



Gene Expression of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) Receptors in Some of the Reproductive Organs of Giant African Land Snail (*Archachatina marginata*)

O. J. Osunkeye^{1*}, O. M. Onagbesan², J. A. Abiona² and S. O. Jinadu²

¹*Department of Animal Science, College of Agriculture, Osun State University, Osogbo, Nigeria.*

²*Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author OJO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OMO and JAA supervised the study. Author SOJ was involved in the laboratory analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2019/v22i330129

Editor(s):

(1) Dr. Kuldip Singh, Department of Biochemistry, Govt. Medical College – Amritsar, Punjab, India.

Reviewers:

(1) Estela Cuevas, Universidad Autónoma e Tlaxcala, Mexico.

(2) Opeyemi Gbenga Oso, University of Ibadan, Nigeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/52853>

Review Article

Received 28 September 2019

Accepted 01 December 2019

Published 13 December 2019

ABSTRACT

The role of receptors in the exertion of the hormonal effect on their target cell creates a better understanding of the mechanism of action of hormones. In the snail, *Archachatina marginata*, there is a dearth of information on the gene expression of FSH and LH receptors. This experiment was conducted to determine the effects of exogenous administration of gonadotropin (PMSG) and some sex steroid hormones (Progesterone (P4) and 17 β -Oestradiol (E2)) on the levels of gene expression of FSH and LH in some selected reproductive organs of *Archachatina marginata*. The experiment was laid out in 4x3x2 factorial design in Completely Randomised Design comprising of 432 snails randomly allotted to 24 treatment groups of 18 snails per group of Control, P4, E2 and PMSG at 3 dosage levels (Low-1 μ g/ml, Medium-5 μ g/ml and High-10 μ g/ml for P4 and E2; and Low-1 IU/ml, Medium-10 IU/ml and High-20 IU/ml for PMSG) at 2 durations of injections (16-days of

*Corresponding author: E-mail: osunkeye.olumuyiwa@uniosun.edu.ng;

four injections and 32-days of eight injections). The gel viewed under the ultra-violet light source showed that the expression of LHR receptor did not appear in all the organs considered. However, FSHR receptor was noted in their appearance in the albumen gland, little hermaphrodite duct, common hermaphrodite duct and vagina of *A. marginata*. Administration of E2 of 5 µg/ml for 16 days and 32 days increase the gene expression of FSH receptors in the common hermaphrodite duct and vagina of *A. marginata* respectively. Also, injections of 5 µg/ml of P4 for 16 days gave a relative increase in the levels of expression of FSH receptors in the little hermaphrodite duct, while the highest level was on the albumen of snails injected with 1 µg/ml of E2 for 32-days of eight injections. Injection of 5 µl/ml of P4 for 16-days of four injections, 5 µl/ml of E2 for 32-days of eight injections, 5 µl/ml of E2 for 16-days of four injections, 1 µl/ml of E2 of 32-days of eight injections increased the gene expression of FSH in the little hermaphrodite duct, vagina, common hermaphrodite duct and albumen respectively. Therefore, it is informed here that administration of P4, E2 and PMSG exogenously to *A. marginata* of reproductive age induces an increase in the levels of gene expression of FSH receptors.

Keywords: Reproductive organs; receptors; gene expression; *Archachatina marginata*.

1. INTRODUCTION

A Giant African Land snail (GALS), *Archachatina marginata*, is a nature of the tropical rainforest [1]. The matured ones can weigh between 600 g and 1600 g. They serve as a source of animal protein, containing about 17% crude protein of high biological value and a source of income especially for the small scale farmer in the rural area. The hormone is a signal molecule which carries a given type of information that is received by a cellular signal receiver (receptor) structure which mediates it into the cell body. The interaction between the hormones and its receptor presuppose that they mutually recognise one another. And cell-hormone recognition is a fundamental phenomenon, in which the receptor-hormone relationship represented the initial step of cell-environment interrelationship [2].

Gonadotropin, Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) are known to be key modulators of normal reproductive function [3,4]. Its role is inevitable in the normal, and assisted reproductive intervention of reproductive physiology and responses in animals. FSH and LH, pituitary glycoprotein hormone, are an integral component of the endocrine axis that regulates gonadal function and fertility. To transmit its signal, FSH must bind to its receptor (FSHR) located on the sertoli cells of the testis and the granulosa cells of the ovary. Thus, both the magnitude and the target hormone response are controlled by mechanisms that determine FSHR levels and cell-specific expression, which are supported by transcription of its gene [5]. Expression of FSHR, both protein and mRNA, is remarkably limited concerning its cellular profile, with sertoli and

granulosa cells being the predominant expressing cell types [6,7]. FSHR expression reported in rodent coincide with primary follicle formation and follicular development through the prenatal stage, with initial full-length transcriptions and hormone-binding observed shortly after birth (around postnatal day 3) and continuing to increase through postnatal day 21 [8,9]. Expression of FSHR is considered to be gonad-specific and restricted to sertoli and granulosa cells in the testis and ovary respectively, but a few notable reports of its presence elsewhere, particularly in the uterus, prostate, bone and ovarian surface epithelial [10, 11,12].

