



## Effect of Clomiphene Citrate on Thyroid Hormones T<sub>3</sub>, T<sub>4</sub> and TSH 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)<sup>®</sup> on albino mice offspring's hormones level. Treating mothers with CC doses 0.2 and 0.3 mg/day caused significant increasing and variation in males offspring TSH and T<sub>3</sub> hormones level only comparing to control and between males & females also of the treated mothers with CC as was clearly noticed. Whereas, the elevations of hormones level was not significant in females offspring of treated mothers with CC.

### Keywords

Clomiphene citrate; Offspring; TSH; T<sub>3</sub>; T<sub>4</sub>

### 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 [1]. Clomiphene citrate or Clomid<sup>®</sup> 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 [2]. The effective dose of CC ranges from 50,100,150 mg /day, doses excess 250 gm/day is not approved by the FDA [3]. In human and animals development thyroid glands and thyroid hormones thyroxine T<sub>4</sub> and triiodothyronine T<sub>3</sub> are too important and central, many previous studies suggested that thyroid hormones plays a big role in immune, nervous, cardiovascular, reproductive system development and function, also in many species they have different widely effects on reproduction and reproductive tract development [4]. Thyroid development pattern in human and rodents is similar. In human fetus thyroid system development happened within hypothalamus, thyroid gland and pituitary gland initial development, that occurs between

embryonic day 10 and gestational week 11[5]. In rats thyroid gland development occurs during pregnancy approximately 3 weeks long, in the same order as human [6]. Hypothyroidism in prepubertal can cause men infertility, previous studies suggested that alterations of semen parameters were induced by hypothyroidism. Patients with thyroid dysfunction were having abnormal values of T<sub>3</sub> and T<sub>4</sub> [7]. In human thyroid dysfunction in fact can cause menstrual abnormalities and pregnancy wastage, therefore, animal *in vitro* studies suggested that thyroid hormones play an important direct role in ovarian physiology [8]. As an important finding of [9]. CC treatment with thyroid hormone replacement therapy is of a great value for luteal-phase defect and ovulation induction in hypothyroxinemia patients. Clomiphene citrate can directly influence thyroid hormones and function [10]. The aim of the present study is to evaluate the effect of CC on thyroid hormones T<sub>3</sub>, T<sub>4</sub> and TSH levels in mice offspring of treated mothers with CC.

### Material 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 week old) and weighing (23-25 gm) 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 ± 1°C), 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 two groups: 1- Control group (five females). 2- Clomiphene citrate treated group (20 females). Mice were oral injected with 0.2 and 0.3 mg /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 26<sup>th</sup> after weaning and 8 week 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 T<sub>3</sub>, T<sub>4</sub> and TSH 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

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## Results

### Males group

Table 1 and Figure 1 as well for the analysed data showed that TSH level were significantly increased in Pre-males vs. Pre-control ( $0.74 \pm 0.01$  vs.  $2.19 \pm 1.24$ ,  $P^1= 0.034$ ), and there were significant differences in TSH level in Pre-males vs. Post-males ( $2.19 \pm 1.24$  vs.

