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Histomorphological Changes in the Ovary of African giant rat (*Cricetomys gambianus,* WATERHOUSE - 1840) subjected to Different light/dark cycles.

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ABSTRACT

The present study was undertaken to investigate the effect of photoperiod on the ovary of African giant rats (AGR), Cricetomys gambianus. A total of 15 captive African giant rats from the wild served as subjects. The rats were randomized into three photoperiodic treatment groups of five rats each. Groups I, II and III were the control (12 hours of darkness (12D), 12 hours of light (12L); total darkness (24D: 0L) and total light (0D: 24L), respectively. The rats were humanely euthanized, and ovaries collected at 4 weeks and 8 weeks of sacrifice for routine histological examination and histomorphometry. Cystic follicles were observed in the ovaries of AGR exposed to constant light. Again, continuous lighting exposure significantly increased ($p < 0.05$) the ovarian follicular diameter when compared with the control. In conclusion, the findings of this study indicate that continuous light exposure could have significant effect in follicular cyst formation in AGR.

Keywords: African giant rat, Photoperiod, Ovary, Histomorphology

INTRODUCTION

Rodents form an important source of meat supply in tropical regions of Africa [1], and the African giant rat (AGR), *Cricetomys gambianus*, is one of the most important [2]. Thus, to provide a sustainable alternative supply, it is imperative to breed them [3][4]. However, the progress in rearing and domestication of the AGR has however been slow due to paucity of information on its reproductive biology [5], particularly as it is influenced by photoperiodism.

It is well established that many animals use day length (photoperiod) to predict and adjust seasonal changes in the environment through predictive changes in physiology and behaviours [6][7]. Together with temperature changes, photoperiod elicit changes in the color of fur and feathers [8][9], migration, entry into hibernation, sexual behaviour, e resizing of gonads [10].

Thus, knowledge of the season of the year is vital to many animals [10]. In mammals, most physiological processes exhibit daily rhythms generated by a system of cell autonomous circadian oscillators located in the brain and peripheral organs and tissues [11]. A master clock in the hypothalamic suprachiasmatic

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nucleus provides circadian output signals that are essential for maintaining synchrony of oscillators within organs and between organ systems and for coupling circadian physiology to environmental light-dark cycles [12].

Photoperiod affects reproduction in several rodent species [13][14]. It has been shown that female Syrian hamster became acyclic following exposure to short days [15]. On the other hand, impaired ovary development has been indicated in mouse exposed to continuous light [16]. However, fewer studies were reported in female AGR [3;17]. Therefore, in view of the increasing need to breed AGR, and provide a sustainable alternative animal protein supply, the present study was designed to investigate the histomorphological changes in the ovary of AGR subjected to controlled experimental photoperiod conditions.

Materials and methods

Study Area

The study was conducted in Ahmadu Bello University, Zaria (11°4′N, 7°42′E), located in the Northern Guinea Savannah zone of Nigeria, with an altitude of 686 m above sea level, and a mean (± Standard Error) monthly photoperiod of 12.13 ± 0.13 h [18][19]. The zone is characterized by three seasons: Wet Season (June-October), Cold dry Season (November-February) and Hot dry Season (March-May) [19][20].

Animals and Management

A total of fifteen (15) adult female African Giant rats (AGR) (*Cricetomys gambianus*) weighing between 1.3-1.6 kg, captured from the wild were purchased from surrounding villages in Zaria, Nigeria during the rainy season. They were transported to the experimental animal unit of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. They were housed in cages with individual cells. Feed (groundnuts, carrots, cucumber, sweet potatoes and onions) and water were provided *ad libitum*. The rats were acclimatized for a period of at least two (2) weeks prior to the commencement of the experiment. The study protocol was approved by the Ahmadu Bello University Institutional Animal Care and Use Committee and ethical clearance number (ABUCAUC/2021/043) was subsequently issued.

Experimental Procedure

Following acclimatization, the rats were randomly divided into three (3) photoperiodic treatment groups of five (5) rats each: Group I-control rats (12 h light/ 12 h darkness); Group II-rats exposed to constant darkness (0 h light/ 24 h darkness); Group III- rats exposed to constant light (24 h light/ 0 h darkness). The experiment was conducted for a period of 8 weeks.

Sample Collection

At the end of the experimental period, the rats were humanely euthanized using sodium pentobarbital at 86 mg/kg I.P. A ventral mid-line incision was made from the base of the neck to the pelvic area to expose the abdominal cavity. The rats were dissected gently to expose the reproductive organs. The ovaries were excised from adjoining structures and trimmed of excess fat for histomorphological studies.