According to Tarrant [13], the vertebrate endocrine system is an exquisite example of compartmentalization and communication. Specialised cells and organs secrete tens to hundreds of distinct bio-regulatory molecules (or hormones), which travel through the circulatory system and regulate physiological processes in the target organs and tissue. Similarly, many invertebrates have been said to possess discrete endocrine organs that share some functions with the corresponding organ in vertebrates [14,15, 16]. Recently, there has been an increasing study on the use of sex steroid hormone [17,18, 19], concerning their effects on the reproductive responses in mollusc and another invertebrate. There is a dearth of information on the use of FSH and another gonadotropin. This could be due to lack of information on its mechanism of action through FSHR in the snail, *A. marginata*. The need to boost production of the giant African land snails (GALs) has led to the search for artificial or hormonal intervention whereby the sustainable and affordable products can be attained, especially during the off-season of this

animal [4]. Therefore, the objective of this study is to determine the gene expression of FSH and LH receptors in the vagina, common hermaphrodite duct, albumen gland and little hermaphrodite duct of *A. marginata*.

2. MATERIALS AND METHODS

2.1 Management of the Experimental Animal

The giant African land snail (*Archachatina marginata*) used in this research were acquired from the same source as much as possible to reduce the variability of biotype. The snails were housed in cages, with a stocking density of 7 snails per cage to give approximately 100 snails per m² as recommended by Ogogo and Ayodele [20]. Each cage padded on the floor with layers of moist humus soil to the depth of 5 cm for the period of the experimentation. They were kept under a photoperiod of 12 hours light: 12 hours darkness (12L: 12D). The cages were sprinkled with water daily. The animals were fed *ad libitum* on broilers' mash and dried pawpaw leaves a meal in ratio 1:1 (weight/weight) at 170 g Feed/day/pen. The leftover feed and faecal material were removed daily to prevent contamination.

2.2 Experimental Layout

The basal population of Four Hundred and Thirty-Two (432) snails ranging between 100 g – 140 g were randomly allotted to 24 treatment groups. Pregnant Mare Serum Gonadotropin (PMSG), 17 β -Oestradiol (E2) and progesterone (P4) were procured from Sigma® (Mississauga, Ontario, Canada). The steroid hormones which include the E2 and P4 were first dissolved in absolute ethanol to yield 200 μ g/ml stock solutions, thereafter distilled water was used to reconstitute into the dosage needed. However, PMSG was dissolved in distilled water to yield 100 IU/ml stock solution and reconstituted to the required dosage. The snails in the control treatment were injected with 2 ml of dH₂O while the reconstituted hormone doses were injected through the neck of the foot muscle of the snail with a syringe and needle. This experiment was laid out in 4 x 3 x 2 factorial arrangement in a Completely Randomized Design with 18 snails per treatment replicated three times. The snails were divided into four groups as follows: Group I: Control; Group II: Progesterone injected group; Group III: Oestradiol injected group; and Group IV: PMSG injected group. The experimental treatments were further subdivided into three

doses (Low, Medium and High), and all these were categorised into two durations of injection that is, 16 days of four injections and 32 days of eight injections on four days interval. The Control of this experiment was injected with 2 ml of distilled water. However, E2 and P4 treatments were injected with three dosage levels of 1 μ g/ml, 5 μ g/ml and 10 μ g/ml, while PMSG treatment consists of three dosage levels of 1 IU/ml, 10 IU/ml and 20 IU/ml.

At the end of the experimental treatments, two snails (*A. marginata*) were selected from the experimental stock according to the treatments, dissected, and vagina, common hermaphrodite duct, albumen gland and little hermaphrodite duct parts of the reproductive organs were removed and labelled for identification. These organs were kept frozen at -70°C in Ultra-low freezer for the analysis of gene expression of FSH and LH receptors. Thereafter, the tissue stored in the Ultra freezer at -70°C were homogenised for PCR procedures. The total RNA was extracted from the tissues using the RNA extraction kit. Primers used were designed using a commercial program on the internet that could retrieve the sequence of the targeted RNA (Integrated DNA Technology, IDT, Inc., USA). For this experiment, the primers designed for Follicle Stimulating Hormone (Forward 5'-AGA AGG CCA ACA ACC TCG TG-3'; Reverse 5'-ACA GCA ATG GCT AGG ATA GCT-3') and Luteinizing Hormone receptor (Forward 5' – AAA AAG GAC GAG TCG CTG C – 3'; Reverse 5' – GAT TCA TTG TGG CGT ATT CA – 3') was adopted. The extracted RNAs were subjected to Reverse Transcriptase Polymerase Reaction (RT-PCR) and the products were subjected to Polymerase Chain Reaction (PCR). The amplicon was subjected to an Agarose Gel Electrophoresis. The gel was viewed and captured under the ultra-violet light source mounted camera.

