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Effect of Age at Breeding on Female African Giant Rat (*Cricetomys gambianus*) Fertility in Captivity

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ABSTRACT

This study aimed to evaluate the influence of age at breeding one female African giant rat (FAGR) fertility. Twenty-four nulliparous FAGR of 6, 8, or 10 months old were allocated to one of the three groups according to their age. They were housed individually and fed ad libitum. After five days of accommodation, a male was transferred into a female cage, and daily vaginal smears were performed to evidence mating for a period not exceeding 30 days. Reproductive parameters were then evaluated. Results showed a significant reduction ($P<0.05$) in pre-coitus cohabitation time in 8 (6.75±4.23days) and 10 (5.13±1.25days) months old females as compared to those aged six months (22.25±1.75days). There was an increase in mating rates, fertile mating, fertility, and stillbirth in 8 and 10 months old FAGR. The litter size significantly increased ($P<0.05$) in 8 and 10 months old compared to 6 months old FAGR. Preimplantation embryo losses rates were 60.00±39.44, 66.66±34.96, and 35.00±21.19% in 6, 8, and 10 months old FAGR, respectively. In this study, fertility increased with age, so it was better in the FAGR older than six months.

Key words: African giant rat, Age at breeding, Mating rate, Fertility.

INTRODUCTION

In many tropical regions of Africa, rodents constitute an important source of meat supply for population. However, population growth led to the over exploitation of wildlife animals (Laurance et al. 2006). The African giant rat or Cricetoma (*Cricetomys gambianus*) is one of the most important rodents consumed in tropical countries in Africa (Asibey and Addo 2000). The quantities provided by hunters have diminished progressively due to its rarity in the wild. The breeding of Cricetoma could be an alternative to supply meat to the population thereby preserving its biodiversity. Unfortunately, its reproductive performances are still low in captivity, with a long period of cohabitation without birth and if any, with very low litter size (Tchoumboue et al. 2002; Tsambou 2020) compared to that of females captured from the bush in a pregnancy state, which give birth to up to 8 kids. In captivity, some factors such as photoperiod (Fonou 2021) and dietary energy level (Tsambou 2020) were suspected to be the cause of those poor performances. It resulted from those studies that the mating rate in FAGR varies from 75.00 to 100.00% (Tsambou 2020; Fonou 2021) and that the rate of fertile mating varies from 25 to 67% (Tsambou 2020; Fonou 2021). These findings demonstrated that the absence or low rate of kidding is not linked to the non-receptivity of males by females. Indeed, the high rate of mating is evidence of regular heats. The subfertility of females could therefore be linked to anovulatory heats or their inability to maintain pregnancy until the term.

In some species of small mammals such as the rabbit (Rommers et al. 2004) and laboratory rat (Rosen et al. 1987)

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the age of female at breeding has been reported as one of the main factors of hypo-fertility. In fact, fertility rate decreases both in oldest and youngest females (Derivaux and Ectors 1986). This phenomenon has been reported in New Zealand white rabbit (Rommers et al. 2004) and in laboratory rat (Rosen et al. 1987). The lack of knowledges on the breeding age for FAGR could be the reason of low reproductive performances observed in captivity. Therefore, the objective of this study is to search the effects of the age at which mating in FAGR would result to a best fertility.

MATERIALS AND METHODS

Ethical Approval

Experimental protocol used was in conformity with the international accepted standard ethical guidelines for laboratory animal use and care as described in the European community guidelines; ECC directive 86/609/EEC, of the 24th November 1986.

Study Area, Animal Housing and Experimental Design

The present study was carried out at the Teaching and Research Farm of the University of Dschang (in Dschang town) between July 2020 and July 2021. This Farm has the following geoclimatic characteristics: altitude: 1420m; latitude 5-7N; longitude: 8-12E; rainfall: 1500-2000mm/year; temperature: 15-25°C; relative humidity: 49-97%. Twenty-four nulliparous FAGR of 6, 8 or 10 months old, weighing 568.10 to 1076.93g were used. Rats were caged individually under natural photoperiod (12h L/ 12h D). The adult rats were obtained from the rat farmers and then bred in the Teaching and Research Farm of the University of Dschang. Animals had free access to water and food made up of resources usually eaten in the wild (sweet potatoes, cassava, ripe banana) and provender with the following bromatological characteristics (Fonou et al. 2021): energy (2700Kcal/kg of DM), crude proteins (21.00%), lipids (3.50%), cellulose (6.00%), calcium (0.80%) and phosphorus (0.80%).

