Original

Highly Active Antiretroviral Therapy (HAART) disrupts estrous cycling pattern following dysregulations of reproductive hormones in female Sprague-Dawley Rat

[1]Bakare AA, [1]Lawal TN, [2]Ohihoin AG

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, PMB 12003 Nigeria.
[2]Clinical Sciences Department, Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria

Corresponding author: Airat Adeola Bakare, *Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos; aabakare@unilag.edu.ng; +2348033691277*

Article history: Received 8 June 2024, Reviewed 3 September 2024, Accepted for publication 10 September 2024

Abstract

Background: The transmission of HIV is a global crisis with females being more vulnerable to the infection. Currently, there is no known cure for HIV but fortunately, highly active antiretroviral (HAART) drugs have enhanced the well-being of infected patients and also reduced mother to child transmission. Hence, high desire of infected couples to procreate. However, there is an emerging trend of infertility indices in HIV infected women on HAART with pronounced miscarriage, menstrual irregularities and reduced pregnancy rate. This study investigated the effects of HAART on some reproductive hormones and the corresponding effects on estrous cycle and fertility.

Method: Twenty female rats were randomly divided into treatment group A and control group B of ten rats each. Group A animals received 1.88mg of HAART dissolved in 0.1mls of normal saline water while group B received 0.1mls of normal saline (placebo) to ensure uniformity. The first five sub-sets were used for experimentation while the second sub-set of five were used to assess the eventual impact on pregnancy outcome.

Result: The findings showed the alterations in estrogen, FSH and LH levels with disruption of estrous cycling and reduction in pregnancy rate in the HAART treated group when compared with the control.

Conclusion: The current study demonstrated dysregulation of reproductive hormones and its consequential disruption on estrous cycle and pregnancy outcome. Further investigation is needed on hormone activation at their receptor sites for comprehensive understanding of the mode of actions of HAART for modification of treatment regimen.

Keywords: HAART, infertility, estrous cycle, estrogen, follicle stimulating hormone, luteinizing hormone.

How to cite this article:

Bakare AA, Lawal TN, Ohihoin AG. Highly Active Antiretroviral Therapy (HAART) disrupts estrous cycling pattern following dysregulations of reproductive hormones in female Sprague-Dawley Rat. The Nigerian Health Journal 2024; 24(3):1446-1452.
https://doi.org/10.60787/tnhj.v24i3.843

Introduction

Acquired immunodeficiency syndrome (AIDS) is a worldwide public health concern caused by human immunodeficiency virus (HIV) that fights the immune system. The transmission of AIDS is ongoing globally even though there is a global strategy of sustainable development goal that targets the ending of HIV pandemic by 2030.[1] Recent reports have shown that two thirds of HIV cases are in Sub-Saharan Africa where Nigeria is ranked third. More redefined reports have shown that the largest group of people affected are females of reproductive age bracket between 15 and 44 years.[2,3]

Women are more vulnerable to HIV infection where a biological factor with a more complex and dynamic reproductive system that exposes the mucosa lining of the female tract poses easier transmission from men to women than vice versa. More so, women are more exposed to the spread of HIV especially in Africa where socio-cultural practices (polygamy, forced marriages, wife inheritance and rape) and economic deprivation (transactional engagements, age-disparate relationship, multiple and concurrent sexual affairs for survival) are disproportionally putting them at the more receiving end than their male counterpart.[4,5]

