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## Research Article

# Effects of contraceptive medicines on some physiological and hormonal parameters of the thyroid gland, blood triglycerides and lipoprotein of male rat

Sabah H. ENAYAH
*Department of Biology, College of Science University of Thi-Qar, Nasiriyah, Iraq.*
**Email: senayah@sci.utq.edu.iq*

**Abstract:** This study was conducted to investigate the effect of contraceptive drugs on some of the physiological parameters of the thyroid gland and its relation to fertility standards of male rats and on triglycerides, lipoproteins, follicle stimulation hormone, and cholesterol levels in the blood. The animals were divided into 3 groups, (1) the control group, (2) T2, administered 0.32mg of levonorgestrel and (3) T3, administer 0.64mg orally for 2 months. The fertility of rats was estimated by administrating 3 females for each male and calculating the number of fetuses and pregnancies. The results showed decreased male desire and mating for females in the T1 and T2 groups. The testosterone and cholesterol testes in the fetuses showed some of them mutilated, and some pre-emptive and some less than normal. Fetuses of the T1 were not different from normal control. At the end of the experiment, animals were killed and dissected, and blood and sample were collected. The level of the T4 increased compared to the control one. In addition, increasing the blood triglycerides in the T2 and decreasing the level in T2 compared to the control were recorded. Decreasing in high-density lipoproteins was also recorded. The results also showed a significant reduction in the sperm and distortion sperm with a significant decrease in the hormone levels of the male ($P$<0.05clearly affected that the pills have a clear effect on males' fertility, causing a pathological hormonal imbalance that can negatively affect the ability of male fertilization.

**Keywords:** Contraceptive, Male, Lipoproteins, Fertility.

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## Introduction

Contraceptive drugs contain artificial female estrogen and progesterone, produced naturally in the ovaries. Females administer them orally to prevent pregnancy without affecting other physiological functions (Stefanick 2005; Bahadur et al. 2007). Contraceptive medicines consist of estrogen, androgen, and progesterone, which can disrupt egg formation in the ovary (Christin-Maitre 2013). Estrogen in contraceptive drugs is lower than the two other hormones (progesterone) and prevents the pituitary gland's effect on ovulation, where stimulating hormones of the reproductive glands are secreted from its anterior lobe (Jain 2015). Progesterone prevents ovulation and makes the mucus fluid secreted by the cervix gland before ovulation as a barrier in the way of male sperm to prevent the fertilization process (Su et al. 2017).

The male produces sperm under the influence of FSH (Follicle Stimulating Hormone) (Okutsu et al. 2006), which induces the entry of iodine into the thyroid gland to produce T3 and T4 hormones and testosterone (Shahid et al. 2018). Administration of any drug that affects the activity of the testis will affect the male hormone leading to changes in the testis (Schaalan et al. 2018). The disturbance in the hormonal balance occurs due to an imbalance in the production of a certain hormone excess or deficiency (Bretveld 2006). Estrogen is secreted by the pituitary gland in a female under the influence of LH and FSH

from the same luteinizing region. It plays an important role in the early stages of spermatogenesis (Chimento et al. 2014). Estrogen in high levels in males leads to negative effects on the reproductive system (Słowikowska-Hilczer 2006). It is found in males but in small quantities and is regulated by the testosterone effect, while when this balance is altered, the estrogen rises higher than the testosterone hormone, causing physiological effects such as weight gain, decreased sexual desire, and increased levels of triglycerides and lipoproteins in the blood and general physical weakness (Horstman et al. 2012). Progesterone is secreted from the corpus luteum, and its increase in the blood leads to a decrease in the hormone (LH) (Yang et al. 2006).

The thyroid gland secretes thyroxine and triiodothyronine (T3 and T4), which are necessary to maintain the balance and work of every body cell (Sobha 2013). Previous studies reported a relationship between thyroid hormones and male sex hormones, on top of which is testosterone, since a decrease in thyroid hormone is directed to the decrease in iodine thyroid hormones (Trokoudes et al. 2006). Furthermore, it causes an increase in fat breakdown, leading to a rise in fatty acid in the blood. Thus increases the formation of ketone bodies and thyroid hormones that help the oxidation of cholesterol to bile acids in the liver, leading to a decrease in the level of cholesterol in the blood (Schleicher et al. 2015). Furthermore, thyroid gland disorders can cause fertility disorders in males and females due to a decline in thyroid function, such as the absence of thyroid secretion leading to slows down of metabolic processes causing a negative effect on the shape and structure of sperms leading to infertility (Krassas et al. 2010). Thus, the present study aimed to investigate the effects of contraceptive drugs on some of the physiological parameters of the thyroid gland and its relationship to fertility standards of male rats and triglycerides, lipoproteins, follicle stimulation hormone, and cholesterol levels in the blood.

