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Effect of Clomiphene Citrate on the Hormonal Profile of Adult Female Wistar Rats with Regular Estrous Cycle

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigates the effect of Clomiphene citrate (CC) on the Hormonal profile of female Adult Wistar rats with regular estrous cycle. A total of thirty six (36) healthy female Wistar rats were reared and screened for the purpose of this study. The animals were divided into 4 groups

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consisting of 4 rats each with regular estrous cycle and were treated as follows; Group 1 served as control while Groups 2, 3 and 4 were treated with 1 mg/kg, 2mg/kg and 5 mg/kg body weight of CC respectively, for 11 consecutive days. Body weight was obtained at commencement and every other day of the experiment using digital weighing scale. There was no statistically significant ($p>0.05$) difference in body weight of rats with regular estrous cycle at day 1, 3, 5 and 7 compared to control. However, there was a statistically significant ($p<0.05$) difference in body weight across the group on day 9 and 11. Progesterone was significantly ($p<0.05$) higher in the 1mg as well as 5mg CC treated groups with a significant decrease in follicle stimulating hormone (FSH) value across treatments. CC could improve the sex hormone changes in women due to its anti-estrogenic properties, which is consistent with our results hence, the significant changes observed in the reproductive hormone levels.

Keywords: Clomiphene citrate (CC); progesterone; follicle stimulating hormone (FSH).

1. INTRODUCTION

Clomiphene citrate (CC) is a selective estrogen receptor modulator (SERM) that is primarily used as a fertility medication in women. It works by blocking the action of estrogen in the hypothalamus, which leads to an increase in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) production. This increase in FSH and LH stimulates the ovaries to produce and release eggs, which can increase the chances of pregnancy [1]. It is also sometimes used off-label as a treatment for male infertility and for other conditions such as polycystic ovary syndrome (PCOS). In men, CC can increase testosterone production by blocking estrogen's negative feedback on the hypothalamus and pituitary gland, which can improve sperm count and motility [2].

CC is considered a tissue-selective SERM because it has different effects on different tissues in the body. While it blocks the action of estrogen in the hypothalamus and pituitary gland, it can act as an estrogen agonist in other tissues such as the bones and liver [3]. It is a medication that is used in the treatment of female infertility. It is also sometimes used off-label to increase testosterone levels in men with hypogonadism [4].

The half-life of CC is approximately 5-7 days, meaning that it takes this amount of time for half of the medication to be eliminated from the body. However, it is important to note that the half-life can vary based on individual factors such as age, liver function, and metabolism [5]. It is recommended that CC be taken once daily for five days, typically beginning on the fifth day of the menstrual cycle in women. In men, it may be prescribed in a daily dose for several weeks. Because of its relatively long half-life, it is possible for CC to accumulate in the body with repeated dosing [6].

As with any medication, it is important to follow the dosing instructions provided by the healthcare provider and to speak with them about any concerns or questions one may have about the medication's half-life or other pharmacokinetic properties [7]. It is a medication used in the treatment of infertility in women by stimulating ovulation. It is a selective estrogen receptor modulator (SERM) and works by blocking estrogen receptors in the hypothalamus, leading to an increase in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, which in turn stimulate the ovary to produce and release an egg [1].

Like all medications, CC can cause side effects. The most common side effects include hot flashes, mood swings, breast tenderness, bloating, and headaches. These side effects are usually mild and temporary, and they typically resolve once treatment is completed [8].

Less common but more serious side effects may include ovarian hyperstimulation syndrome (OHSS), which can cause abdominal pain, bloating, nausea, vomiting, and shortness of breath. In rare cases, CC may increase the risk of developing ovarian or breast cancer [9].

It is important to discuss any potential side effects and risks with ones doctor before starting treatment with CC. The aim of this study is to evaluate the Effect of Clomiphene on the Hormonal profile of female Adult Wistar rats with regular estrous cycle, hence the importance of this study will help guide in the use and administration of CC as observed in a similar study by de Ronde *and* Smit, [10].

2. METHODOLOGY

2.1 Vaginal Smear Method

Vaginal smear method to determine the estrous cycle of the rats was as documented by McLean et al. [11]. Briefly, sterile pipette was use to draw up about 100 µl of Normal saline. The rat was lifted and placed on the cage lid with her hind/rear not on the lid by firmly grasping the tail and elevate the rear end. The mouse had only her front paws grasping the hopper (at this point the rat may urinate and if so, we waited until the rat urination stops and if there be urine left at the entrance to the vaginal canal, the opening was rinsed with excess distilled water using a separate tip). The normal saline filled tip was then place at the opening of the vaginal canal taking care to not penetrate the orifice as vaginal and the bulb gently depressed to expel a quarter to half of the volume of normal saline at the opening of vaginal canal and the liquid was spontaneously aspirated into the pipette without tip insertion. This was repeated (3-5 times) using the same tip, bulb, and fluid to obtain a sufficient number of cells in a single sample. The fluid was then placed on glass slide and viewed under the microscope to determine cell types present. The ratio of cells present was used to determine the estrous stage of the rat at the time of sample collection.

