



**BIOMETRIC AND HISTOLOGICAL STUDY OF THE OVARY OF THE FEMALE AFRICAN GIANT RAT (*CRICETOMYS GAMBIANUS*, WATERHOUSE)**

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**ABSTRACT**

This study was carried out to investigate the morphometric parameters of the ovaries of the African giant rat. A total of ten mature female AGRs were used in this study. The rats were sacrificed by cervical dislocation lightly and weighed. The ovaries were collected for biometrical, histological analysis. The length, width, thickness and weight of the right and left ovaries were measured. The result showed that the mean length, width, thickness and weight of the right and left ovaries were  $0.78 \pm 0.05$ ,  $0.02 \pm 0.03$ ,  $0.24 \pm 0.03$  cm and  $0.78 \pm 0.04$ ,  $0.02 \pm 0.01$ ,  $0.25 \pm 0.03$  cm respectively. The mean weight of the ovaries was observed to be  $0.08 \pm 0.02$  and  $0.08 \pm 0.03$  g respectively. Sections of 5µm were cut in paraffin blocks, stained with haematoxylin and eosin stain. Histologically, the ovary was composed of two zones, cortex and medulla. Primary, secondary, tertiary follicles, corpus luteum and atretic follicles were seen in the cortex of the ovaries. Theca interna and externa were evident in tertiary and developed secondary follicles. The medulla consisted of loose connective tissue which contained blood and lymph vessels of varying sizes. The mean diameter of the ovarian follicles measured with ocular micrometer, varied between 16 - 440µm. This study has provided a base-line data of the histology and ovarian parameters of the AGR.

**Keywords:** African giant rat, Biometry, Histology, Ovary,

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**INTRODUCTION**

The African Giant Rat (AGR) also known as the giant pouch rat resembles a hamster in having a storage pouch inside of each cheek. The pouch rats are huge and are among the largest of the murid rodents [1]. There are two species of the genus *Cricetomys*; *Cricetomys gambianus* and *Cricetomys emini*. They have a wide distribution occurring everywhere in tropical Africa. AGR's meat has relatively higher protein content than beef, pork and mutton and can be used as alternative meat source [2]. The AGR by size is one of Africa's largest rodents and is arguably becoming Africa's most intriguing rodent because of its scientific attributes such as the detection of landmines [3] and also in the medical diagnosis of pulmonary tuberculosis [4], disease vectors [5], potential pest species status [6] among many others.

Although domestication of the AGR has been encouraged in Africa, progress work has however been slow due to paucity of information on its reproductive biology. Thus, it is necessary that the biology including the biology of reproduction be thoroughly investigated as a means of providing adequate information to aid the process of domestication. At present, a lot of research has already been done on the reproductive biology of the

male AGR by Oke *et al.* and Adeyemo & Oke [7, 8] while existing reports on the reproductive biology of the female appears to be limited. Ali *et al.* [9] reported few work on the female reproductive organs of AGR.

The ovary is the major female reproductive organ of most mammals. In rats, the ovaries are lentiform, lie on each side of bicornuate uterus in relation to the lateral abdominal wall. Ovaries mainly produce haploid female gametes (oogenesis) and steroid hormones [10]. Tunica albuginea, is a poorly vascularized, dense, irregular collagenous connective tissue capsule of the ovary. Each ovary is subdivided into the highly cellular cortex and medulla, which consisted mostly of a richly vascularized loose connective tissue. The cortex, which is surrounded on the outside by the surface epithelium, contained germ cell clusters, some primordial, primary, secondary, tertiary and atretic follicles, and dilated blood vessels. Primordial follicles in the ovary of the porcupine were found to be distributed under tunica albuginea and extended deeply for a short distance into the cortical tissue. The primordial follicle was composed of a primary oocyte surrounded by a single layer of flattened follicular cells cuboidal in shape. Primordial follicles were approximately 80 µm in diameter [11]. Primary

follicles were composed of a primary oocyte surrounded by a simple cuboidal and a simple columnar epithelium of follicular cells. Primary follicles were approximately 150  $\mu\text{m}$ . Secondary follicles were composed of a primary oocyte surrounded by a stratified epithelium of granulosa cells and were approximately 210  $\mu\text{m}$  in diameter. Tertiary follicles were composed of a primary oocyte surrounded by a stratified epithelium of follicular cumulus cells. The stratum granulosum was surrounded by the theca, which in tertiary follicles differentiates into two layers: an inner vascular theca interna and an outer supportive theca externa. The theca interna cells were spindle shaped and located in a delicate reticular fibre. The theca externa consisted of a thin layer of loose connective tissue with fibroblasts arranged concentrically around the theca interna. In these follicles, PAS positive reaction was seen in the zona pellucida. Tertiary follicles were approximately 1600  $\mu\text{m}$  in diameter [11]. The detection of ovarian function is very hard without a thorough understanding of the normal morphology, and so, the purpose of this study was to evaluate the histology and biometry of the ovary of the AGR.