## Materials and methods

The contraceptive pill was obtained from pharmacies (Microgynon ED Fe, Germany) with the scientific name of Levonorgestrel. It is the most effective drug; its failure rate is weak and has a strong effect on preventing pregnancy (Glasier et al. 2011). The sexually active adult rats at ages 6-8 weeks and relatively same weights (250-300g) were obtained from the animal house in the Department of Biology, College of Science, Dhi Qar University. Animals were placed in plastic cages in animal housekeeping, and each with a metal mash, a clip fitted with a bottle of clean water, and a place to get food that provides a high-protein. The concentrated commercial diet was used to feed the rate. The floor was covered with sawdust, which was changed weekly to keep the cage clean. After 2 weeks of the adaptation period, the animals were randomly divided into three. The temperatures in the animal housekeeping ranged light 18-26°C with a photoperiod of 14L/10D.

A total of 18 males were divided into three groups, 6 animals per group as (1) The control group (C) was administered with physiological saline solution (normal saline) prepared by dissolving 0.9g of pure NaCl in 100 ml of distilled water and feed without any additives for 60 days, (2) treatment 1 (T1), administrated with oral contraceptive pills with 0.32mg daily for 60 days, and treatment 2 (T2), administrated with oral contraceptive pills with a concentration 0.64mg daily for 60 days. After 60 days of the experiment, males were introduced to females to determine the sexual ability of males. The fertility of rats was estimated by administrating 3 females for each male and calculating the number of fetuses and pregnancies. The females were left with the males for 21 days. Then the animals were taken and examined by ultrasound at the veterinary hospital (Fig. 1).

Males of all the groups were anesthetized with chloroform and dissected. The abdominal cavity was opened and extraction surgery of the male genital organs (testes, penis, and sperm duct) was removed and pieces of them were placed in 10% formalin

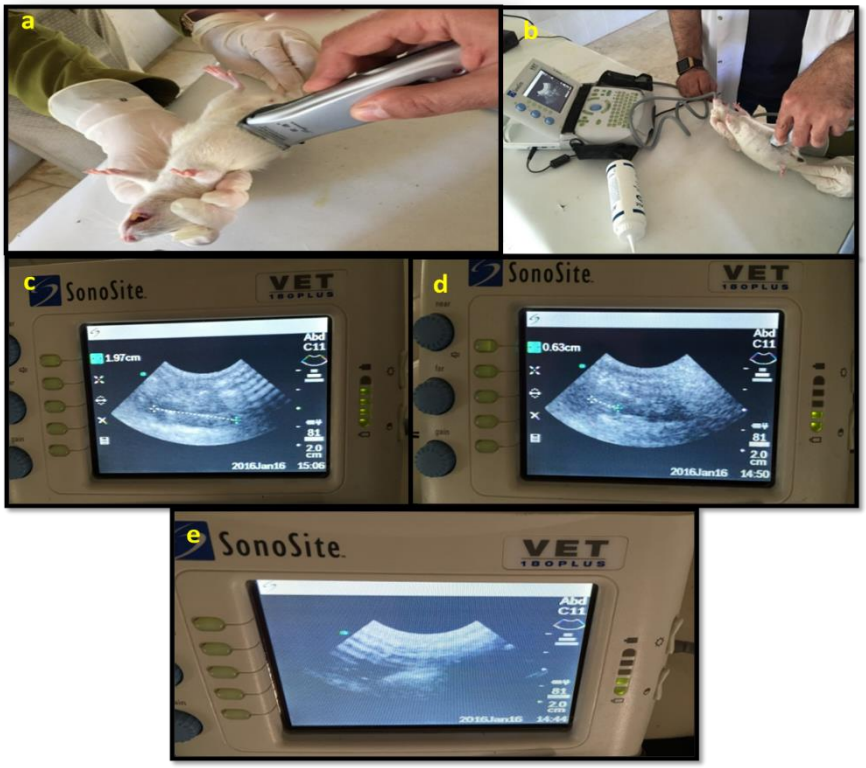


**Fig.1.** (a) Hair removal before ultrasound examination, (b) ultrasound examination from female mice that fertilized by males which administrated contraceptive, (c) of female mice fertilized by males without contraceptive administration the pregnancy appears clearly by the ultrasound monitor with normal fetus is recognized, (d) ultrasound examination of female mice fertilized by males with contraceptive administration a first concentration of 0.32mg, and pregnancy is clear by ultrasound, where the fetus is indicated, but it is small in size co, and (e) female mice vaccinated by males who took the contraceptive pill with a double concentration of 0.64mg, and the uterus is completely empty.

solution. After removing the fatty tissue, they were dried with filter paper.