2.2 Experimental Animals and Grouping

Sixteen (16) healthy female Wistar rats with regular estrous cycle were selected from the total of thirty six (36) adult female wistar rats. The animals were allowed free access to water and were fed with standard rat chow.

They were allowed free access to food and clean water plus the test drug for 11 days during the experimental period many times for all groups.

2.3 Drug Treatment

The test group (group 2, group 3, group 4) rats were treated with CC (50 mg body weight daily as Mong-Ting et al. (1986) dissolved in required quantity of normal saline.

2.4 Sample Collection

At the end of the 11^{th} day of drugs, vaginal smear was carried out and threats were sacrificed by cervical dislocation. Blood sample was collected into sample bottle (plain) from the jugular vein.

2.5 Sample Analysis

- **Body weight**

Body weight: Rats in all groups were weighed before the study and at 1, 3, 5, 7, 9 and 11 days of the study period using a digital weighing scale.

- **Hormonal Assay**

Determination of Testosterone, Progesterone, luteinizing hormone and follicle stimulating hormone levels (Butt and Blunt, 1988)

Sample Preparation: The blood sample is collected from the patient and allowed to clot. Serum is then separated from the clot by centrifugation.

Coating: The wells of a microtiter plate are coated with a specific antibody against testosterone.

Blocking: Non-specific binding sites on the microtiter plate are blocked using a blocking solution.

Standard and Sample Addition: A series of standard solutions containing known concentrations of testosterone and the patient serum samples are added to the wells.

Incubation: The plate is incubated to allow the testosterone in the sample to bind to the antibody on the plate.

Washing: The wells are washed to remove any unbound proteins or contaminants.

Detection: A second antibody that is conjugated to an enzyme is added to the wells, which binds to the testosterone-antibody complex. Then, a substrate is added, which reacts with the enzyme to produce a colorimetric signal.

Measurement: The absorbance of the colorimetric signal is measured using a spectrophotometer, and the concentration of testosterone in the sample is calculated by comparing the signal to the standard curve.

Analysis: The results are analyzed and interpreted by the physician or laboratory technician.

3. RESULTS

Table 1 shows the number and percentages of diestrus, estrus, proestrus and metestrus period in control and test groups.

The summary of the result which is shown in Table 2 shows that in the control groups 24.51 and 22.89 % underwent diestrus and estrus period respectively while 0% underwent proestrus and metestrus when compared to their respective test groups.

The result on the effect of CC on the hormonal profile of adult female rats on the regular estrus cycle is represented below.

Table 4 shows the body weight for regular estrous cycle at day 1, 3, 5, 7, 9 and 11 of control and test subjects in which the Mean±SEM of day 1, 3, 5, 7, 9 and 11 were 136.60±4.78, 139.60±5.74, 146.90±6.89, 147.40±5.87, 150.70±5.58 and 152.0±4.93 respectively for control subjects. Of all the test groups none were found statistically significant ($p<0.05$) when compared with their respective control.

The effect of different doses of CC on reproductive (FSH & LH) and female sex hormones (Progesterone and estrogen) in regular estrous cycle is represented in Table 4. There was an apparent change in Progesterone level from 15.13±2.86 in control group to 19.97±8.80, 14.95±4.05.29, 30.47±13.40 in group B, C and D respectively. However, these changes were not statistically significant ($p>0.05$). Also, there was an apparent decrease in estrogen level which not significant from 38.73±1.57 in the control group to 36.10±4.21, 31.50±4.20 in group B and C respectively except for group D which increased to 41.40±5.54 when compared to control group. As for FSH, there was a significant decrease from 3.57±0.62 in control group to 0.93±0.52, 0.30±0.30 and 0.83±0.52 in group B, C and D respectively. LH also decreased non significantly from 6.73±1.78 in control group to 2.07±1.09 and 0.002±0.00 in group B and C respectively and then later increase to 12.83±12.44 in group D.

4. DISCUSSION

CC is a medication used to stimulate ovulation in women who have difficulty conceiving due to anovulation or oligoovulation. In rats, CC has been shown to increase the number of ovulations, and also affects the estrous cycle.

