IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 15, Issue 3 Ser. III (March 2022), PP 15-21
www.iosrjournals.org

17-α-hydroxyprogesterone caproate improves female African giant rats *(Cricetomys gambianus)* fertility in captivity

Fonkem Severin[1*], Tsambou Megnimeza Astride Martine[1], Fonou Tadiesse Lavoisier[1], Foda Fopa constant[1], Vemo Bertin Narcisse[3], Takam Mbogne Boris, Kouamo Justin[2], Kenfack Augustave[1]

[1]*Department of Animal Science, Faculty of Agronomy and Agricultural Science, University of Dschang PO Box: 188 Dschang-Cameroon*
[2]*Department of Surgery and Medical pathology, School of Veterinary Medicine and Science, The University of Ngaoundere PO Box: 454 Ngaoundere- Cameroon*
[3]*Department of Animal Science, Faculty of Agriculture and Veterinary Medicine, University of Buea. PO Box: 63 Buea-Cameroon*
**Corresponding author: fonkem1988@yahoo.fr*

Abstract
This study aimed to evaluate the effects of 17-α- hydroxyprogesterone caproate (HPC) on female African giant rats (FAGR) fertility in captivity. After mating confirmation by the observation of spermatozoa in vaginal smears, mated females were assigned to three groups of 16 animals. They were housed singly and had free access to water and feed. Groups 2 and 3 received daily an intramuscular injection of 16 and 20 mg/kg.bw of HPC respectively, in a volume of 0.3ml while group 1 received a ricin oil at 0.3ml/kg.bw. Injections stated at day 3 and ended at day 27 post coitus. At day fifteen, eight rats per group were sacrificed and the remaining female rats of each group was observed until the parturition. Main results showed a significant reduction (P<0.05) in the rate of pre (9.82±5.11%; 23.55±9.95%; 47.29±16.66%) and post (13.75±6.94%; 14.49±4.03%; 58.33±26.72%) implantation embryo losses and stillbirth rate (3.57±1.12%; 0.00±0.00%; 8.33±3.74%) in 16 and 20 mg/kg HPC- treated FAGR compared to the control. The rate of implanted embryos (91.73±09.59; 76.44±09.95; 52.70±16.66), fertile mating rate (100.00±00%; 87.50±23.14%; 50.00±26.72) and litter size (7.00±0.92; 5.83±0.63; 3.25±0.32) significantly increased in females treated with 16 and 20 mg/kg of HPC (P<0.05) compared to the control, respectively. In conclusion, HPC administration improved fertility of FAGR, with best reproductive performances at dose of 16 mg/kg.

Key words: *Female African giant rats, 17-α-hydroxyprogesterone caproate, embryo losses, fertility*

Date of Submission: 15-03-2022 Date of Acceptance: 31-03-2022

I. Introduction

African giant rat (AGR) or Cricetoma is one of the widely hunted and consumed rodents in African tropical countries (Asibey et *al.*, 2000). Its breeding remains an alternative for its meat supply. Unfortunately, AGR exhibits poor reproductive performances in captivity (Tsambou, 2020; Fonou, 2021) and reasons remain unknown. On the other side, it is regularly observed that, females captured from the wild in pregnant state gave birth to great litters (up to 8 kids). This evidence shows that AGR reproductive performances in captivity are still to be improved. For this reason, at the Teaching and Research Farm (TRF) of the University of Dschang, factors such as photoperiod (Fonou, 2021) and dietary energy level (Tsambou, 2020) were suspected to be the causes of low performances recorded in captivity and studied. It resulted from those studies that mating rate in domestic FAGR was not far to 100% (Tsambou, 2020; Fonou, 2021) and that, the rate of fertile mating ranges between 25 to 67% only (Tsambou, 2020, Fonou, 2021). These findings clearly evidenced that the absence or low fertility observed in captivity is not linked to the non-receptivity of males by females. Indeed, the high rate of mating is a proof of regular heats. In consequence, the subfertility of females could therefore be linked to an anovulatory heat or their inability to maintain pregnancy until the parturition.

