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Effect of Age on the Levels of Growth Hormone, Leptin Hormone, And Some Biochemical variables among a number of Infertile Women in Mosul City .

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Abstract

The study included an analysis of the impact of aging on the levels of Growth Hormone and Leptin Hormone, and their significance as new indicators that increase the likelihood of infertility. The study also examined the correlation between these hormones and various hormonal and biochemical variables, including Follicular Stimulating Hormone (FSH), Luteinizing Hormone (LH), Lipid Profile, which measures the levels of Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein cholesterol (HDL-c), Low-Density Lipoprotein cholesterol (LDL-c), Very Low-Density Lipoprotein cholesterol (VLDL-c), and Body Mass Index (BMI). A total of 60 blood samples were collected from women with both primary and secondary infertility, and 20 blood samples were collected from healthy women as a control group. The samples were divided into two groups: the first group was based on Body Mass Index (BMI) and included four subgroups, each consisting of 10 samples, including the Control group, BMI 1 (19-24), BMI 2 (25-30), and BMI 3 (32) ≤ kg/m2. The second group was based on age and also included four subgroups, each consisting of 10 samples, including the Control group, Age 1 (17-27), Age 2 (28-37), and Age 3 (38-47) years.

All relevant information about the infertile and healthy women, such as name, age, height, weight, type of infertility, number of children, medical and genetic history, was included in the study. The study relied on the diagnosis of cases by obstetricians and gynecologists specializing in infertility from Al Batool Teaching Hospital, Al Khansaa Women's and Maternity Hospital, Al Salam Teaching Hospital, and some private clinics, based on clinical examination, biochemical laboratory tests, and ultrasound examination. Samples were collected from September 2022 to February 2023. The study showed the impact of aging on female fertility, as the results indicated a significant decrease in the levels of Growth Hormone (GH) and High-Density Lipoprotein cholesterol (HDL-c) at a probability level of ($P \leq 0.01$) with the lowest hormone levels found in the Age 3 group compared to the control group. The results also showed a significant increase in the levels of Leptin, FSH, LH, Total Cholesterol (TC), Triglycerides (TG), very-low-density lipoprotein cholesterol (c-VLDL), and Low-Density Lipoprotein cholesterol (LDL-c) at a probability level of ($P \leq 0.01$) compared to the control group.

Key words : BMI, , Leptin hormone , Growth hormone

Introduction

Infertility is defined as the inability to conceive or become pregnant within a year. Statistics indicate that one in every seven couples in the Western world suffers from infertility, and this rate increases in developing countries to one in every four couples. Infertility rates can reach up to 30% in certain regions of the world such as South Asia, some sub-Saharan African countries, the Middle East, Central and Eastern Europe, and Central Asia (1). Global estimates suggest that 8-12% of couples of reproductive age experience fertility issues. It has also been found that males are responsible for 20-30% of infertility cases, while females are involved in 50% of cases overall, with 10% attributed to unexplained infertility (2). There are two types of infertility: primary infertility, which refers to a woman who has never been pregnant before, and secondary infertility, which occurs when a woman has previously had a live birth, stillbirth, or miscarriage but is unable to conceive again (3). Delaying pregnancy

due to economic or other reasons reduces the chances of conception. When a woman is younger than 30 years old, she has an 85% chance of conceiving within one year, and this percentage gradually decreases as she reaches 30 years old, with a 75% chance of conceiving within 12 months. The chances of conception further decrease to 66% at age 35 and 44% at age 40, due to the effects of aging on the ovaries and eggs, as well as an increased risk of miscarriage in older women (4) (5) (6). The decline in growth hormone levels is associated with aging in women (7). Studies have shown a positive and proportional relationship between the concentration of Leptin hormone in serum and aging, as its levels are lower in youth and begin to increase with age and increased fat mass. This causes what is known as central and peripheral Leptin resistance, as evidenced by its failure to reduce food intake and increase metabolic rate, thereby promoting weight loss. This resistance is similar to insulin resistance (8). Aging has also been associated with an increase in fat mass due to a lack of physical activity, especially in age groups approaching menopause. This increase has been linked to an increase in the concentration of low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c), as well as triglycerides (TG), in addition to an increase in total cholesterol (TC) and a significant decrease in high-density lipoprotein cholesterol (HDL-c) (the "good" fats), which increases the risk of developing arterial sclerosis and heart disease (9). Estrogen hormone promotes the production of beneficial fatty proteins, and as age advances, the concentration of this hormone decreases, leading to an increase in harmful fatty proteins (10) (11).

