

EFFECT OF CLOMIPHENE CITRATE ON GONADOTROPIN HORMONES FSH, LH AND TESTOSTERONE LEVELS IN MICE OFFSPRING

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ABSTRACT

Infertility can cause considerable social, emotional and psychological stress. Ovulatory dysfunction is one of the most common causes of reproductive failure in sub-fertile and infertile women. There are several approaches to ovulation induction therapy for the management of women with ovulatory disorders. Fertility drugs are spreading worldwide fast and therefore many studies have reviewed the association between the use of these drugs and physiological, biochemical and histopathological alterations. The results of present study showed that there were observed effects of Clomiphene citrate (Clomid)[®] on albino mice offspring's hormones level. Treating mothers with CC doses 0.2 and 0.3 mg/day caused high increasing and variation in gonadotropin hormones FSH, LH and testosterone (T) levels comparing to control and between males & females also as was clearly noticed on the new offspring of the treated mothers with CC.

KEYWORDS: Offspring, Testosterone, FSH, LH, Clomiphene citrate.

INTRODUCTION

There are many ovulation inducing drugs is prescribed nowadays, Clomiphene citrate (CC) is the first choice for women treatment with ovulatory disorder or those with polycystic ovaries (PCOS) and have been widely used since 1962 until today (2-3). Clomiphene citrate or Clomid[®] tablets are orally administered, nonsteroidal and usually given on the third or the fifth day of cycle after spontaneous or progesterone induced withdrawal bleeding with 50 mg

for five days. The effective dose of CC ranges from 50,100,150mg /day, doses excess 250gm /day is not approved by the FDA.^[1,3] During CC treatment which binds to estrogen receptors(ER) in reproductive system the levels of both (FSH & LH) is indicated to rise and effect ovarian stimulation to produce one or more dominant follicles emerge and mature.^[2] Anterior pituitary gonadotropin hormones, FSH and LH plays an essential role in mammalian reproduction by regulating germ cell proliferation and differentiation. In females they controlling estrogen, progesterone secretion and oocyte maturation leading to ovulation. In males FSH considered as an essential hormone for spermatogenesis and viability and motility of the sperms. Spermatogenesis is also under the control of testosterone while LH controlling testosterone production.^[4,5,6] Testosterone is gonadal sex steroid hormone, it has reproductive function and it also responsible about develop and maintain the secondary male characteristics. Its takes a part in nervous system development.^[7,8] Previous study suggested that a daily oral dose of CC 50 mg/day to 44 nomogonadotrophic subfertile men for 3 months resulted a significant increases in FSH, LH and T levels.^[9] In treated female rats with 100mg/day for 2 days recorded an increase in FSH level and decrease in T level, whereas LH level was similar to saline group.^[10] The aim of the present study is to evaluate the effect of CC on gonadotropin hormones FSH, LH and Testosterone (T) levels in mice offspring of treated mothers with CC.

MATERIALS AND METHODS

Animals

All experimental procedures with mice were approved by the ethical guidelines of the animal care and use committee of King Abdulaziz University. Twenty five virgin albino mice of SWR strain female, at age (8 wk old) and weighing (23-25gm) were used in the present study. Mice were obtained from animal house unit of king Fahad Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were acclimatized to laboratory conditions for one week before to the initiation of experimental treatments and were housed in standard plastic cages and maintained in controlled laboratory conditions room, temperature (20±1C°), light: dark cycle (12:12h) and humidity (65%). Mice were feed ad libitum with standard diet and had free access to tap water.

Experiment design

Animals were divided in to tow groups:1- Control group(five females).2- Clomiphene citrate treated group (20 females). Mice were oral injected with 0.2 and 0.3mg/day of CC daily for

2wk, after 2wk every female were housed with a male for mating after female get pregnant males were taken out. On the day 26th after weaning and 8 wk blood samples were taken from orbital sinus of mice offspring (22 pre-pubertal, 22 post- pubertal male & 23 pre-pubertal, 23 post- pubertal female) for FSH, LH and T levels were determined by using (Elba Science) Eliza kits, according to the manufacturer's instructions.

Statistical analysis

Statistical Package for Social Sciences (SPSS version 20) was used. Data were presented as mean (standard deviation). The continuous variables between more than 2 groups as comparison between control, male and female were compared using Onaway ANOVA (LSD) test, and between 2 groups as pre-pubertal and post-pubertal were made using unpaired student "t" test. A probability (*P*) <0.05 was considered significant. Graphs were made using Prism software for statistics version 5.