Uterus quiescence is essential for pregnancy establishment in mammal species. Progesterone (P4) is a key hormone involved in uterine preparation for implantation and pregnancy maintenance (Stevenson and Lambt, 2016, Aguilar and Mitchell, 2010). In fact, the efficacy of P4 in improvement of fertility has been shown in some mammal species such as human (Shruti et *al.*, 2018; Joan et *al.*, 2019), cow (Stevenson and Lambt,

2016; Wiltbank et *al.*, 2014), rabbit (Kwum and Emmens, 1974) and laboratory rats (Kota et *al.*, 2013). P4 inhibits myometrial contractility during pregnancy (Anderson et *al.*, 2009, Zongzhi et *al.*, 2018), promotes uterine relaxation at early pregnancy by decreasing prostaglandin F2α production and gene expression of oxytocin (Edey et *al.*, 2018). It is therefore possible that the inability of FAGR to maintain gestation after mating be linked to insufficiency of progesterone. This suspicion needed to be verified and it is the reason why the aims of this study was to evaluate the effect of 17-α-hydroxyprogesterone caproate on FAGR fertility.

II. Material And Methods

2.1 Study area

The study was conducted at the Teatching and research Farm (TRF) and at the Laboratory of Animal Health of the University of Dschang (Cameroon) between July and December 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%.

2.2 Animals and housing

Forty eight nulliparous FAGR aged 8 months, weighing 860 to 974,5g were used. They were housed individually under natural photoperiod (12h light/ 12h dark). The adult rats were obtained from the rat farmers and then bred in the TRF of the University of Dschang.

Experimental protocols used were 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.

2.3 Feeding

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%).

2.4 Chemical

The source of the progesterone used was 17-α-hydroxyprogesterone caproate, a synthetic progestational agent manufactured by BAYER HEALTHCARE Laboratory.

2.5 Assay

A group of FAGR was mated with males. Daily vaginal smears were performed to evidence mating. Once confirmed, mated females were gradually assigned to each of the three groups that have been formed. From days *post coïtus* 3 to 27, FAGR were daily treated with intra-muscular injections of ricin oil or 17-α-hydroxyprogesterone caproate in ricin oil at the rate of 0.3ml.Kg.bw. Thus, group 1 rats received only ricin oil whereas groups 2 and 3 were treated with 16 and 20 mg/kg.bw of HPC respectively in a volume of 0.3ml.kg bw. The half of animals in each group was sacrificed at day 15 and the second half was further treated and observed until day 27 *post coitus*.

2.6 Data collection and studied parameters

Sexual organs weight

Rats were sacrificed, uterus and ovaries were removed and weighed using an electronic balance (capacity160g and 10^{-3} of sensibility).

Rate of pre and post implantation embryo losses

Fifteen days after mating, rats were anesthetized using chloroform, sacrificed and peritoneal cavity was opened. The uterus was removed, then opened longitudinally, submerged in NaOH 2% for 15 min and implantation sites were counted. Corpora lutea were counted on the ovarian surface as a yellow protuberance (Garcia et *al*., 2005). The rate of pre implantation embryo losses (RPreIEL) was then determined as follow: RPreIEL (%) = (number of corpora lutea- number of implantation sites/number of corpora lutea) X 100. The rate of post implantation embryo losses (RPIEL) was determined as: RPIEL (%) = (number of implantation sites- number of viable fetus/ number of implantation sites) X 100.

Rate of implanted or resorbed embryos

The rate of implanted embryos was calculated as the numbers of implanted embryos divided by the number of corpora lutea X 100. The number of resorbed embryos was determined by counting the number of implantation sites that underwent scar**.** Then, the rate of resorbed embryos was determined as the number of resorbed embryos divided by the number of corpora lutea X 100.

Percentage of live embryos (%)

The number of live embryos was counted in each uterine horn. Then, the percentage of live embryos was calculated by diving the number of live embryos by the total number of implantation sites X 100

Pregnancy duration

The pregnancy duration was calculated by counting the number of days between the day spermatozoa were detected in vaginal smear and the day of parturition.

Rate of fertile mating

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

Litter size and stillbirth rate

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

2.7 Statistical analysis

Results were expressed as mean ± standard deviation. Analysis of data 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 HPC on the studied reproductive parameters, followed by DUNCAN TEST for the mean separation. Significance difference was fixed at 0.05.

III. Results

3.1 Effect of 17α-hydroxyprogesterone caproate on reproductive organs weight

It appears from the table 1 that, weights of uterus significantly decreased (P<0.05) in females treated with HPC at dose 20 mg/kg.bw compared to those receiving 16 mg/kg.bw and the control group.

