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Seasonal Fluctuations In Luteinizing Hormones (lh), Follicle Stimulating Hormones (FSH) And Testosterone Levels In Male African Giant Rats.

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ABSTRACT

As part of the several efforts to understand the biology of the African Giant rats (AGR) (Cricetomys gambianus), seasonal changes in the gonadotropin and testosterone levels of the adult male African giant rats of about 1 – 1½ years of age in captivity were investigated during wet and dry seasons in the tropics. Male giant rats (n = 10) were kept for 12 months and blood samples were collected monthly (on 15th of each month), during the dry (November – February) and wet (March – June) seasons for Luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone assays. The hormones' levels were evaluated to suggest the best breeding season for giant rat in South-West Nigeria. The result showed that there were significant variation ($p<0.001$) in LH and FSH levels in the giant rat in the wet season when compared with the dry season. However, testosterone level was significantly lower ($p<0.001$) in the wet season than in the dry season.

The study showed that sexual activities in the male AGR might be higher in the dry season with peak activities in December in the tropics while the gonadotropins prepare the animals for sexual activities in the dry season. Further studies on the seasonal activities of gonadotropins in the female will give more insight into the sexual receptivity and performance in these animals.

Keywords: *African giant rats (Cricetomys gambianus), season, Gonadotropin, Testosterone.*

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INTRODUCTION

The African giant pouched rat (AGR) is a wild subterranean rodent found in Africa including Nigeria. [1,2] African giant rats possess long fur, which is buff-grey with slightly paler under parts. The gestation period in AGR is 27 days and the rat has a good potential for use as a laboratory animal.[3] The rats have been shown to be a good host for the laboratory passage of *Schistosoma mansoni* and *Trypanosoma evansi*. [4] A number of farmers in Africa trade or breed the wild rodents [5,6], but research studies on their domestication are producing conflicting results. [6,7] Therefore the potential of the African giant pouched rat as a laboratory model for biomedical research has not been fully exploited.

Domestication of this rat has been recognized as a possible way of achieving the objective. [8] But attempts to breed these rats on a large scale have not been successful because of paucity in knowledge of their reproductive biology. African giant rats are seasonal breeders, which make the domestication of the species quite a difficult task. [9]

Gonadotropins – LH (luteinizing hormone) and FSH (follicle stimulating hormone) are anterior pituitary hormones responsible for ovulation and folliculogenesis, respectively to facilitate breeding in female animals irrespective of their species. [10] Luteinizing hormone, also known as interstitial cell stimulating hormone in males enhances production of sex steroids from the gonads. [11] Follicle stimulating hormone potentiates the development of ovarian follicles in female and stimulates for spermatogenesis in male. [12] It enhances the performance of Sertoli cells necessary for numerous aspect of sperm cell maturation [10]

It has however been observed that gonadotropin levels vary from one season to the other. [13] To be able to achieve full domestication of this animal therefore, there is a need to study the seasonal variation of Gonadotropins – LH (luteinizing hormone) and FSH (follicle stimulating hormone) in the African giant rat in order to establish appropriate breeding season in South West Nigeria. Meanwhile, the domestication of the African giant rat has proved to be difficult owing partly to their wild nature, and little understanding of their reproductive biology.[14] The present study was aimed at contributing to the resolution of this problem investigating the fluctuations in the levels of gonadotrophins and testosterone in the male African giant rat across two major seasons in South-West, Nigeria.

MATERIALS AND METHODS

The study utilized 67 apparently healthy adult male wild African giant rats (*Cricetomys gambianus*, Waterhouse) with age range 1 - 1½ years throughout the course of the experiment. The rats were purchased from a local market in Ibadan, South West Nigeria. They were housed in a separate cage in the experimental animal house in the Department of Veterinary Physiology and Biochemistry, University of Ibadan, Ibadan, Nigeria. The rats were fed on a commercially available rat chow diet of pelletized growers feed (containing 15% crude protein; 7% fat; 10% crude fibre; 1.0% calcium; 0.35% phosphorus; 2,550 kcal/kg metabolizable energy). The feeds and water were provided *ad libitum*. Feeding was supplemented with palm kernel fruits and yam peelings. The rats were stabilized and allowed to acclimatize for 2 weeks before the commencement of the study and they were exposed to 12 hours of light and 12 hours of dark cycles throughout the period of the experiment.