Blood samples (3-4ml) were taken directly from heart puncture using 5ml syringes; then each blood sample was divided into two parts. The first part (2ml) was placed in tubes containing anticoagulant (EDTA), and the remaining (3 ml) into free anticoagulant tubes to separate the serum using a centrifuge at a speed of 5000rpm for 10min. Blood serum samples were transferred using a Pasteur pipette to special plastic tubes and kept at -20°) for further biochemical tests (Landau 1980). Red blood cell count, hemoglobin level estimation, compacted cell volume, average cell volume, average hemoglobin, white blood cell count, and erythrocyte sedimentation rate were measured. The radioimmunoassay (RIA) method was used to measure the level of thyroid hormones (T3, and T4) and testosterone (Corparanan CA90045) in the Institute of Embryology and Sterility Research, Baghdad). The biochemical parameters, including glucose, cholesterol, and protein in the blood serum, were also measured using commercial kits.

**Dara analysis:** The results were subjected to statistical analysis using the Completely Randomized Design; CRD to know the significant differences between the groups. The least difference test was used under the probability level of 0.05.

## Results and Discussion

The results indicated physiological and pathological changes in the rate treated with oral contraceptives as accent or presence of laziness, lethargy, increased appetite, and decreased movement, especially in the first 30 days of the experiment, especially in T2. The weight of the animals was recorded before a time (60

**Table 1.** Difference in weights of males treated with oral contraceptive pills.

| Timing | Average weights control group | Average weights treatment 1 | Average weights treatment 2 |
|---|---|---|---|
| Before treatment | 250-300 g | 250-300 g | 250-300 g |
| After two months | 260-305 g | 265-325 g | 275 - 355 g |
| P-value | 0.3 | 0.02 | 0.000 |

**Table 1.** The differences in cholesterol level between groups treated with oral contraceptive pills.

| Standard | Treatment groups | | |
|---|---|---|---|
| | Control | T1 | T2 |
| Chol | 78-91 | 70-86 | 53-68 |

**Table 3.** The level of triglycerides in control and treated groups with oral contraceptive pills.

| Standard | Treatment groups | | |
|---|---|---|---|
| | Control | T1 | T2 |
| Trig | 61-71 | 69-80 | 43-57 |

**Table 4.** The level of lipoproteins in control and groups treated with oral contraceptives.

| Standard | Treatment groups | | |
|---|---|---|---|
| | Control | T1 | T2 |
| HDL | 37-43 | 28-33 | 25-32 |

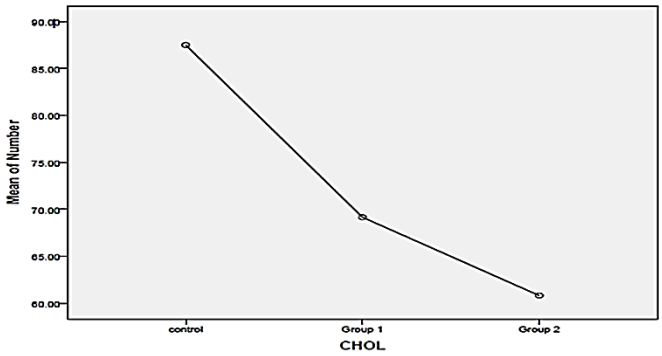


**Fig.2.** The difference in cholesterol level between control and treated groups with contraceptive pills.

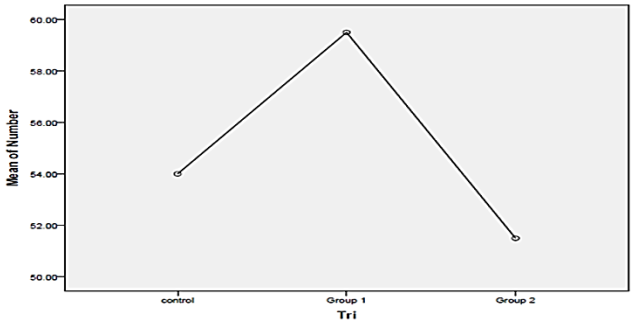


**Fig.3.** Differences in the level of triglycerides between studied groups with oral contraceptives.

days), and a significant increase in the weight of animals treated with contraceptive pills was recorded (Table 1) due to the imbalance of hormones. Previous studies have proven that the imbalance of hormones causes obesity in males or females (Basar & Avci 2021).