In this study, the number and percentages of the various stages of the estrous cycle that is, diestrus, estrus, proestrus and metestrus period in control and test groups were evaluated. The summary of the result as shown in Table 4 shows that in the control groups 24.51 % and 22.89 % underwent diestrus and estrus period respectively while 0 % underwent proestrus and metestrus when compared to their respective test groups. The results of previous work showed that 42.1 % of the CC treated female rats were in the estrus phase of the oestrus cycle [12].

CC has been shown to affect the estrous cycle in female rats by prolonging the duration of the estrus phase [13]. This effect is thought to be due to the medication's ability to stimulate the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn stimulates the ovary to produce and release an egg [14].

Prolonging the estrus phase can have a significant impact on fertility and the ability to conceive, as it may increase the chances of successful mating and fertilization. However, it is important to note that the effects of CC on the estrous cycle may vary depending on the dose and duration of treatment, and may also be influenced by other factors such as age, health, and hormonal status [15].

This study shows no statistically significant ($p<0.05$) difference in the body weight for experimental animals with regular estrous cycle in the test groups at day 1, 3, 5 and 7. However, there was a statistically significant ($p<0.05$) difference in body weight across the groups on day 9 and 11. Although it has been reported that there is no direct mechanism by which CC causes increased body weight and that weight gain is not a common side effect of Clomid, and the medication does not directly affect metabolism or the storage of fat [16]. There was a decrease in body weight for the treated animals in the latter days of the experimental period.

However, some women may experience weight gain as a result of the hormonal changes that occur during treatment with Clomid. In particular, Clomid can increase levels of estrogen in the body, which can cause water retention and bloating in some women [17]. This temporary water weight gain may be mistaken for actual weight gain and this could be the reason for the observed result in the body weight in this present study. Additionally, some women may

Table 1. Comparison of number and percentages of diestrus, estrus, proestrus and metestrus period in control and test groups

Drugs		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Control	Diestrus(%)	2 (8.00)	2 (8.00)	4 (16.00)	4 (16.00)	1 (4.00)	1 (4.00)	1 (4.00)	3 (12.00)	3 (12.00)	2 (8.00)	2 (8.00)
	Estrus (%)	2 (10.53)	2 (10.53)	-	-	3 (15.79)	3 (15.79)	3 (15.79)	1 (5.26)	1 (15.79)	2 (10.53)	2 (10.53)
	Proestrus(%)	-	-	-	-	-	-	-	-	-	-	-
	Metestrus(%)	-	-	-	-	-	-	-	-	-	-	-
1mg	Diestrus(%)	4 (14.29)	3 (10.71)	3 (10.71)	2 (7.14)	3 (10.71)	2 (7.14)	3 (10.71)	2 (7.14)	2 (7.14)	3 (10.71)	1 (3.57)
	Estrus (%)	-	2 (7.69)	2 (7.69)	3 (11.54)	2 (7.69)	3 (11.54)	2 (7.69)	3 (11.54)	3 (11.54)	2 (7.69)	4 (15.38)
	Proestrus(%)	1 (100.00)	-	-	-	-	-	-	-	-	-	-
	Metestrus(%)	-	-	-	-	-	-	-	-	-	-	-
2mg	Diestrus(%)	-	1 (6.25)	3 (18.75)	1 (6.25)	-	2 (12.50)	2 (12.50)	-	2 (12.50)	3 (18.75)	2 (12.50)
	Estrus (%)	3 (13.64)	4 (18.18)	1 (4.55)	3 (13.64)	2 (9.09)	2 (9.09)	2 (9.09)	3 (13.64)	1 (4.55)	-	1 (4.55)
	Proestrus(%)	2 (50.00)	-	-	-	2 (50.00)	-	-	-	-	-	-
	Metestrus(%)	-	-	-	-	-	-	-	-	-	-	-
5mg	Diestrus(%)	4 (12.12)	1 (3.03)	3 (9.09)	4 (12.12)	4 (12.12)	4 (12.12)	4 (12.12)	3 (9.09)	2 (6.06)	2 (6.06)	2 (6.06)
	Estrus (%)	-	4 (25.00)	2 (12.50)	1 (6.25)	1 (6.25)	1 (6.25)	1 (6.25)	2 (12.50)	2 (12.50)	1 (6.25)	1 (6.25)
	Proestrus(%)	1 (100.0)	-	-	-	-	-	-	-	-	-	-
	Metestrus(%)	-	-	-	-	-	-	-	-	-	-	-

Table 2. Summary of comparison of numbers and percentages of diestrus, estrus, proestrus and metestrus period in control and test groups

