AGE-RELATED CHANGES IN THE TESTICULAR AND EPIDIDYMAL SPERM PARAMETERS IN THE AFRICAN GREATER CANE RAT (*THRYONOMYS SWINDERIANUS*, TEMMINCK, 1827)

[1]OMIRINDE, Jamiu Oyewole, [2]OLUKOLE, Samuel Gbadebo and [2]OKE, Bankole Olusiji

[1]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Jos, Plateau State, Nigeria.

[2]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

Corresponding Author: Omirinde, J. O. Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Jos, Jos, Nigeria. **Email:** omirindej@unijos.edu.ng **Phone:** +234 8069735125

Received: *February 20, 2019* ***Revised:*** *March 01, 2019* ***Accepted:*** *April 08, 2019*

ABSTRACT

This study investigated age-related changes in the testicular and epididymal sperm characteristics and spermiogram profiles in fifteen African greater cane rats (Thryonomys swinderianus) of different age-groups; pubertal: 5 – 11 months, adult: 12 – 30 months and aged: > 30 months, obtained from commercial cane rat farm, Badagry, Lagos State, Nigeria. Following a ventral abdominal incision of anaesthetisized cane rats, testes and epididymides were exteriorized for the determination of spermatozoa morphology, motility, concentration, live-dead ratio and morphometry. Spermatozoa head from pubertal cane rat onwards were ovoid in shape and lack visible acrosomal hook. From pubertal to aged cane rat, percentage abnormal sperm cell was not significantly different (p>0.05). Sperm morphometric parameters (sperm length and width or diameter, mid-piece length, tail length and complete spermatozoa length) were not significantly (p>0.05) different with age. There was consistent remarkable increased epididymal sperm motility in the adult cane rat relative to other groups. Testicular and epididymal sperm counts were markedly elevated in the adult relative to other age groups. However, both the testicular and epididymal spermatozoa live-dead ratio were not significantly different (p>0.05) from pubertal to aged cane rat. This study has demonstrated that sperm motility and concentration were remarkably elevated in adult cane rat. Therefore, cane rat breeders are advised to utilize adult rat in breeding.

Keywords: Sperm characteristics, Spermiogram, Testis, Epididymis, Cane rat, *Thryonomys swinderianus*

INTRODUCTION

The African greater cane rat (AGCR) (*Thryonomys swinderianus* Temminck, 1827) also known as grasscutter is a wild hystricomorphic herbivorous rodent found majorly in several savanna biotic zones as well as areas on the fringes of the rainforest of sub-Saharan Africa (Happold, 1987; Monadjem *et al*., 2015). Cane rat is a recognized excellent source of protein which accounts for its vigorous exploitation for meat through aggressive hunting and bush burning (Jori *et al*., 1995; Juste *et al*., 1995; Fayenuwo *et al*., 2003). It is known to contribute to both local and foreign earnings of most West African countries where its meats are expensively sold and preferentially demanded over other wild rodents (Baptist and Mensah, 1986; Ntiamoa-Baidu, 1998; Asibey and Addo, 2000). The comprehensive insight into the adaptive significance of the sperm morphophysiology has been a challenge to

biologists, because of the highly specialized nature of spermatozoa and coupled with their operations at a microscopic level in a complex environment (Gage, 1998). The important parameters considered when evaluating sperm qualities in a reproductively active mammalian male subject include: sperm morphology, motility and concentration (Malmgren, 1997; Tabatabaei *et al.,* 2009). Sperm morphological features are reliable indicators in predicting the fertilizing capacity of sperm and could also reflect certain spermatogenetic disorders (Barth and Oko, 1989). The presence of abnormal shaped spermatozoa in the ejaculate of mammals at a normal acceptable range of up 20 % in cat and less than that in rodents is normally encountered (Moss *et al*., 1979; Wilde *et al*., 1999). It might be associated with impairment of fertility when they are present in large numbers (Moss *et al*., 1979). The significance of the usage of sperm morphology in determining reproductive success has been documented in humans (Menkveld *et al*., 1991; Zamboni, 1992) and domesticated animals such as the bull (Barth and Oko, 1989; Freneau *et al*., 2010), stallion (Brito, 2007), boar (Briz *et al*., 1996) and dog (Martínez, 2004). It has equally been reported that ageing is an important factor in semen quality and is usually impaired in the aged animal due to morphological alteration in the epididymal epithelium most especially in the caudal region of the duct with probable consequential effect of disrupted sperm maturation (Calvo *et al*., 1999).

