Int. J. Adv. Res. Biol. Sci. (2024). 11(6): 83-93

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 6-2024

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.06.009

Effects of Clomiphene Citrate on Progesterone, Estrogen and Testosterone of Female Albino Wistar Rats Administered at Various Concentrations

Airhomwanbor, K.O.[1], Ugege, C.[2], Omolumen, L.E.[1], Animasaun, O.P.[3,4], Usiobeigbe, O.S[1]., Obohwemu, K.O.[5], Oikerhe, E.G.[6], Ibanga, I.E.[7], Oboh, M.M.[6], Asibor, E.[8], Ogobuegwu, I.O.[1]

[1]Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[2]Department of Medical Laboratory Science, Edo University, Uzairue, Edo State, Nigeria.
[3]Biochemistry Department, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[4]Biochemistry Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[5]Department of Health, Wellbeing & Social Care, Global Banking School, London, United Kingdom.
[6]Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[7]Chemical Pathology Department, Federal College of Medical Laboratory Science and Technology, Jos, Plateau State, Nigeria.
[8]Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Abstract

Clomiphene citrate is the treatment of first choice in the management of infertility in normally oestrogenized, anovulatory women. The aim of this study is to determine the effect of clomiphene citrate on Estrogen, Progesterone and Testosterone levels of female albino wistar rats administered at various concentrations. A total of fifty (50) female albino Wistar rats of comparable sizes (150-200 gram) were used for this study. They were divided into five equal groups (A–E) with ten (10) rats each. Group A served as the control and the rats were given distilled water and feed only. In addition to feed and water, Wister rats in group B received 1ml of 50mg/100ml, C received 2ml of 50mg/100ml, D received 3ml of 50mg/100ml and E received 4ml of 50mg/100ml oral administration of clomiphene citrate preparations. The drug administration was given daily for 14 days (2 weeks). After the administration, the rats were put under light chloroform anaesthesia and the blood samples were collected. Estrogen, Progesterone and Testosterone were measured by enzyme-linked immunoassay test (ELISA). The results obtained in mean ± standard deviation showed that the Testosterone (ng/ml) in group A (control), group B, C, D and E was 0.72±0.18, 0.58±0.20,

0.53±0.12, 0.36±0.11 and 0.34±0.10, the Progesterone (ng/ml) was 3.68±2.12, 3.12±1.53, 2.58±1.22, 2.10±1.27 and 1.67±0.64, while the Estrogen (pg/ml) was 239.41±38.74, 319.43±45.77, 372.71±85.09, 427.00±95.22 and 432.00±69.63 respectively. Testosterone was significantly higher ($p<0.05$) in control group compared with test groups, Progesterone was higher in control group compared with test groups but not significant ($p>0.05$), while Estrogen was significantly ($p<0.05$) lower in control group compared with test groups respectively. The study concludes that oral administration of clomiphene citrate have significant effect on estrogen, progesterone and testosterone levels in Wistar rats and the effect is dosage dependent.

Keywords: Clomiphene, Citrate, Progesterone, Estrogen, Testosterone

Introduction

Infertility is observed in approximately 10 to 15% of couples of reproductive age (Speroff *et al.,* 1999). Ovulation disorders account for about 30 to 40% of female infertility and about 20% of infertility in couples. Several treatment modalities have been suggested for women with ovulation disorders, but the first-line regimen for medical induction of ovulation is Clomiphene Citrate for most patients (Horstein and Schust, 1996). Anti-estrogenic drugs, such as clomiphene citrate, are the first-line therapy for ovulation induction in these patients and are capable of producing ovulation in 70-75% of cases. Satisfactory response is seen in 70% of patients when clomiphene citrate is given at a dosage of 50-100 mg day^{-1} (Sohrabvand *et al.,* 2006).

Clomiphene, an established clinical agent for the induction of ovulation in sub-fertile women, is a substituted triphenylethylene that is considered to be an antiestrogen, based on ability to antagonize uterine growth and vaginal cornification induced by estrogen in immature rodents (Massai *et al.,* 1993; Borges *et al.,* 2000). Chemically, Clomiphene Citrate (like tamoxifen) is a nonsteroidal triphenylethylene derivative that exhibits both estrogen agonist and antagonist properties. In general, estrogen agonist properties are manifest only when endogenous estrogen levels are extremely low. Otherwise, clomiphene citrate acts solely as a competitive estrogen antagonist. Clomiphene citrate is cleared through the liver and excreted in stool. About 85% of an administered dose is eliminated after approximately 6 days, although traces may remain in the circulation for much longer (Clark and Markavench, 2012).

