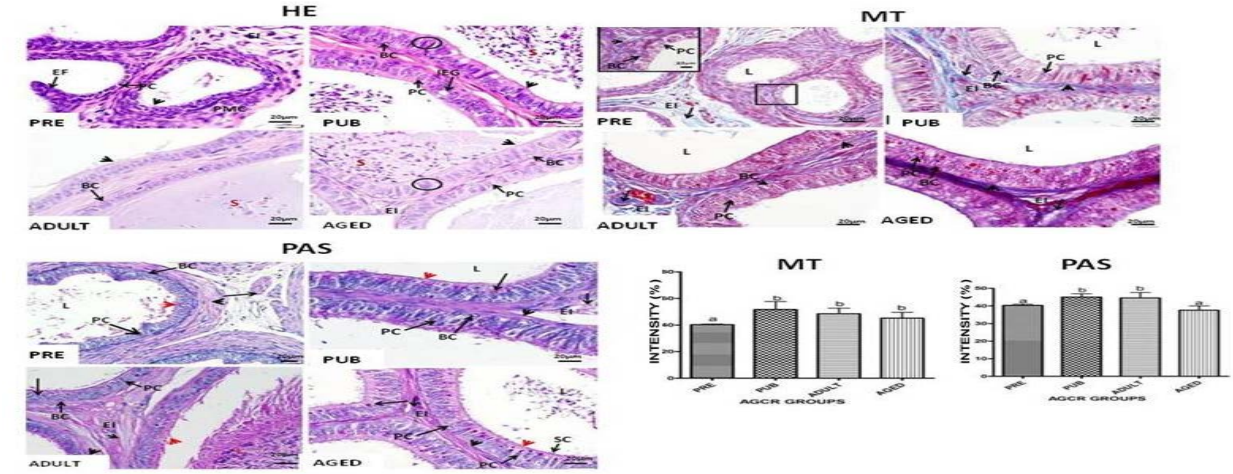

Figure 7. Photomicrographs of the cauda segment of the epididymis in different age groups of AGCR. Note in the HE sections the display of reduced stereocilia height (arrowhead), almost roundish ductal luminal (L) shape and copious epithelial fold (EF) in pre-pubertal rats when compared to the numerous intraepithelial glands (IEG), large roughly around ductal lumen shape containing spermatozoa (S), ductal lining bears a markedly reduced pseudostratified columnar epithelium (oval) with prominent stereocilia (arrowhead) as well as the dominating population of basal cells (BC) and Principal cells (PC) present in other groups. Also, note in MT sections the bluish staining collagen fibres (arrow) in the epididymal ductal interstices (EI) together with pink-staining smooth muscles (arrowheads) surrounding the epididymal ducts in inset A and other groups. Observe in the PAS sections positive areas to PAS staining in the interstitium (short arrow), lamina propria (arrowhead), epithelial supranuclear region (long arrow) and lumina (L) spermatozoa especially from pubertal to aged. Scale bar: 20µm. Bars with different superscripts (a, b) are significantly different.

Table 1. Age-related changes in epididymal parameters of African greater cane rat

