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Stereological Studies on the Testis of Adult Male African Giant Rat *(Cricetomys gambianus,* Waterhouse*)*

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ABSTRACT

The science of Stereology deals with a body of methods for the exploration of three-dimensional space, when only two-dimensional sections through solid bodies or projections on a surface are available. It provides practical techniques (model- or design-based techniques) for extracting quantitative information about a three-dimensional material from measurements made on two-dimensional planar sections of the material. African giant rat (AGR) is in general appearance, much like an extremely large rat. The tail however, is distinctive; it is long and robust with the proximal portion dark, like the body of the animal, while the terminal part is white. Cheek pouches are present hence the animal is sometimes referred to as the pouched rat. However, there is dearth of information regarding the animal's testis, hence the purpose of this study was to obtain morphometric information on some parameters of its testis. In this study, the testicular volume and weight, total number of seminiferous tubules and numerical density of seminiferous tubules, as well as volume fraction of interstitium and volume fraction of testicular capillaries were investigated. The results showed that the mean body weight and testes weight of the adult AGR was 848.14g and 0.95g respectively, with no significant correlation ($p<0.05$) between these parameters ($r = 0.62$). The mean weight observed for the left and right testes were very similar ($p<0.05$), with a significant correlation ($r=0.99$). Similarly, the mean volume for the right and left testes, were very similar ($p<0.05$) and a positive significant correlation was observed ($r = 0.97$). The mean numerical density of seminiferous tubules of the left and right testes was approximately $6.54 \times 10^{-9} \mu m^{-3}$ and $6.06 \times 10^{-9} \mu m^{-3}$ respectively with a significant correlation ($p<0.05$) between the left and right testes ($r = 0.88$). The mean of the total number of seminiferous tubules of the left and right testes was approximately 55 and 49 respectively, with no significant correlation ($p> 0.05$) observed between the left and right testes ($r = 0.36$). A negative correlation with no statistical significance ($p> 0.05$) was observed for the mean fraction of interstitium of the left and right testes ($r = -0.31$). The results also showed a significant correlation ($p< 0.05$) for the mean fraction of capillaries of the left and right testes ($r = 0.64$). This study provides baseline data that could be used for further research on the testis of the AGR.

Keywords: African giant rat *(Cricetomys gambianus, Waterhouse)*; Stereology; Testis

INTRODUCTION

Conventional Morphometry is the measurement of biological objects using classical (Man-made) models, formulas and assumptions about the shape, size, orientation and spatial distributions of biological objects. It is an approach more applicable to religion than to science, because morphometry is based on faith in models, assumptions and formulas used in estimations. Morphometry is usually biased as classical models do not neatly fit into biological objects which on the other hand, are non-classical and posses both inter- and intra-variations[1]. Stereology on the alternative is the theoretically unbiased estimation of objects based on designs. It deals with a body of methods for the exploration of three-dimensional space, when only two-dimensional sections through solid bodies or projections on a surface are available[1,2]. Stereology provides practical techniques (design-based methods) for extracting quantitative information about a three-dimensional material from measurements made on two-dimensional planar sections of the material[1]. It applies methodology that avoids assumptions about the shape, size, orientation and distribution of objects; avoids the use of incorrect formulas; avoids the use of models; avoids the application of wrong mathematics amongst others, in the estimation of objects. It is more precise and accurate, and provides information on biological objects in numbers, rather than expressing biological information in terms of " increasing" or "decreasing", an approach that is less precise but well utilized in morphometry[2].

In this study, some stereological principles, which include Physical disector, Cavaleri's point-counting methods, were applied to investigate some parameters of the testes of African giant rat *(Cricetomys*

gambianus, Waterhouse*)*. Booth[3] stated that giant rats and grass cutters have a wide distribution, occurring everywhere in Africa. Thus the species is adapted to a wide variation of climate and ecological zones. This kind of adaptation to various ecological conditions is an attribute which Bigalke[4] recommended as an important criterion in selecting animals for domestication. Asibey[5] found that there was no data for giant rat consumption in Ghana, but that it is as important as the grass cutter. Bates[6], reported that hunters in Southern Cameroon went on distant camping trips for the expressed purpose of seeking and smoking grass cutters and giant rats for meat. However in Southern Nigeria, the African giant rat has been reported to have a wide social acceptance and have been kept successfully in captivity as pets[7]. According to Adebayo *et al.,* the gross morphometry of the vesicular, prostate and the bulbourethral glands of the Greater cane rat *(Thryonomys swinderianus,* Termminck*)* showed similarity to that of the rabbit and African giant rat[8]. Osinubi *et al.,*[9] applied stereology to determine the long-term morphometrical response of the testis of rat to long-term administration of Quinine. Katoh *et al.,*[10] showed in their study that paired parameters of the testes of a rat (*Thryonomys swinderianus*) have similar values. The Greater cane rat is a comparable but larger rodent than the African giant rat[11] which has an average liveweight of 800-1400g[12]. *Cricetomys gambianus* Waterhouse is aggressively hunted for meat and local earnings in most parts of West African countries[13], posing a threat to the ultimate survival of the species[14]. Moreover, domestication of the giant rat has not been all that successful so far, and this may be a possible reason for the paucity of information on the animal's testes[14].