Hormones are chemical substances having a specific regulatory effect on the activity of a certain organ or organs. Hormones travel through the blood to distant tissues and organs, where they can bind to specific cell sites called receptors. By binding to receptors, hormones trigger various responses in the tissues/cells containing cognate receptors (Rebar *et al.,* 2012). On the basis of their chemical nature, hormones are classified as peptides, steroids, and amino acid derivatives. Mechanism of action of hormones comprises two components. Protein hormones interact with a receptor on the outer surface of cell membrane and they signal via second messengers generated by interacting with receptors at the cell surface (Kleerekoper, 2015). Steroid hormones pass through cell membrane and interact with intracellular receptors and the hormone receptor complex eventually binds to a segment of chromatin, which induces formation of messenger RNA that in turn enters the cytoplasm and initiates the synthesis of protein or peptides that carry out the action attributed to the hormone (Arneson *et al.*, 2007). Their functions can be broadly grouped into several categories: reproduction and sexual differentiation; development and growth; maintenance of the internal environment; and regulation of metabolism and nutrient supply. A single hormone may affect more than one of these functions and each function may be controlled by several hormones. Progesterone is a female sex hormone which, in conjunction with estrogens, regulates the accessory organs during the menstrual cycle (Bozza *et al.,* 2017). Testosterone is the primary androgen responsible for the development and maintenance of male sexual characteristics. It also stimulates anabolic processes in non-sexual tissues (Azziz *et al.,* 2016). Estrogen is the primary female sex

hormone responsible for the development and regulation of the female reproductive system and secondary sex characteristics (Abraham, 2001).

Reproductive function is controlled by a very sophisticated system composed by the perfect synchronization of neural and endocrinological functions (Genazzani, 2005). Hormone replacement strategies have limited benefit because they do not promote recovery from these allostatic endocrine adjustments in the Hypothalamus-Pituitary-Ovarian (HPO) axis. Although the menstrual pattern can be restored with exogenous administration of estrogen/progesterons, the long-term deleterious consequences of stress-induced anovulation may lead to increased risk of cardiovascular disease, osteoporosis, depression and other psychiatric conditions (Massai *et al.*, 1993). Clomiphene citrate has been applied as the first-line treatment in anovulatory women since the 1960s, due to its low cost and minor side-effects or complications (Van-Santbrink *et al.,* 2005). It is recognized that Clomiphene citrate has partial antagonist/partial agonist properties and it dominant effect on the HPO axis is related to its antiestrogenic properties (Fiad *et al.,* 2008).

Dehbashi et al, (2006) found that treatment with Clomiphene citrate is associated with higher rates of pregnancy if started early (days 1-5) in the menstrual cycle. Although Clomiphene citrate induced ovulation rates are between 80 and 85% conception rates are only around 40% (Dehbashi *et al.,* 2006; Clark & Markaverich, 2012). This could be due to negative effects of Clomiphene citrate on ocyte or granulose cells, or because of prolonged antiestrogenic effects on the endometrium and cervical mucosa. These negative effects are augmented by the relatively long half-life of Clomiphene citrate, which is known to be 5 days. If treatment is started late in the cycle, those negative effects are more likely to extend into the sensitive peri-implantation period (March, 2012). However, the effect of various concentrations of clomiphene citrate on female hormones has not been fully elucidated. Hence, this study was carried out to determine the effect of clomiphene citrate on Estrogen, Progesterone and Testosterone levels of female albino wistar rats administered at various concentrations.

Materials and Methods

Experimental Animals/Housing Condition

Fifty (40) female albino Wistar rats weighing 150-200 grams were procured from the animal house of the college of Medicine, Ambrose Alli University, Ekpoma, Edo State and transferred to the experimental site where they were allowed two (2) weeks of acclimatization. They were housed in well ventilated labeled wooden cages at the site of the experiment. The cages was designed to secure the animals properly especially from wild animals/insects and cleaned daily. During this period of acclimatization, the rats were fed growers' mash and water provided *ad libitum*. Animals were maintained and experimental procedures complied with the guide for care and use of laboratory animals.