Table 1: Effects of 17-α- hydroxyprogesterone caproate on sexual reproductive organ weight (g/100g.bw) of FAGR

	Doses of HPC (mg/kg. bw)			
Organs	0	16	20	P
Uteri	0.052±0.031[a]	0.101±0.051[a]	0.041±0.017[b]	0.000
Ovaries	0.013±0.005[a]	0.013±0.005[a]	0.017±0.005[a]	0.066

[a, b] In the same row, data with different letters are significantly different. P<0.05

3.2 Effect of 17-α- hydroxyprogesterone caproate in some female African giant rat gestational parameters

The numbers of corpora lutea, implantation site, live embryos and the rate of implanted embryos significantly increased (P<0.05) in females treated with HPC at doses of 16 and 20 mg/kg.bw compared to those of control. Furthermore, the rate of resorbed embryos significantly reduced (P<0.05) in HPC-treated groups compared to the control (Table 2).

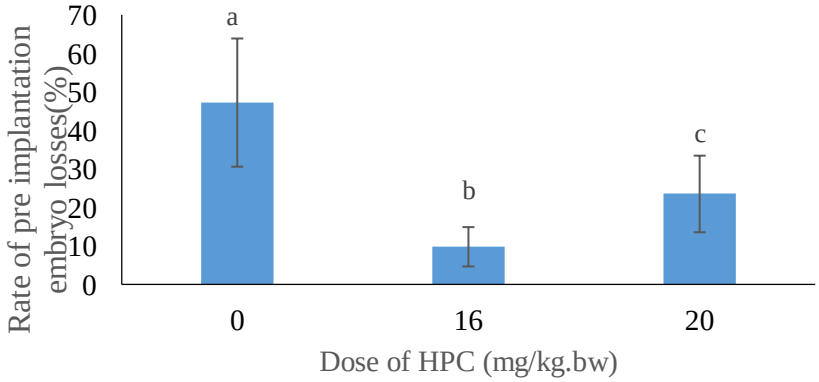
Table 2: Effects of 17-α- hydroxyprogesterone caproate on gestation parameters in female African giant rat

	Doses of HPC (mg/kg .bw)			
Parameters	0	16	20	P
Number of corpora lutea	04.50±0.92[a]	07.50±0.75[b]	06.38±01.30[c]	0.00
Number of implantation sites	02.38±0.91[a]	06.88±0.99[b]	04.88±1.24[c]	0.00
% of live embryos	41.66±09.25[a]	86.23±09.63[b]	85.50±09.45[c]	0.00
Rate of implanted embryos (%)	52.70±16.66[a]	91.73±09.59[b]	76.44±09.95[c]	0.00
Rate of resorbed embryos (%)	29.37±11.08[a]	13.20±01.53[b]	11.50±02.64[b]	0.00

[a, b, c] In the same row, data with different letters are significantly different. P<0.05

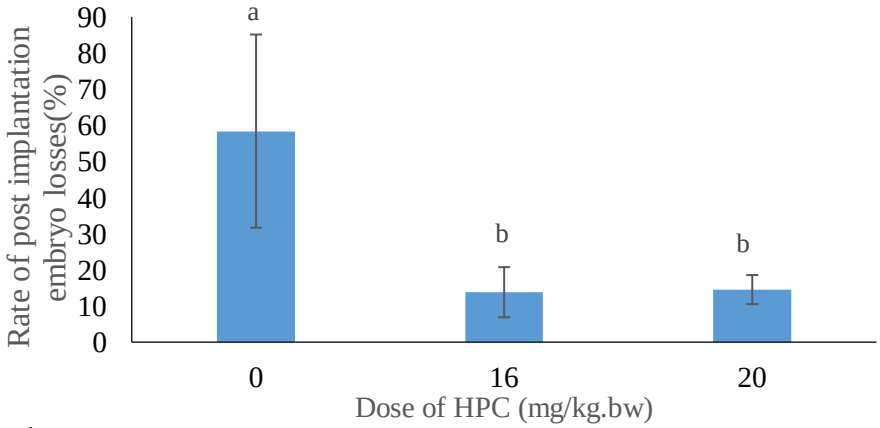
3.3 Effect of 17-α- hydroxyprogesterone caproate on the rate of embryo losses

It observed from the figure 1 that, the rate of pre implantation embryos losses significantly reduced (P<0.05) in females receiving HPC at doses of 16 and 20 mg/kg.bw compared to the control group. Similarly, females treated at these doses exhibited significant reduction of the rate of post implantation embryos losses compared to the control group (Figure 2). However, the rate of pre-implantation embryos losses was significantly increased (P<0.05) in females treated with 20 mg/kg.bw compared to those receiving 16 mg/kg.bw of HPC.