Increasing estrogen is led to weight gain in males. This hormone is present in males but in small quantities in the testicles and fat cells. Its proportion is balanced by testosterone, which is important in forming proteins and impacts the balancing process. The increase in weight as a result of taking female hormones through contraceptive pills and the decrease in movement and lethargy cause an increase in body temperature, and the testicles heat up due to the expansion of the veins around the testicle, which negatively affects spermatogenesis (Reyes & Farias 2012).

This manipulation of hormones in the male greatly also affects the change in the level of cholesterol and triglycerides (Tables 2 and 3, Figs. 2, 3). The increase in body fat is resulted from hormonal imbalance due to contraceptive pills (Table 4, Figs. 4) and a negative impact on the thyroid gland and metabolism (McMurray & Hackney 2005) and, consequently, lethargy and decreased movement and activity. This reduces high-density cholesterol levels (lipoproteins), disturbing their natural ability, especially on male sex hormones (Edwards & Li 2013). Estrogen has a role in building protein synthesis and increasing calcium concentration in the blood, and it is associated with cells to activate sensory receptors. Therefore, it is found in a small percentage of the male bloodstream.

The decreases in the level of the thyroid hormone T3, and the increase in T4 occurred as a result of

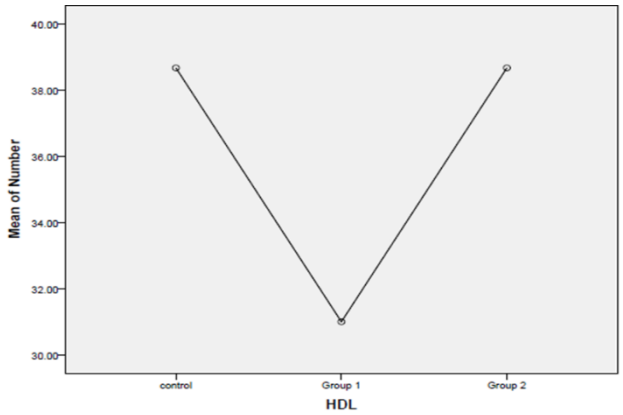


**Fig.4.** Differences in lipoproteins between the studied groups treated with contraceptive pills.

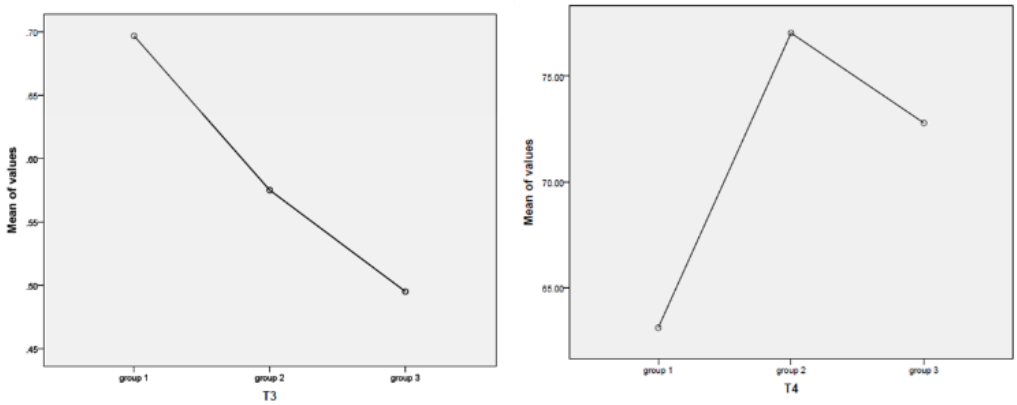


**Fig.5.** The differences between the studied groups treated with the contraceptive pill in (left) T3 and (right T4 hormones.

**Table 5.** The percentages of deformed sperms in the treated males.

| Groups | Normal sperms% | non-normal sperms% |
|---|---|---|
| control | 79-88 | 12-21 |
| T1 | 77-83 | 17-28 |
| T2 | 70-81 | 19-30 |

taking oral contraceptive pills to male rats (Fig. 5). This causes impaired proliferation of Leydig cells, a decrease in the production of male androgens, and reducing the level of the testicular hormone (von Deutsch et al. 2012). The low level of thyroid hormone directly affects changes in the testicles, negatively affecting its physiological process. The T3 hormone is necessary and important in the maturation of Sertoli cells and the initiation of the differentiation of mesenchymal cells into primary cells.