Experimental Design

A total of fifty (50) female albino Wistar rats of comparable sizes (150-200 gram) were used for this study. They were divided into five equal groups (A–E) with ten (10) rats each. Group A served as the control and the rats were given distilled water and feed only. In addition to feed and water, Wister rats in group B, C, D and E were orally administered with different concentrations of clomiphene citrate drug dilutions. The drug administration was given daily for 14 days (2 weeks) and the weights of both the test and control animals was monitored before and after administration of clomiphene citrate. After the administration, the rats were put under light chloroform anaesthesia and the blood samples were collected. All data was presented in table as mean ± standard deviation (SD).

Animal Grouping

The experimental animals were separated into five groups (A–E) with each group having ten rats (n = 10) each using 5 big cages to house them. Group A served as the control and received only

the normal feed (grower's mash) and water with no administration of clomiphene citrate, while Group B-E received different doses of clomiphene citrate and were equally fed with grower's mash and water.

Study Duration

The preliminary studies, animal acclimatization, drug procurement and preparation, actual animal experiment and evaluation of results, lasted for a period of three months. However, the experiment lasted for lasted for four (4) weeks: two weeks of acclimatization and two weeks administration of clomiphene citrate to the test animals.

Substance Preparation

The drug clomiphene citrate which is the brand name of CLOMID was procured from Ehi Care Pharmacy in Ekpoma, and stored in a cool dry place pending usage. One tablet of the drug contains 50 mg of clomiphene citrate. The Clomiphene citrate was prepared by grinding into powder using mortar and pestle. The mixture was filtered and stored in closed bottle until required for administration.

Substance Administration

The administration of the clomiphene citrate was given to the test animals orally as follows:

Group A (Control) received only normal feed (growers' mash) and distilled water daily for 28 days.
Group B received 1ml of 50mg/100ml of clomiphene citrate, feed and distilled water daily for 14 days.
Group C received 2ml of 50mg/100ml of clomiphene citrate, feed and distilled water daily for 14 days.
Group D received 3ml of 50mg/100ml of clomiphene citrate, feed and distilled water daily for 14 days.
Group E received 4ml of 50mg/100ml of clomiphene citrate, feed and distilled water daily for 14 days.

Sample Collection

Weight was measured before and after acclimatization. Similar weight measurements were done at the end of the treatment periods and the average weight recorded accordingly. Five (5.0) mls of blood sample was collected from each rat at the end of the experiment under chloroform anaesthesia. The blood was dispensed into a plain container without any additive for the determination of hormonal profile. It was allowed to stand for one hour to clot. It was then centrifuged at 3000g for 10 min in order to separate blood cells and suspended particles from serum. The serum was aliquoted and stored at 4^0c until required for analysis.

Sample Analysis

Estrogen, Progesterone and Testosterone were measured by enzyme-linked immunoassay test (ELISA) kits (Bio Check Inc., Vintage Park Dr, California, USA) using stat fax. All analysis was carried out in the Clinical Chemistry Laboratory at University College Hospital, Ibadan, Oyo State, Nigeria.

Estrogen (Tietz, 1986) - Principle: The principle of Estrogen ELISA is base on competitive immunoassay. Competition occurs between total estrogens (estrone, estradiol, and estriol) present in standards, controls and patient samples and an enzyme-labelled antigen (conjugate) for a limiting number of anti-estrogen antibody binding sites on the microplate wells. After a washing step that removes unbound materials the enzyme substrate is added and approximately 15–20 minutes later the enzymatic reaction is terminated by addition of stopping solution. The resulting optical density(OD), measured with a microplate reader, is inversely proportional to the concentration of total estrogens in the sample.Astandard curve is plotted with a provided set of standards to calculate directly the concentration of total estrogens in patient samples and controls.