Blood samples were collected once monthly from each animal from the retro-orbital venous plexus under anaesthesia during the dry and wet seasons on the same day and period of each month (15th of each month at 08:00 am). During each sampling period, the meteorological data (ambient temperature and relative humidity) in the experimental animal house were recorded using the digital thermo-hygrometer (Shanghai Total Industrial Co, Ltd. Shanghai, China). The rainfall pattern data for the entire community was collected during the same period from the Department of Geography, University of Ibadan, Ibadan, Nigeria. For the dry season, the months are November and December, 2013, January and February, 2014 while the wet season was between March and June 2014.

The animals were pre-anaesthetized in a glass desiccator using chloroform and the blood samples were collected from the retro-orbital sinus plexus of each rat into plain bottle for serum samples. The concentration of reproductive hormones (Luteinizing hormones (LH), Follicle stimulating hormones (FSH) and Testosterone) were determined following manufacturer's instruction.

Estimation of Follicle Stimulating, Luteinizing Hormones and Testosterone

Serum FSH, LH and Testosterone were estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure used was as described by the manufacturer of the kit (Randox Laboratories Limited, UK). A 50 µl of serum sample was added to microtitre wells and 100 µl of the kit (containing the horseradish conjugated antibody against each of the hormone being evaluated) were also added to the wells which were labeled appropriately. They were incubated at room temperature (25°C) for 1 hour. The wells were washed 3 times with deionized water to remove unbound antibodies. A 100 µl tetramethylbenzidine (TMB) reagent (colour developer) was added and incubated at room temperature for 20 minutes for the colour change to develop. Exactly 100µl of hydrochloric acid (HCL) was added to each of the wells to stop further development of colour and absorbance was read at 450nm using ELISA plate reader (Robonic 11-2000, United Kingdom).

Statistical Analysis

Data was presented as mean SEM. The means were compared using One-way ANOVA with Dunnett's post-hoc test using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, (www.graphpad.com).

RESULTS

Table 1 shows the seasonal variations in the weather parameters, viz a viz the average ambient temperature, rainfall pattern and relative humidity during the study period. The mean ambient temperature and relative humidity did not vary significantly across the wet and dry seasons, while the rainfall pattern was significantly higher ($P<0.001$) in the wet season than the dry season.

The Fluctuations in the levels of LH, FSH and Testosterone in the Giant Rats between Dry and Wet Seasons

The result of seasonal fluctuation in the gonadotropins and testosterone levels shown Fig 1 reveals that both LH and FSH levels in the giant rat were significantly ($p<0.001$) higher in the wet season than the dry season. However, testosterone level was significantly lower ($p<0.001$) in the wet season than the dry season. The monthly variations in the LH, FSH and testosterone values were also calculated as shown in Fig 2 where we observed a monthly fluctuation in the plasma gonadotropin and testosterone levels in the male AGR (Fig. 2). For example, LH and FSH levels were at a constant basal level in January – LH (8.441.41 IU/L), FSH (6.220.88IU/L); February (6.000.68 IU/L), FSH (4.110.57 IU/L) till March LH (9.880.69 IU/L), FSH (7.000.68 IU/L). These values peaked in April LH (29.133.86 IU/L), FSH (20.753.61 IU/L) while

the levels dropped towards the basal values in the month of May LH (10.870.76 IU/L), FSH (8.250.52 IU/L) and rose again in June – LH (23.004.35 IU/L), FSH (17.503.33 IU/L). Testosterone on the other hand maintained a basal level in January (0.800.16) and February (0.870.09), rose slightly in March (1.610.12) before the serum levels came down to the basal values. The final increase in serum concentration of testosterone was observed in November (2.000.36 IU/L), while the highest value of 3.500.25 IU/L was reached in December during the hot dry season.

Table 1: Temperature, relative humidity and rainfall pattern in the animal house in the dry and wet seasons during the study period.