Tissue preparation

The ovaries were processed by immersing in 10 % neutral buffered formalin. Post-fixation, the tissues were dehydrated through a series of graded concentrations of ethanol (70%, 80%, 90%, and 100%) with gentle shaking and then soaked in absolute ethanol overnight. The tissues were immersed in xylene for 2 hours and infiltrated in molten paraffin wax [21]. Sections of 5 µm thick were cut from the embedded tissues and mounted on clean grease-free glass slides and stained using Haematoxylin and Eosin (H & E). The stained slides were examined using light microscope (Amscope, T120B California) and photographed using Amscope Digital Camera

(Tokyo, Japan) for microscope version 2.0 for histological and histometric evaluations.

Histomorphometry evaluations

Histomorphometry of the ovaries was performed in the Department of Veterinary Anatomy in Federal University of Agriculture, Abeokuta, Nigeria using Digitizer Image analysis software (Medcalc Software™, Oostende, Belgium). From the photographed sections (x10 objective lens), follicular diameter and follicular epithelial height were measured using an ocular micrometer calibrated with a stage micrometer. Data were finally expressed in micrometers.

Data Analyses

Histomorphometric data generated were expressed as mean (± standard error of the mean), and were subjected to statistical analysis using IBM-SPSS (Statistical Package for Social Science), Version 17.0, Chicago, IL, USA. Significant differences were assessed using one- way analysis of variance (ANOVA) Value of $p < 0.05$ was considered significant.

Results

Histology

The present study revealed that the ovarian cortex contained follicles at various stages of development and these included primary, secondary, tertiary and atretic follicles, and *corpora lutea*. Ovaries from rats in the control group displayed few Graafian follicles (tertiary follicles), in addition to atretic, pre-antral follicles and increased size and number of corpora lutea (Plate 1). On the other hand, rats exposed to constant light exhibited large cystic follicles, although secondary follicles and corpus luteum were also seen (Plate 2). However, there was conspicuous presence of Graafian follicles, in addition to developing follicles and decreased size and number of corpora lutea in the ovaries of rats exposed to total darkness (Plate 3). Moreover, corpus luteum was completely absent in the ovaries of rats exposed to total darkness for 8 weeks.

Histomorphometry

There was no significant difference ($P > 0.05$) in mean follicular diameter among all the treatment groups regardless of photoperiod at the end of 4 weeks exposure (Table 1). Contrastingly, after 8 weeks, mean follicular diameter was significantly ($P < 0.05$) reduced in rats exposed to total darkness and significantly increased ($P < 0.05$) in the group exposed to constant light when compared with those in the control and total darkness groups (Table 1).

The mean epithelial height was significantly ($P < 0.05$) increased at 4 weeks in rats exposed to total darkness when compared to other treatments. However, at the end of 8 weeks exposure, the mean epithelial height was significantly ($P < 0.05$) reduced in rats exposed to total darkness and significantly ($P < 0.05$) increased in those exposed to total light (Table 1).

Table 1: Mean values of ovarian follicular diameter and epithelial height in African Giant Rats, *Cricetomys gambianus,* exposed to different photoperiods

Parameter	Duration of exposure (Wks.)	Control (n=5)	Total light (n=5)	Total darkness (n=5)
Follicular diameter (μm)	4 weeks	229.05 ± 35.45^{ab}	263.17 ± 33.55^{ab}	247.88 ± 40.11^{ab}
	8 weeks	236.67 ± 34.05^{ab}	317.56 ± 30.47^{b}	216.56 ± 20.57^{a}
Epithelial height (μm)	4 weeks	24.55 ± 4.17^{a}	24.48 ± 2.09^{a}	42.02 ± 3.61^{c}
	8 weeks	36.00 ± 3.16^{b}	45.80 ± 3.33^{c}	25.10 ± 2.57^{a}

[a,b,c] Values with the different superscript alphabets in the same row differ significantly at $p < 0.05$.

Discussion

The present study revealed the presence of ovarian follicles at various stages of development. The presence of large cystic follicles observed in the ovaries of rats exposed to continuous light could be a consequence of reduced ovarian activity and the stressful effect of constant light exposure on the AGR. This observation corroborates the findings of [3] in AGR and [22] in Wistar rats. In addition, [23] reported the presence of light induced stress in female AGR subjected to >12h of light. On the hand, the conspicuous presence of Graafian follicles and small follicles at different phases of development in the ovaries of rats exposed to total darkness suggests an active ovary. The aforementioned observation could be hinged on the fact that the AGR is nocturnal and lengthening of the dark period could be pro-gonadotropic in the AGR. Moreover, the AGR can see well and feed better in the dark [17].

The increased mean follicular diameter in ovaries of rat exposed to constant light could be attributed to the presence of large cystic follicles. A similar finding of increased mean follicular diameter was reported in Wistar rats [22]. Again, the increased mean epithelial height observed in ovaries of rats exposed to constant darkness for 4 weeks agrees with the findings of [17] in AGR. Conversely, increased mean epithelial height observed in the ovaries of rats exposed to constant light for 8 weeks could be attributed to the effect of photo-refractoriness.