Procedure

1. Prepare the working solution of wash buffer.
2. Remove the required number of microplate strips. Reseal the bag and return unused strips to the refrigerator.
3. Pipette 25µL of each calibrator, control and specimen sample into planned wells in duplicate.
4. Pipette 150 µL of the Estrogen-HRP conjugate into each well.
5. Gently shake the plate by hand for ten seconds (or tap it on the side with your hand) to mix the contents of the wells.
6. Incubate for 2hours at room temperature (no shaking). Cover the plate to avoid any contamination.
7. Wash the wells 3 times with 350 µL of diluted wash buffer per well. Tap the plate firmly against absorbent paper to ensure that no droplets remain in the wells.
8. Pipette 150 µL of TMB substrate into each well at timed intervals.
9. Incubate for 15 to 20 minutes at room temperature (or until calibrator A attains dark blue colour).
10. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 8. Gently tap the side of the microplate to mix the contents of the wells.
11. Read in a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

Testosterone (Tietz, 1986): **Principle:** The DIA source Testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a monoclonal [mouse] antibody directed towards an unique antigenic site on the Testosterone molecule. Endogenous Testosterone of a patient sample competes with a Testosterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of Testosterone in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Testosterone in the patient sample.

Procedure

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense 25 µL of each Calibrator, Control and samples with new disposable tips into appropriate wells.
3. Dispense 200 µL Enzyme Conjugate into each well. Thoroughly mix for 10 seconds.
4. Incubate for 60 minutes at room temperature (without covering the plate).
5. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
6. Add 200 µL of Substrate Solution to each well.
7. Incubate for 15 minutes at room temperature.
8. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.
9. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.

Progesterone (Engvall, 2000) – **Principle:** The Progesterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the Progesterone molecule. Endogenous Progesterone of a patient sample competes with a Progesterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of Progesterone in the sample. After addition of the substrate solution, the intensity of colour developed is reverse proportional to the concentration of Progesterone in the patient sample.

Procedure

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense 25 µL of each Standard, controls and samples with new disposable tips into appropriate wells.
3. Incubate for 5 minutes at room temperature.
4. Dispense 200 µL Enzyme Conjugate into each well. Thoroughly mix for 10 seconds.
5. Incubate for 60 minutes at room temperature.
6. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
7. Add 200 µL of Substrate Solution to each well.
8. Incubate for 15 minutes at room temperature.
9. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.
10. Determine the absorbance (OD) of each well at 450±10 nm with a microtiter plate reader.

Data Analysis: The results were presented using tables. Data was presented as mean ± S.D (standard deviation). Comparison was made between subjects and control groups using one-way analysis of variance (ANOVA) and the student's t-test. Significant difference was accepted at $p<0.05$.

Results

Weight of the test animals before and after acclimatization

The Mean ± S.D of Group A for Day 1 (weight before acclimatization) was 108.8 ± 27.6^{ab}, Day 14 (before administration) was 122.3 ± 29.8, Day 21 (4th week) was 148.9 ± 36.5^{a}, while Day 28 (before sacrifice) was 130.3 ± 34.0^{b}. The Mean ± S.D of Group B for Day 1 (weight before acclimatization) was 114.3 ± 28.8, Day 14 (before administration) was 130.6 ± 16.1, Day 21 (4th week) was 132.8 ± 16.7, while Day 28 (before sacrifice) was 134.3 ± 20.9. The Mean ± S.D of Group C for Day 1 (weight before acclimatization) was 107.1 ± 12.2^{ab}, Day 14 (before administration) was 126.8 ± 31.7, Day 21 (4th week) was 135.9 ± 20.1^{a}, while Day 28 (before sacrifice) was 138.4 ± 25.2^{b}. The Mean ± S.D of Group D for Day 1 (weight before acclimatization) was 112.1 ± 14.4^{ab}, Day 14 (before administration) was 132.7 ± 23.5, Day 21 (4th week) was 140.3 ± 28.2^{a}, while Day 28 (before sacrifice) was 154.6 ± 17.0^{b}. The Mean ± S.D of Group E for Day 1 (weight before acclimatization) was 103.9 ± 12.8^{abc}, Day 14 (before administration) was 132.0 ± 30.4^{a}, Day 21 (4th week) was 135.4 ± 26.2^{b}, while Day 28 (before sacrifice) was 143.0 ± 24.7^{c}. However, there is a statistical significance (p 0.05) across the Days for Group A, Group C, Group D and Group E respectively while there is no statistical significance (p 0.05) across the Days in Group B (Table 1).