Therefore, this study was conducted to investigate some testicular parameters of the giant rat to provide useful research data in the comparative regional anatomy and reproductive biology of the animal, which may also subsequently aid in the domestic rearing and domestication of rat, using unbiased and efficient stereological methods that provide reliable and precise data of parameters, rather than providing data in "ranges", as in the case of conventional morphometry.

MATERIALS AND METHODS

Animal Model: Seven sexually mature male (20-24 weeks of age, weighing 653-990g) African giant rats were obtained from Mushin General Market Mushin, Lagos and carried to the Department of Zoology, University of Lagos, for certification. On arrival at the animal room, Department of Anatomy, University of Lagos, the animals, were housed in perforated iron cages, in a specific pathogen-free environment, under standard conditions of temperature (25±2^{O}C), relative humidity (50±10%) and light (12hr light / 12 hr dark). They were fed with peanuts, maize and baked cake made from ground beans. They were allowed to acclimatize for two weeks (14days), in the animal room, before the onset of experimentation.

Animal Sacrifice and Tissue Processing: After 14days of acclimation to the environment, the rats were placed inside a desiccator containing chloroform for brief anaesthesia. They were brought out of the desiccator, and longer-time anaesthesia was induced by intra-abdominal injection of 0.5ml ketamine hydrochloride (per animal) with an insuline syringe.
Whole-body Perfusion.

The animals were subsequently perfusion-fixed by whole body perfusion, based on the method proposed by Sprando (see review[15]) as follows: a midline abdominal incision was made on each rat, while the heart was still beating. The incision was made through the abdomen to the thorax, gaining access to the heart. A butterfly needle, connecting the line of normal saline, was placed into the apex of the beating heart, and a nitch (cut) was quickly made on the inferior vena cava (IVC), to allow a free flush, of the entire blood of the animal.

Through this line containing normal saline, about 20-25cl of normal saline, was run into the animal through its heart. About same quantity of Bouin's fluid was also passed through the animal, via same line, after the normal saline, to initiate the process of anti-post mortem changes.

Organ Removal: Through this same abdominal incision, the testes were dissected out, cleared of adhered connective tissue.

Tissue Processing: After removal, the testes were refixed in Bouin's fluid for 24 hours. After 24 hours, they were dehydrated in ascending grades (70% - 100%) of ethanol. The testes were fixed for 2 hours in each grade of ethanol.

Sampling: According to Gundersen HJG and Jensen EB[16], a systematic sampling provides equal representation of every parameter under investigation and increases efficiency in stereology and stereological prediction. A systematic sampling was carried-out on each testes to obtain a sample of tissue sections for stereological estimations to determine stereological parameters. Samples of the tissue sections were obtained by; sectioning each tissue into slabs of 2mm, embedded, and finally every 5th section (of 40µm thickness) was picked from each slab as follows: An exhaustive serial sectioning of the testes was done to produce parallel slices (slabs) of 2mm thickness.

The slabs were divided into ten (10) sampling fractions, f, (f = 1)

10.
Every 6th (sixth) slabs from each of these sampling fractions was picked (i.e. 6th-,12th-, 18th-, 24th-, 30th- , 36th- slab, etc) and cleared-off ethanol, with xylene, before embedding, in paraffin wax.

Sectioning, Mounting and Staining: Sections of 40µm thick[2] were obtained and every 5th section was picked, mounted and stained with Haematoxylin and Eosin (H&E).

Determination of Testicular Weight and Volume: The testes were initially dissected out, cleared of connective tissue. Their weights were measured on a sensitive digital balance. While the testicular volume was obtained by Archimedes' fluid (water) displacement method, using 10ml measuring cylinder, before they were subjected to further tissue processing.