The Aim is to study the effect of Age On the Levels of Growth Hormone, Leptin Hormone and several biochemical parameters in Some Infertile Women in Mosul City.

Materials and Methods

Blood Sample Collection

Blood samples were taken from both healthy and infertile women using a sterile syringe from the cubital vein . This was performed by specialized personnel on days 2-5 of the menstrual cycle after a continuous 12-hour fast to ensure the accuracy and success of the test results. The

collected samples were placed into clot activator gel tubes (Gell Tubes) and left for 15 minutes at room temperature or 5 minutes in a water bath. Subsequently, they were centrifuged at 5000 revolutions per minute for 10 minutes in a centrifuge to ensure complete separation of the serum, obtaining a pure serum. The serum was transferred using a sterile micropipette and placed in sterilized Eppendorf tubes. It was stored by freezing at -20 degrees Celsius until the required tests were conducted.

The blood samples were collected from various hospitals in Nineveh Province, including Al-Batool Teaching Hospital, Al-Salam Teaching Hospital, and Al-Khansaa Women's and Maternity Hospital, within the scheduled period. The collection process was carried out under the supervision of specialized gynecologists .

Study groups :

1. Control group: Included (10) samples of healthy women aged between(17-27) year within Body mass index (BMI) (19-26) Kg/m^2 .
2. Age 1 group : Included (10) samples of infertile women aged between (17-27) year within Body mass index(BMI) (19-26) Kg/m^2 .
3. Age 2 group : Included (10) samples of infertile women aged between (28-37) year within Body mass index (BMI) (19-26) Kg/m^2 .
4. Age 3 group : Included (10) samples of infertile women aged between (38-47) year within Body mass index (BMI) (19-26) Kg/m^2 .

Serum hormonal tests :

Determination of Growth Hormone Concentration in Blood Serum

Basic Principle

The concentration of growth hormone efficiency was estimated using several processed analyses prepared by DRG International Inc. Elisa (ELISA) Enzyme-Linked Immunosorbent Assay. This method is based on an enzyme immune system associated with the double-body

antibody. By competing between the antibodies found in the drilling of the micro-fouling chip and the antibodies associated with the horseradish peroxidase on the site of association with the growth hormones found in the serum. After washing with an orderly washing solution to remove unconnected antibodies, the base substance solution for the hormone associated with a blue solution is added directly to the yellow solution after the stop solution is added. The severity of the color is perpendicular to the concentration of the hormone antigens in the serum, for which the absorption is measured at 450 nanometers, and the final results were obtained from the ELISA system after comparing them with the standard curve values installed in the device.

(12) (13) (14) .

Determination of Leptin Hormone Concentration in Blood Serum

Basic Principle :

The concentration of the Leptin hormone efficiency using several processed analyses by BT LAB is estimated to be based on an Enzyme-linked Immunosorbent Assay system associated with the double antibody. After washing with the orderly washing solution, the antibodies found in the micro-fouling and the antibodies associated with HRP are competitively combined to form a blue-colored solution that converts to yellow after the addition of the Stop Solution. The severity of the color is proportional to the concentration of the hormone antigens in the serum for which the absorption is measured at wavelength (450 nanometers) and the final results were obtained from the ELISA system after comparing it with the standard curve values installed in the device.

Determination of (FSH) Follicle Stimulating Hormone Concentration in Blood Serum

Basic Principle :

The concentration of the hormone (FSH) in the blood serum was estimated using the German-born Cobas e411 device, which relied on the mechanical method using the Electrochemilumescence technique (ECL).

Determination of serum (LH) Luteinizing Hormone Concentration in Blood Serum

The concentration of Luteinizing hormones (LH) in the blood serum was estimated using the German-born Cobas e411 device, which relies on the mechanical method using the Electrochemilumescence technique (ECL).