Testosterone, Progesterone and Estrogen in test animals (B, C, D and E) compared with control (A)

Table 2 showed the Testosterone, Progesterone and Estrogen of test animals (A, B, C, D and E) compared with control (A). The results obtained in mean ± standard deviation showed that the Testosterone (ng/ml) in group A (control), group B, C, D and E was 0.72±0.18, 0.58±0.20, 0.53±0.12, 0.36±0.11 and 0.34±0.10, the Progesterone (ng/ml) was 3.68±2.12, 3.12±1.53, 2.58±1.22, 2.10±1.27 and 1.67±0.64, while the Estrogen (pg/ml) was 239.41±38.74, 319.43±45.77, 372.71±85.09, 427.00±95.22 and 432.00±69.63 respectively. Testosterone and Progesterone was significantly higher ($p<0.05$) in control group compared with test groups, while Estrogen was significantly lower in control group compared with test groups.

Post-Hoc test for Table 2 showing ANOVA statistics for various levels of Testosterone, Progesterone an Estrogen of Wistar rats administered with various concentration of Clomiphene and control group

Table 3 showed the post-Hoc test for Table 2 showing ANOVA statistics for various levels of Testosterone, Progesterone an Estrogen of Wistar

rats administered with various concentration of Clomiphene and control group. The results obtained showed that there was significant difference (p<0.05) in Testosterone in group A compared with D, A compared with E, B compared with D, E compared with E, C compared with D and C compared with E respectively. On the other hand, Progesterone was only show significant difference in A compared with C and E compared with D. Others were not significant (p>0.05). There was significant difference (p<0.05) in Estrogen in A compared with B, A compared with C, A compared with D, A compared with E, B compared D, and B compared with E respectively.

Table 1: Weight of the test animals before and after acclimatization

GROUPS	Day 1 (weight before acclimatization) (Mean ± S.D)	Day 14 (before administration) (Mean ± S.D)	Day 21 (4th week) (Mean ± S.D)	Day 28 (before sacrifice) (Mean ± S.D)	F-value	P-value
Group A	108.8 ± 27.6^{ab}	122.3 ± 29.8	148.9 ± 36.5^{a}	130.3 ± 34.0^{b}	2.680	0.069*
Group B	114.3 ± 28.8	130.6 ± 16.1	132.8 ± 16.7	134.3 ± 20.9	1.339	0.289
Group C	107.1 ± 12.2^{ab}	126.8 ± 31.7	135.9 ± 20.1^{a}	138.4 ± 25.2^{b}	2.892	0.055*
Group D	112.1 ± 14.4^{ab}	132.7 ± 23.5	140.3 ± 28.2^{a}	154.6 ± 17.0^{b}	4.518	0.013*
Group E	103.9 ± 12.8^{abc}	132.0 ± 30.4^{a}	135.4 ± 26.2^{b}	143.0 ± 24.7^{c}	3.571	0.030*

***Values are significant at p<0.05.**
Keys:

Similar Superscript: A statistical significance p 0.05 occurred with each other
Group A (control) received only distilled water and feed only for 28 days.
Group B (test groups) received feed, water and 1mls of 50mg/100ml of Clomiphene citrate for 14 days.
Group C (test groups) received feed, water and 2mls of 50mg/100ml of Clomiphene citrate for 14 days.
Group D (test groups) received feed, water and 3mls of 50mg/100ml of Clomiphene citrate for 14 days.
Group E (test groups) received feed, water and 4mls of 50mg/100ml of Clomiphene citrate for 14 days.

Table 2: Testosterone, Progesterone and Estrogen in test animals (B, C, D and E) compared with control (A)

Parameters	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	Group D Mean ± SD	Group E Mean ± SD
TESTO (ng/ml)	0.72±0.18^{a}	0.58±0.20^{a}	0.53±0.12^{a}	0.36±0.11^{b}	0.34±0.10^{b}
PROG (ng/ml)	3.68±2.12^{a}	3.12±1.53^{a}	2.58±1.22^{b}	2.10±1.27^{b}	1.67±0.64^{b}
ESTRO (pg/ml)	239.41±38.74^{a}	319.43±45.77^{b}	372.71±85.09^{c}	427.00±95.22^{d}	432.00±69.63^{e}