Determination of Stereological Parameters: The numerical density of seminiferous tubules, total number of seminiferous tubules, volume fraction of interstitium, and volume fraction of testicular capillaries, were determined using image analyzer program "Leica 1CC50 HD", version 4.1.0 (Build: 1264) under a x4/0.10 objective lens [obtaining counts between the range of 124 - 176 points, to satisfy the guilding principle for the number of points to be counted per structure, which is between 100 – 200 (see review[16,17])], connected to a desktop computer, at the Department of Anatomy, College of Medicine, University of Lagos, Lagos Nigeria, as follows:

- An Area of Interest (AOI) was captured from the testicular tissue section; and this was constant for each tissue structure.
- A point counting grid (test system) and a dissector frame (test system), were per-turn superimposed on the tissue section to estimate:

(a) Numerical density (N_v) of seminiferous tubules. This is the number of objects per unit volume and it was determined by the equation:

$$N_v = \frac{\Sigma Q^-}{\Sigma \text{Vol.}_{samp}}.$$

proposed by D.C Sterio, 1984[1], after sampling the tissue sections using a test frame according to the unbiased forbidden line rule[17].

Where, ΣQ^- is the sum of objects counted and Σ Vol. $_{samp}$. is the sum of volume of all disectors. Σ Vol. $_{samp}$. = n X [a (frame] X h], where n is the number of disector sampled, h is height of disector probe in z-axis, a (frame) is area of disector frame, in x- X y-axis[1].

(b) Total number (N) of seminiferous tubules. The total number of seminiferous tubules was determined, using the equation:

$N = N_v \text{ X } V_{ref}$. proposed by Gundersen (see review[16]) where V_{ref}. is the volume of reference space, and it was calculated:

$V_{ref} = a(p) \text{ X } t \text{ X } \Sigma P$, where, a(p) is the area per point in μm^2 (40µm in this work), and Σ P, is sum of points hitting the reference space[1].

(c) Volume Fraction of Interstitium. The volume fraction of interstitium was estimated (using Cavaleri-point counting method) by superimposing a transparent test point system (point grid) of regularly spaced points, on the samples section (reference section), counting the points that fell on testicular interstitium, and dividing it by the total points that fell on the entire sample (AOI) of testis section. The calculation was: $\Sigma P_{int.}$

$\Sigma P_{test.}$,

and it is represented in percentage (%) where; $\Sigma P_{int.}$ is the sum of points hitting the interstitium, and $\Sigma P_{test.}$ is the sum of points that fell on the entire sample (AOI) of testis section[1].

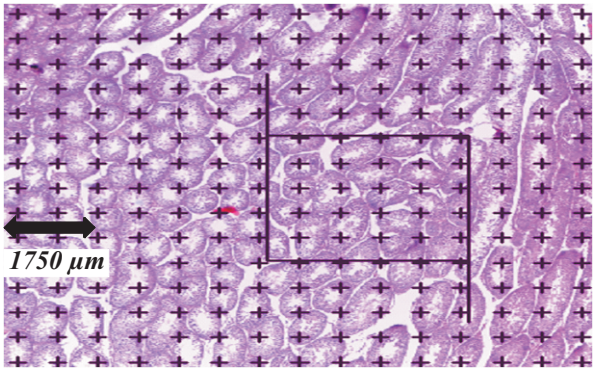
(d) Volume Fraction of Testicular capillaries. The fraction of testicular capillaries was obtained using Cavaleri-point counting method of superimposing a transparent test point system (Point grid) of regularly spaced points, on the sampled section (reference section), and counting the points that fell on testicular capillaries divided by the total points that fell on the entire sample of testis section. The calculation was:

$\Sigma P_{cap.}$

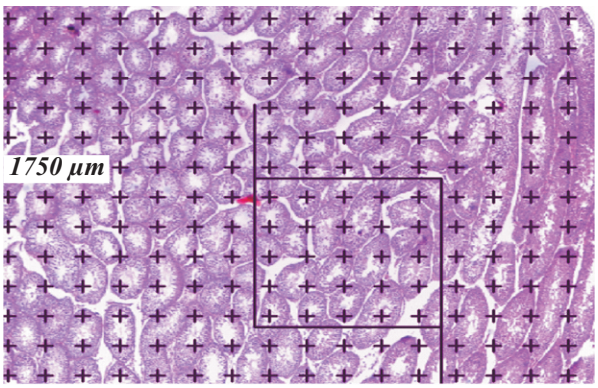
ΣP_{testis}

and represented in percentage (%).

Where, $\Sigma P_{int.}$ is the sum of points that fell on the capillaries, and $\Sigma P_{test.}$ is the sum of points hitting the testicular sample of testis[1].



(a) Reference section



look up section

Figure1. Photomicrographs of sections of testes of *Cricetomys gambianus* Waterhouse. The test system superimposed in (a) and (b) was used in the estimation of the numerical density (N_v) of seminiferous tubules. Scale bar = 1,750µm.