2.3 Experimental Procedure

The samples from each treatment were prepared by obtaining ten to twenty milligram of frozen tissue of *Archachatina marginata* from each of the required sections of the reproductive tract after dissection, and stored in Eppendorf tube and labelled according to the experimental treatment for easy identification. 10 μ l of β -Mercaptoethanol was added to each weighed tissue and grind to homogenized using a mortar and pestle. An additional 600 μ l of a mixture of Lysis Buffer RL was added to the homogenised

sample and was vortexed for 15–30 seconds. The lysate was transferred into an RNase-free microcentrifuge tube. The lysate was spun for 2 minutes to pellet any debris. Thereafter, the supernatant was transferred into another RNase-free micro-centrifuge tube. An equal volume of 70% ethanol was added to the lysate collected (100 µl of ethanol was added to every 100 µl of lysate) then the sample was vortexed to mix up together.

The lysate was purified using the procedures which include binding RNA to the column, column washing and RNA elution. 600 µl of lysate with 70% ethanol was transferred into spin column tube and was centrifuged for 1 minute at $\geq 3,500 \times g$. RNA binds to the column leaving the residue in the flow-through which was discarded. This was repeated three times to ensure that the entire lysate volume has passed through into the collection tube. Thereafter, 400 µl of primary washing solution was applied to the spin column and was centrifuged for 1 minute at $100 \times g$, and the flow-through discarded. This step of the procedure was repeated two more times. Then the column was spun again for 2 minutes to dry the resin and the collection tube discarded. The column was placed into a fresh 1.7 ml Elution tube and 50 µl of the elution solution was added to the column and centrifuge for 2 minutes at $200 \times g$, followed by 1 minute at $14,000 \times g$. The second elution was performed using a separate microcentrifuge tube for maximum recovery of RNA. The purity and dosage of RNA were quantified using the Nanodrop spectrophotometer set at the absorbance of 260 nm and 280 nm. Thereafter, the RNA dosage was dilute further to 50% for standardisation. The purified RNA sample for each treatment was stored at -20°C .

The extracted and purified RNA were mixed with the RT-PCR primer and master mix (Norgen Biotek Corp., Ontario, Canada)– which consists of the buffer and other nucleotide and subjected to Reversed Transcription Polymerase Chain Reaction (RT-PCR) in the PCR machine for 2 hours at an annealing temperature of 94°C , which is the average of the temperature stated on the primer. The amplicons product from the RT-PCR were mixed with the DNA primer and master mix for FSH-R and LH-R, and subjected to PCR in the PCR machine for 2 hours at an annealing temperature of 56.9°C for FSH and 52.9°C for LH. For this experiment, the primers designed for Follicle Stimulating Hormone (Forward 5'-AGA AGG CCA ACA ACC TCG TG-

3'; Reverse 5'-ACA GCA ATG GCT AGG ATA GCT-3') and Luteinizing Hormone receptor (Forward 5' – AAA AAG GAC GAG TCG CTG C – 3'; Reverse 5' – GAT TCA TTG TGG CGT ATT CA – 3') (Integrated DNA Technology, IDT, Inc., USA) was adopted. Twenty-five microlitres of the final PCR product were run out on a 1% agarose gel and viewed for band verification.

One hundred millilitres of working Tris Acetic Acid EDTA (TAE) buffer solution was added to 1 g of Agarose powder and mixed properly before placing the mixture into Microwave Oven to melt at $37\text{--}40^{\circ}\text{C}$. Afterwards, One drop of Ethidium Bromide was added to the melted mixture, mixed properly, poured into the agarose chamber with the comb in it and allowed to cool. The solid gel was formed after cooling, leaving wells in it. The expression of the gene encoding the protein of the receptors was derived from the PCR and Agarose Gel Electrophoresis through the imaging that was viewed under Ultra Violet light and captured with the digital camera. Thereafter, the expressivities of the receptors were quantified using ImageJ software.

3. RESULTS

The result showed that there was no expression of LH gene receptor in all the selected reproductive organs considered. However, receptors for FSH were present in different parts of the reproductive tract selected. It was also obvious that levels of expression vary with hormone type, hormone dosage and period of administration (Plates 1 – 4, Fig. 1).