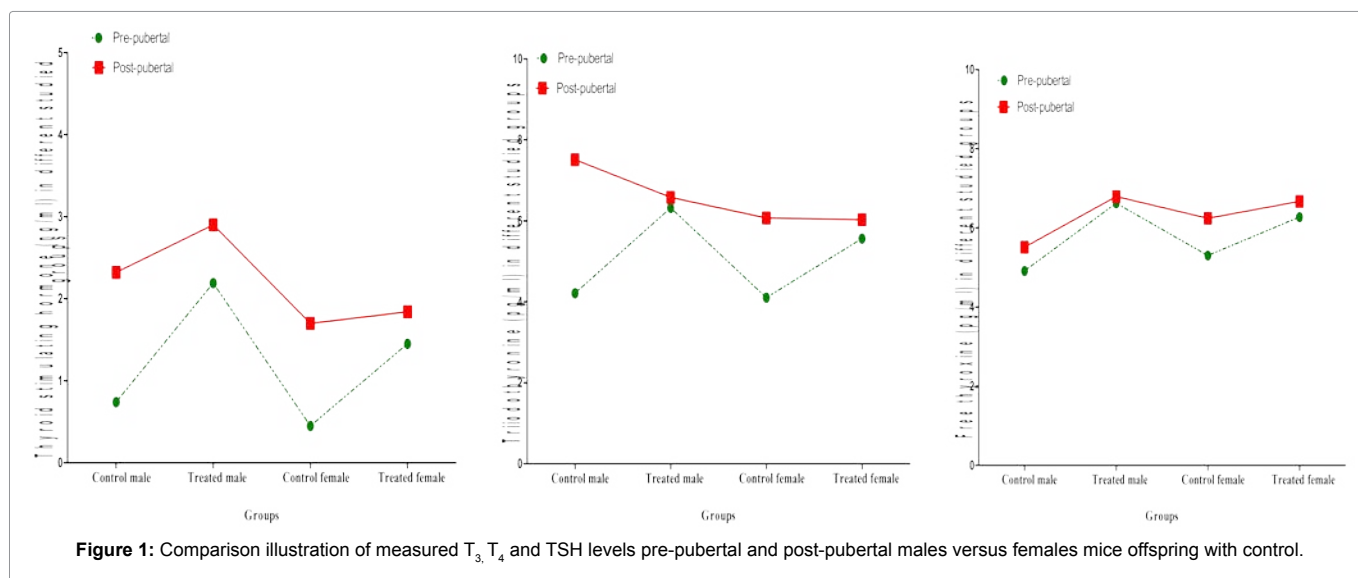
$2.90 \pm 1.01$ ,  $P^2=0.031$ ). Whereas, for  $T_3$  levels were high significant increase in Pre-males vs. Pre-control only. As for  $T_4$  levels there were no significant differences in this group Pre or Post.

### Females group

In Table 2 and Figure 1 analysed data showed that there were no statistically significant difference in Pre- females vs. Pre- control,

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

Parameters	Pre-pubertal		Post-pubertal	
	Control	Treated	Control	Treated
Thyroid stimulating hormone (ng/ml)	$0.74 \pm 0.01$	$2.19 \pm 1.24$	$2.32 \pm 0.03$	$2.90 \pm 1.01$
Significance		$^1P= 0.034$		$^1P= 0.385$ ; $^2P= 0.031$
Triiodothyronine (pg/ml)	$4.21 \pm 0.01$	$6.32 \pm 1.39$	$7.51 \pm 0.01$	$6.58 \pm 1.44$
Significance		$^1P= 0.015$		$^1P= 0.267$ ; $^2P= 0.512$
Free thyroxine (pg/ml)	$4.91 \pm 0.01$	$6.62 \pm 1.96$	$5.51 \pm 0.02$	$6.79 \pm 1.90$
Significance		$^1P= 0.139$		$^1P= 0.264$ ; $^2P= 0.752$



**Figure 1:** Comparison illustration of measured  $T_3$ ,  $T_4$  and TSH levels pre-pubertal and post-pubertal males versus females mice offspring with control.

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

Parameters	Pre-pubertal		Post-pubertal	
	Control	Treated	Control	Treated
Thyroid stimulating hormone (ng/ml)	$0.45 \pm 0.01$	$1.45 \pm 1.02$	$1.70 \pm 0.087$	$1.84 \pm 1.00$
Significance		$^1P= 0.106$		$^1P= 0.814$ ; $^2P= 0.180$
Triiodothyronine (pg/ml)	$4.10 \pm 0.01$	$5.56 \pm 1.35$	$6.07 \pm 0.41$	$6.03 \pm 1.38$
Significance		$^1P= 0.076$		$^1P= 0.957$ ; $^2P= 0.234$
Free thyroxine (pg/ml)	$5.30 \pm 0.01$	$6.27 \pm 1.73$	$6.24 \pm 0.23$	$6.67 \pm 1.73$
Significance		$^1P= 0.349$		$^1P= 0.678$ ; $^2P= 0.419$

**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
Thyroid stimulating hormone (ng/ml)	$2.19 \pm 1.24$	$1.45 \pm 1.02$	$2.90 \pm 1.01$	$1.84 \pm 1.00$
Significance		$^1P = 0.035$ ; $^4P=0.0001$		$^2P= 0.001$ ; $^3P=0.312$
Triiodothyronine (pg/ml)	$6.32 \pm 1.39$	$5.56 \pm 1.35$	$6.58 \pm 1.44$	$6.03 \pm 1.38$
Significance		$^1P= 0.072$ ; $^4P=0.016$		$^2P= 0.184$ ; $^3P=0.488$
Free thyroxine (pg/ml)	$6.62 \pm 1.96$	$6.27 \pm 1.73$	$6.79 \pm 1.90$	$6.67 \pm 1.73$
Significance		$^1P = 0.527$ ; $^4P=0.327$		$^2P= 0.813$ ; $^3P=0.933$

Post-females vs. Post- control and in Pre- females vs. Post- females groups.