## MATERIALS AND METHODS

### Study area and site

The experiment was conducted at the Department of Veterinary Anatomy, Ahmadu Bello University, Zaria (11°N, 742°E), located in the Northern Guinea Savannah zone of Nigeria, at an altitude of 686 m above maximum sea level, and has monthly equatorial photoperiod of  $12.13 \pm 0.13$  h [12]. The zone is characterized by three major seasons: harmattan (November - February), hot-dry (March - May) and rainy (June - October) seasons [12, 13].

### Animal husbandry and management

Mature female AGRs (*Cricetomys gambianus*) were obtained from rural farmers in the surrounding villages around Zaria, Nigeria were used for the study. They were transported via vehicle to the experimental animal unit of the Department of Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The animals were housed in metal standard laboratory cages, measuring 50 x 40 x 40 cm. Throughout the experiment, the rats were fed with feed supplement, mixtures comprising of maize grains, ground-nuts, dried cassava tubers and sweet potatoes and drinking water provided ad libitum. All animal procedures and protocols were carried out according to the strict guidelines prescribed by the Animal Care and Use Committee of the American Society of Mammalogists for the use of wild mammals in research [14].

### Organs collection

The AGRs were sacrificed by cervical dislocation and weighed using a weighing balance (Ohaus scale crop) with a sensitivity of 0.1g and then sacrificed by severing the jugular vein. The animals were then placed on a dorsal recumbence and a mid-line incision was made, starting from the xiphoid cartilage to the pubic symphysis in order to expose the structures in the peritoneal cavity. The peritoneum was reflected and the intestines exteriorize to gain access to the reproductive organs. The ovarian samples, trimmed of fat and extraneous tissues were measured. The length, width, thickness and weight of the right and left ovaries were measured using Venire caliper and a mettler balance P1210 (Mettler Instrument AG, Switzerland) with a sensitivity of 0.01g respectively.

### Histological preparation

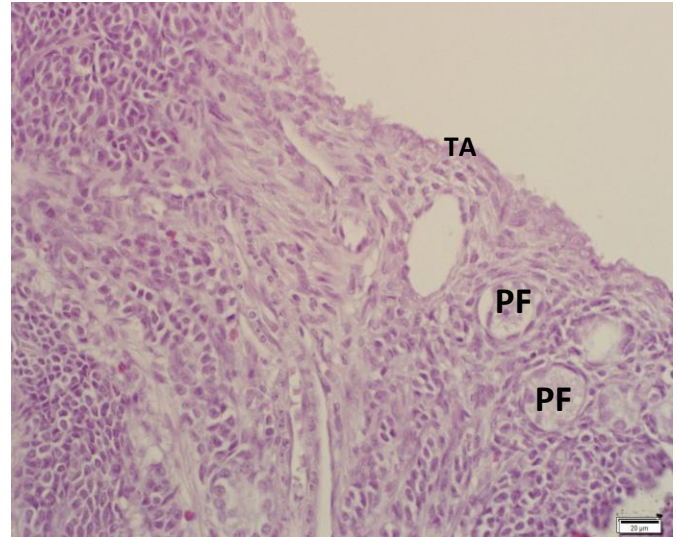
The harvested ovarian tissues from each rat were cut into thin slices, were kept in labelled bottles containing 10% buffered neutral formalin (for 24 hours). After fixation, the tissues were subsequently processed through a series of graded ethanol solution (70%, 80%, 90%, and 100%) for 1.5h with gentle shaking and the tissues were soaked in absolute ethanol overnight, then cleared in xylene (three rinse, 30min each) and embedded in molten paraffin [15]. Transverse sections (5  $\mu\text{m}$ ) were cut and stained at room temperature using hematoxylin and eosin (H and E). The stained slides were examined under a bright-field microscope (OlympusTM Corporation, Japan) at magnification of  $\times 40$ ,  $\times 100$  and  $\times 400$  and digital photographs were taken using Sony® cCyber-short digital still camera N50. The diameters of ovarian follicles were measured using an ocular micrometer. The data generated were expressed as mean  $\pm$  standard error of the mean (mean  $\pm$  SEM).