In the snail vagina, the level of FSH gene expressed was more in P4 injected snails (169.20 pixels) and E2 injected snails (173.79 pixel) compared to that level of expression observed in the Control (153.15 pixel). Comparing the level of FSH gene expression by the Control and PMSG injected snails revealed that the latter had lower expressivity (153.15 vs 150.67 pixel). The effect of dosage of hormone administration showed that as the dosage increases from low dosage to medium dosage, the level of FSH gene expression increases but reduces as the dosage was increased from medium to high (165.00 vs 167.89 vs 153.40 pixel for low, medium and high respectively). For the period of hormone injection, the level of FSH gene expressed increased as the days or number of injection of hormone increases. The peak expressivity of FSH receptor gene showed with the injection of E2 at $5 \mu\text{g/ml}$ for 32 days of eight injections while the least was seen in snails

injected with PMSG at 20IU/ml for 16 days of four injections (176.51 and 113.18 pixel respectively). In the common hermaphrodite duct, there was an increasing level of expression in P4 and E2 injected snail compared with the Control treatment. But the expression of FSH gene reduces in PMSG injected snails about the Control. The level of expression increases as the dosage level of hormones increases from the Low to High (79.09, 79.09 and 80.40 pixel) respectively). FSH gene expression was more on 32 days of eight injections than that expressed in 16 days of four injections. In all these treatments on the common hermaphrodite duct, the highest expression of FSH receptor gene appeared with the injection of E2 at 5 µg/ml for 16 days of four injections and lowest with the injection of PMSG

at 20 IU/ml for 32 days of eight injections (88.49 and 68.15 pixel respectively). In the albumen gland, the expressions of FSH gene were more with all hormone used (that is P4, E2 and PMSG) compared to the control in the first 16 days of the experiment. The level of expression in the Low dosage level of hormone was lower compared to the others in the Medium dosage level, and High dosage level (191.74 and 189.76 pixel respectively). Also, the level of expression of FSH gene in 32 days of eight injections was more than 16 days of four injections. The appearance of FSH receptor gene in the albumen was highest with the administration of E2 at 1 µg/ml for 32 days of eight injections (207.70 pixel), which is also the highest for all the organs selected for this study.

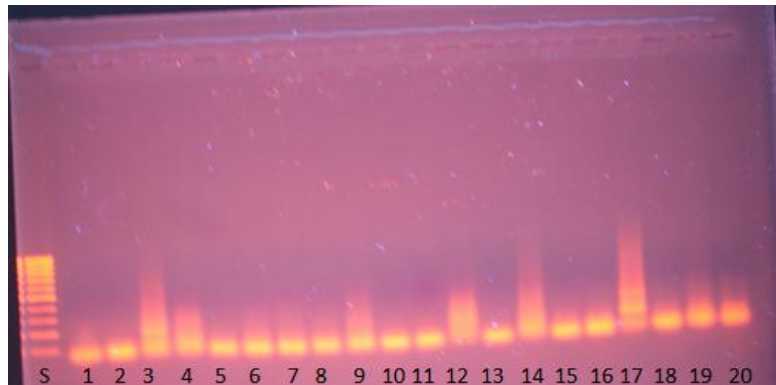


Plate 1. Gene expression of FSH receptor for albumen gland

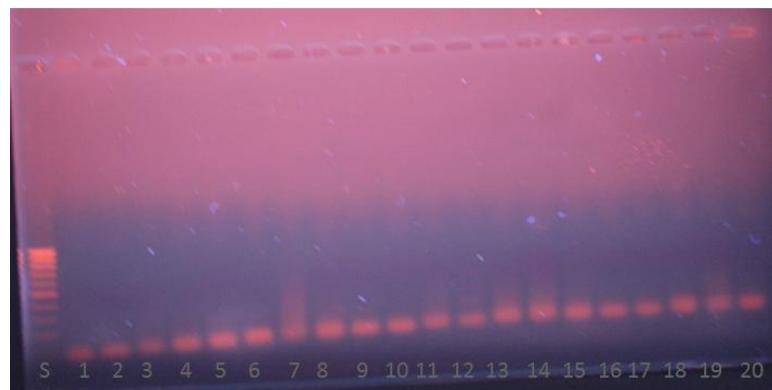


Plate 2. Gene expression of FSH receptor for the little hermaphrodite duct

S = Standard marker, 1 = Normal or control (for 16 days of four injections), 2 = Normal or control (for 32 days of eight injections), 3 = P411 is 1 µg for 16 days of four injections, 4 = P412 is 1 µg for 32 days of eight injections, 5 = P451 is 5 µg for 16 days of four injections, 6 = P452 is 5 µg for 32 days of eight injections, 7 = P4101 is 10 µg for 16 days of four injections, 8 = P4102 is 10 µg for 32 days of eight injections, 9 = E211 is E2 1 µg for 16 days of four injections, 10 = E212 is E2 1 µg for 32 days of eight injections, 11 = E251 is 5 µg for 16 days of four injections, 12 = E252 is 5 µg for 32 days of eight injections, 13 = E2101 is E2 10 µg for 16 days of four injections, 14 = E2102 is 10 µg for 32 days of eight injections, 15 = PMSG11 is 1IU for 16 days of four injections, 16 = PMSG12 is 1 IU for 32 days of eight injections, 17 = PMSG101 is 10 IU for 16 days of four injections, 18 = PMSG102 is 10 IU for 32 days of eight injections, 19 = PMSG201 is 20 IU for 16 days of four injections, 20 = PMSG202 is 20 IU for 32 days of eight injections