Sperm morphometry refers to some linear dimensions of the head, mid-piece and the tail of a typical spermatozoon (Banaszewska *et al*., 2011). There is a strong relationship between sperm function and morphometry and has been empirically validated in different mammalian species (Immler *et al*., 2010). For instance in bulls, sperm head morphometric parameters have been regarded as excellent indicators of semen quality (Phillips *et al*., 2004). Also, the age of a male animal has equally been identified as an essential factor in causing variation in spermatozoa morphology and morphometric dimensions (Gregor and Hardge, 1995; Kondracki *et al.,* 2005; Quintero-Moreno *et al.* 2009). In majority of the mammalian species, sperm morphometric analysis revealed that the tail was the longest portion and represents about 89 % of the total sperm length (Ogbuegbu *et al*., 1985; Meisner *et al*., 2005; Batalha and Oba, 2006; Brito *et al*., 2010).

With the exception of reports of Olukole *et al.* (2014) on semen characteristic and spermiogram, and Olukole *et al.* (2010) on gonadal and extragonadal sperm reserves in the sexually matured adult of this rodent, there is dearth of information on age-related changes in the testicular and epididymal sperm characteristics and spermiogram profiles in AGCR. Hence, this study seeks to investigate age-related changes in testicular and epididymal spermatozoa of the AGCR.

MATERIALS AND METHODS

Animals and Experimental Design: Fifteen (15) cane rats of different age groups were obtained from a commercial farm, Badagry, Lagos State, Nigeria. Records relating to the ages of the rats were obtained at the point of purchase. They were stabilized for seven days in the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. During the period, the animals were fed with dry corn feed daily and water *ad libitum*. The cane rat grouping reported by Soro *et al*. (2014) in a similar age-related study was modified and used for sorting the AGCR into three (3) groups of five (5) animals each; pubertal (5 – 8 months), adult (12 – 30 months) and aged (>30 months) and their average body weights recorded as 1.95 ± 0.08 kg, 3.08 ± 0.15 kg and 4.63 ± 0.24 kg respectively.

Anaesthesia and Organ Excision: The rats were anaesthetized using intramuscular injection of xylazine and ketamine combination (20 : 80 mg/kg body weight respectively). Thereafter, the abdominal wall of each rat was dissected through a ventral midline incision on the linea alba using the method of Olukole *et al.* (2010). The testes together with their attached epididymis were removed via the incision site on the tunica vaginalis and then placed in Petri

dishes containing normal saline to prevent organ desiccation.

Morphological Characteristics: Morphological characteristics of the spermatozoa architecture was observed from a total of 400 sperm cells using the method of Wells and Awa (1970). Briefly, a drop each of Wells and Awa stain (0.2 g of Eosin and 0.6 g of Fast green dissolved in distilled water and ethanol in ratio 2:1) and semen were placed on a warm slide mixed and smeared with another slide. The stained smear was then air dried and viewed under the light microscope. Normal spermatozoa and site of defects in the abnormal spermatozoa (head, neck/mid-piece, tail) were recorded and classified as described by Bloom (1973) and Parkinson (2001).

Sperm Morphometrics: Spermatozoa histomorphometry was carried out using the method of Sousa *et al.* (2013). Parameters measured were; sperm head length (SHL, the vertical distance between the tip of the acrosome and the boundary with the neck of spermatozoa), sperm head diameter (SHD, longest horizontal distance between the two edges of sperm head), sperm mid-piece length (SML , distance between the commencement and the end of the mid-piece), sperm tail length (STL, distance between the proximal end of the neck and the tip of the tail), sperm whole length (SWL, the distance between rostral tip of the sperm head and the tip of the spermatozoa tail). Sperm morphometric analysis of sections captured for morphological study was performed using GIMP2 Software. For each of the five animals per group, 10 spermatozoa devoid of any morphological defects were selected totalling 50 spermatozoa per group of AGCR.

Determination of Sperm Motility, Concentrations and Live-Dead Ratio: The percentage of spermatozoa in a unidirectional progressive movement over a field on a slide was observed with a light microscope fitted with camera using the method of Zemjanis (1977). Briefly, the excised testis and epididymal segments (caput, corpus and cauda) from each of the different groups of AGCR were incised on the surface and a small drop of semen was taken and mixed with 2.9 % warm sodium citrate buffer on a warm clean slide. The percentage of motile spermatozoa moving in a straight forward unidirectional rectilinear motion were counted by quick observation at x10 low power microscope objective; while, sperm cells moving in circular, backward directions or those showing pendulous movement pattern were ignored.

For the estimation of testicular and epididymal sperm counts, the method described by Olukole *et al.* (2010) was used. Briefly, testicular and epididymal sections (cauda, corpus, caput) were minced separately using scissors, washed out with 10 ml of saline and homogenized at 6000 rpm for two minutes. Eosin was then added to stain the sperm heads in the obtained homogenate. The gonadal and extragonadal sperm reserves were then estimated as the total number of late spermatids and spermatozoa in the epididymal and testicular sperm samples. All samples were finally made up to 1:20 before counting on the improved Neubauer haemocytometer counter.