Currently, there is no known cure for HIV but fortunately, antiretroviral drugs which basically is highly active antiretroviral therapy (HAART) usually a combination of tenofovir, emtricitabine and efavirenz have enhanced the well-being of infected patients and also reduced the transmission from mother to child during pregnancy.[6] Unexpectedly, there is an emerging trend of infertility indices in HIV infected women on HAART.[7] Clinical cross-sectional studies have documented infertility challenges in HIV-positive patients on HAART. The modalities of menstrual cycle were investigated in HIV-positive women on HAART as a practical reproductive tool that is central to ovulation, fertilization and pregnancy. High incidence of menstrual irregularities was discovered where the most common menstrual abnormality observed was oligomenorrhea.[8,9] It is important to know that generally, viral infections can induce several physiological effects on the endocrine system particularly the HPG-axis by direct viral invasion into the endocrine organs or by systemic disturbances resulting into over-excited and under-excited functions that alter system physiological events in the body.[10] Additionally, several reports have shown endocrine alterations of HAART where the mode of actions leading to infertility indices are lacking[9]. Hence, investigating the adverse impact that accompany the usage of the HAART drugs in the management of HIV infection especially on female fertility, has become important for more improved management and future treatment discovery. The major fundamental aspect of reproductive capacity in female mammals is cyclical activity which includes the menstrual cycle in women that is synonymous to the estrous cycle in experimental mammals. Estrous cycle comprises recurring physiological reproductive cycle that pronounces changes that are coordinated by reproductive hormones in most female mammals, each phase of estrous cycle is tightly regulated by hormone.[11] Since more HIV-positive patients now live longer with the advent of HAART and reproductive challenges are becoming increasingly prominent in their healthcare, It is important to carry out investigations that will unravel the basis behind the cascade of events leading to infertility which is seen to be unclear. Hence the calls for urgent attention as reproduction is one of the fundamental motives of existence. Therefore, this study investigated the effects of HAART on reproductive hormones which include follicle stimulating hormone, luteinizing hormone and estrogen. This study will provide the understanding of the mode of action of HAART on endocrine regulations and consequently its effects on estrous cycle that is synonymous with menstrual cycle in women and the overall impact on pregnancy sustenance.

Method

Source of HAART and dose estimation

Combined HAART was purchased from Lagos University Teaching Hospital AIDS clinic, Idi-Araba Lagos State, Nigeria. The human dosage was extrapolated to the weight of the experimental animals and administration was carried out for 28 days through forceful feeding with the use of an oropharyngeal cannula.

Procurement of Animals

Twenty adult female Sprague-Dawley rats obtained from Ilogbo Eremi Oko-Afo Farm in Badagry. The weight of the animals ranges from 122-135 grams. The animals were housed in well-ventilated cages in the experimental house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Lagos with 12:12 light and dark periods. The animals were fed on standard rat diet, allowed free water access and allowed to acclimatize

Print ISSN: 0189-9287 Online ISSN: 2992-345X

to experimental conditions by housing them for 14 days prior to the commencement of the experiment.

Experimental protocol

Ethical approval for this study was obtained from the College of Medicine Ethical Committee of the University of Lagos with approval number CMUL/ACUREC/5/24/1468 and the experiment complied with the international standard of Health Guideline Principles of Laboratory Animal in Biomedical Research.

Twenty female rats were randomly divided into treatment groups A and control group B of ten rats each. Group A animals received 1.88mg of HAART dissolved in 0.1mls of normal saline water while group B received 0.1mls of normal saline (placebo) to ensure uniformity. The administration was done by the use of an oropharyngeal cannula daily for 28 days. The first five sub-sets were used for experimentation while the second sub-set of five were used to assess the eventual impact of HAART on pregnancy sustenance

Staging of estrous cycle

The phases of the estrous cycle were established by daily microscopic investigation of fresh vaginal smear between 8:00 to 10:00 am. Approximately 0.2 ml of normal saline was drawn into the suction pipette. The tip of the pipette was pushed gently into the vagina canal to a depth of 2 mm, the normal saline was flushed into the vagina and back up into the pipette. The collected smear was dropped onto a clean glass slide and viewed under the light microscope with 40X objective lens.[12,13] The microscopic presentation of the collected smear was used to establish the phases of the estrous cycle. The first day was designated as metestrus, the vagina smear histology on this stage presented leukocytes amidst few nucleated and squamous cells. The second day was tagged the diestrus phase and this showed predominantly leukocytes. The third day was seen with large nucleated cells and this was designated as the proestrus phase. The fourth day was designated as the estrus phase and the smear microscopic view showed large flakes of squamous cells.[14]

Serum level of Estrogen

The procedure for estradiol serum profiling was done in accordance with guidelines from Monobind Inc. (2012) using AccuBind ELISA microwells. Before the commencement of the assay, serum for treatment of the first five animals of sub-set of group A and B were brought to room temperature (20-27◦C). The microplates' wells were formatted for each serum treatment and control specimen to be assayed in duplicate. Afterwards, 0.025ml of the serum was pipetted into the labeled wells. 0.050ml of Estradiol Biotin reagent was added to all wells, the microwells were swirled gently for 20-30 seconds to mix. It was covered and incubated for another 30 minutes at room temperature. Further 0.050ml of estradiol enzyme reagent was added to all wells, the microplate was swirled gently for 20-30 seconds to mix, and thereafter covered and incubated for 90 minutes at room temperature. The contents of the microplate were discarded by decantation. Next, 350µl of was buffer was added and decanted. The process was repeated two more times to make a total of three washes. Thereafter, 0.100ml of substrate solution was added to all wells and were incubated at room temperature for 20 minutes. This is followed by adding 0.050ml of stop solution to each well and gently mixing for 15-20 seconds. The absorbance in each well at 450 nm (using a reference wavelength of 620-630nm) was read within 30 minutes of adding the stop solution. Afterwards, the results of the analysis were calculated.