Parameters (means ± SEM)	Dry Season	Wet Season
Temperature (^{0}C)	30.13 ± 1.34	30.08 ± 1.52
Relative Humidity (%)	63.50 ± 5.07	67.50 ± 4.77
#Rainfall (mm)	0.41 ± 0.10*	4.36 ± 0.89*

Values are mean ± SEM

Mean values with the same superscripts asterick along the same row are significantly different (p< 0.001)

#Rainfall data obtained from the Department of Geography, University of Ibadan, while Temperature and relative humidity were recorded with the aid of a digital thermo-hygrometer in the experimental house.

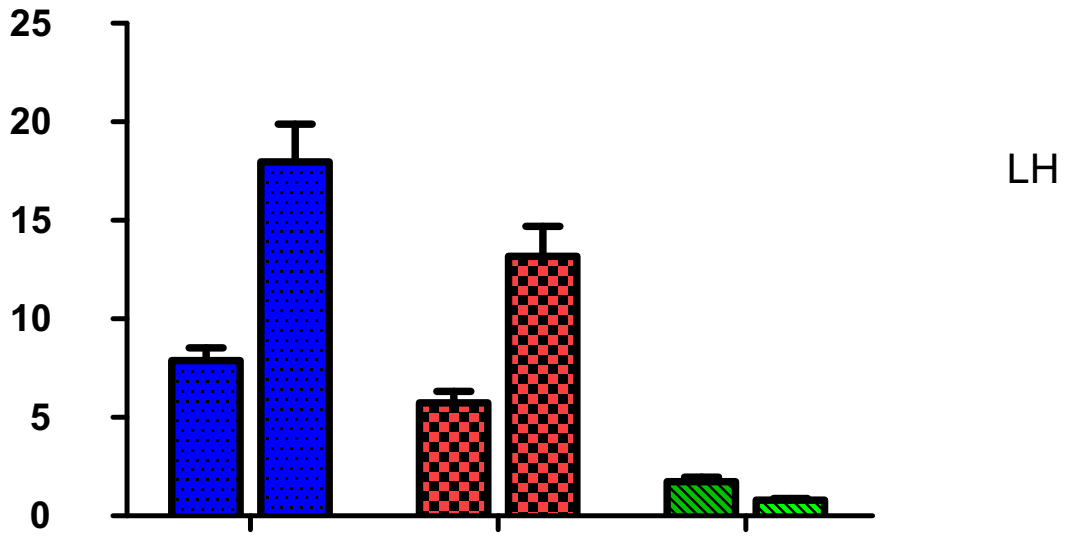

Fig. 1. Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and Testosterone levels in the adult male giant rats during the dry and wet seasons.
Values are means ± SEM

Vertical bars represent SEM, Mean values with different superscripts alphabets in each parameter are significantly different (p < 0.001)