The live-dead ratio was estimated in accordance to Zemjanis (1977). Briefly, from each of the incised testicular and epididymal tissues, a drop of semen was placed on a well labelled warm slide, mixed with a drop of warm Eosin-Nigrosin stain and then observed under the microscope at x40 objective. It was performed immediately to avoid wrong results. Live sperm cells fail to pick up the stain and appeared as clear cells, while dead sperm cells picked up the stain and are seen to be purplish in colour. The live and the dead sperm cells were separately counted from a total of 600 spermatozoa in smears stained with Eosin-Nigrosin and the ratio was determined according to the method of Zemjanis (1977).

Statistical Analysis: Data obtained from this study were analyzed using GraphPad Prism Statistical Package, Version 4.00 for Window (GraphPad Software Incorporated, California, USA). The variations in sperm parameters in pubertal cane rat onwards were compared using one-way analysis of variance (ANOVA). Results

were expressed as group mean ± standard error of mean (SEM). The level of significance for all analyses was set at $p<0.05$.

RESULTS

Spermatozoa Morphological Characteristics: The spermatozoa head of AGCR were typically flat and ovoid in shape with indistinct neck (Figure 1a-c). The acrosomal head of sperms in all AGCR bear no evidence of hook (Figure 1a-c). There is no age-related difference in the division of the spermatozoa tail of AGCR which is characteristically made up of three distinct segments, namely; the mid-piece, principal piece and end piece. The base of the head continued with the mid-piece, the first segment of the tail (Figure 1a-c).

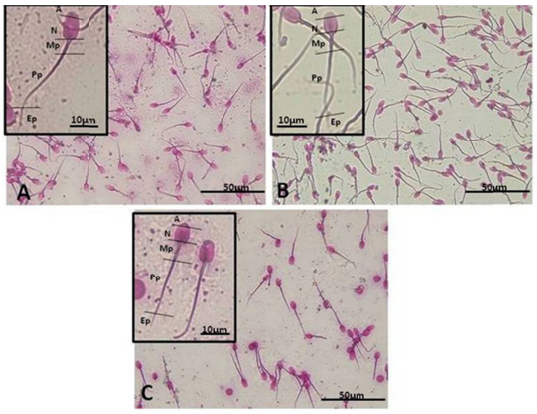

Figure 1 a-c: Photomicrographs of the spermatozoa morphology in the different age groups of African greater cane rat. A (Pubertal), B (Adult) and C (Aged). Note the absence of acrosomal hook on spermatozoa head. A - Acrosome, N - Nucleus, Mp - Mid-piece, Pp - Principal piece, Ep-End piece. Stain: Nigrosin-Eosin

In addition, the percentage of the different types of abnormal sperm cells in both testis and epididymis were not significantly different ($p>0.05$) in pubertal AGCR onwards (Table 1). Across all age groups, curved and bent mid-pieces as well as curved and bent tail defects appeared to be present in greater amount relative to other types of abnormalities. It was also evident that roughly 85.0 % of the spermatozoa of the pubertal, adult and aged AGCR displayed the normal morphology outlined in the inserts of Figure 1 a-c.

Sperm Motility, Count and Liveability (Live-Dead Ratio): Testicular spermatozoa were observed to be immotile in all the age group of AGCR. There was consistent significant increase ($p<0.05$) in the epididymal sperm motility of the pubertal and adult AGCR relative to the aged (Table 2). The sperm motility profile displayed in the epididymis appears to increase with age increment. Motility values in the caudal epididymal segment of all age groups were markedly higher relative to other segments and were more remarkably elevated in the cauda epididymis of adult cane rats.

Similarly, sperm count as shown in Table 2 followed similar pattern described for motility. Though, testicular sperm count (TSC) was significantly reduced ($p<0.05$) in the aged AGCR relative to other groups, the TSC values were not significantly different ($p>0.05$) in adult and pubertal AGCR. In the caput and corpus epididymal segments, sperm count values were not significantly different ($p>0.05$) in pubertal to aged AGCR, while in the caudal segment, spermatozoa counts of aged cane rats were significantly lowered ($p<0.05$) relative to the spermatozoa counts in pubertal and adult rats.

With respect to sperm livability, there was no significant difference ($p>0.05$) among the age groups (Table 2).

Sperm Morphometrics: There was no significant difference ($p>0.05$) in the morphometric parameters in all the different age groups of AGCR (Table 3), although, the parameters tended to increase with advancement in age. The mean lengths of the spermatozoa in the different age group of AGCR were 55.92 ± 1.39 µm, 57.06 ± 0.95 µm, and 58.41 ± 0.67 µm respectively for pubertal, adult and aged. The tail lengths were 45.22 ± 1.14 µm, 46.16 ± 0.84 µm and 48.47 ± 1.08 µm respectively for pubertal, adult and aged. The mean sperm head lengths were 9.52 ± 0.44 µm, 10.26 ± 0.45 µm and 10.46 ± 0.50 µm respectively for pubertal, adult and aged, while; the mean sperm widths or diameters include 5.04 ± 0.23 µm, 5.18 ± 0.18 µm and 5.63 ± 0.19 µm in pubertal, adult and aged accordingly.