Serum level of gonadotropins (FSH and LH)

This procedure was carried out using the method described by ElabScience Biotechnology (2017). The standard working solution was added to the first two columns, each concentration of the solution was added in duplicate to one well each, side by side (100 uL for each well). Afterwards, the samples were added to the other wells (100 uL for each well). The plate was covered with the sealer provided in the kit and incubated for 90 min at 37℃. Next, the liquid was removed out of each well, but not washed. 100 µL of biotinylated detection Ab working solution was immediately added to each well, covered with the plate sealer, gently mixed, and incubated for 1 hour at 37°C.

Furthermore, the solution was decanted from each well, and 350 uL of wash buffer was added to each well. It was then soaked for 1~2 min, solution was decanted from each well and pat dry against clean absorbent paper. This wash was repeated 3 times. 100 µL of HRP conjugate working solution was added to each well, covered with plate sealer and incubated for 30 min at 37°C. Next, it was decanted from each well, and the wash process was repeated for 5 times. 90 µL of substrate reagent was added to each well, covered with a new plate sealer and incubated for about 15 min at 37°C. It was ensured to protect the plate from light. Lastly, 50

Print ISSN: 0189-9287 Online ISSN: 2992-345X

µL of stop solution was added to each well in the same order as the substrate solution and the optical density (OD value) of each well was determined at once with a microplate reader set to 450 nm.

Statistical Analysis

The data obtained was analyzed using GraphPad Prism Statistics version 8.0.1. Collected values were presented as mean ± SD. Statistical comparisons between groups were made by One-way analysis of variance (ANOVA). The differences between the treated and the control groups was analyzed statistically by Bonferroni's post-hoc test to evaluate the level of statistical significance.

Results

Effect on the number of completed estrous cycles

The group treated with HAART showed a lower number of completed estrous cycles that is statistically significant when compared with the control group as shown in figure 1. A total of approximately seven cycles were completed within the 28 days duration in the control group. However, a lower number of completed estrous cycles was shown in the treated group that is statistically significant.

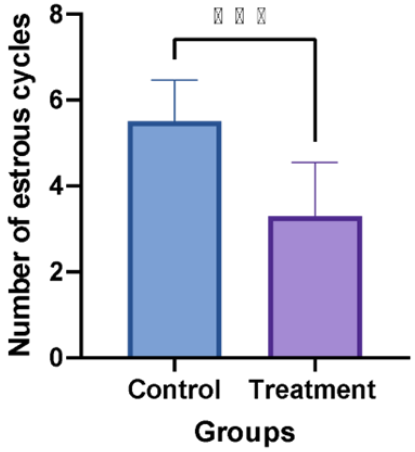

Figure 1: Showing the number of completed estrous cycle in HAART treated and control groups
Values are represented as mean ± SD. significant level of difference at *p < 0.05 when compared to the control

Effect on the number of days spent on each phase of estrous cycles

The number of days spent on the estrus and metestrus phases of the estrous cycle were significantly higher in the treatment group A when compared with the control group B while the number of days spent on the diestrus phase of the estrous cycle was significantly lower in the treated group when compared with the control as shown in figure 2.

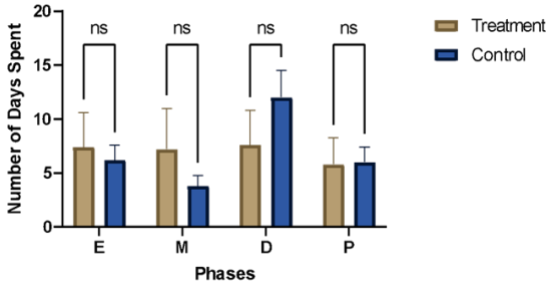

Figure 2: Showing the number of days spent on each phase of estrous cycle in HAART treated and control groups
Values are represented as mean ± SD. significant level of difference at *p < 0.05 when compared to the control.