The T3 hormone is one of the components of the neurohormonal system that regulates the seasonal cycles of reproductive activity (Krajewska-Kulak & Sengupta 2013). The process of spermatogenesis is regulated by hormones starting from the hypothalamus gland that secretes stimulating hormone-releasing the luteinizing hormone, GnRH, which affects the pituitary to release the two hormones LH, FSH, as the latter stimulates the Leydig cells to produce the testicular lipoprotein hormone responsible for the production of sperm. The former stimulates the Sertoli cells to complete the process (Table 5).

The results also showed a significant reduction in the sperm and distortion sperm with a significant decrease in the hormone levels of the male ($P$<0.05) (Table 5). Sperm maturation is occurred by maturating the spermatids (Kaprara & Huhtaniemi 2018; Alabedi et al. 2021). The results showed a non-significant increase in the levels of FSH in the animals treated with the contraceptive compared to the control group, the reason for this difference is due to the lack of the hormone (Inhibit) secreted from

**Table 6.** The results of the hormonal analysis in control and groups treated with oral contraceptives.

| Parameter | Control group | | | | |
|---|---|---|---|---|---|
| | Testosterone | FSH | T3 | T4 | TSH |
| C1 | 0.61 | <0.10 | 0.76 | 61.78 | <0.05 |
| C2 | 0.90 | <0.10 | 0.77 | 58.22 | <0.05 |
| C3 | 0.83 | <0.10 | 0.56 | 69.35 | <0.05 |
| C4 | 0.99 | <0.10 | 0.70 | 58.20 | <0.05 |
| C5 | 0.90 | <0.10 | 0.66 | 60.19 | <0.05 |
| C6 | 1.1 | <0.10 | 0.77 | 67.40 | <0.05 |
| Group 1 dose 0.32mg | | | | | |
| A1 | 0.65 | <0.9 | 0.70 | 79.88 | 2.55 |
| A2 | 0.41 | <0.8 | 0.57 | 79.20 | <0.05 |
| A3 | 0.06 | <0.9 | 0.54 | 81.09 | <0.05 |
| A4 | 0.53 | <0.7 | 0.58 | 71.85 | <0.05 |
| A5 | 0.18 | <0.10 | 0.50 | 81.18 | <0.05 |
| A6 | 0.34 | <0.9 | 0.56 | 68.99 | <0.05 |
| Group 2 dose 0.64 mg | | | | | |
| B1 | 0.33 | <0.7 | <0.40 | 86.09 | <0.05 |
| B2 | 0.08 | <0.6 | 0.74 | 68.92 | <0.05 |
| B3 | 0.18 | <0.8 | <0.40 | 84.50 | <0.05 |
| B4 | 0.31 | <0.6 | <0.40 | 72.70 | <0.05 |
| B5 | 0.39 | <0.6 | 0.45 | 59.55 | <0.05 |
| B6 | 0.42 | <0.8 | 0.50 | 64.97 | <0.05 |

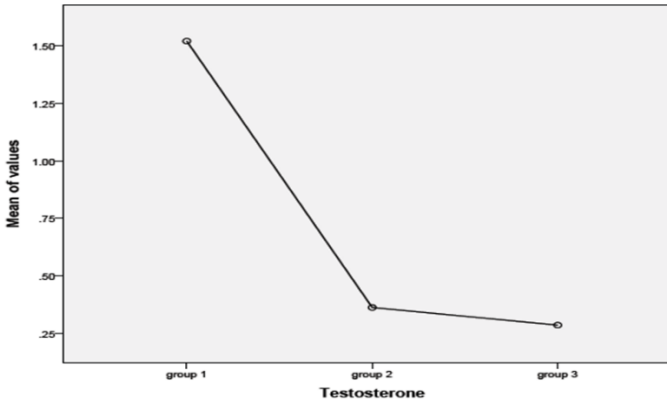


**Fig.6.** The differences that occurred between studied groups treated with contraceptive pills and its effect on the testosterone.

Sertoli, which is the main regulator of follicle hormone secretion and production. Thus, this may be because the contraceptive played an important role in lowering the level of the third thyroid hormone and raising the concentration of T4 (Wiegratz et al. 2003). It showed that an increase in progesterone and estrogen in male rats orally leads to a decrease in T3 and an increase in the level of T4 leads to a halt in the process of differentiation and proliferation of interstitial Leydig cells, and thus a decrease in the production of male androgens (Wagner et al. 2008; Bustani et al. 2021).

The results of the hormonal test clearly showed that the contraceptive given to males led to an overall decrease in the levels of the testicular fat hormone (Table 6) and a decrease in FSH (Fig. 6) due to the

decrease in the T3, which in turn caused a decrease in the level of luteinizing hormone and follicle-stimulating hormone. It can be concluded that the pills clearly affect males' fertility, causing a pathological hormonal imbalance that can negatively affect the ability of male fertilization.

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