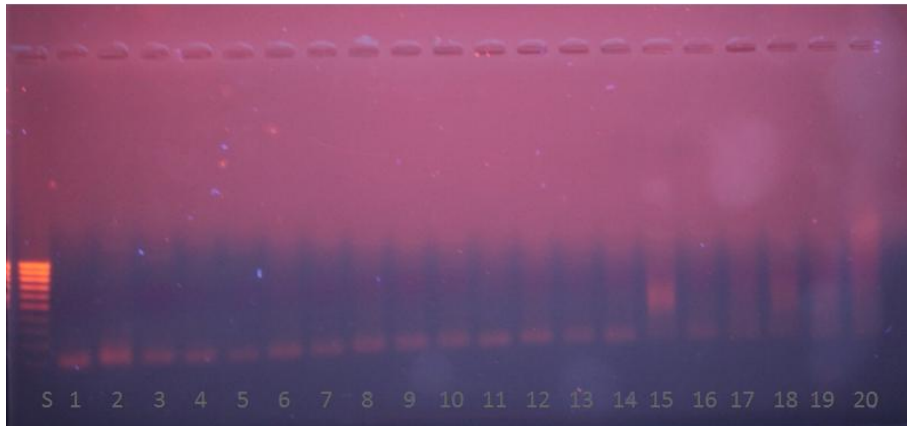


Plate 3. Gene expression of FSH receptor for common hermaphrodite duct

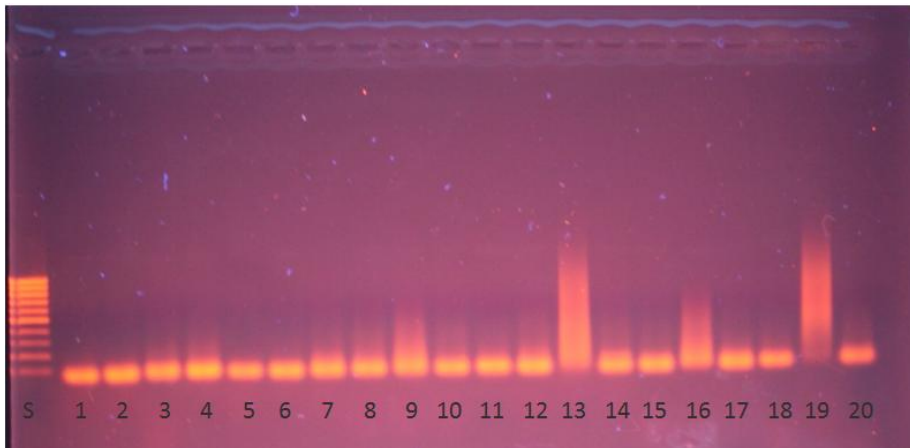


Plate 4. Gene expression of FSH receptor for vagina

S = Standard marker, 1 = Normal or control (for 16 days of four injections), 2 = Normal or control (for 32 days of eight injections), 3 = P411 is 1 µg for 16 days of four injections, 4 = P412 is 1 µg for 32 days of eight injections, 5 = P451 is 5 µg for 16 days of four injections, 6 = P452 is 5 µg for 32 days of eight injections, 7 = P4101 is 10 µg for 16 days of four injections, 8 = P4102 is 10 µg for 32 days of eight injections, 9 = E211 is E2 1 µg for 16 days of four injections, 10 = E212 is E2 1 µg for 32 days of eight injections, 11 = E251 is 5 µg for 16 days of four injections, 12 = E252 is 5 µg for 32 days of eight injections, 13 = E2101 is E2 10 µg for 16 days of four injections, 14 = E2102 is 10 µg for 32 days of eight injections, 15 = PMSG11 is 1IU for 16 days of four injections, 16 = PMSG12 is 1 IU for 32 days of eight injections, 17 = PMSG101 is 10 IU for 16 days of four injections, 18 = PMSG102 is 10 IU for 32 days of eight injections, 19 = PMSG201 is 20 IU for 16 days of four injections, 20 = PMSG202 is 20 IU for 32 days of eight injections

Considering little hermaphrodite duct, the highest level of expression of FSH gene was recorded in E2 (113.04) compared with that of P4, PMSG and the Control (109.58, 101.70 and 82.45 pixel respectively). The Medium dosage level of the hormone administered had the highest level compared with Low dosage level (100.83 pixel) and with High dosage level (103.54 pixel). However, the result showed that the highest value of the FSH receptor gene expression in the little hermaphrodite duct was observed with the administration of P4 at 5 µg/ml for 32 days of eight injections (119.89 pixel). The administration

of P4, E2 and PMSG hormones tend to increase the level of expression of FSH gene receptor in the albumen gland and little hermaphrodite when compared to the Control. It was obvious that Medium dosage administration of hormone gave the highest level of FSH expression in albumen gland and little hermaphrodite duct. Considering the period of injection of the hormone type, the same trend was followed in FSH gene expression by all the reproductive organs which revealed that 32 days of eight injections had more expression than 16 days of four injections.