***Values in a row with different superscript are significant at p<0.05**
Keys: TESTO – Testosterone**; PROG –** Progesterone**; ESTRO –** Estrogen**; SD –** Standard deviation

Table 3: Post-Hoc test for Table 2 showing ANOVA statistics for various levels of Testosterone, Progesterone an Estrogen of Wistar rats administered with various concentration of Clomiphene and control group

Parameters	TESTO (ng/ml)	PROG (ng/ml)	ESTRO (pg/ml)
AvsB	0.255(NS)	0.589(NS)	0.015(S)
AvsC	0.061(NS)	0.041(S)	0.013(S)
AvsD	0.001(S)	0.095(NS)	0.001(S)
AvsE	0.000(S)	0.061(NS)	0.002(S)
BvsC	0.594(NS)	0.471(NS)	0.144(NS)
BvsD	0.045(S)	0.007(S)	0.028(S)
BvsE	0.035(S)	0.096(NS)	0.013(S)
CvsD	0.031(S)	0.235(NS)	0.131(NS)
CvsE	0.003(S)	0.124(NS)	0.090(NS)
DvsE	0.639(NS)	0.609(NS)	0.746(NS)

Keys: **TESTO –** Testosterone**; PROG –** Progesterone**; ESTRO –** Estrogen

Discussion

Clomiphene, an established clinical agent for the induction of ovulation in sub-fertile women, is a substituted triphenylethylene that is considered to be an antiestrogen, based on ability to antagonizeuterine growth and vaginal cornification induced by estrogen in immature rodents (Massai *et al.,* 1993). The study was carried out to determine the Estrogen, Progesterone and Testosterone levels of female albino Wistar rats administered with various concentrations of clomiphene citrate.

The results of this study showed that clomiphene citrate significantly decrease ($p<0.05$) estrogen level and remarkably increase progesterone and testosterone level in test animals compared to control group (with no treatment) which indicate the useful effect of clomiphene citrate in improving hormonal changes in rats. These results were supported by previous studies (Kerin *et al.*, 1985; Dickey *et al.,* 1993; Shekoufeh *et al.,* 2017). Estrogen and progesterone in females are produced mostly in the gonads (ovaries) under the influence of FSH and LH secreted by the anterior pituitary gland. The secretions of sex hormones are known to be regulated by hypothalamic GnRH which acts on the pituitary gland, and thus regulate the secretion of gonadotropins which in turn influence the secretions of gonadal hormones (Rhoades & Bells, 2013).

Clomiphene citrate has some structural similarity to estrogen compounds and by occupying estrogen receptors could decrease the function of estrogen and increase progesterone performance (Dickey *et al.,* 1993). Furthermore, clomiphene by having an anti-estrogenic effect could stimulate the pituitary Gonadotropin-releasing hormone secretion, regulate gonadotropin secretion and ultimately stimulate ovulation (Gerli *et al.,* 2000). Therefore, it is likely that clomiphene could improve the sex hormone changes in women due to having anti-estrogenic properties, which is consistent with our results. Other studies had suggested that clomiphene citrate will improve ovulation and fertility in women (Yarali *et al.,* 2002; Brown & Farquhar, 2016).

Also, previous studies suggested that clomiphene citrate is more effective than similar drugs in the treatment of polycystic ovarian syndrome (PCOS) (Misso *et al.,* 2012). In the current study, improvement in sex hormone serum level was higher in rats receiving clomiphene citrate than the control group, which indicates that clomiphene is more effective in improving sex hormone changes in patients with PCOS. In their

study, Shekoufeh *et al.* (2017) observed that ginger extract could have positive and dose-dependent effect in improving serum level changes of LH, FSH, estrogen and progesterone in PCOS. Although ginger extract is more effective in improving hormonal changes at higher doses, even at the highest dose, it has less improving effect compared to clomiphene. It is also indicated that in PCOS rats some changes in estrogen and progesterone level will occur as progesterone to estrogen ratio will reduce and balance of these hormones can be one way of treating this disorder (Jelodar & Karami, 2013).