Effects on serum estrogen level

The serum level of estradiol in the treated group was lower when compared to the Control group but not statically significant as shown in figure 3

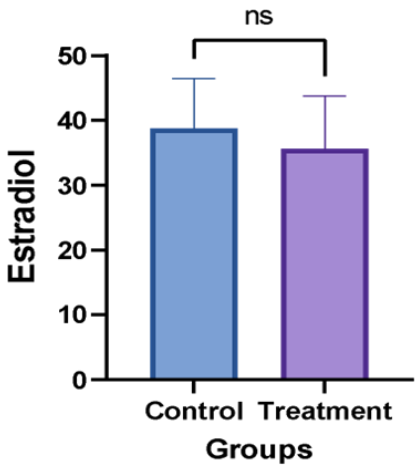

Figure 3: Showing serum level of estrogen in HAART treated and control groups
Values are represented as mean ± SD.

Effects on serum FSH level

The serum FSH level in the HAART-treated group A showed a lower level of FSH when compared with the

Print ISSN: 0189-9287 Online ISSN: 2992-345X

control group B1 but was not statistically significant as shown in figure 4.

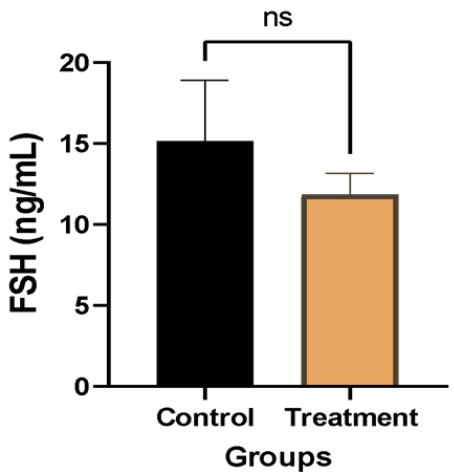

Figure 4: Showing serum level of FSH in HAART treated and control groups
Values are represented as mean ± SD.

Effects on serum LH level

The serum LH level in the HAART-treated group A showed a lower level of FSH when compared with the control group B1 but was not statistically significant as shown in figure 5.

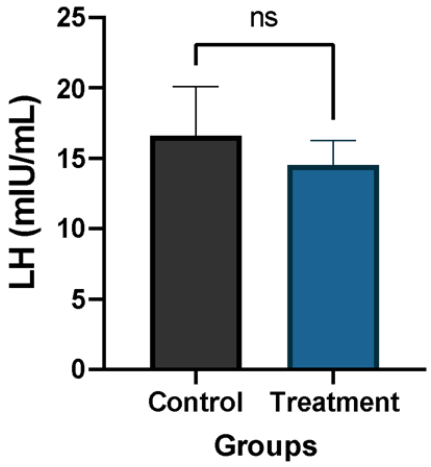

Figure 5: Showing serum level of LH in HAART treated and control groups
Values are represented as mean ± SD.

Effects on the number of litters

There was a statistically significant difference in the number of litters in the HAART-treated group A when compared to the control group B2. The number of litters in the treated group was significantly lower when compared to the control group as shown in figure 6 below.

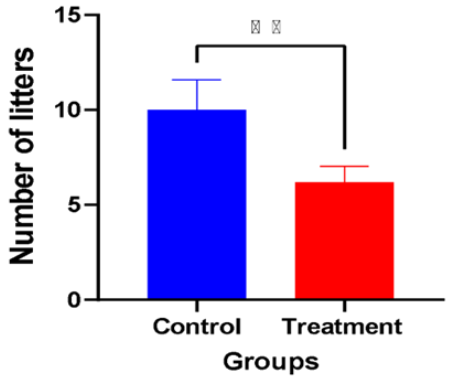

Figure 6: Showing the number of litters in HAART treated and control groups
Values are represented as mean ± SD. significant level of difference at *p < 0.05 when compared to the control

Discussion

This study examined the effects of HAART on reproductive hormones and its consequential impact on estrous cycling pattern and pregnancy outcome. The results showed alterations in estrogen, FSH and LH levels that exert disruptive effects on estrous cycling pattern. This study's observation on the alterations of estrogen, FSH and LH levels were in close conformity with the study of Ohihoin et al,[15] who found that antiretroviral drugs altered serum levels of anti-Mullerian hormone, estrogen and FSH in female Wistar rats. Another study on hormonal analysis in both male and female rodents revealed significant reductions in LH, FSH, testosterone, estrogen and progesterone levels. These confirm that the administration of combined antiretroviral drugs have potential toxic effects on reproductive functions.[16] The disruptions of reproductive hormones in this present study and also confirmed in other studies were believed to be responsible for the acycling pattern exhibited by the HAART treated animals as transition from one phase of estrous cycle to the other are tightly regulated by hormones.