Parameter	AGCR Group	Pro. Ini. Seg	Middle Ini. Seg	Distal Ini. Seg	Caput	Corpus	Cauda
Ductal Diameter (µm)	Prepub.	166.90 ± 8.89^{a}	151.20 ± 6.98^{a}	168.40 ± 15.51^{a}	106.50 ± 5.47$^{a\#\#}$	167.60 ± 11.91^{a}	239.20 ± 13.18$^{a\#}$
	Pub.	329.50 ± 16.42$^{b\#\#}$	250.50 ± 6.65^{b}	257.50 ± 8.58^{b}	282.10 ± 11.06^{b}	252.30 ± 7.60^{b}	430.10 ± 21.86$^{b\#}$
	Adult	309.00 ± 18.50$^{b\#\#}$	274.80 ± 7.64^{b}	289.70 ± 16.86^{b}	270.50 ± 13.70^{b}	272.20 ± 16.28^{b}	440.80 ± 25.86$^{b\#}$
	Age	329.00 ± 8.78^{b}	295.80 ± 9.87^{b}	305.70 ± 12.13^{b}	370.80 ± 10.49^{b}	308.80 ± 12.30^{c}	685.60 ± 34.26$^{c\#}$
Luminal Diameter (µm)	Prepub.	75.27 ± 6.73 $^{a\#\#\#}$	60.14 ± 3.99^{a}	53.32 ± 3.08^{a}	49.95 ± 4.39^{a}	112.30 ± 8.80$^{a\#\#}$	149.40 ± 12.84$^{a\#}$
	Pub.	133.50 ± 10.57$^{b\#\#\#}$	118.60 ± 5.38^{b}	110.40 ± 6.35^{b}	171.80 ± 9.36$^{b\#\#}$	158.90 ± 9.44$^{b\#\#}$	379.90 ± 30.18$^{b\#}$
	Adult	187.80 ± 12.77$^{c\#\#}$	117.40 ± 7.15^{b}	107.50 ± 8.25^{b}	161.90 ± 10.64$^{b\#\#}$	183.20 ± 7.56$^{c\#\#}$	396.00 ± 23.47$^{b\#}$
	Age	213.70 ± 9.53^{c}	190.60 ± 13.89^{c}	190.20 ±8.82^{c}	219.90 ± 16.20^{c}	208.80 ± 10.52^{d}	576.30 ± 36.66$^{d\#}$

Epithelial Height (µm)	Prepub.	41.74 ± 1.90$^{a\#}$	47.32 ± 2.21$^{a\#}$	47.80 ± 0.95$^{a\#}$	33.33 ± 1.65$^{a\#\#}$	39.23± 2.42$^{a\#\#\#}$	26.56 ± 3.39$^{a\#\#\#}$
	Pub.	65.43 ± 2.41$^{b\#\#}$	76.54 ± 2.35$^{b\#}$	73.41 ± 4.11$^{b\#}$	61.58± 2.37$^{b\#\#}$	55.95± 2.53$^{b\#\#\#}$	50.03 ± 1.66$^{b\#\#\#}$
	Adult	85.75 ± 3.39$^{c\#}$	86.43 ± 4.37$^{b\#}$	77.86 ± 2.55$^{b\#}$	66.61 ± 3.09$^{b\#\#}$	51.10 ± 1.64$^{b\#\#\#}$	55.01 ± 3.07$^{b\#\#\#}$
	Age	90.77 ± 4.06$^{c\#}$	64.50 ± 1.75$^{c\#\#}$	72.47 ± 5.01$^{b\#\#}$	70.24 ± 2.93$^{b\#\#}$	50.61 ± 2.52$^{b\#\#\#}$	23.50 ± 1.16$^{a\#\#\#\#}$
Stereocilia Height (µm)	Prepub.	3.96 ± 0.24$^{d\"{}}$	2.76 ± 0.09$^{a\"{}}$	2.59 ± 0.12$^{a\"{}}$	2.44 ± 0.10$^{a\"{}}$	2.53 ± 0.23$^{a\"{}}$	2.11 ± 0.13$^{a\"{}}$
	Pub.	8.51 ± 0.45$^{c\"{}\"{}}$	10.51 ± 0.47$^{c\"{}}$	8.71 ± 0.24$^{b\"{}\"{}}$	7.62 ± 0.30$^{b\"{}\"{}}$	7.94 ± 0.31$^{c\"{}\"{}}$	5.59 ± 0.48$^{b\"{}\"{}\"{}}$
	Adult	8.73 ± 0.49$^{c\"{}}$	7.74 ± 0.19$^{b\"{}\"{}}$	7.62 ± 0.24$^{b\"{}\"{}}$	6.47 ± 0.14$^{b\"{}\"{}}$	7.24 ± 0.14$^{c\"{}\"{}}$	5.03 ± 0.13$^{b\"{}\"{}\"{}}$
	Aged	6.95 ± 0.25$^{b\"{}}$	7.45 ± 0.38$^{b\"{}}$	6.99 ± 0.36$^{b\"{}}$	6.21 ± 0.29$^{b\"{}}$	5.79 ± 0.34$^{b\"{}\"{}}$	4.72 ± 0.24$^{b\"{}\"{}}$
Perimuscular Coat Thickness (µm)	Prepub.	13.25 ± 0.90$^{a\"{}\"{}\"{}\"{}\"{}}$	16.17 ± 1.21$^{a\"{}\"{}\"{}\"{}}$	10.54 ± 0.65$^{a\"{}\"{}\"{}\"{}\"{}}$	23.3 ± 2.40$^{a\"{}\"{}}$	21.12 ± 1.40$^{a\"{}\"{}}$	46.01 ± 4.30$^{a\"{}}$
	Pub.	14.74 ± 0.80$^{a\"{}}$	17.93 ± 1.01$^{a\'{}}$	13.20 ± 0.75$^{a\'{}}$	11.36 ± 0.91$^{a\'{}}$	16.87 ± 1.60$^{a\"{}}$	51.30 ± 5.70$^{a\"{}}$
	Adult	20.00 ± 0.90$^{a\`{}}$	21.03 ± 1.90$^{a\`{}}$	17.10 ± 1.40$^{a\`{}}$	14.28 ± 1.30$^{a\`{}}$	15.90 ± 1.40$^{a\"{}}$	60.99 ± 8.20$^{a\"{}}$
	Aged	17.22 ± 1.70$^{a\"{}\"{}\"{}}$	12.40 ± 0.91$^{a\"{}\"{}}$	16.10 ± 1.90$^{a\`{}\"{}\"{}}$	8.97 ± 0.44$^{a\"{}\"{}\"{}}$	14.17 ± 0.88$^{a\"{}\"{}}$	51.20 ± 3.70$^{a\"{}}$