RESULTS

Males group

Table 1 and figure 1 as well for the analyzed data showed that **Pre-males vs. Pre-control** significant difference increase in LH (2.08 ± 0.59 VS. 0.50 ± 0.01 , $P^1=0.002$) and T (14.43 ± 3.01 VS. 9.50 ± 0.01 , $P^1=0.017$) only. **Post- males vs. Post- control** there were high significant increase in LH level (3.44 ± 0.97 vs. 1.60 ± 0.01 , $P^1=0.0001$), and lower significant increase in T (16.39 ± 3.68 vs. 12.11 ± 0.01 , $P^1=0.036$) level comparing to control. In **Pre- males vs. Post- males** analyzed data for this group showed high significant increase in LH (2.08 ± 0.59 vs. 3.44 ± 0.97 , $P^2= 0.0001$) level, and lower significant increase in FSH (5.32 ± 1.91 vs. 6.76 ± 1.72 , $P^2=0.007$) and T (14.43 ± 3.01 vs. 16.39 ± 3.68 , $P^2=0.046$) levels.

Table: 1 Comparison of the measured hormones in different studied groups of male mice.

Parameters	Pre-pubertal		Post-pubertal	
	Control	Treated	Control	Treated
Luteinizing hormone (ng/ml)	0.50 ± 0.01	2.08 ± 0.59	1.60 ± 0.01	3.44 ± 0.97
Significance		¹ P= 0.002		¹ P= 0.0001 ; ² P= 0.0001
Follicle stimulating hormone (ng/ml)	6.70 ± 0.01	5.32 ± 1.91	7.31 ± 0.02	6.76 ± 1.72
Significance		¹ P= 0.201		¹ P= 0.606 ; ² P= 0.007
Testosterone (ng/ml)	9.50 ± 0.01	14.43 ± 3.01	12.11 ± 0.01	16.39 ± 3.68
Significance		1P= 0.017		1P= 0.036 ; 2P= 0.046

Data are expressed as mean +/- standard deviation. ¹P: significance versus control; ²P: significance pre-pubertal versus post-pubertal. Comparison between groups was made using OneWay ANOVA test.

Females group

In table 2 and figure 1 analyzed data showed that there were no statistically significant difference in **Pre- females vs. Pre- control, Post-females vs. Post- control and in Pre-females vs. Post- females groups.**

Table (2): Comparison of the measured hormones in different studied groups of female mice.

Parameters	Pre-pubertal		Post-pubertal	
	Control	Treated	Control	Treated
Luteinizing hormone (ng/ml)	3.10±0.01	3.80±1.63	5.90±0.69	4.58±1.33
Significance		¹ P= 0.435		¹ P= 0.140; ² P= 0.069
Follicle stimulating hormone (ng/ml)	6.90±0.01	5.67±1.83	8.24±0.29	6.53±1.76
Significance		¹ P= 0.250		¹ P= 0.112; ² P= 0.099
Testosterone (ng/ml)	8.71±0.01	9.54±1.29	9.10±0.35	10.00±1.34
Significance		1P= 0.286		1P= 0.252; 2P= 0.225

Data are expressed as mean +/- standard deviation. ¹P: significance versus control; ²P: significance pre-pubertal versus post-pubertal. Comparison between groups was made using One-way ANOVA test.

Males & Females group

In Table 3 and Figure 1 as well Analyzed data for **Pre-males vs. Pre- females** group showed high significant difference in LH (2.08±0.59 vs. 3.80±1.63, P¹=0.0001) and T (14.43±3.01 vs.9.54±1.29, P¹=0.0001) levels only. In **Post- males vs. Post- females** there were high significant difference in T level (16.39±3.68 vs. 10.00±1.34, P²=0.0001), and low significant difference in LH (3.44±0.97 vs. 4.58±1.33, P²=0.002) level. **Pre-males vs. Post- females** analyzed data for the same hormones in this group were high significant difference in LH (2.08±0.59 vs. 4.58±1.33, P³=0.0001) and T (14.43±3.01 vs. 1.00±1.34, P³=0.0001) levels. But lower significant difference in FSH (5.32±0.59 vs. 6.53±1.76, P³=0.033).

Table (3): Comparison of the measured hormones in treated pre-pubertal and post-pubertal male versus female mice.

Parameters	Pre-pubertal		Post-pubertal	
	Male	Female	Male	Female
Luteinizing hormone (ng/ml)	2.08±0.59	3.80±1.63	3.44±0.97	4.58±1.33
Significance		¹ P= 0.0001; ⁴ P=0.375		² P= 0.002; ³ P=0.0001
Follicle stimulating hormone (ng/ml)	5.32±1.91	5.67±1.83	6.76±1.72	6.53±1.76
Significance		1P= 0.527; 4P=0.042		2P= 0.650; 3P=0.033
Testosterone (ng/ml)	14.43±3.01	9.54±1.29	16.39±3.68	10.00±1.34
Significance		1P= 0.0001; 4P=0.0001		2P= 0.0001; 3P=0.0001

Data are expressed as mean +/- standard deviation. ¹P: significance pre-pubertal male versus pre-pubertal female; ²P: significance post-pubertal male versus post-pubertal female; ³P: significance pre-pubertal male versus post-pubertal female; ⁴P: significance post-pubertal male versus pre-pubertal female. Comparison between two different groups was made using independent sample "t" test.