[a,b] bars with different letters are significantly different, P<0.05

Figure 1: Effects of 17-α- hydroxyprogesterone caproate on pre implantation embryo losses in female African giant rat.

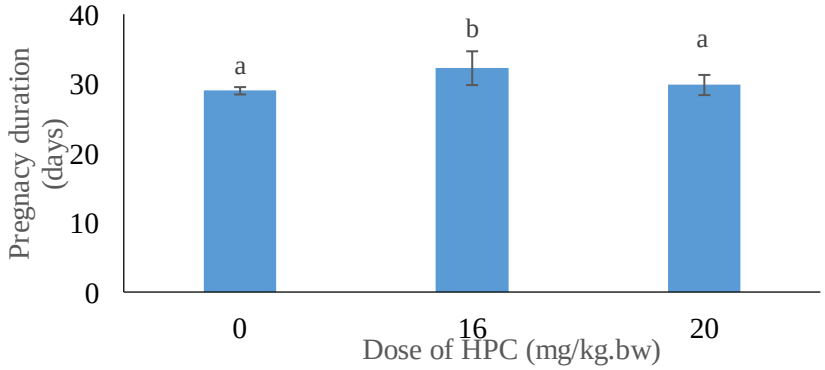


[a,b] bars with different letters are significantly different, P<0.05

Figure 2: Effects of 17-α-hydroxyprogesterone caproate on post implantation embryo losses in female African giant rat.

3.4 **Effect of 17-α-hydroxyprogesterone caproate on pregnancy duration in female African giant rat**

The pregnancy duration of rats varied independently of HPC doses with a significant increase (P<0.05) observed with 16 mg/kg compared to the 20 mg/kg HPC- treated FAGR and control group (Figure 3).

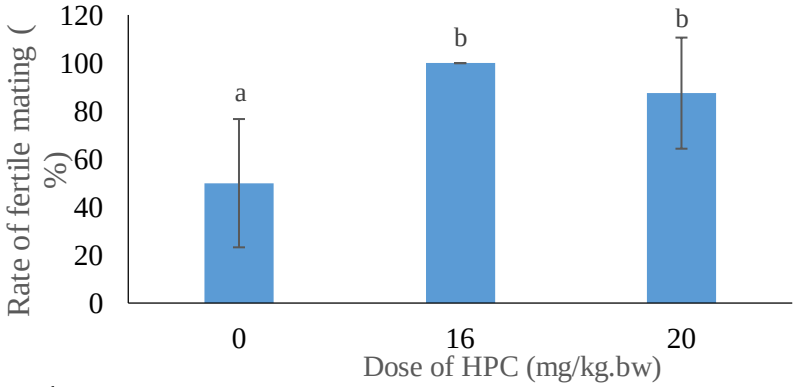


[a,b] bars with different letters are significantly different, P<0.05

Figure 3: Effects of 17-α- hydroxyprogesterone caproate on pregnancy duration in female African giant rat.

3.5 Effects of 17-α- hydroxyprogesterone caproate on the rate of fertile mating

The rate of fertile mating significantly increased (P<0.05) in HPC treated-female groups as compared to the control group (Figure 4). However it was comparable between females receiving 16 mg/kg.bw and those treated with 20 mg/kg.bw of HPC.

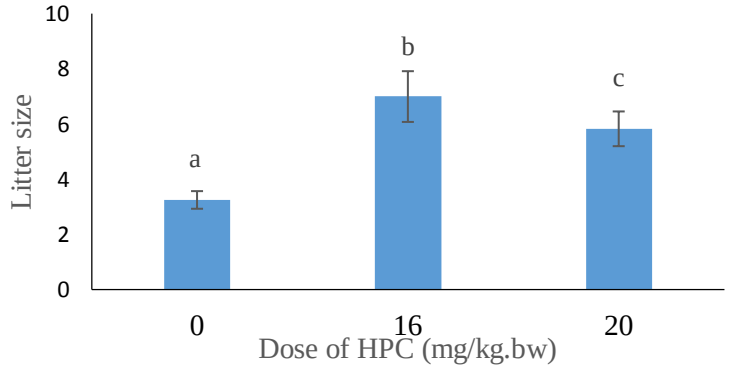


[a,b] bars with the different letters are significantly different, P<0.05

Figure 4: Effects of 17-α- hydroxyprogesterone caproate on the rate of fertile mating in female African giant rat.