## RESULTS

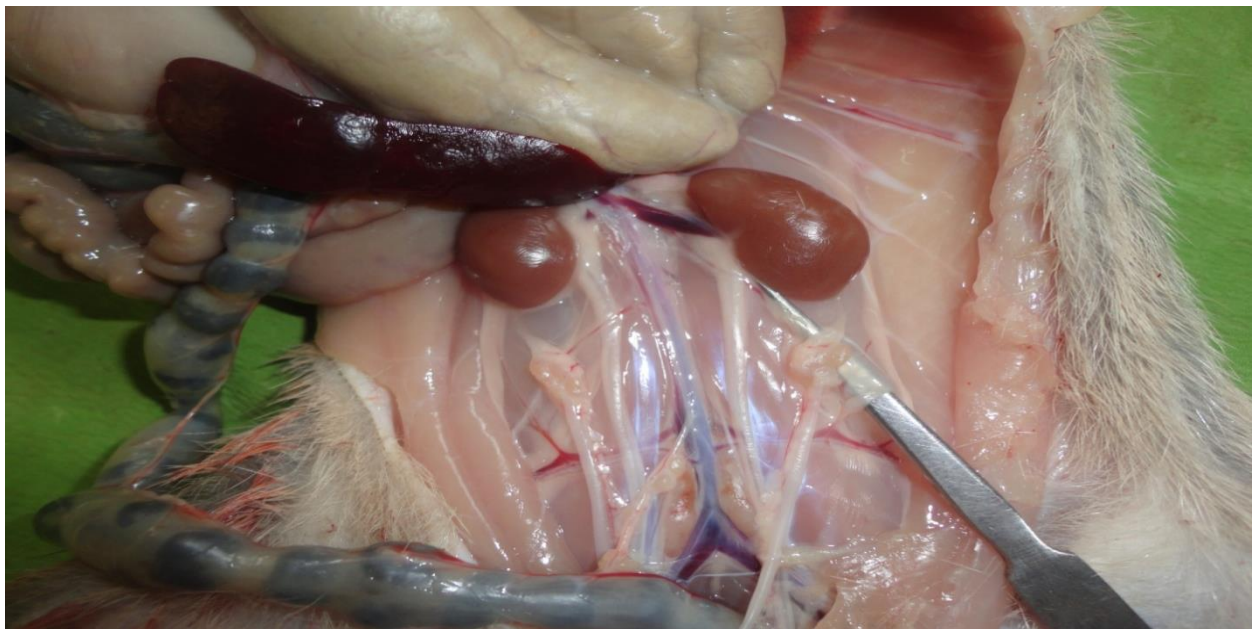
Plate I shows that the ovaries of the AGR are located in the sublumbar area, caudal to the kidneys, with the right ovary being situated more anteriorly than the left and were connected by proper ligament to the lumbar muscle cranial to the kidneys. Plates II and III shows the histological features of the ovary. It was subdivided into the highly cellular cortex and medulla, which consisted mostly of richly vascularized loose connective tissue. The cortex, which was surrounded on the outside by surface epithelium, contained germ cell clusters, some primordial, primary, secondary, tertiary, corpus luteum and atretic follicles. Immediately beneath the surface epithelium was a dense connective tissue sheath, the tunica albuginea. Primary follicles were found to be distributed under tunica albuginea and extended deeply for a short distance into the cortical tissue. The primary follicles were composed of primary oocyte surrounded

by a simple cuboidal and a simple columnar epithelium of follicular cells. The primary follicles were approximately 16  $\mu\text{m}$ . the secondary follicles were composed of primary oocyte surrounded by a stratified epithelium of granulosa cells and were approximately 230  $\mu\text{m}$  in diameter. The tertiary follicles were composed of a primary oocyte surrounded by a stratified epithelium of follicular cumulus cells. The stratum granulosum was surrounded by the theca, which was differentiated into two layers: an inner vascular *theca interna* and an outer supportive *theca externa*. The tertiary follicles were approximately 440  $\mu\text{m}$  in diameter.

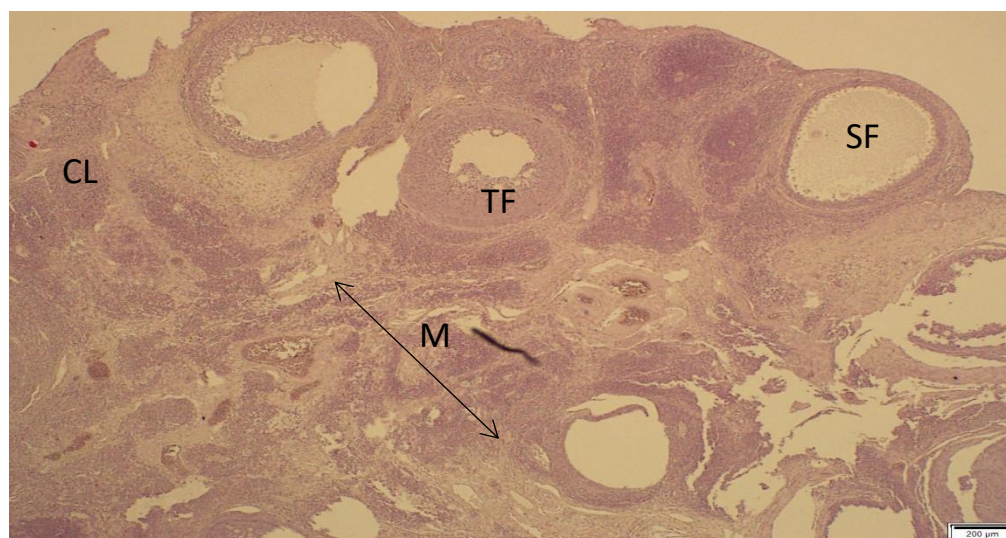
Table 1 shows the mean length, width and thickness of the right and left ovaries which were  $0.78 \pm 0.05$  cm,  $0.02 \pm 0.03$  cm,  $0.24 \pm 0.03$  cm, and  $0.78 \pm 0.04$  cm,  $0.02 \pm 0.01$  cm,  $0.25 \pm 0.03$  cm respectively.