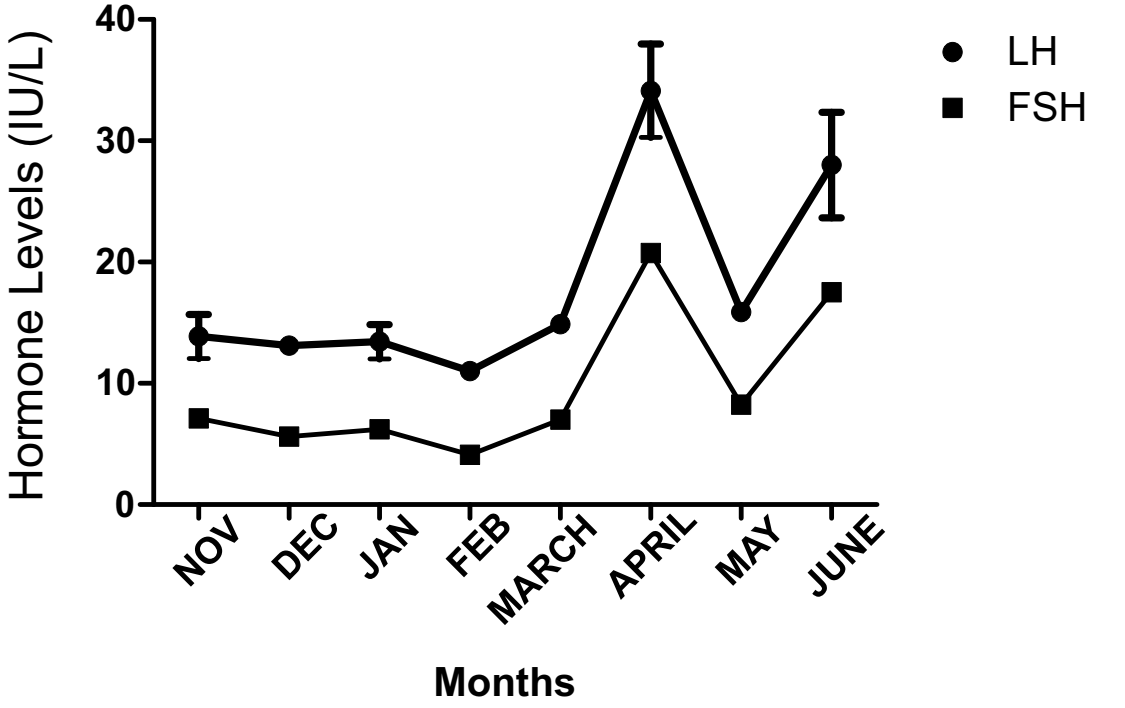

Fig. 2. Monthly fluctuations in the plasma levels of gonadotropins in the adult male African giant rats.

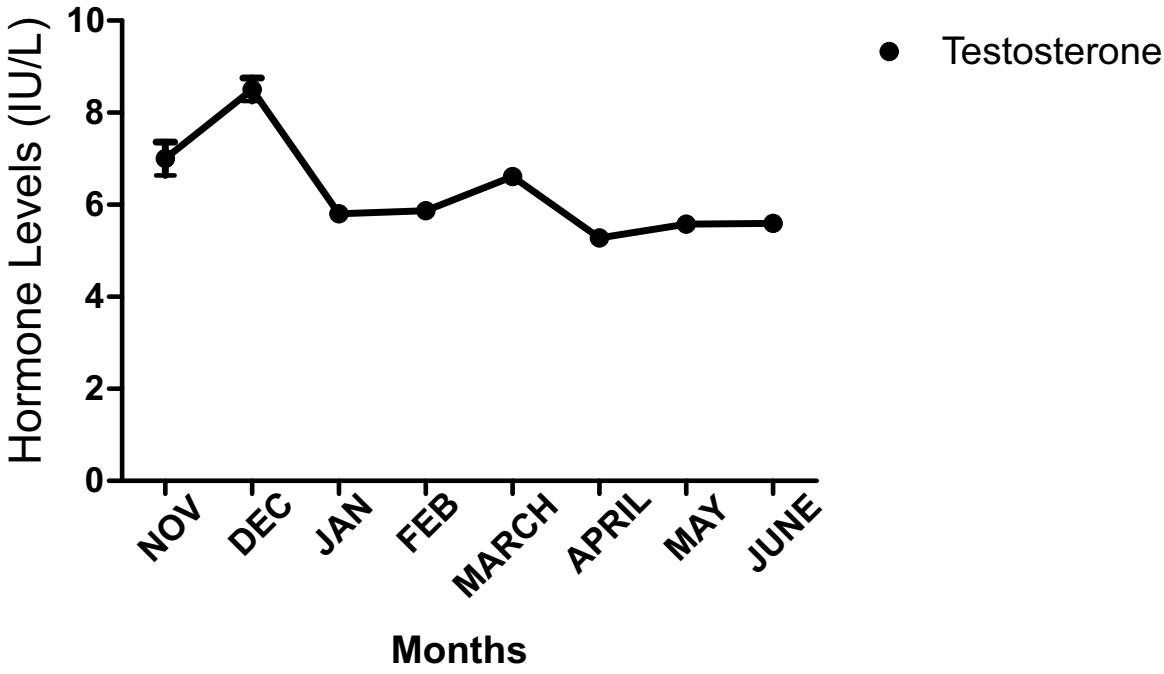

Fig 3. Monthly fluctuations in the plasma levels of testosterone in the adult male African giant rats.