Estrous cycle comprises sequential phases characterized by different cell types in vaginal smears; diestrus,

Print ISSN: 0189-9287 Online ISSN: 2992-345X

metestrus, proestrus and estrus controlled by hypothalamic GnRH, pituitary gonadotropins (FSH and LH) and ovarian estradiol.[17] The diestrus and metestrus phases are regulated by increase in the release of LH and FSH by the secretory cells of the pars distalis of the anterior pituitary gland. These hormones have an impact on other reproductive organs while regulating the estrous cycle for complementary reproductive effects. The increase in FSH stimulate follicular development in the ovary and LH initiates the synthesis of androstenedione by the ovarian theca cells that is utilized by the granulosa cells as raw substrate for estrogen production.[18] In the proestrus phase, high levels of LH and FSH in sustaining further transition from metestrus occur to also initiate characteristic structural changes in the wall of the vagina that will facilitate fertilization and endometrial wall of the uterus for implantation. There is a suppression of the rise in estrogen during late proestrus with inhibition of FSH production to subdue folliculogenesis and surge in LH to initiate ovulation. This drives the cycle into the estrus phase that is the heat phase when the animals are highly receptive to male. The estrus phase is characterized by the formation of corpus luteum secreting progesterone in preparing the uterine wall for implantation. It is evident that estrous are exclusively controlled by the highlighted hormones.[19,20] The overall impact on pregnancy achievement and sustenance were established in this study with decrease in the number of litters at the end of projected pregnancy in the experimental animals.

The findings from this study suggest systematic evidence of potential interference of hormonal regulation on estrous cycling pattern by HAART, possibly affecting the hypothalamic release of GnRH, pituitary secretion of FSH and LH and the secretion of estrogen and progesterone by the ovaries compromising the HPG axis and subsequently inducing infertility by reducing pregnancy rate as shown in this study. These outcomes have provided the understanding of reproductive events from the brain axis dysregulating functional events at the gonadal level.

However, the expressions of these hormones at the cellular levels are crucial as hormone activation at receptor sites imply effective synchronized functional action, hence should be the next point of target in the comprehensive understanding of the mode of action of HAART.

Implications of the findings of this study

The study has demonstrated compromised fertility potential in female Sprague-Dawley rats fed with anti-retroviral medications (HAART). The implication is far reaching for HIV positive women within the reproductive age range who are desirous of conception and receiving HAART. There is the need to further interrogate these findings in human population and possibly review the current therapeutic protocol for HAART in HIV positive women within the reproductive age range.

Strength and limitations of the Study

The finding from this study is consistent with earlier work that demonstrated menstrual irregularities in HIV positive pregnant women receiving HAART. This study however has certain limitations since antral follicular count and Anti-Mullerian Hormone (AMH) which are strong predictors of fertility were not evaluated in this rodent model. Furthermore, findings from animal models should be interpreted with caution in humans.

Conclusion

The current study demonstrated the negative effects of HAART on reproductive hormones and its consequential disruption on estrous cycle and reduction of pregnancy rate. Further investigation is needed on hormone actions at their receptor sites for detailed understanding of the mode of actions of these hormones needed for the modification of treatment regimen and/or discovering of treatment agent for the infertility issue in HIV positive women.

Declarations

Ethical Consideration: Ethical approval for the study was obtained from College of Medicine Ethical Committee of the University of Lagos with ethical approval number CMUL/ACUREC/5/24/1468 and the experiment complied with the international standard of Health Guideline Principles of Laboratory Animal in Biomedical Research.

Authors' Contribution: The conception and design of the research: AAB, TNL and AGO, The conduct of the research: TNL, Analysis and interpretation of data: AAB and TNL. All authors were involved at different stages of manuscript writing.

Conflict of interest: The authors declare no competing interest.

Funding: No funding was received for this study.