4. DISCUSSION

Researches on the FSH and LH receptors in snails have been conducted, and it was concluded that FSH receptors were expressed in normal snails but there is little or no information on those treated with hormones. However, the present study revealed that even with administration of hormone, FSH receptors were expressed in some of the reproductive organs, and the magnitude of expression varied with the injected hormones in each reproductive organ considered. The result of this study showed that the albumen gland, little hermaphrodite duct, common hermaphrodite duct and snails' vagina contain FSH receptor. This is to say that all the parts of the reproductive organ studied were

directly under the influence of FSH hormone. Comparatively, with the normal snails in the Control group, it was discovered that the expression of FSH receptor was slightly higher in snails' little hermaphrodite duct when treated with 5µg/ml of P4 either for 16 days of four injections or 32 days of eight injections. Administration of E2 had a positive influence on the quantity of FSH receptors expressed in most reproductive organs studied. To be precise, 5µg/ml of E2 for 32 days of eight injections gave the highest quantity of FSH receptors in the snails' vagina. Also, the quantity of FSH receptors in snails treated with 5µg/ml of E2 for 16 days of four injections and 1µg/ml of E2 for 32 days of eight injections gave the best in common hermaphrodite duct and albumen respectively.

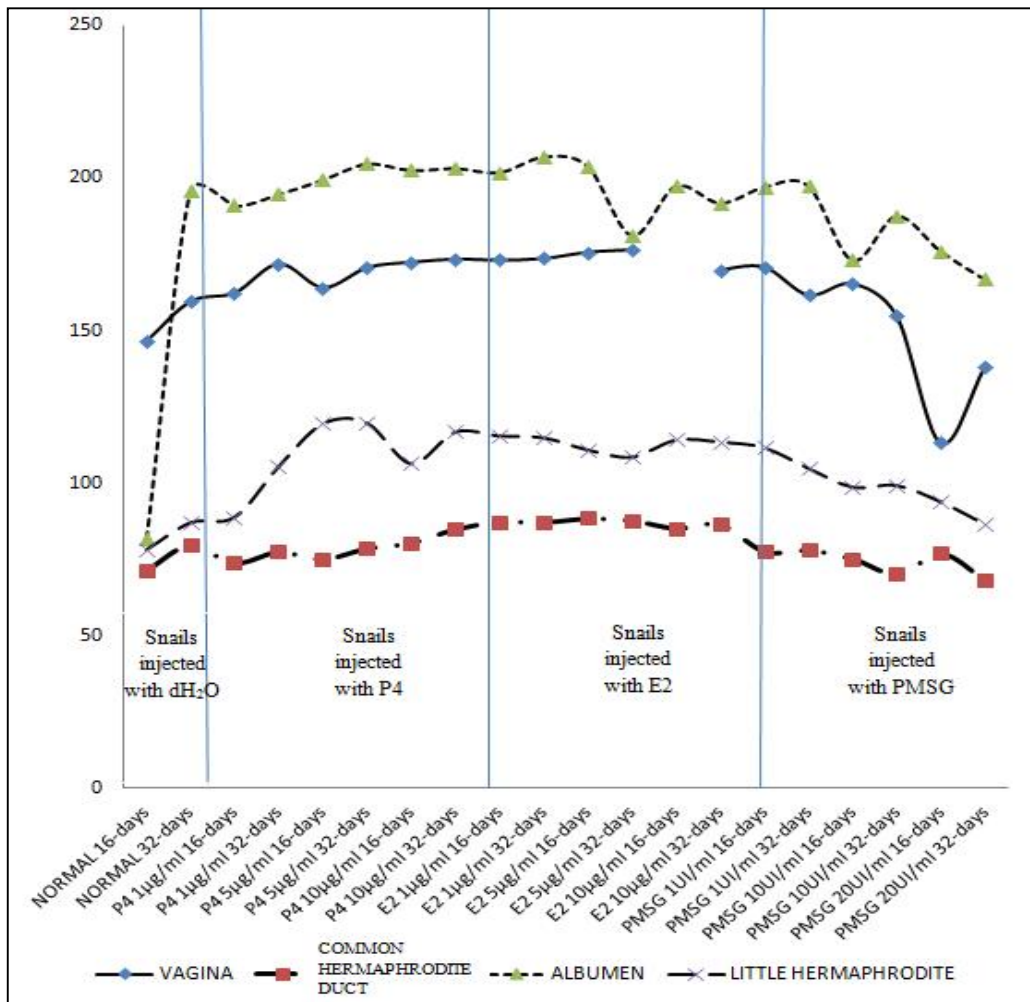


Fig. 1. The quantity of FSH gene receptors expressed by the selected reproductive organs of *A. marginata*