Conclusion

The study concludes that oral administration of clomiphene citrate have significant effect on estrogen, progesterone and testosterone levels in Wistar rats and the effect is dosage dependent. Testosterone was significantly higher ($p<0.05$) in control group compared with test groups, Progesterone was higher in control group compared with test groups but not significant ($p>0.05$), while Estrogen was significantly ($p<0.05$) lower in control group compared with test groups respectively. There has been report that prolonged use of clomiphene citrate is associated with an increased risk of a borderline or invasive ovarian tumour. Taking into consideration these observations, we recommend that further research be carried out with special focus on the use of plants instead of chemical agents like clomiphene citrate.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

The entire study procedure was conducted with the involvement of all authors.

Acknowledgements

The authors would like to acknowledge the management of the Department of Chemical Pathology, Faculty of Medical Laboratory Science, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria for creating the enabling environment for this study. Thanks to all the technical staff of St Kenny Research Consult, Ekpoma, Edo State, Nigeria for their excellent assistance and for providing medical writing/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

References

Abraham, G.E. (2001). Radioassay Systems in Clinic. Endocrinol., Marcel Dekker, Inc., New York, Pp. 125-130.

Arneson, W., Chandler, K. & Brickell, J. (2007). Endocrine disorders and function. In: W. Arneson, J. Brickell, editors. Clinical Chemistry: A Laboratory Perspective 1st ed. Philadelphia: F.A Davis. Pp. 371-427.

Azziz, R., Nestler, J. & Dewailly D. (2016). Androgen excess disorders in women. Second ed. Totowa, NJ: Humana Press Inc, Pp. 206-220.

Borges, L.E., Morgante, M.C., Musacchio, F., Petraglia, G.a nd Leo, V.D. (2000). New protocol of clomiphene citrate treatment in women with hypothalamica menorrhea. *Gynecology & Endocrinology*,23(6),343-346.

Bozza, V., D'attilio, L., Mahuad, C., Giri, A., Del Rey, A. & Besedovsky, H. (2017). Altered Cortisol/DHEA Ratio in Tuberculosis Patients and its Relationship with Abnormalities in the Mycobacterial driven Cytokine Production by Peripheral Blood Mononuclear Cells. *Scandinavian Journal of Immunology,* 66 (1), 97-103.

Brown, J. & Farquhar, C. (2016). Clomiphene and other antioestrogens for ovulation induction in polycystic ovarian syndrome. *Cochrane Database Systemic Review,* 12 (2), 249-254.

Clark, J.H. &Markaverich, B.H. (2012). The agonistic-antagonistic properties of clomiphene: A review. *Pharmacology Therapy*,15 (2), 469-479.

Dehbashi, S., Vafaei, H. & Parsanezhad, S. (2006). Time of initiation of clomiphene citrate and pregnancy rate inpolycystic ovarian syndrome. *International Journal of Gynaecology & Obstetrics*,9 (3), 44-48.

Dickey, R.P., Olar, T.T., Taylor, S.N., Curole, D.N. & Rye, P.H. (1993). Sequential clomiphene citrate and human menopausal gonadotrophin for ovulation induction: comparison to clomiphene citrate alone and human menopausal gonadotrophin alone. *Human Reproduction*, 8 (1), 56-59.

Engvall, E. (2000). Methods in Endocrinology, Volume 70, Van Vunakis, H. and Langone, J.J. (eds.), Academic Press, New York, 419-492.

Fiad, T., Smith, T.P., Cunningham, M. and McKenna, S.K. (2008). Declinein insulin-likegrowth factor I levels after clomiphene citrate does not correct hyperandrogenemiain polycystic ovary syndrome. *Journal of Clinical Endocrinology &Metabolism*, 83(8), 2394-2398.

Genazzarri,A.D. (2005). Neuroendocrine aspects of amenorrhea related to stress. *Pediatric Endocrinology Review*, 2(18), 661-668.

Gerli, S., Gholami, H., Manna, C., Di-Frega, A.S., Vitiello, C. &Unfer, V. (2000). Use of ethinyl estradiol to reverse the antiestrogenic effects of clomiphene citrate in patients undergoing intrauterine insemination: a comparative, randomized study. *Fertility & Sterilization*, 7 (3), 85-89.

Horstein, M.D. & Schust, D. (1996.) Infertility Novaks Gynecology. 18th Edn. Philadelphia, PA: Lippin Cott Williamsa Wilkins CO.,Pp:9053-1066.