DISCUSSION

Our present study reveals some degree of seasonality in the plasma levels of gonadotropins and testosterone in the African Giant rat (Fig 1). For example, it was observed that LH and FSH levels were higher and testosterone level was lower in the wet season than in dry season in adult male African giant rats. The LH and FSH level was lower in February than April while LH was higher in April than March or May. The study further showed higher testosterone level in December than April. Similar seasonal fluctuations in FSH and LH and testosterone have been previously reported in domestic animals such as goats, [15] ram [16] and in wild animals such as ground squirrel , fox and badgers. [17] Physiologically, steroid hormone levels such as testosterone are expected to follow that of the FSH and LH because gonadotropins are the stimulants for steroidogenesis in the gonads. The testosterone thereafter serves as the inhibitor of further LH and FSH synthesis via feedback negative inhibition of gonadotropin releasing hormone (GnRH) in the hypothalamus. Concomitant increase in testosterone and decreased LH and FSH in the dry season in the present study might not be unconnected with negative feedback inhibition of gonadotropins release in the anterior pituitary and vice versa. The testosterone level in AGRs was lowest in April and highest in December while FSH and LH levels were highest in April and lowest in December. This has also been observed in laboratory rats, in which a fall in the level of testosterone stimulated increase in the plasma concentration of LH while increased plasma testosterone led to significant decrease in the LH level even in castrated male rats. Our observation of seasonal variation in gonadotropin and steroid levels in AGR are in agreement with the observations of [9] who reported that these rats are seasonal breeders in the United State of America. However, it was not in agreement with the earlier reports of [14] while investigating the effects of season on the reproductive organs of the male African giant rats in Nigeria. This author reported that the animals were not seasonal breeders. Ajayi [2] also reported that giant rats in captivity reproduced throughout the year and did not show any seasonal peak in breeding.

Meanwhile, Rosevear [1] had earlier suggested that West African rodents including AGR in the wild have breeding seasons, despite being raised in the tropics where the four different seasons and seasonality are not well delineated. The conflicting reports might not be unconnected with the difference in the environment where the animals were reared because adaptation to various environmental conditions is an attribute considered to be an important criterion in selecting animals for domestication. [18]

The study reveals that both LH and FSH levels in the male giant rat was significantly lower in the dry season than the wet season. Seasonality in gonadotropin levels and their receptors have also been observed in other rodents. According to the report by Wang *et al* [19] and Zhang *et al* [20] LH and FSH levels in the plasma and the number or level of expression of their receptors follows the breeding season closely in the testes of ground squirrel (*Citellus dauricus*) and the scented gland of male muskrat (*Ondatrazib ethicus*). The findings in the present study are also similar to the observations of [9] who reported a seasonal variation in the morphometry of the female reproductive tract in the AGRs. Furthermore, Claudia *et al.*[21] reported that the adult male Viscacha (*Lagostomus maximus maximus)* – a rabbit-like rodent native to South America belonging to the *Chincillidae* family showed testicular involution during the shorts days of winter (July-August) and maximum gonadal activity during the long days of summer and autumn (December-March) under natural condition. This is in consonance with the findings from this present study. Fuentes *et al.* [22,23] have also reported seasonal variations in the plasma testosterone level and in the concentrations of testicular LH, FSH and PRL receptors in Viscacha (*Lagostomus maximus maximus*). The authors reported that the testosterone levels were higher in the active period (summer) than in the period of regression. Viscacha, being a nocturnal rat like

African giant rat exhibited important seasonal morphological change in synchrony with the external environment, such as long periods of light.[24]

The observations from the present study support our personal observation and field reports by hunters that African giant rats were more active during dry season in their burrows and rarely seen moving about in search of food and warmth, unlike in the wet season when they are easily caught while looking for food and warm places, mostly around human habitation.

In seasonal breeders, photoperiod (day length) tightly regulates reproduction to ensure birth occurs at the most favourable time of the year [25] while [26] reported that LH, FSH and testosterone are necessary for quantitatively normal spermatogenesis in non-seasonal species. Different environmental factors such as ambient temperature, food availability, rainfall periods and natural photoperiod may be considered important variables that control the reproductive processes in rodents [27] and may be important sources of influence, especially the average rainfall pattern, on the blood gonadotropins and testosterone levels in male AGR in the present study.

Conclusion

The presence of higher plasma testosterone level in the captive male giant rats in the dry season indicates higher sexual activity than in the wet season and suggests that AGRs are seasonal breeders. This finding is particularly helpful for breeding and domestication programs for the African Giant rats on a large scale.

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