**Plate II:** Section of the ovary of the AGR showing primary follicles (PF) and tunica albuginea (TA). Haematoxylin and Eosin, Scale bar = 20 $\mu\text{m}$ .



**Plate I:** Photograph of the ovaries of the AGR and other related structures *in situ*: Left kidney (LK), left ovary (LO), stomach (S), left uterine horn (LU), spleen (SP), right kidney (RK), right ovary (RO), colon (C) and right uterine horn (RU).



**Plate III:** Section of the ovary of the AGR showing secondary follicles (SF) tertiary follicle (TF), corpus luteum (CL) and medulla (M). Haematoxylin and Eosin, Scale bar = 200  $\mu$ m.

**Table 1:** Mean values of right and left ovarian parameters of female African Giant rats (Mean  $\pm$  SEM).

Parameters	Right Ovary	Left Ovary
Length (cm)	0.78 $\pm$ 0.05 <sup>a</sup>	0.78 $\pm$ 0.02 <sup>a</sup>
Width (cm)	0.02 $\pm$ 0.01 <sup>a</sup>	0.02 $\pm$ 0.01 <sup>a</sup>
Thickness (cm)	0.24 $\pm$ 0.03 <sup>a</sup>	0.25 $\pm$ 0.03 <sup>a</sup>
Weight	0.08 $\pm$ 0.02 <sup>a</sup>	0.08 $\pm$ 0.03 <sup>a</sup>

a, b Values with same letter superscript on same row does not differ significantly ( $P > 0.05$ ).

## DISCUSSION

The ovaries of the AGR were found to be small, pinkish and kidney shaped, with two distinct poles, cranial and caudal poles. This finding agreed to what was reported by [16] in the Mongolian gerbil. However, the finding was contrary to the report of Hebel and Stromberg [17] in the laboratory rats, where the ovary was reported to be a follicular mass with an irregular nodular surface. The weight ( 0.08  $\pm$  0.03 g ) of the ovary of the AGR varied considerably compared with those of the laboratory rats (0.06 g) reported by Hebel and Stromberg [17], and the rabbit (0.23  $\pm$  0.022 g) as presented by Bitto *et al.* [18]. The differences in the dimension obtained above may be due to the variation in the size of the animals. The cortex of the ovaries had follicles at various stages of development and many corpora lutea and interstitial cells. Williams [19] reported that the ovaries of domestic mammalian and laboratory animals, having both exocrine and endocrine functions, consists of an outer cortex and an inner

medulla; the cortex contained follicles, which were in various stages of development, while the medulla consisted of loose connective tissue, as well as blood and lymph vessels. In the present study we observed similar histological findings in the ovaries of the AGR. Polyovular follicles in the ovaries of some rodents have been described previously, for instance, in hamster and guinea pig [20]. However, Ozdemir and Dinc [21] did not observed polyovular follicles in the guinea pig and the mouse. In the present study, polyovular follicles were not observed in the ovaries of the AGR, which is consistent with previous reports. Many studies measuring follicular diameter have been reported in various animals and was found to be: 18 - 300  $\mu$ m in guinea pig [21], 100 - 399  $\mu$ m in the mouse [22] and 0.1 - 0.8 mm in rat. In the present study, the follicular diameter was found to be 16 - 440  $\mu$ m in diameter. During this study, the epithelial cells vary in shape over the surface of same ovary, from squamous to low columnar. The differences in shape appear to result from simple mechanical deformation of otherwise identical



cells. It was ascertained that ovarian volume and number of mitoses in the surface epithelium varies in adult with the estrous cycle.

## CONCLUSION

This study documents baseline data on the morphometry of the left and right ovaries and the ovarian follicular diameter of the AGR, thus contributing to the knowledge of comparative anatomy in rodents.

## ACKNOWLEDGEMENTS

Special thanks to God Almighty and the Department of Veterinary Anatomy.

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