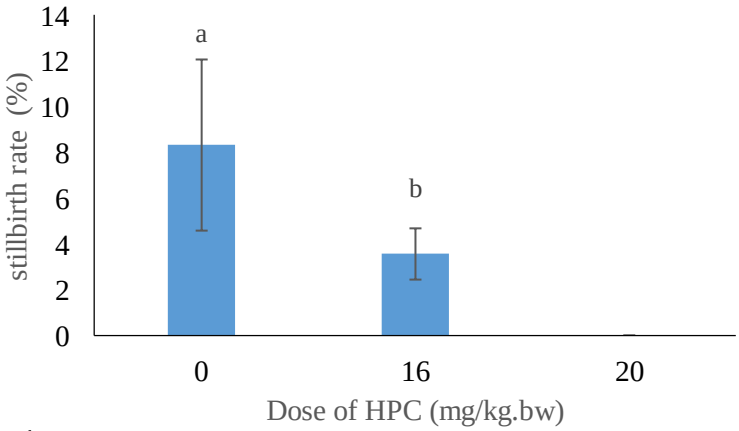
3.6 Effect of 17-α- hydroxyprogesterone caproate on the litter size and stillbirth rate

The litter size significantly increased (P<0.05) in HPC treated-groups as compared to the control group. However, females receiving HPC at the dose of 20 mg/kg.bw showed low litter size (P<0.05) compared to those treated with 16 mg/kg.bw (Figure 5). The stillbirth rate in HPC injected-FAGR was significantly reduced (P<0.05) compared to that of the control group, with 0% mortality at 20 mg/kg.bw (Figure 6).



[a,b,c] bars with different letters are significantly different, P<0.05

Figure 5: Effects of 17-α- hydroxyprogesterone caproate on litter size in female African giant rat.



[a,b] bars with different letters are significantly different, P<0.05

Figure 6: Effects of 17-α- hydroxyprogesterone caproate on stillbirth rate in female African giant rat.

IV. Discussion

African giant rat generally exhibits poor fertility in captivity. Results obtained in previous studies have demonstrated that in captivity, the mating rate ranged between 75 and 100% (Tsambou, 2020; Fonou, 2021). However, the fertile mating rate is low, varying from 25 to 67%, and indicating that after mating there is a low fecundation rate or a great number of embryos undergo resorptions. It is the reason why in the present study, we investigated the effect of exogenous progesterone on mated FAGR fertility and the 17-α-hydroxyprogesterone caproate (HPC) was used.

The result of the present study showed that, although weights of uteri increased at 16 mg/kg. bw, at the highest dose (20 mg/kg) as soon as in the control group, they were the lowest. This could suggest that at high dose, HPC inhibit development of sexual organs. The ability of progesterone to reduce sexual organs weight has been reported in male rats exposed *in utero* to progesterone and could be due to the induction of cells apoptosis (Harini et *al.*, 2009; Samy et *al.*, 2016). In the present study, the mechanism by which HPC at high dose induced decrease in uterus weight remains unknown.

When administered at doses of 16 and 20 mg/kg.bw, FAGR showed significant reduction of the still birth rate, the rates of pre and post implantation embryos losses, as well as the rate of resorbed embryos compared to the control group. Moreover, the numbers of corpora lutea, implantation site, live embryos and the rate of implanted embryos significantly increased ($P<0.05$) in females treated with the same doses of HPC compared to those of control. The reduction of pre and post implantation embryo losses could be linked to the inhibitory and relaxing effects of HPC on myometrium contraction and further justify the high rate of implanted embryos, of fertile mating rate and litter size. In fact, during pregnancy, increase of progesterone concentration is necessary for the inhibition of myometrium contractility (Anderson et *al.*, 2009, Zongzhi et *al.*, 2018, Edey et *al.*, 2018).This action of progesterone enhances implantation, maintenance and development of *conceptus*. Thus, the diminution of circulating progesterone concentration during pregnancy could result to lack of implantation or to preterm birth. Similar findings have been obtained in human (Shruti et *al.*, 2018; Joan et *al.*, 2019), cow (Stevenson and Lambt, 2016; Wiltbank et *al.*, 2014), rabbit (Kwum and Emmens, 1974) and laboratory rats (Kota et *al.*, 2013). HPC used in this study is a structural analogue of progesterone. It is therefore possible that it acts through genomic mechanism by binding to intracellular receptors of progesterone (Leo and Lin, 2008) or through a non-genomic mechanism which involves interaction with membrane receptor and kinase pathway (Thoresen et *al.*, 2020).