Rats were grouped in 6-, 8- and 10-months old weighing 587.11±19.01, 849.16±100.27 and 954.16± 122.77g respectively. Males were introduced in different female cages for mating and transferred to another female of different age group every two days, in order to minimize the effect linked to the male. Females were maintained in cohabitation with a male until the positive mating test (detection of spermatozoa in vaginal smears). In the absence of mating, FAGR were maintained in cohabitation with a male until 30 days, a duration corresponding to 3 estrous cycles. The mated females were isolated from the males and observed until the birth. Among them, those that didn't give birth after 33 days were sacrificed.

Evaluation of Pre-Coitus Cohabitation Time and Pre-Coitus Number of Sexual Cycles

The pre coitus cohabitation time (PCCT) was determined by counting the number of days between the date a male was transferred into the female cage and the date spermatozoa were detected in its vaginal smear. The presumed number of an estrous cycle was determined by dividing the pre coitus cohabitation time by the duration of estrous cycle in FAGR.

Mating Rate, Fertile Mating Rate and Fertility Rate

Female was considered mated when spermatozoa were detected in its vaginal smears. Then, the rate of mating was calculated as the number of females mated/number of females presented to male X 100.

The rate of fertile mating was calculated as the number of females that delivered/number of females mated X 100.The fertility rate (%) was evaluated as the number of females that delivered/ number of females submitted to male X 100.

Litter Size and Stillbirth Rate

The litter size was determined as the total number of kids (dead or alive) born. The rate of stillbirths was calculated as the number of stillbirth/litter size X 100

Rate of Pre-implantation Embryo Losses

Once mated FAGR at the presumed end of pregnancy were sacrificed, peritoneal cavity was opened. The uterus was removed, then opened longitudinally and submerged in NaOH 2% for 15min. The implantation sites were then counted. The corpora lutea once recognized, were counted on the ovarian surface as a yellow protuberance (Garcia et al. 2005). The rate of preimplantation embryo losses (RPreIEL) was then determined as: RPreIEL (%) = (number of corpora lutea- number of implantation sites/number of corpora lutea) X 100.

Statistical Analysis

Results were expressed as mean ± standard deviation. Analysis was carried out using a statistical software SPSS. 20.0 (statistical package for social science). One way ANOVA was used to appreciate the effect of age on the studied reproductive parameters, followed by Duncan Test for the mean separation. Significance difference was fixed at 0.05.

RESULTS

In this study, the pre coitus cohabitation time (Fig. 1) broadly decreased with an increasing age of FAGR, with a significant difference ($P<0.05$) at 8 and 10 months old as compared to 6 months old. Consequently, the presumed number of estrous cycles (Fig. 2) significantly ($P<0.05$) decreased in 8- and 10-months old females compared to those of 6 months. Therefore, no significance difference was observed in pre-coitus cohabitation time as well as in the presumed number of estrous cycles of 8 and 10 months old FAGR. The mating rate (Fig. 3) and fertile mating rate (Fig. 4) increased ($P<0.05$) when the animal age increased with a maximum rate of mating at 8 months old. Therefore, in 8- and 10-months old females, no significant difference was observed between the rates of mating as well as the fertile mating rates. The fertility rate (Fig. 5) was lower ($P<0.05$) in females bred at 6 months compared to those of 8 and 10 months old. A significant increase ($P<0.05$) was observed in the litter size (Fig. 6) of 8 and 10 months old compared to the female bred at 6 months old. Therefore, females bred at 8- and 10-month-old showed no significant difference in the litter size. Concerning the still birth rate (Fig. 7), although no significant difference was observed in females of different ages, it augmented with an increase age of FAGR. The rate of preimplantation embryo losses decreased with the increasing of FAGR age (Fig. 8).

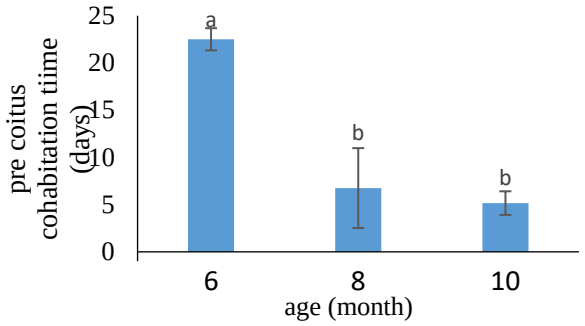

Fig. 1: Effects of age at breeding on pre coitus cohabitation time in female African giant rat. Bars with different letters differ significantly (P<0.05).