Table 1: Age-related changes in the frequency of morphological abnormalities in the testicular and epididymal spermatozoa of African greater cane rat

Organs	Groups	Percentage Abnormal Cell (%)									
		Head		Mid-Piece		Tail				Total	
		TH	HT	CMP	BMP	CT	RT	BT	LT	TAC	TNC
Testes	Pub	1.35 ± 0.11	1.03 ± 0.09	3.21 ± 0.16	2.55 ± 0.14	2.54 ± 0.06	0.43 ± 0.13	2.71 ± 0.24	0.41 ± 0.13	14.42 ± 0.76	85.58 ± 0.24
	Adult	1.43 ± 0.06	1.12 ± 0.12	2.74 ± 0.10	2.49 ± 0.14	2.68 ± 0.06	0.50 ± 0.10	2.68 ± 0.16	0.38 ± 0.07	14.02 ± 0.44	85.98 ± 0.66
	Aged	1.48 ± 0.17	1.07 ± 0.16	2.97 ± 0.24	2.84 ± 0.23	2.75 ± 0.15	0.67 ± 0.08	3.00 ± 0.14	0.33 ± 0.05	15.11 ± 0.97	84.89 ± 0.03
	P-value	0.7673	0.8877	0.2318	0.3765	0.3753	0.3054	0.3054	0.8368	0.6088	0.5033
Caput	Pub	1.48 ± 0.10	1.30 ± 0.08	3.06 ± 0.20	2.62 ± 0.13	2.38 ± 0.47	0.79 ± 0.03	2.79 ± 0.03	0.70 ± 0.08	15.86 ± 0.32	84.14 ± 0.70
	Adult	1.35 ± 0.12	1.23 ± 0.19	2.85 ± 0.24	2.46 ± 0.15	2.58 ± 0.19	0.53 ± 0.12	2.45 ± 0.16	0.57 ± 0.06	14.03 ± 1.00	85.97 ± 0.91
	Aged	1.41 ± 0.06	1.08 ± 0.06	2.57 ± 0.06	2.30 ± 0.04	2.39 ± 0.12	0.68 ± 0.06	2.86 ± 0.06	1.40 ± 0.87	13.60 ± 0.32	86.42 ± 0.68
	P-value	0.6627	0.4935	0.2372	0.2041	0.5786	0.1286	0.347	0.4894	0.0734	0.082
Corpus	Pub	1.15 ± 0.08	1.49 ± 0.10	2.45 ± 0.04	2.56 ± 0.11	3.21 ± 0.19	0.53 ± 0.02	2.65 ± 0.08	0.45 ± 0.07	14.21 ± 0.33	85.79 ± 0.67
	Adult	1.12 ± 0.13	1.09 ± 0.18	2.43 ± 0.08	2.49 ± 0.05	2.75 ± 0.21	0.64 ± 0.07	2.75 ± 0.14	0.51 ± 0.14	13.69 ± 0.39	86.31 ± 0.62
	Aged	1.47 ± 0.04	1.15 ± 0.09	2.73 ± 0.10	2.45 ± 0.06	2.65 ± 0.13	0.68 ± 0.06	2.89 ± 0.29	0.53 ± 0.12	14.55 ± 0.36	85.45 ± 0.70
	P-value	0.1101	0.1197	0.0682	0.5857	0.1135	0.2055	0.6849	0.8840	0.2849	0.4012
Cauda	Pub	1.25 ± 0.10	1.05 ± 0.12	2.48 ± 0.17	2.88 ± 0.15	2.29 ± 0.25	0.64 ± 0.06	2.46 ± 0.26	0.58 ± 0.11	13.90 ± 0.88	86.12 ± 0.91
	Adult	1.32 ± 0.06	1.14 ± 0.18	2.52 ± 0.07	2.39 ± 0.18	2.52 ± 0.14	0.82 ± 0.06	2.39 ± 0.11	0.32 ± 0.07	13.41 ± 0.46	86.59 ± 0.54
	Aged	1.09 ± 0.11	1.23 ± 0.07	2.16 ± 0.16	2.62 ± 0.13	2.37 ± 0.12	0.81 ± 0.11	2.68 ± 0.35	0.58 ± 0.16	13.53 ± 0.94	86.57 ± 0.11
	P-value	0.0733	0.6878	0.1913	0.1406	0.6695	0.2732	0.7212	0.2564	0.9026	0.3020

TH – Tailless head, HT- Headless tail, RT- Rudimentary tail, BT- Bent tail, CT- Curved tail, CMP- Curved mid-piece, BMP- Bent mid-piece, LT- Looped tail, TAC- Total abnormal cell, TNC- Total normal cell, Pub- Pubertal