Conclusion

The findings of the current study indicate that, exposure of AGR to continuous lighting conditions could play a significant role in the formation of large cystic follicles. It is therefore not recommended to subject female AGR to continuous lighting under rearing conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

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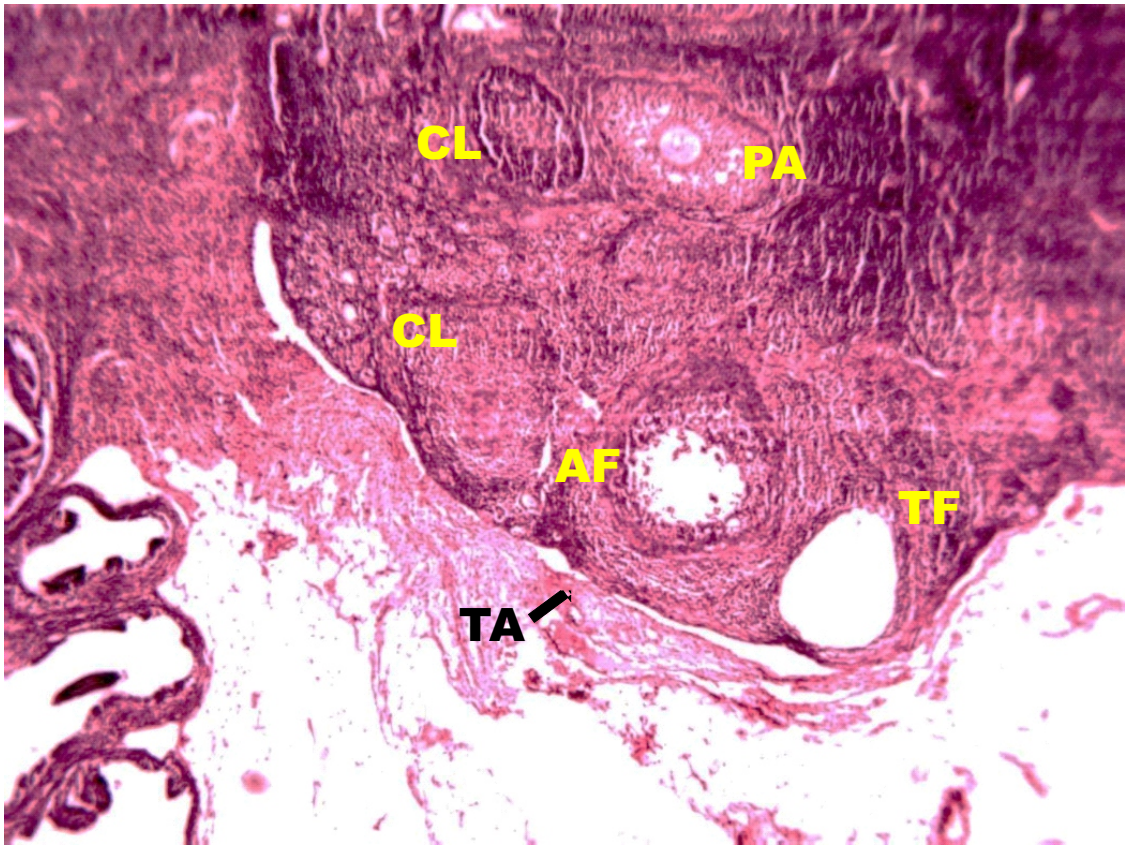

Plate 1: Photomicrograph of section of ovary from control African Giant Rat showing preantral follicle (PA), atretic follicle (AF), corpora lutea (CL), tertiary follicle (TF) and tunica albuginea (TA). Haematoxylin and Eosin Stain (x100).