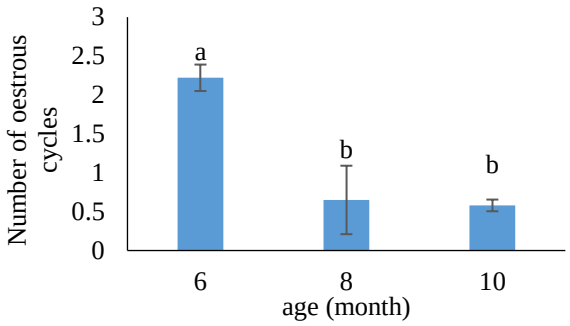

Fig. 2: Effect of age at breeding on the number of estrous cycles in female African giant rat. Bars with different letters differ significantly (P<0.05).

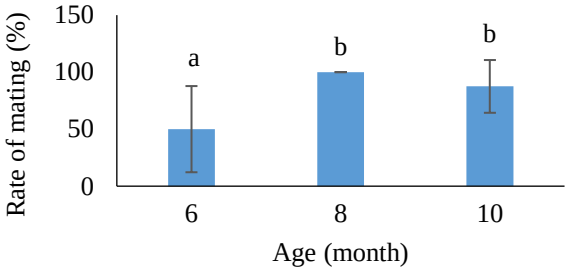

Fig. 3: Effect of age at breeding on the rate of mating in female African giant rat. Bars with different letters differ significantly (P<0.05).

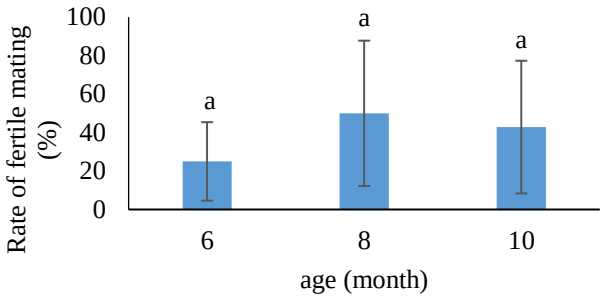

Fig. 4: Effect of age at breeding on the rate of fertile mating in female African giant rat. Bars with different letters differ significantly (P<0.05).

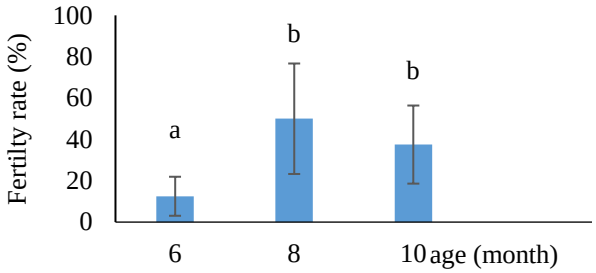

Fig. 5: Effect of age at breeding on fertility rate in female African giant rat. Bars with different letters differ significantly (P<0.05).

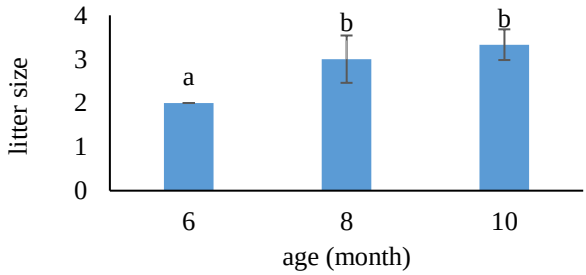

Fig. 6: Effect of age at breeding on the litter size in female African giant rat. Bars with different letters differ significantly (P<0.05).

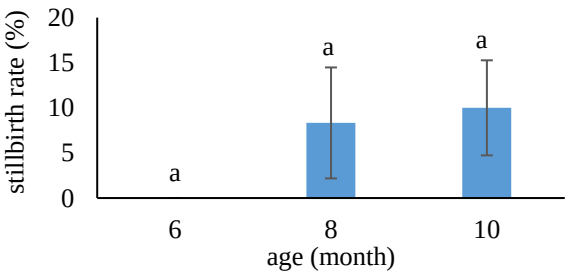

Fig. 7: Effect of age at breeding on the stillbirth rate in female African giant rat. Bars with different letters differ significantly (P<0.05).