The rate of implanted embryos and consequently the litter size significantly increased ($P<0.05$) in females treated with 16 and 20 mg/kg of HPC ($P<0.05$) compared to the control. Tchoumboue et *al.* (2002) early reported the litter size of 3.20 ± 0.19 in FAGR. Later, the litter size of 4.00 ± 1.41 has been reported under the photoperiod of 12h light/24h dark (Fonou, 2021). In this study, a litter size of 7.00 ± 0.92 obtained in FAGR receiving HPC at dose of 16 mg/kg.bw was comparable to those (7 to 8) generally observed in females captured from the wild in pregnant state. This demonstrated that the low rate of fertile mating or kidding is owed to the inability of females to maintain pregnancy, due to progesterone insufficiency. FAGR treated with 20 mg/kg.bw showed low fertility compared to those exposed to 16 mg.kg.bw. This could suggest that at high dose, HPC may be detrimental to reproductive function of FAGR. In fact, it has been reported that in mice, subcutaneous injection of high concentration of progesterone (8 mg) is harmful for endometrial receptivity and decidualization (Yu et *al.*, 2018).

V. Conclusion

The present study demonstrated that, administration of 17-α-hydroxyprogesterone caproate in mated FAGR improves their fertility with best reproductive performances at 16 mg/kg.bw.

References

[1]. Aguilar, H. N., & Mitchell, B. F. (2010). Physiological pathways and molecular mechanisms regulating uterine contractility.*Hum Reprod Update*, 16, 725–44.

[2]. Anderson, L., Martin, W., Higgins, C., Nelson, S. M., & Norman, J. E. (2009). The effect of progesterone on myometrial contractility, potassium channels, and tocolytic efficacy. *Reprod Sci*, 16, 1052–1061.

[3]. Asibey, E., & Addo, P. (2000). The glasscutter, a promising animal for meat production. African perspective practice and policies supporting sustainable development, Turnhan, D. (ed.). Zimbabwe.Waever press, 251-263

[4]. Edey, L. F., Georgiou, H., O'dea, K. P., Mesiano S., Herbert, B. R, Lei, K., Hua, R., Markovic, D., Waddington, S. N., Macintyre, D., Bennett, P., Takata, M. & Johnson, M. R. (2018). Progesterone, the maternal immune system and the onset of parturition in the mouse. *Biol reprod*, 98, 376-395

[5]. Fonou, T. L. (2021). Effets de la durée de la photopériode sur les performances de croissance et de reproduction chez le rat de Gambie en captivité. Thèse de Doctorat/PhD en biotechnologie et Productions Animales. Faculté d'Agronomie et des sciences Agricoles. Université de Dschang, P119

[6]. Fonou, T. L., Fopa, F. C., Vemo, B. N., Tsambou, A. M., Fonkem S., Takam, M. B., & Kenfack A. (2021). Effect of photoperiod on the estrus and reproductive organs in female African giant rat (*Cricetomys gambianus waterhouse*). *International journal of sustainable agricultural research*, 8, 87-92.

[7]. Garcia, D. M. T., Nilson, D., Nilka, F. D., Alessandra, C. B., & Benjamin, H. (2005). Comparison of embryo implantation in wistar rats that underwent ovarian stimulation using exogenous gonadotrophins associated with cetrorelix acetate or leuprolide acetate. *Fertility and infertility*, 85, 1235-1240

[8]. Harini, C., Sainath, S. B., & Sreenivascula, R. P. (2009). Recovery of suppressed male reproduction in mice exposed to progesterone during embryonic development by testosterone. *Reproduction*, 137, 439-448.