	Control	1mg	2mg	5mg
Diestrus(%)	25 (24.51)	28 (27.45)	16 (15.69)	33 (32.35)
Estrus (%)	19 (22.89)	26 (31.33)	22 (26.51)	16 (19.28)
Proestrus(%)	-	1 (16.67)	4 (66.67)	1 (16.67)
Metestrus(%)	-	-	-	-

Table 3. Comparison of mean ± S.E of body weight measurement on different days in test and control groups using ANOVA

Days	Group A (Control) (gram)	Test Groups			F value	p-value
		Group B (gram)	Group C (gram)	Group D (gram)		
Day 1	136.6 ±4.78	155.5 ±7.61	155.1 ±7.95	158.3 ±4.40	2.424	0.1163
Day 3	139.6 ±5.74	154.0 ±4.33	159.4 ±9.86	157.7 ±3.12	2.027	0.1638
Day 5	146.9 ±6.89	162.6 ±8.00	152.9 ±9.69	157.9 ±3.97	0.816	0.5095
Day 7	147.4 ±5.87	158.7 ±9.06	155.3 ±11.05	150.0 ±3.24	0.416	0.7446
Day 9	150.7 ±5.58	150.4 ±9.39	159.5 ±11.47	142.6 ±2.47	0.786	0.5264
Day 11	152.0 ±4.93	154.7 ±10.04	151.4 ±10.96	146.9 ±3.46	0.156	0.9237

*Numbers represent Mean±S.E, *mean significant p≤0.05*

Table 4. Comparison of Mean ± S.E of progesterone, estrogen, follicle stimulating hormone (FSH) and luteinizing hormone (LH) measurement in test and control groups using ANOVA

Hormones	Group A (Control)	Test Groups			F value	p- value
		Group B	Group C	Group D		
Progesterone (ng/ml)	15.13±2.86	19.97±8.80	14.95±4.05	30.47±13.40	0.64	0.6119
Estrogen (ng/ml)	38.73±1.57	36.10±4.21	31.50±4.20	41.40±5.54	0.88	0.4977
Follicle stimulating hormone (ng/ml)	3.57±0.62	0.93±0.52*	0.30±0.30*	0.83±0.52*	0.77	0.0149*
Luteinizing hormone (ng/ml)	6.73±1.78	2.07±1.09	0.002±0.00	12.83±12.44	0.64	0.6153

*Numbers represent Mean±S.D, *mean significant p≤0.05*

experience changes in appetite or cravings while taking Clomid, which could potentially lead to weight gain if they are consuming more calories than they are burning [18]. This could have also been the reason for this increased body weight.

In this study, the effect of different doses of CC on reproductive (follicle stimulating hormone and LH) and female sex hormones (Progesterone and estrogen) in irregular estrous cycle is represented in Table 4. There was an apparent significant decrease in FSH levels and non significant variation in the progesterone, estrogen and LH levels however increased levels were observed mainly in the group D administered with higher concentration of clomid. Normally, FSH and LH are produced by the pituitary gland in the brain and are responsible for stimulating the ovaries to produce and release eggs. However, in some women, the production of FSH and LH may be insufficient, leading to ovulation problems and infertility [19]. This observation was in contrast with the study of Dozortsev et al., [20] who noted that by increasing FSH levels, Clomid can help to promote the growth and development of follicles in the ovaries, which contain the eggs. This can increase the chances of ovulation and improve fertility. This was in similarity with the study of Miller et al., [21] who reported that in some cases, Clomid may cause a temporary decrease in FSH levels after the medication is discontinued. This is because the medication can disrupt the normal feedback loop that regulates FSH production. However, this decrease is usually temporary and does not typically have any significant long-term effects on fertility Clomid works by binding to estrogen receptors in the brain, which tricks the body into thinking that estrogen levels are low. This causes the pituitary gland to increase its production of FSH and LH, which in turn stimulates the ovaries to produce and release eggs [22].

5. CONCLUSION

i. There was no significant effect of CC on the total body weight of female wistar rats on regular estrous cycle.
ii. CC has no significant effect on the hormonal profile of female wistar rats on regular estrus cycle with exception to the follicle stimulating hormone.

6. RECOMMENDATION

Before this time, it has been believe that CC is recommended for female having hormonal imbalance. However the result from this study has shown that it's most effective only for follicle stimulating hormone (FSH).

7. LIMITATION

a. Some of the adult female wistar rats became aggressive during the process of drug administration and to a large extent, affecting the handlind of the animals.

CONTRIBUTION TO KNOWLEDGE

From this study, it was observed that CC which is a hormonal drug does not really have any significant effect/changes in the body weight in those that take it.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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