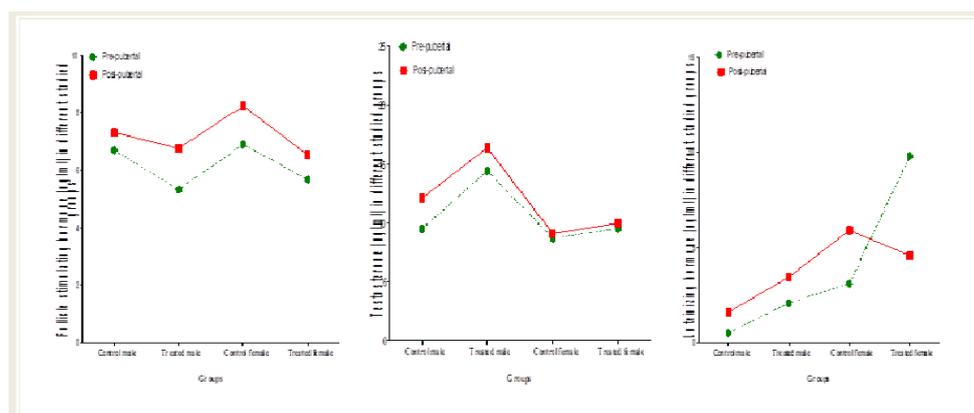


Figure 1: Comparison illustration of measured FSH, LH and T levels pre-pubertal and post-pubertal males versus females mice offspring with control.

DISCUSSION

Although, Clomiphene citrate (CC) considered one of the safest drug given to women with evolutionary disorder since the 60s, rises concern in the medical field on its effects on the newly offspring. Results of the current study shows a consistent and statistically significant extended effects of CC in increasing FSH, LH and T levels in both pre and post male offspring comparing to control. Whereas in females pre and post offspring hormones level

remained unchanged comparing to control also. On other hand there were significant differences in LH, FSH and Testosterone levels in pre and post males comparing to pre and post females. This finding is agree and consistent with previous studies on males that recorded significant increase in FSH, LH and T levels in obese hypogonadal young men patients after 3 months of CC treatment.^[11] Similarly finding were reported increasing in FSH, LH and T levels in uremic men treated with long- term CC.^[12] In other study indicates that there were no significant differences in FSH, LH and T levels between patients with idiopathic oligospermia and control during CC treatment.^[13] Whereas, previous study recorded highly significant increase in FSH, LH and T levels during CC 50mg/day treatment in preselected infertile men for 3 months.^[14] Also 10 oligozoospermic men treated with CC were showed significant increase in FSH, LH and T levels as an CC effect on sex hormone binding globulin in normospermic and oligozoospermic men study.^[15] As treatment in hypogonadal infertile men by using CC to raising testosterone hormone recorded significantly increased in T level at 6 and 12 weeks of CC 25mg/day.^[16] In study of hormonal response and profile of CC in hypogonadal and infertile men reported high significant increased T level with improvements in androgen deficiency by using 50mg CC every other day for 2 weeks.^[17] Although, as acceleration of puberty in 29 boys with delayed puberty were treated with CC 50 mg/day for 3 months, CC therapy caused a rise in T level in the treatment groups.^[18] Another increased of T level were found as a result of oral administered of CC 25mg/day for male testosterone deficiency.^[19] On other hand in women CC treatment showed a reverse result to the present study as where confirmed reported increases in FSH and LH levels several days after CC administration.^[20] Whereas a recorded increasing in FSH and LH levels in other study on unexplained infertile women treated with low doses of CC 50mg/day and 100mg/day.^[21] The present study result for females was inversely to rises of FSH and LH were found in PCOS patients treated with CC150mg/day for 5 days.^[22] Previous study also recorded that both of FSH and LH were similar in natural and medicated cycles with CC 50mg/day and letrozole in 19 normal ovulatory women.^[23] A recorded increased in FSH and LH levels also were found in 60 sexually immature female rats(24-25 days old) treated with 10mg/kg BW comparing to control group.^[24] Blood transmission form the treated mothers to their offspring may cause these highly significant increases and disparity changes in hormones concentrations levels.

CONCLUSION

In conclusion, blood biochemical analysis indicated that CC causes alterations on FSH, LH and Testosterone hormones in treated mother's offspring in albino mice.

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