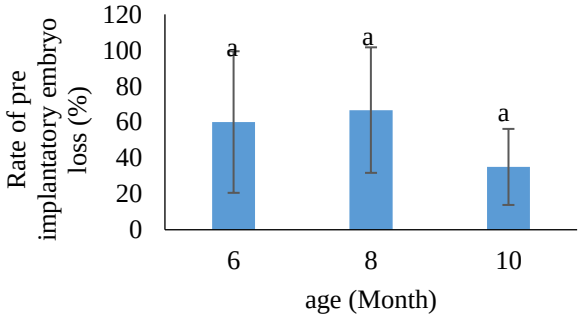

Fig. 8: Effect of age at breeding on the rate of preimplantation embryo losses in female African giant rat. Bars with different letters differ significantly (P<0.05).

DISCUSSION

Female African giant rat is a mammal with good reproductive characteristics in the wild. On the other hand, in captivity its reproductive performances are still poor, especially it fertility. To explain those poor performances, many factors were suspected and studied. It resulted from those studies that heats are regular in FAGR and that the absence or low rate of kidding was not linked to the non-receptivity of males by females (Tsambou 2020; Fonou 2021). It is now certain that the subfertility recorded in captivity would be due either to anovulatory heat or to the inability of females to maintain their gestation until the term.

As in captivity, FAGR are generally younger than in the wild, its age at the breeding was also suspected to be the cause of low fertility in farms. Indeed, it is known that

in small mammal species, the low fertility rate is observed both in the youngest and oldest females (Derivaux and Ectors 1986).

Our result showed a significant ($P<0.05$) reduction of PCCT and consequently the presumed number of sexual cycles in cohabitation in 8- and 10-months old females as compared to that of the 6 months. The PCCT obtained in this study are in agreement with others findings which previously reported the PCCT ranging from 3 to 24 days in FAGR reared under different photoperiods (Fonou 2021). In contrary, the PCCT ranging from 2 to 6 days was reported in FAGR reared under different dietary energy levels Tsambou (2020). The age at puberty in FAGR was previously reported as 7.5 months (Tchoumboue et al. 2002). Others research workers recently reported the age at puberty as varying from 4 to 5 months (Ali et al. 2011). The higher PCCT and number of sexual cycles during cohabitation observed in 6-month-old rat could be due to their young age and perhaps, the puberty is not yet achieved, although in FAGR puberty onset is around 4 to 5.

The rates of mating, fertile mating and fertility augmented with the increase of animal age. Similar results were obtained in New Zealand rabbits (Rommers et al. 2004), Wistar rat (Rosen et al. 1987) and *Rattus argentiventer* (Rahmini et al. 2003). In fact, in small mammal species with estrous cycle, the first cycles are generally without ovulation, the embryo losses are more important in the younger females and fertility rate decreases in older females (Derivaux and Ectors, 1986; Maeda et al. 2000). The lower rates of mating, fertile mating and fertility obtained in 6 months old female could be associated either with the absence of estrous cycle, the anovulatory cycle or the implantation failure after fertilization.

The litter size increased with an increasing female age in this study, although it is still low compared to the average litter size observed in the wild. This result is in agreement with others findings (Tchoumboue et al. 2002). Litter size is linked to the sexual maturity and ovulation rate. Indeed, the ovulation rate in polytocous mammals is positively correlated with age (Kirk 1986).

The stillbirth rate increased with increasing age and with the liter size. Similar result has been reported in sow (Pardo et al. 2013; Ward et al. 2020) and in *Cavia porcellus* (Czarnecki and Adamski 2015). In fact, the increase of the litter size may be positively correlated with stillbirth due to the intrauterine overcrowding and insufficiency of nutrient supply.

The rate of preimplantation embryo losses decreases with increasing FAGR age. The rates of preimplantation embryo losses of 60 and 66, 66% were observed in 6- and 8-months old females respectively. These values are 3 times higher than that of 20% generally reported in polytocous mammals (Sharkey and Smith 2003). In European brown hare (*Lepus europaeus)*, the rate of embryo loss was reported as 42% (Schroeder et al. 2013). The mechanism of embryo loss in mice is owed to endogenous apoptosis which takes place without maternal interaction (Drews et al. 2020). In this study the responsible mechanism remains unknown. Nevertheless, progesterone secreted by the *corpus luteum* at early pregnancy plays an important function during embryo implantation (Stevenson and Lamb 2016).

Conclusion

The youngest female African giant rat showed the poor reproductive performances in this study. Although the 8 months old FAGR showed the best reproductive performances, the fertility of female at this age was comparable to those of 10 months old females. It can then be concluded that, breed FAGR at 8 months old will be beneficial.

Author Contributions

All authors were involved in the conceptualization, writing and review of manuscript draft. They have read and approved the final version of the manuscript.

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Conflict of Interest

The authors declare that there are not conflict of interest.

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