FSH has a direct influence on the parts of the reproductive organs studied. This contradicts the report of Fasoro [21] who illustrated FSH receptor only in Ovo-testis of *A. marginata* among other parts such as Ovotestis, vasdeferens, oviduct (common hermaphrodite duct), albumen gland and spermatheca considered. FSH receptor was found in albumen gland and common hermaphrodite duct in this present study. The disparity may be due to the techniques involved. The non-expressivity of LH in this study may be localised or restricted to the tissues understudied because FSH and LH were expressed in the cerebral ganglia of *B. alexandrian* snail [22]. Also, it could be that LH does not have any significant role or involved in the development and growth of the reproductive activities of *A. marginata*. More so, there is a dearth of information on the influence of LH on the reproductive system of giant African land snails and snails in general. PMSG contains both FSH and LH, and they have been reportedly used in small and large vertebrates such as rat, fish, chicken, sheep and goat, to increase, improve and/or control their reproductive activities. But its influence was slightly seen in this study. This could be due to the non-expressivity of LH receptor, which means that the fraction of LH in PMSG will be ineffective, whereas it supposed to compliment the effect of FSH in these snails.

The expressivity of FSH in the Control group showed that the receptors were present in the tissues of the selected reproductive organs of this study, but the observable increase after administration of P4, E2 and PMSG endogenously could be explained in two ways. Firstly, it could be as it was reported that, even within unicellular organisms, the extracellular hormonal environment of a cell can determine the development of receptor systems in the particular cells [23] and it was discovered that the response of the organism to the particular hormone increased on re-exposure [24]. Secondly, it could be as a result of hormonal imprinting. Hormonal presence is an indispensable prerequisite of receptor formation. The first encounter of a particular hormone with a primordial binding site will result in the Imprinting of this binding site into the complementary shape of the hormone. According to Csaba [23], this memory can fade eventually if the receptor in deprived of repeated contact with the hormone [25] leading to receptor desensitisation. The receptors remain sensitised only as long as it has repeated (sometimes pulsatile) contact with the

hormone. However, in the case of excess hormonal activity, membrane receptors may be down-regulated, possibly as a result of endocytosis, thus avoiding hyper-reaction of the cell. The same may also occur at the primordial binding site of hormones of protein and glycoprotein origins within the cellular membrane. Nevertheless, all these depend on the particular sensitive phase during development or reproduction of the animals, and it depends on the concentration of the interacting hormones.

Dankbar, et al. [6] gave a report of a comprehensive study on FSH receptor expression performed on non-human primate, in which 38 different tissue and organs were screened for the presence of FSH receptor transcripts, using the RNase protection assay technology. It was discovered that no transcript could be detected in organs of tissues other than the testis. Thus, unlike the LH receptor, the expression of the FSH receptor seems to be strictly gonad-cell specific. Quantification of FSH receptor mRNA levels in the human and monkey testis showed that 0.05 to 0.1 pg/ μ g testis RNA encode the FSH receptor. Simoni, et al. [26] also said that FSH decreases remarkably in the absence of FSH, suggesting a mechanism of receptor up-regulation by FSH, whereas in mice and rat FSH deprivation led to an increase in FSH binding, indicating instead of down-regulation action of the hormones in these species [9]. Also, an introduction of E2 has been said to cause an increase in FSH binding and FSH receptor mRNA levels [26].

5. CONCLUSION

Gene expression for LH receptors was not seen in albumen, little hermaphrodite duct, common hermaphrodite duct and vagina of *A. marginata*, but that of FSH was observed in all the reproductive organs considered (albumen, little hermaphrodite duct, common hermaphrodite duct and vagina) though at varying levels. Injection of 5 μ l/ml of P4 for 16-days of four injections, 5 μ l/ml of E2 for 32-days of eight injections, 5 μ l/ml of E2 for 16-days of four injections, 1 μ l/ml of E2 of 32-days of eight injections increased the gene expression of FSH in the little hermaphrodite duct, vagina, common hermaphrodite duct and albumen respectively. The highest expression of FSH receptors was recorded in the albumen of *A. marginata*. It was observed that the effect of injecting PMSG at 10 IU/ml for 16-days of four injections increased the