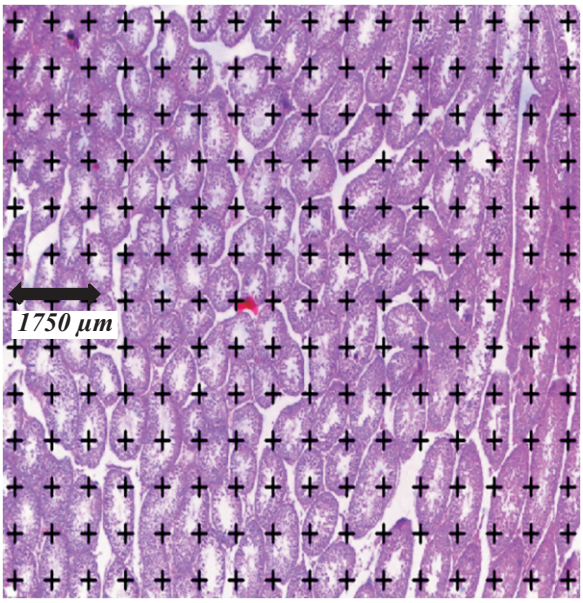


Figure2. Photomicrograph of a section of testis of *Cricetomys gambianus* Waterhouse. The test system superimposed on this section was used in the estimation of fraction of interstitium of the testis and fraction of testicular capillaries. Scale bar = 1,750µm.

Statistical Analysis.
All data are presented as Mean±SEM. Analysis of correlation was made using the program STATISTICA for Windows (Statsoft, Inc. Tulsa, Ok). The significance level considered was P<0.05.

RESULTS

The mean body weight and testes weight found for the adult African giant rat (AGR), was approximately 848.14g and 0.95g respectively (Table 1 below), a significant correlation (p<0.05) was observed between these parameters (r = 0.62). The mean weight observed for the left and right testes were very similar (p<0.05) and a positive significant correlation was observed between them (r = 0.99). Similarly, the mean volume, for the right and left testes, were very similar (p<0.05) and a positive significant correlation was observed (r = 0.97). Mean numerical density (N_v) of seminiferous tubules observed for the left and right testes were approximately 6.54 x 10^{-9} µm^{-3}and 6.06 x 10^{-9} µm^{-3} respectively (Table 1). A positive significant correlation (p<0.05) was observed between the right and left for this parameter (r = 0.88). No significant correlation (p>0.05) was observed between the left and right testes for the mean total number (N) of seminiferous tubules (r = 0.36). Similarly no significant correlation (p>0.05) was observed for the mean volume fraction of interstitium (see Table 1) in the left and right testes of the AGR (r = -0.31). The result also indicates that there is a significant correlation (p<0.05) for the mean volume fraction of capillaries, of the left and right testes (r = 0.64).

Table 1. Biometric and morphometric data of sexually mature African giant rat, expressed as mean ± SEM (n =

Parameter	Left testis	Right testis	Statistical significance (Analysis of Correlation, r)[b]
FT_c (%)	0.04 ± 0.01	0.04 ± 0.01	*
FT_i (%)	0.12 ± 0.01	0.1 ± 0.01	Ns
N	55 ± 3	49 ± 3	Ns
N_v(x10^{-9} µm $^{-3}$)	6.54 ± 0.97	6.06 ± 0.91	*
V_T (ml)	1.21 ± 0.24	1.13 ± 0.22	*
W_T (g)	1.01 ± 0.24	0.89±0.20	*
W_T (g)[a]	0.95 ± 0.22		*
W_B (g)	848.14 ± 44.11		

SEM, Standard error of mean
n, Number of animals utilized
FT_c, Volume fraction of testicular capillaries
FT_i, Volume fraction of testicular interstitium
N, Total number of seminiferous tubules
N_v, Numerical density of seminiferous tubules
V_T, Volume of testis
W_T, weight of testis
[a]W_T, Weight of right testis plus left testis divided by two.
W_B, Body weight of rat
LT, left testis; RT, right testis.
[b]ns: not significant; * p< 0.05.

DISCUSSION

A mean testicular weight of 0.95g and an average body weight of 848.14g obtained in this study shows that AGR has a testicular/body weights similar to other large rodents like the African great cane rat (see review[11]). The significant similarity (p<0.05) and correlation (r=0.99) observed between the right and left testes in this study is typical of most paired organs of similar rodents[10]. Similarly, the average liveweight of the AGR in this study (848.14g) was within the range (800-1400g) reported by Ajayi[12]. Results of the present study were obtained using unbiased counting rules and unbiased counting frame proposed by Gundersen[17], unbias stereology. These results show divergence to those obtained for the control animals in the experiment by Osinubi *et al.,*[9] in which biased/old stereology was applied.

CONCLUSION

The findings of this study provide baseline information on the testes of the African giant rat, thereby making available useful research data in the comparative regional anatomy and reproductive biology of the animal which may subsequently aid in its domestic rearing and domestication.

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