### Males and Females group

In Table 3 and Figure 1 as well Analysed data showed high significant differences for TSH level in Pre-males vs. Pre-females ( $2.19 \pm 1.24$  vs.  $1.45 \pm 1.02$ ,  $P^1=0.035$ ), Post-males vs. Post-females ( $2.90 \pm 1.01$  vs.  $1.84 \pm 1.00$ ,  $P^2=0.001$ ) and Pre-females vs. Pre-males ( $1.45 \pm 1.02$  vs.  $2.90 \pm 1.01$ ,  $P^4=0.0001$ ). As for T<sub>3</sub> level also was significantly lower in pre-females vs. Post-males only ( $5.56 \pm 1.35$  vs.  $6.58 \pm 1.44$ ,  $P^4=0.0001$ ). Whereas, T<sub>4</sub> level there was no significant differences in all males & females groups.

### 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 show a consistent and statistically variations extended effects of CC on thyroid hormones and TSH in mice offspring. These affects clearly appeared as an increasing of hormones levels in general, but the significant increase was in TSH level in per-males and post-males offspring comparing to control and to each other and a significant decreased in TSH level was found in females groups comparing to males groups, while it was no significant difference in females group. Also, there was a significant increase in T<sub>3</sub> level of pre-males only comparing to control whereas, not in females group. However, there were no significant differences in T<sub>4</sub> level in all males and females groups in this study finding.

This finding is in agreement with the previous studies that recorded increased TSH serum concentration after and during ovaries stimulation (OS), also they were recorded an increase in T<sub>4</sub> level in infertility women within 1 month after OS. While, T<sub>3</sub> level did not change after CC treatment [11,12]. Another increasing in TSH level affected by patients who used of CC was reported as well [13]. Similarly, TSH was increased in 15 boys with delayed adolescence and 6 males with isolated gonadotropin deficiency (IGD) compared adult controls male and was similar to adult female as a response of using CC in IGD patients [14]. But disagree with another study when he reported that no change found in TSH level in women after OS [15]. Also it has been suggested that TSH, T<sub>3</sub> and T<sub>4</sub> levels were remained unchanged in women after OS [16]. In similar study indicates that TSH level was increased, while T<sub>4</sub> level was not changed in patients during OS [17]. Also De Leo reported that no significant difference in TSH level was found in CC administration group and CC plus L-thyroxine group in women [18]. Similar result in previous study although reported that there were no statistically significant differences in TSH level between two groups of Clomiphene citrate challenge test(CCCT) in 70 patients with menstrual cycle disturbances [19].

Some finding of a previous study on patients with primary testicular failure recorded an increase in TSH level and no changes in both T<sub>3</sub> and T<sub>4</sub> levels after CC administration [20]. Although, it has been suggested that TSH, T<sub>3</sub> and T<sub>4</sub> levels were normal as a result of CC administration for male infertility with chromosomal abnormalities [21]. Another study was done on healthy men, they were given 100 mg /day of CC for 5and 12 consecutive days, the finding of this study recorded small but statistically significant decreased in T<sub>3</sub> and T<sub>4</sub> levels on day 4of CC and a slight increase in TSH level was found in day 5 of CC administrated [10]. Recent study on albino rats indicates to TSH level significantly decreased in males treated with CC and CC+H and in females treated with CC+ H while

TSH level was increased in treated females with CC+H comparing to controls, in the same study T<sub>3</sub> level significantly elevated in males and females treated with CC alone and decreased in males and females treated with CC+H comparing to control also they recorded significant reduction of T<sub>4</sub> level in both groups of males and females [22]. Blood transmission form the treated mothers to their offspring may cause these highly significant increases and disparity changes in hormones concentrations levels.

### Conclusions

In conclusion, blood biochemical analysis indicated that CC causes TSH, T<sub>3</sub> disturbance in treated mother's offspring in albino mice.

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