Prepub – Prepubertal, Pub – Pubertal, Pro. Ini. Seg – Proximal Initial Segment

- Values with the different alphabet superscripts (a,b,c,d) within the column (comparison within an epididymal segment) are significantly different
- Values with the different number of harsh tag (#) in the same row (comparison along each epididymal segment i.e Pro. Ini. Seg to Cauda) are significantly different.

4. Discussion

The epididymal segments and zones of the AGCR in this study are in agreement with documentations on the epididymis of adult cane rats (Adebayo and Olurode, 2010) as well as the boar (Wrobeland Fallenbacher, 1974). These observations, however, contrasted the documentations of 3 segments in dog and camel (Chandler et al., 1981; Ruhl, 2001), 4 segments in rat and cat (Hamilton, 1975; Sànchez et al., 1998), 5 segments in the hamster, mouse, African giant rat and buck (Flickinger et al., 1978; Takano, 1980; Goyal and Williams, 1991; Oke, 1982). It is, therefore, suggested that the segments and zones of the epididymis of animals vary based on species.

The simple cuboidal to the columnar epididymal epithelial lining of the pre-pubertal unlike the classical pseudostratified stereociliated columnar epithelium observed in other groups conforms with earlier reports on the epididymal epithelial lining in immature and mature mammals (Oke, 1982; Adebayo and Olurode, 2010; Elzoghby et al., 2014). The functional relevance of this variation in epididymal epithelial lining could be correlated with the ongoing active secretory and absorptive activities on the epithelium of the pubertal to aged cane rats as opposed to the quiescent nature of the prepubertal rats.

Also, the observed progressive age-related increase in epididymal histomorphometric parameters in the different age groups of cane rat, as well as craniocaudal alterations (increase or decrease) to some of the parameters along the epididymal duct in this study, agree with the reports of Adebayo and Olurode (2010) in adult cane rat as well as Kishore et al. (2012) in the goat. The variations noticed in the histomorphometric parameters between the segments and within cane rat groups might be to accommodate the varied physiological activities of the different segment of the epididymis across age groups. For instance, the increase in stereocilia height of the initial segment functionally has been attributed to an additional resorptive ability of the epithelium in this segment (Alkafafy, 2005) or aiding the movement of spermatozoa along the duct.