Table 2: Age-related changes in the sperm parameters of African greater cane rat

Organs	Pubertal	Adult	Aged
	Sperm Motility (%)		
Testes	Nm	Nm	Nm
Caput	55.00 ± 5.00[a]	65.00 ± 0.89[a]	37.50 ± 2.50[b]
Corpus	60.00 ± 7.07[a]	65.00 ± 2.89[a]	37.50 ± 4.79[b]
Cauda	62.50 ± 4.79[a]	80.00 ± 4.08[b]	55.00 ± 5.00[a]
	Sperm Count (x10^9 ml)		
Testes	37.25 ± 2.49[a]	37.75 ± 2.02[a]	28.50 ± 1.04[b]
Caput	43.25 ± 0.85	44.25 ± 1.11	42.50 ± 1.04
Corpus	48.00 ± 1.08	50.00 ± 0.71	46.50 ± 0.96
Cauda	101.5 ± 7.96[a]	135.3 ± 6.42[b]	91.25 ± 2.56[c]
	Live Dead Ratio		
Testes	92.75 ± 1.03	94.25 ± 0.85	96.75 ± 0.75
Caput	97.25 ± 0.75	98.00 ± 0.25	96.25 ± 063
Corpus	97.25 ± 0.75	98.00 ± 0.00	96.00 ± 0.71
Cauda	97.50 ± 0.50	97.25 ± 0.75	97.30 ± 0.75

Values with different superscripts on a row are significantly different. Nm – Not motile

Table 3: Age-related changes in the sperm morphometric parameters of African greater cane rat

Age	Sperm Morphometrics (µm)				
	SHL	**SHD**	**MPL**	**STL**	**SWL**
Pubertal	9.52 ± 0.40	5.04 ± 0.23	12.82 ± 0.33	45.22 ± 1.14	55.92 ± 1.39
Adult	10.26 ± 0.45	5.18 ± 0.18	13.21 ± 0.27	46.16 ± 0.84	57.06 ± 0.95
Aged	10.46 ± 0.50	5.63 ± 0.19	13.86 ± 0.35	48.47 ± 1.08	58.41 ± 0.67

SHL- Sperm head length, SHD- Sperm head diameter, MPL- Mid-piece length, STL-Sperm tail length, SWL-Sperm whole length

Both the spermatozoa mean length and width linear measurements were comparatively shorter when compared to their tail counterpart and also increased insignificantly with age.

DISCUSSION

Sperm morphological characteristics are essential parameters that reveal the extent of normality and maturity of the sperm population in the ejaculate and could as well correlate with fertility status of a mammalian species (Memon *et al.,* 1986). The shape of spermatozoon head in the different age groups of AGCR observed in this study is in agreement with the report of Olukole *et al*. (2014) and also concurs with spermatozoa head of mammals (Villalpando *et al*., 2000; Breed, 2005; Oyeyemi and Babalola, 2006). The absence of acrosomal hook on the sperm head in all the age groups also confirmed earlier report of Olukole *et al*. (2014). This observation distinguished it from the other rodents in which sperm head folds back onto itself to give a hook-like appearance (Blandau, 1951; Leblond and Clermont, 2000; Breed, 2005). The non-significant difference in the linear dimensions; sperm head length and width, mid-piece length, tail length and the complete spermatozoa lengths observed among the three age-groups of this rodent were similar to the sperm morphometric data reported in age-related study conducted in boar (Quintero-Moreno *et al.,* 2009; Banaszewska *et al.,* 2011). Although insignificant increment occurred in the sperm dimensions with increasing age, this was not enough to conclude that there was an age-related alteration in sperm morphometrics, in spite of the fact that age of male animal has been identified as an important cause of variation in spermatozoa morphometric dimensions (Gregor and Hardge, 1995; Kondracki *et al.,* 2005; Quintero-Moreno *et al*., 2009).

The observed uniformly low percentage (roughly 15 %) of abnormal sperm cells in both testes and epididymis of the different age groups of cane rat concurred with the normal acceptable range reported for mammals (Moss *et al*., 1979; Wilde *et al*., 1999). Bearing this in mind, the level of these abnormalities might not

affect the breeding soundness of all the age category of cane rats. Also, the higher proportions of the curved and bent mid-pieces as well as bent tail defects displayed across all age groups has been suggested to be due to the disorganization of structural components of the sperm tail with resultant weakness of the structure and folding of the flagellum (Briz *et al*., 1996). The finding on the percentage abnormal spermatozoa was similar to the report of Olukole *et al*. (2014) on matured adult of this rodent species and also agreed partially with the report of Varesi *et al.* (2013) on canine spermatozoa.