[9]. Joan, T. P., Bellington, V., Bethany, L. F., Stephen, R. C., Helen, B. M., Jennifer, W., Felistas, M. M., Elwyn, C., Lynne, M. M., Dwight, J. R., Robert, L. G., & Jeffrey, S. A. S. (2019). Intramuscular 17-hydroxyprogesterone capraote to prevent preterm birth among HIV- infected women in Zambia: study protocol of the IPOP randomized trial. *BMC pregnancy and childbirth.* 19/81. Doi.org/10.1186/s12884-019-222-8

[10]. Kota, S. K., Gayatri, K., Jammula, S., Kota, S. K., Krishna, S. V., Meher, L. K., & Modi, K. D. (2013). Endocrinology of parturition. *Indian J Endocrinol Metab*, 17, 50-59.

[11]. Kwum, J. K., & Emmens, C. (1974). Hormonal requirement for implantation and pregnancy in the ovarioectomized rabbit. *Australian journal of Biological Science*, 27, 275-83

[12]. Leo, J. C., & Lin, V. C. (2008). The activities of progesterone receptor isoform A and B are differentially modulated by their ligands in a gene-selective manner. *Int J cancer*, 122, 230-243

[13]. Samy, I. A., Adil, S. E., Gamal, A., & Tahir, O. A. (2016). Prenatal progesterone exposure of male rats induces morphometric and histological changes in testes. *Asian Pacific Journal of Reproduction* , 5, 204-209

[14]. Shruti, S., Rashmi, B., Pallavi, B., Jasvinder, K. &Praveen, K. (2018). A randomised trial to compare 200 mg micronised progesterone effervescent vaginal tablet daily with 250 mg intramuscular 17 alpha hydroxy progesterone caproate weekly for prevention of recurrent preterm birth. *Journal of Obstetrics and Gynecology*, 38, 800-806.

[15]. Stevenson, J.S., & Lamb, G. C. (2016). Contrasting effects of progesterone on fertility of dairy and beef cows. *Journal of Dairy Science*, 99, 5951–5964.

[16]. Tchoumboue, J., Niba, A. T., Zango, P., Dafem, & Téguia, A. (2002). Reproductive and growth performance of cricetoma *(Cricetomys gambianus*) under captivity. *Tropicultura*, 20,130-134.

[17]. Thoresen, E. S., Smaglyukova, N., Ane, O., & Sager, G. (2020). Expression of nuclear progesterone receptor (nPRs), membrane progesterone receptors (mPRs) and progesterone receptor membrane components (PGRMCs) in the human endometrium after 6 months levonorgestrel low dose intrauterine therapy. *Journal of steroid Biochemestry and molecular biology*. 202: doi.org/10.1016/j.jsbmb.2020.105701

[18]. Tsambou, M. M. A. (2020). Effets du niveau d'énergie de la ration sur la croissance et les performances de reproduction du rat de gambie (*Cricetomys gambianus*) en captivité. Thèse de Doctorat/PhD en biotechnologie et productions Animales. Université de Dschang. Faculté d'Agronomie et des Sciences Agricoles. P135

[19]. Wiltbank, M. C., Souza, A. H., Carvalho, P. D., Cunha, A. P., Giordano, J.O., Fricke, P. M., & Diskin, M. G. (2014). Physiological and practical effects of progesterone on reproduction in dairy cattle. *Animal*, 8, 70–81.

[20]. Yu-Xiang, L., Li, L., Zhi-Yong, J., Xiao-Huan, L., Yong-Sheng, F., Xiao-Wei, G., & Zeng-Ming, Y. (2018). The high concentration of progesterone is harmful for endometrial receptivity and decidualization. Scientific report. 8:712 DOI: 10.1038/s41598-017-18643-w

[21]. Zongzhi, Y., Yun, L., Wenzhu, H., Dan, L., Hongyan, L., Yuanyuan, Y., Bing, S., Xi, W., Yunxia, C., & Raouf, A. K. (2018). Progesterone inhibits contraction and increases TREK-1 potassium channel expression in late pregnant rat uterus. *Oncotarget*, 9, 651–661.

Fonkem Severin, et. al. "17-α-hydroxyprogesterone caproate improves female African giant rats (Cricetomys gambianus) fertility in captivity." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 15(03), 2022, pp. 15-21.