The increased periductal muscle coat thickness towards the caudal segment in each group of cane rat is in accord with the pattern previously reported for the epididymal segments in most mammals (Delhon and von Lawzewitsch, 1994; Sànchez et al., 1998; Calvo et al., 1999; Ruhl, 2001). The functional implication of periductal smooth muscular coat presence along the epididymal segments has been suggested to be essential in the movement of the sperm toward the terminal segment (Goyal, 1985; Zayyed et al., 2012). The pronounced thickness of the PMC in the cauda epididymis could be linked to ejaculation. Also, the presence of an age-dependent increase in the thickness of the epididymal coat of the different groups of the cane rat more particularly in the cauda segment of the pubertal group could be suggestive of morphological compensation needed for the initiation of ejaculatory activity of the rats in this group.

The observation of round ductal luminal shape in all the epididymal segments of prepubertal rats, as well as the display of variable luminal shape (stellate to roundish) in other rat groups, agree with the numerous reports of round luminal shape in the lower segments of the epididymal duct of most mammals (Sànchez et al., 1998; Alkafafy, 2005; Shagufta et al., 2012). The roundish shape of epididymal ducts has been attributed to the regular nature of the epithelium in lower segments especially the cauda segment where the epithelium is uniformly low and luminal diameter is at maximum thereby favouring adaptation for sperm storage and maturation (Delhon and von Lawzewitsch, 1994; Zayyed et al., 2012). However, the irregularly long nature of the epithelium in the initial segments has been suggested to contribute to the stellate shape of their lumen (Sànchez et al., 1998; Alkafafy, 2005). Based on the above morpho-functional assumptions regarding the ductal luminal shape, it is understandable to attribute the fairly uniform epididymal epithelium height in prepubertal rats to their roundish ductal luminal shape.

The presence of positive PAS staining in the epididymal interstitium, lamina propria region, the peri-nuclear region of the epithelium, ductal stereocilia and lumen of different age groups of cane rat is consistent with glycogen-rich parts of the epididymis previously reported

in most mammals (Goswami et al., 1990; Oke et al., 1988; Kishore et al., 2012; Kumari, 2013). The significantly higher PAS staining intensity observed in all the epididymal segments of pubertal cane rat relative to others implies the active functional status of this age group. The demonstration of intense PAS staining in pubertal rats is consistent with the reports of Kishore et al. (2012) and Kumari (2013) on similar age-related studies in the epididymis of goats.

The demonstration of MT-positive epididymal duct interstices and the smooth muscles surrounding the epididymal ducts is consistent with the reported sites of collagen fibres in the epididymis (Shagufta et al., 2012). Owing to the resilience of collagen fibres (Bacha and Bacha, 2000), it suffices to assume that its presence in the epididymal interstices and periductal muscle coat correlates with its functional roles of maintaining the ductal architecture. The demonstration of exceptionally high MT intensity in nearly all the epididymal segments of the pubertal rat can be linked to the reproductive activeness of this age group.

In conclusion, this study has demonstrated that epididymal light microscopic details in cane rats were remarkably influenced by age increment as evidenced in the changes in the epididymal epithelium from simple cuboidal in prepubertal to pseudostratified columnar in other cane rat groups, significantly higher glycogen and connective tissue components in the pubertal and adult rats and progressive age-related increase in the histomorphometric parameters. These sets of data could probably be associated with reproductive quiescence in the prepubertal and remarkable vigor more particularly in the adult cane rat investigated. Even though previous authors have described light microscopic changes in the epididymis of the adult AGCR, this work is possibly the first report of the age-related changes in histology, histochemistry and histomorphometric changes in the epididymis of cane rat.

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