The percentage motility of live spermatozoa has been reported to positively correlate with the fertilizing capability of sperm cells (Oyeyemi and Ubiogoro, 2005). Therefore, the significant increased motility observed in the epididymis of the pubertal and adult cane rats more remarkably in the adult could be suggested to reflect the excellent fertilizing ability of this age group. Conversely, the reduced percentage sperm motility displayed by the aged AGCR could be linked to the progressive ageing process within the epididymal segments more particularly the cauda segment and may as well account for most of the reduction in the fertility potential of most aged animals. The profile of sperm motility seen in this study was consistent with the pattern documented in similar age-related study in hamster rat (Calvo *et al*., 1999) and in humans (Kidd *et al*., 2001; Jung *et al*., 2002).

The non-significant difference in the gonadal and extragonadal percentage sperm livability from the pubertal to aged AGCR implies that the ratio of the live spermatozoa to dead counterparts in the ejaculate of each group was uniformly higher across the groups. This is expected because the groups of the cane rat studied were not exposed to toxicant that could have markedly disrupted the balance in the livability ratio.

Furthermore, the marked increased testicular and gonadal sperm concentrations observed in the pubertal and adult cane rat more particularly in the latter age group may be attributed to the morphophysiological activeness of this group. However, the markedly decreased gonadal and extragonadal sperm concentrations observed in aged AGCR was consistent with the widely documented decline profile of sperm concentration in aged animals (Humphrey and Ladds, 1975; Carvalho *et al*., 1988; Calvo *et al*., 1999) and in men (Neaves *et al*., 1985).

Conclusion: This study has demonstrated that the profiles of sperm morphology, morphometry and livability parameters were not different across the entire cane rat investigated. However, sperm motility and concentration were remarkably elevated in the adult cane rat. Owing to the significance of the two parameters in determining the fertilizing ability, cane rat farmers are encouraged to make use of adult rat in breeding programme.

ACKNOWLEDGEMENTS

We thank the management and staff of the cane rat farm in Badagry, Lagos State, Nigeria for breeding the cane rats used in this study and provision of the chronological data of the rats. We equally appreciate the management and staff of the Experimental Animal Unit, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, for care and management of the experimental animals and Mr. Omoko Ejiro of the Department of Veterinary Theriogenology, University of Ibadan, Oyo State for his technical expertise in sperm analysis.

REFERENCES

ASIBEY, E. O. A. and ADDO, P. G. (2000). The grasscutter, a promising animal for meat production. *In:* TURNHAM, D. (Ed.). *African Perspective, Practices and Policies Supporting Sustainable Development.* Scandinavian Seminar College, Denmark, in Association with Weaver Press Harare, Zimbabwe. www.cdr.dk/sscafrica/asad-gh.ht.m

BANASZEWSKA, D., KONDRACKI, S. and WYSOKIŃSKA, A. (2011). Effect of age on the dimensions and shape of spermatozoa of large white Polish boars *Archiv fur Tierzucht*, 54(5): 504 – 514.

BAPTIST, R. and MENSAH, G. A. (1986). Benin and West Africa: the cane rat farm animal of the future. *World Animal Review,* 60: 2 – 6.

BARTH, A. D. and OKO, R. J. (1989). *Abnormal Morphology of Bovine Spermatozoa*. Iowa State University Press, Ames, USA.

BATALHA, L. M. and OBA, E. (2006). Caracterização morfométrica e morfológica de célula espermática de capivara (*Hydrochoerus hydrochaeris*). *Archives of Veterinary Science*, 11: 66 – 72.

BLANDAU, R. J. (1951). Observations on the morphology of rat spermatozoa mounted in media of different refractive indices and examined with the phase microscope. *The Anatomical Record*, 109(2): 271 – 275.

BLOOM, E. (1973). The ultrastructure of some characteristic sperm defects. *Nordisk Veterinary Medicine,* 25: 283.

BREED, W. G. (2005). Evolution of the spermatozoon in muroid rodents. *Journal of Morphology*, 265(3): 271 – 290.

BRITO, L. F. C. (2007). Evaluation of stallion sperm morphology. *Clinical Technique in Equine Practice*, 6: 249 – 264.

BRITO, L. F. C., SERTICH, C. P. L., STULL, G. B., RIVES, W. and KNOBBE, M. (2010). Sperm ultrastructure, morphometry, and abnormal morphology in American black bears (*Ursus americanus*). *Theriogenology,* 74(8): 1403 – 1413.

BRIZ, M. D., BONET, S., PINART, B. and CAMPS, R. (1996). Sperm malformations throughout the boar epididymal duct. *Animal Reproductive Science,* 43: 221 – 239.

CALVO, A., PASTOR, L. M., MARTINEZ, E., VAZQUEZ, J. M. and ROCA, J. (1999). Age-related changes in the hamster epididymis. *Anatomical Record,* 256: 335 – 346.

CARVALHO, T. L., FAVARETTO, A. L., KOMESU, M. C., LOPES, R. A., PETENUSCI, S. O. and SILVA-NETTO, C. R. (1988). Influence of age on the production of rat spermatozoa, on their concentration in the cauda epididymidis, and on FSH, LH and testosterone plasma levels. *Histology and Histopathology,* 3(4): 413 – 417.