Jelodar, G. & Karami, E. (2013). Effect of Hydroalcoholic Extract of Vitex Agnus-Castus Fruit on Ovarian Histology in Rat with Induced Polycystic Ovary Syndrome (PCOS). *Journal of Babola University of Medical Science*, 15 (3), 96-102.

Kerin, J.F., Liu, J.H., Phillipou, G. & Yen, S.S. (2015). Evidence for a hypothalamic site of action of clomiphene citrate in women. *Journal of Clinical Endocrinology & Metabolism,* 6 (1), 265–268.

Kleerekoper, M. (2015). Hormones. In: Burtis CA, Bruns DE, editors. Tietz fundamentals of clinical chemistry and molecular diagnostics. 7th ed. ed. Philadelphia: Saunders; Pp. 430-442.

March, C.M. (2012). Induction of Ovulation. In: Infertility, Contraception and Reproductive Endocrinology, Lobo, R.A., D.R. Mishell and R.J. Paulson (Eds.).4thEdn. Oxford, UK: Blackwell Science Ltd.,pp:60.

Massai, M.R., DeZiegler, V.,Lesobre, C., Bergeron, R., Frydman, H. & Bouchard, P. (1993). Clomiphene citrate affects cervical mucus and endometrial morphology independently of the changes in plasma hormonal levels induced by multiple follicular recruitment. *Fertility & Sterility,*5 (9), 1179-1186.

Misso, M.L., Teede, H.J., Hart, R., Wong, J., Rombauts, L. & Melder, A.M. (2012). Status of clomiphene citrate and metformin for infertility in PCOS. *Trends in Endocrinoloy & Metabolism,* 23 (9), 533-543.

Rebar, R.W., Erickson, G.G. & Yen, S.S.C. (2012). Hormones: An overview. *Fertility &Sterility*, 37 (2), 35-40.

Rhoades, R.A. & Bells, D.R. (2013). Medical Physiology: Principles for Clinical, 4th edition. Lippincott Williams and Wilkins, a Wolters Kluwer business. 351 West Camden Street Two Commerce Square Baltimore, Philadelphia, PA, Pp. 523-49.

Shekoufeh, A., Hossein, K.J., Zahra, K.J. & Mozhgan, M.D. (2017). Comparison of the effects of Ginger extract with clomiphene citrate on sex hormones in rats with polycystic ovarian syndrome. *International Journal of Reproductive BioMedicine*, 15 (9), 561-568,

Sohrabvand, F., Ansari, S.H. & Bagheri, M. (2006). Efficacy of combined metformin-letrozolein comparison with metformin-clomiphene citrate in clomiphene-resistant infertile women with polycystic ovarian disease. *Human Reproduction*,21(6),1432-1435.

Speroff, L., Glass, R.N. & Kase, N.G. (1999). Female Infertility. Clinical Gynaecology, Endocrinology and Infertility, 6th Edn. Philadelphia, PA: Lippincott Williams and Wilkins Co., Pp:1041-1043.

Tietz, N.W. (1986). Textbook of Clinical Chemistry. Saunders, Pp. 456-470.

Van-Santbrink, E.J., Eijkemans, M.J., Laven, J.S. & Fauser, B.C. (2005).Patient-tailored conventional ovulation induction algorithms in an ovulatory infertility. *Trends in Endocrinology & Metabolism*, 16 (8),381 -389.

Yarali, H., Yildiz, B.O., Demirol, A., Zeyneloglu, H.B., Yigit, N. & Bukulmez, O. (2002). Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial. *Human Reproduction*, 17 (2), 289-294.

Access this Article in Online

Quick Response Code

Website: www.ijarbs.com

Subject: Endocrinology

DOI:10.22192/ijarbs.2024.11.06.009

How to cite this article:
Airhomwanbor, K.O., Ugege, C., Omolumen, L.E., Animasaun, O.P., Usiobeigbe, O.S., Obohwemu, K.O., Oikerhe, E.G., Ibanga, I.E., Oboh, M.M., Asibor, E., Ogobuegwu, I.O. (2024). Effects of Clomiphene Citrate on Progesterone, Estrogen and Testosterone of Female Albino Wistar Rats Administered at Various Concentrations. Int. J. Adv. Res. Biol. Sci. 11(6): 83-93.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.06.009