Print ISSN: 0189-9287 Online ISSN: 2992-345X

Acknowledgment: Department of Anatomy, College of Medicine, University of Lagos for providing the facilities for this project

References

1. Nigeria HIV & AIDS statistics 2019 https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2019/march/20190314_nigeria.
2. Global HIV & AIDS statistics 2022; fact sheet https://www.unaids.org/en/resources/fact-sheet
3. Kharsany AB and Karim QA. HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. Open AIDS J. 2016; 10:34-48.
4. Jan L and Marcus A. Sex Differences in Infectious Diseases–Common but Neglected. The Journal of Infectious Diseases. 2014; 209:(3) S79–S80.
5. Murewanhema G, Musuka G, Denhere K, Mulqueeny D, Dzinamarira T. The HIV epidemic and the COVID-19 pandemic: A double tragedy for sub-Saharan African women. Afr J Prim Health Care Fam Med. 2022;14(1):e1-e3.
6. Miriam A. Fertility Desires of People Living With HIV in Enugu State Nigeria. International Journal of Science and Research (IJSR) ISSN (Online): Index Copernicus Value. 2013; 6.14: 4.438.
7. Mkwashapi D, Renju J, Mahande M, Changalucha J, Urassa M, Todd J. Fertility trends by HIV status in a health and demographic surveillance study in Magu District, Tanzania 1994-2018. PLoS One. 2023;18(2):e0281914.
8. Adebimpe WO, Adebimpe MA, Adeoye OA, Omisore A. Menstrual changes among HIV positive women on antiretroviral treatment in southwestern Nigeria. South American Journal of Medicine. 2014; 2(2): 198 – 211.
9. Ohihoin AG1, Ohihoin EN, Bakare A, Olarenwaju O, Okafor A, Ojetunde MM, Oji K, Kalajaiye O, Ujomu I, Aina O, Ajibaye S, Okechukwu A, Kiladejo A, Medobi F, Oladele D, Ezechi O, Iranloye B, Taylor-Robinson S. The Effect of First Line Antiretroviral Drugs on the Menstrual Cycle of HIV Positive Patients. Annals of Medical and Health Sciences Research 2021; 11:1328-1331.
10. Liu LB, Yang W, Chang JT, Fan DY, Wu YH, Wang PG, An J. Z. Virus infection leads to hormone deficiencies of the hypothalamic-pituitary-gonadal axis and diminished fertility in mice. J Virol. 2023;97(10):e0100623.
11. Xianzhi HE, LI Y, LI J, Sun B, LI Y, Zhai Q. Secretion of reproductive hormones and estrous cycle in rats interfered by combined exposure to beta-cypermethrin and emamectin benzoate. Journal of Environmental and Occupational Medicine. 2023;40(1): 83-88.
12. Marcondes FK, Bianchi FJ and Tanno AP. Determination of the estrous cycle phases of rats: some helpful considerations. Brazilian Journal of Biology. 2002; 62: 609-614.
13. Bakare AA, Idaguko CA, Sogbesan ZA, Adeoye F. The effects of variations in length of suction pipette inserted during vaginal smear cytology procedure on estrous cycle in Sprague–Dawley rats. Journal of Biological research and biotechnology 2022; 20(2):1600-1605.
14. Ajayi, AF and Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res Pract. 2020; 6:5.
15. Ohihoin AG, Ohihoin EN, Ujomu I, Bakare A, Olanrewaju O, Okafor A, Ojetunde M, Ayoola JB, Aina O, Ajibaye O, Taylor-Robinson SD. Contemporary Antiretroviral Drugs (ARVDs) Disrupt Follicular Development in Female Wistar Rats. J Exp Pharmacol. 2023; 15:267-278.
16. Awodele O, Popoola TD, Idowu O, Bashua BM, Awolola NA, Okunowo WO. Investigations into the Risk of Reproductive Toxicity Following Exposure to Highly Active Antiretroviral Drugs in Rodents. Tokai J Exp Clin Med. 2018;43(2):54-63.
17. Smith MJ and Jennes L. Neural signals that regulate GnRH neurones directly during the oestrous cycle. Reproduction. (2001); 122:1–10
18. Danuzia AM, Débora C, Glauber SF, Raphael ES, Janete AA, Kênia CB, Luciane HG. Influence of estrous cycle hormonal fluctuations and gonadal hormones on the ventilatory response to hypoxia in female rats. Eur J Physiol. 2017; 469:1277–1286
19. Cristóbal AD, Enrique H, Abril ZG, Miguel AG, Marisela H. Rats in proestrus-estrus present more attention behaviors toward males and exhibit higher prefrontal-parietal EEG synchronization, Physiology & Behavior. 2023; 263:114136
20. Wood GA, Fata JE, Watson KL, Khokha R. Circulating hormones and estrous stage predict cellular and stromal remodeling in murine uterus. Reproduction. 2007; 133:1035–1044.

Print ISSN: 0189-9287 Online ISSN: 2992-345X