FAYENUWO, J. O., AKANDE, M., TAIWO, A. A., ADEBAYO, A. O., SAKA, J. O., LAWAL, B. O., TIAMIYU, A. K. and OYEKAN, P. O. **(**2003). *Guidelines for Grasscutter Rearing*. Technical Bulletin, Institute of Agricultural Research and Technology, Ibadan.

FRENEAU, G. E., CHENOWETH, P. J., ELLIS, R. and RUPP, G. (2010). Sperm morphology of beef bulls evaluated by two different methods. *Animal Reproductive Sci*ence, 118: 176 – 181.

GAGE, M. J. G. (1998). Mammalian sperm morphometry. *Proceeding of Royal Society of London,* 265: 97 – 103.

GREGOR, G. and HARDGE, T. (1995). The influence of gene variants at the ryanodine-receptor on sperm quality of boars. *Archiv fur Tierzucht,* 38: 527 – 538.

HAPPOLD, D. C. D. (1987). *The Mammals of Nigeria*. Clarendon Press, Oxford.

HUMPHREY, J. D. and LADDS, P. W. (1975). A quantitative histological study of changes in the bovine testis and epididymis associated with age. *Research in Veterinary Science*, 19(2): 135 – 141.

IMMLER, S., PRYKE, S. R., BIRKHEAD, T. R. and GRIFFITH, S. C. (2010). Pronounced with in-individual plasticity in sperm morphometry across social environments. *Evolution,* 64(6): 1634 – 1643.

JORI, F., MENSAH, G. A. and ADJANOHOUN, E. (1995). Grasscutter farming: an example of rational utilization of wildlife. *Biodiversity and Conservation,* 4: 257 – 265.

JUNG, A., SCHUPPE, H. C. and SCHILL, W. B. (2002). Comparison of semen quality in older and younger men attending an andrology clinic. *Andrologia*, 34(2): 116 – 122.

JUSTE, J., FA, J. E., PEREZ DEL VAL, J. and CASTROVIEJO, J. (1995). Market dynamics of bush meat species in

Equatorial Guinea. *Journal of Applied Ecology,* 32(3): 454 – 467.

KIDD, S. A., ESKENAZI, B. and WYROBEK, A. J. (2001). Effects of male age on semen quality and fertility: a review of the literature. *Fertility and Sterility*, 75(2): 237 – 248.

KONDRACKI, S., BANASZEWSKA, D. and MIELNICKA, C. (2005). The effect of age on the morphometric sperm traits of domestic pig (*Sus scrofa domestica*). *Cellular and Molecular Biology Letter,* 10: 3 – 13.

LEBLOND, C. P. and CLERMONT, Y. (1952). Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchs in sulfurous acid" technique. *American Journal of Anatomy*, 90(2): 167 – 215.

MALMGREN, L. (1997). Assessing the quality of raw semen: a review. *Theriogenology*, 48: 523 – 530.

MARTÍNEZ, A. I. P. (2004). Canine fresh and cryopreserved semen evaluation. *Animal Reproductive Science*, 82: 209 – 224.

MEISNER, A. D., KLAUS, A. V. and O'LEARY, M. A. (2005). Sperm head morphology in 36 species of artiodactylans, perissodactylans, and cetaceans (Mammalia). *Journal of Morphology,* 263: 179 – 202.

MEMON, M. A., BRETZLAFF, K. N. and OTT, R. S. (1986). Comparison of semen collection techniques in goats. *Theriogenology*, 26(6): 823 – 827.

MENKVELD, R., OETTLÉ, E. E., KRUGER, T. F., SWANSON, R. J., ACOSTA, A. A. and OEHNINGER, S. (1991). *Atlas of Human Sperm Morphology*. Williams and Wilkins, Baltimore.

MONADJEM, A., TAYLOR, P. J., DENYS, C. and COTTERILL, F. P. (2015). *Rodents of Sub-Saharan Africa: A Biogeographic and Taxonomic Synthesis.* Walter de Gruyter GmbH, Berlin, Germany.

MOSS, J. A., MELROSE, D. R., REED, H. C. B. and VANDEPLASSCHE, M. (1979). Semen and artificial insemination. *In*: LAING, J. A. (Ed.). *Fertility and Infertility in Domestic Animals.* 3rd Edition, Bailliere Tindal, London.

NEAVES, W. B., JOHNSON, L. and PETTY, C. S. (1985). Age-related change in numbers of other interstitial cells in testes of adult men: evidence bearing on the fate of Leydig cells lost with increasing age. *Biology of Reproduction*, 33(1): 259 – 269.

NTIAMOA-BAIDU, Y. (1998). *Sustainable use of Bush Meat. Wildlife Development Plan: 1998 - 2003.* Wildlife Department, Accra, Ghana.