FSH receptor expression in the albumen gland of this snail. These imply that administration of P4, E2 and PMSG may have a positive influence on the reproductive organs of the snail by increasing the levels of FSH receptors which produce the protein that exerts its effect on the target cell.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fred-Jaiyesimi AA, Awobajo O. Biochemical and histopathological investigation of the effect of *Archachatina marginata* haemolymph in normal rats. International Journal of Applied Biology and Pharmaceutical Technology. 2011; 2(4):30-33.
2. Csaba G. Why do hormone receptors arise? As introduction. In: Development of hormone receptors. II Series, Experimentia; 53.
3. Gromoll J, Ried T, Holtgreve-Grez H, Nieschlag E, Gudermann T. Localization of the human FSH receptor to chromosome 2 p21 using a genomic probe comprising exon 10. Journal Mol. Endocrinol. 1994; 12:265-271.
4. Abiona JA, Adeleke SO, Ladokun AO, Abioja MO, Daramola JO, Osinowo OA, Onagbesan MO. Effect of *Mucuna pruriens* on gene expression of progesterone and follicle stimulating hormone receptors in the reproductive tract of giant African land snail (*Archachatina marginata*). Journal of Molluscan Research. 2015;1:24-33.
5. George JW, Dille EA, Heckert LL. Current concepts of follicle-stimulating hormone receptor gene regulation. Biology of Reproduction. 2011;84:7-17.
6. Dankbar B, Brinkworth MH, Schlatt S, Weinbauer GF, Nieschlag E, Gromoll J. Ubiquitous expression of the androgen receptor and testis-specific expression of the FSH receptor in the cynomolgus monkey (*Macaca fascicularis*) revealed by a ribonuclease protection assay. Journal Steroid Biochemistry and Molecular Biology. 1995;55:35-41.
7. Heckert LL, Griswold MD. Expression of follicle-stimulating hormone receptor mRNA in rat testes and Sertoli cells. Mol Endocrinol. 1991;5:670-677.
8. Dunkel L, Tilly JL, Shikone T, Nishimori K, Hsueh AJ. Follicle stimulating hormone receptor expression in the rat ovary: Increases during prepubertal development and regulation by the opposing actions of transforming growth factors beta and alpha. Biol. Reprod. 1994;50:940-948.
9. O'Shaughnessy PJ, Marsh P, Dudley K. Follicle-stimulating hormone receptor mRNA in the mouse ovary during postnatal development in the normal mouse and in the adult hypogonadal (hpg) mouse: structure of alternate transcripts. Mol Cell Endocrinol. 1994;101:197-201.
10. Ben-Josef E, Yang SY, Ji TH, Bidart JM, Garde SV, Chopra DP, Porter AT, Tang DG. Hormone-refractory prostate cancer cells express functional follicle-stimulating hormone receptor (FSHR). Journal of Urol. 1999;161:970-976.
11. Mariani S, Salvatori L, Basciani S, Arizzi M, Franco G, Petrangeli E, Spera G, Gnessi L. Expression and cellular localization of folliclestimulating hormone receptor in normal human prostate, benign prostatic hyperplasia and prostate cancer. Journal of Urol. 2006;175:2072-2077.
12. Sun L, Peng Y, Sharrow AC, Iqbal J, Zhang Z, Papachristou DJ, Zaidi S, Zhu LL, Yaroslavskiy BB, Zhou H, Zallone A, Sairam MR. FSH directly regulates bone mass. Cell. 2006;125:247-260.
13. Tarrant AM. Endocrine-like Signaling in cnidarians: Current understanding and implications for ecophysiology. Integr. Comp. Biol. 2005;45:201-214.
14. Klowden MJ. Contributions of insect research toward our understanding of neurosecretion. Arch. Insect Biochem. Physiol. 2003;53:101-114.
15. Segal D. Prospects of using Drosophila for insect neuroendocrine research. Arch. Insect Biochem. Physiol. 1993;22:199-231.
16. Swevers L, Iatrou K. The ecdysone regulatory cascade and ovarian development in lepidopteran insects: Insights from the silkworm paradigm. Insect Biochem. Mol. Biol. 2003;33:1285-1297.
17. Lafont R. Reverse endocrinology, or "hormones" seeking functions. Insect Biochem. 1991;21:697-721.
18. Lafont R, Mathieu M. Steroids in aquatic invertebrates. Ecotoxicology. 2007;16: 109-130.
19. Lehoux JG, Sandor T. The occurrence of steroids and steroid metabolizing enzyme systems in invertebrates (review). Steroids. 1970;16:141-171.

20. Ogogo AU, Ayodele IA. Optimal stocking densities of snails (*Archachatina marginata* Swainson) in the swamp forest zone of Nigeria. African Journal of Livestock Extension. 2003;2:81-85.
21. Fasoro PM. Follicle stimulating hormone receptor expression in the reproductive system of *A. marginata*. B. Agric. Project, UNAAB. 2011;23.
22. Omran NS. Testosterone, gonadotropins and androgen receptor during spermatogenesis of *Biomphalaria alexandrina* snails (Pulmonata: Basommatophora). Reproductive Biology. 2012;12(3):301-308.
DOI: 10.1016/j.repbio.2012.10.002
23. Csaba G. Receptor ontogeny and hormonal imprinting. Experimentia. 1986; 42:750-759.
24. Csaba G, Bierbauer J. Effect of cortisol, deoxycorticosterone and 19-nortestosterone on the gametogenesis of the snail *Helix pomatia*. Acta Biol. Acad. Sci. Hung. 1981;32:15–18.
25. Dohler KD. The special case of hormonal imprinting, the neonatal influence of sex. Experimentia. 1986;42:759-769.
26. Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology and pathophysiology. Endocr. Rev. 1997;18:739–773.

© 2019 Osunkeye et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/52853>