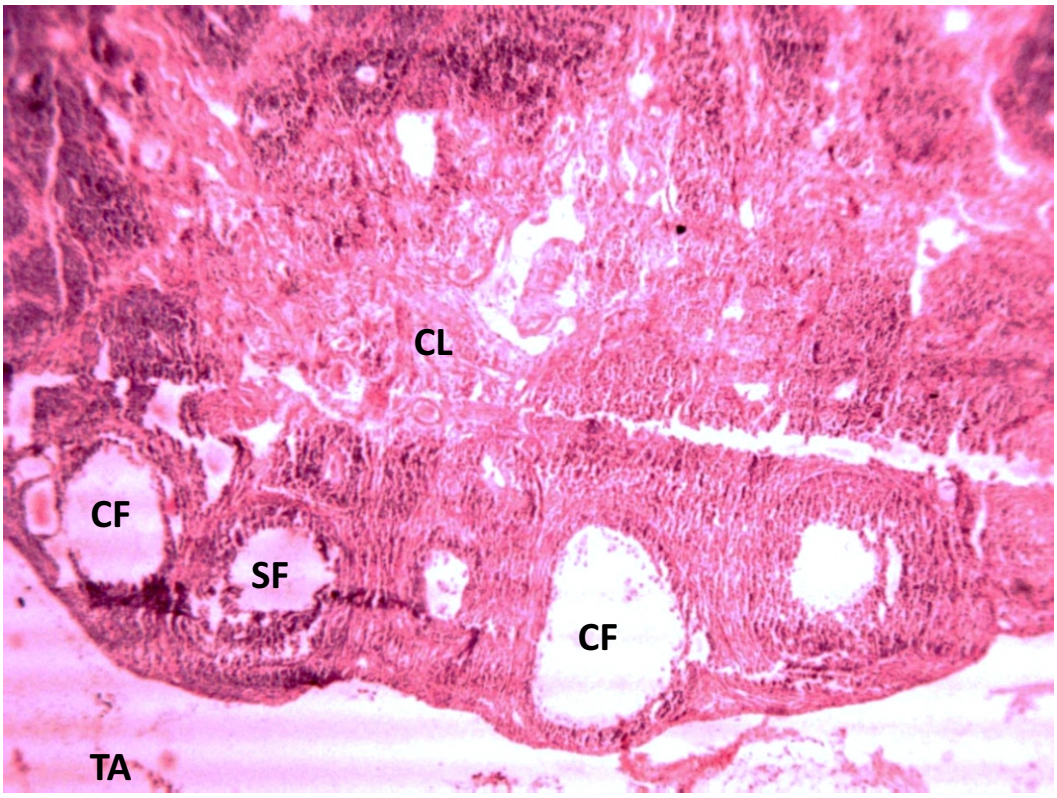

Plate 2: Photomicrograph of the ovary of African Giant Rat from the group exposed to total light showing secondary follicles (SF), Cystic follicle (CF), tunica albuinea (TA) and corpus luteum (CL). Haematoxylin and Eosin Stain (x100).

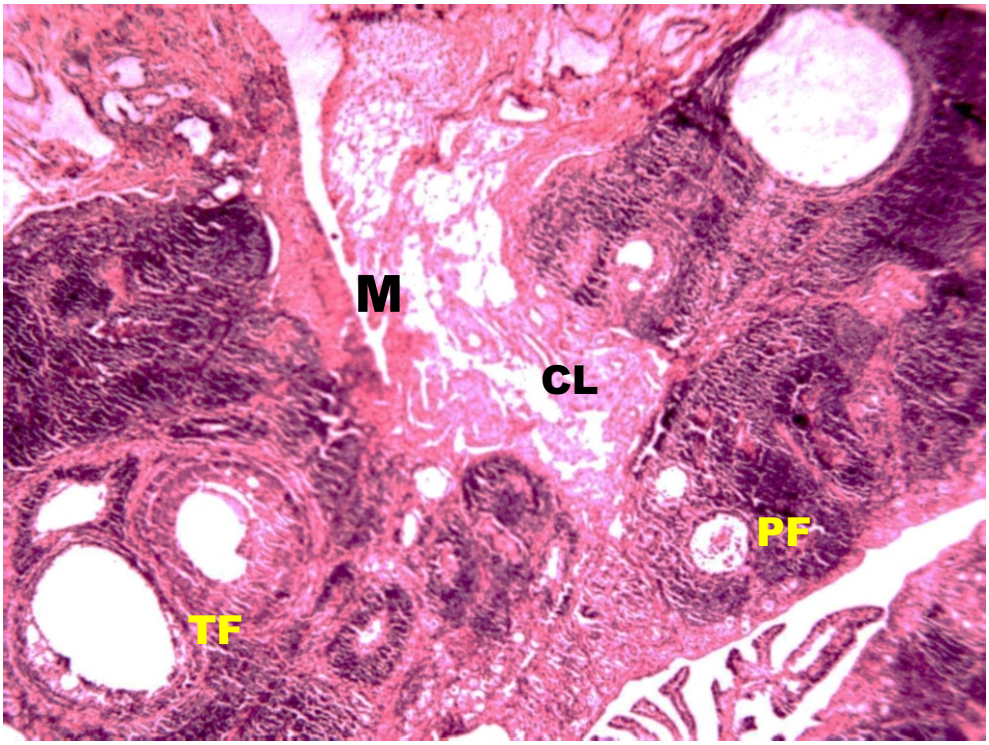

Plate 3: Photomicrograph of the Ovary of African Giant Rat from the group exposed to total darkness showing preantral follicles (PF), tertiary follicles (TF) corpora lutea (CL) and medulla (M). Haematoxylin and Eosin Stain (x100).