OGBUEGBU, S. O., OKE, B. O., AKUSU, M. O. and AIRE, T. A. (1985). Gonadal and extragonadal sperm reserves of the Maradi (Red Sokoto) goat. *Bulletin of Animal Health and Production in Africa,* 33: 139 – 141.

OLUKOLE, S. G., OYEYEMI, M. O. and OKE, B. O. (2010). Gonadal and extragonadal sperm reserves of the domesticated adult African greater cane rat (*Thryonomys swinderianus*). *Reproductive Biology*, 10(2): 155 – 158.

OLUKOLE, S. G., OYEYEMI, M. O. and OKE, B. O. (2014). Semen characteristics and spermiogram of the African greater cane rat (*Thryonomys swinderianus,* Temminick). *Slovak Journal of Animal Science*, 47(3): 125 – 131.

OYEYEMI, M. O. and BABALOLA, E. T. (2006). Testicular parameters and morphological characteristics of testicular of testicular and epididymal spermatozoa of white Fulani bulls in Nigeria. *International Journal of Morphology*, 24(2): 175 – 180.

OYEYEMI, M. O. and UBIOGORO, O. (2005). Spermiogram and morphological characteristics in testicular and epididymal spermatozoa of large white boar in Nigeria. *International Journal of Morphology*, 23(3): 235 – 239.

PARKINSON, T. (2001). Fertility and infertility in male animals. Pages 695 – 750. *In:* NOAKES, D. E., PARKINSON, T. J. and ENGLAND, G. C. W. (Eds.). *Arthur's Veterinary Reproduction and Obstetrics.*

8th Edition, Saunders Publishers, Edinburgh.

PHILLIPS, N. J., MCGOWAN, M. R., JOHNSTON, S. D. and MAYER, D. G. (2004). Relationship between thirty post-thaw spermatozoal characteristics and the field fertility of 11 high-use Australian dairy AI-sires. *Animal Reproductive Sci*ence, 81(1/2): 47 – 61.

QUINTERO-MORENO, A., GONZALEZ-VILLALOBOS, D., LOPEZ-BREA, J. J. G., ESTESO, M. C., FERNANDEZ-SANTOS, M. R., CARVALHO-CROCIATA, J. L., MEJIA-SILVA, W. and LEON-ATENCIO, G. (2009). Morphometric evaluation of sperm head of domestic pig according their age. *Revista Cientifica FCV-LUZ*, 19: 153 – 158.

SORO, D., KARAMOKO, Y., KIMSE, M. and FANTODJI, A. (2014). Study of basic haematological parameters: indicators of the general state and immune competence in the male grasscutter (*Thryonomys swinderianus*, Temminck, 1827) bred in captivity in Côte d'Ivoire. *Journal of Animal and Plant Sciences*, 22(1): 3379 – 3387.

SOUSA, P. C., SANTOS, E. A. A., SOUZA, A. L. P., LIMA, G. L., BARROS, F. F. P. C., OLIVEIRA, M. F. and SILVA, A. R. (2013). Sperm morphological and morphometric evaluation in captive collared peccaries (*Pecari tajacu*). *Pesquisa Veterinária Brasileira,* 33(7): 924 – 930.

TABATABAEI, S., BATAVANI, R. A. and TALEBI, A. R. (2009). Comparison of semen quality in indigenous and Ross broiler breeder roosters*. Journal of Animal and Veterinary Advances*, 8: 90 – 93.

VARESI, S., VERNOCCHI, V., FAUSTINI, M. and LUVONI, G. C. (2013). Morphological and acrosomal changes of canine spermatozoa during epididymal transit. *Acta Veterinaria Scandinavica*, 55(1): 17.

VILLALPANDO, I., VILLAFAN-MONROY, H., AGUAYO, D., ZEPEDA-RODRIGUEZ, A., ESPITIA, H. G. and CHAVEZ-OLIVARES, A. (2000). Ultrastructure and motility of the caudal epididymis spermatozoa from the volcano mouse (*Neotomodon alstoni alstoni* Merriam, 1898). *Journal of Experimental Zoology*, 287(4): 316 – 326.

WELLS, M. E. and AWA, O. A. (1970). New technique for assessing acrosomal characteristics of spermatozoa. *Journal of Diary Science,* 53: 227.

WILDE, D. E., BROWN, J. L. and SWANSON, W. F. (1999). Cats. Pages 497 – 510. *In*: KNOBIL, E. and NEILL, J. (Eds.). *Encyclopedia of Reproduction.* Academic Press, New York.

ZAMBONI, Z. (1992). Sperm structure and its relevance to infertility: an electron microscopic study. *Archive of Pathology and Laboratory Medicine*, 116: 325 – 344.

ZEMJANIS, R. (1977). *Diagnostic and Therapeutic Technique in Animal Reproduction*. William and Wilkins Company, Baltimore.