Determination of serum lipid profile concentrations in blood serum :

Blood samples were analyzed using the Auto Chemical Analyzer from Respons 920 of German origin using Reflectance Spectrophotometry to measure the concentration of each of the following tests: Total cholesterol (TC),Triglycerides (TG) , High density lipoproteins (HDL-c), Low density lipoproteins (LDL-c) , and Very low density lipoproteins (VLDL-c) .

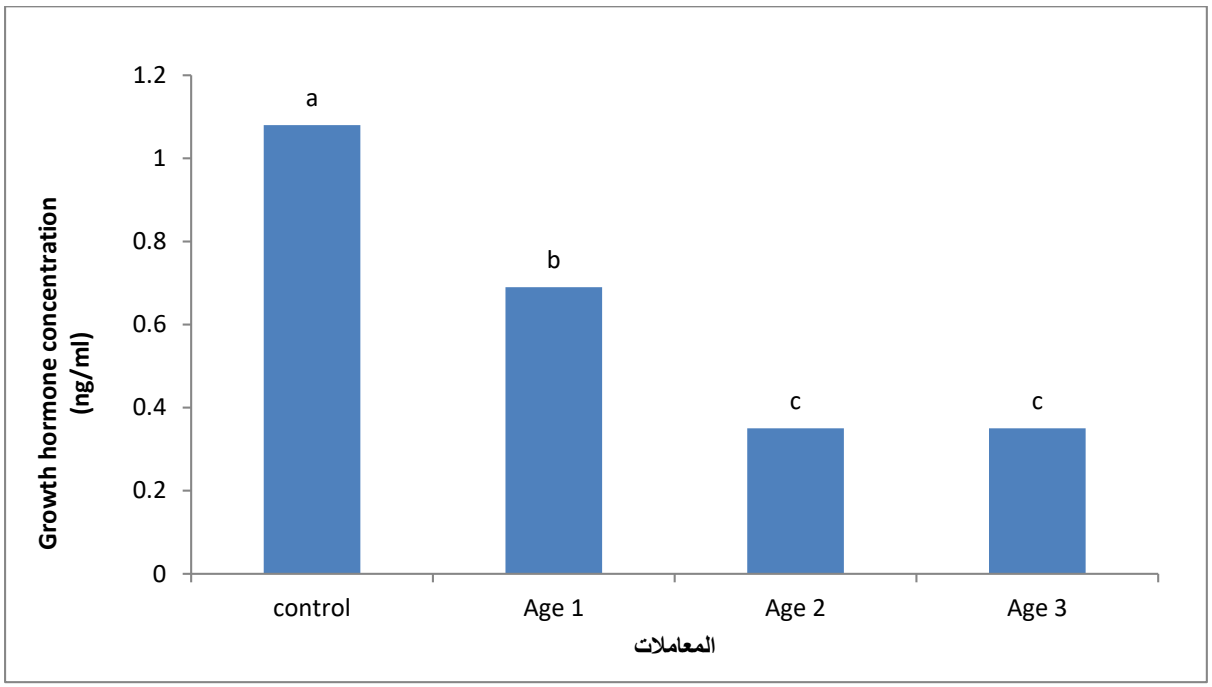
Statistical Analysis

The data analysis was conducted using the Simple Experiments design in the SPSS software, version 16. This analysis aimed to calculate the mean and standard error. A completely random method was employed. Different letters were used to denote significant differences among the variables at the 1% and 5% significance levels based on the Duncan test, a multiple-range test . (Duncun , 1955)

Results and Discussion

Effect of Age on Growth hormone concentration in Infertile women

Figure 1 illustrates the impact of Age on Growth hormone concentration. The results show a significant decrease in hormone concentration at a probability level of ($P \leq 0.01$) in the Age 3 group (0.35 ± 0.05 ng/ml) compared to the Control group (1.08 ± 0.03 ng/ml). Similarly, the results indicate a significant decrease in the Age 2 group (0.38 ± 0.07 ng/ml) and the Age 1 group (0.69 ± 0.01 ng/ml) compared to the Control group knowing that no significant different between Age 2 and Age 3 .



The values are expressed as the mean (±) standard deviation, with sample sizes of 10 in each group. Different letters associated with the figures indicate statistically significant differences at a probability level of ($P \leq 0.01$).

Figure 1 illustrates the effect of Age on the concentration of Growth hormone (ng/ml) in the following groups: Control, Age 1, Age 2, and Age 3.

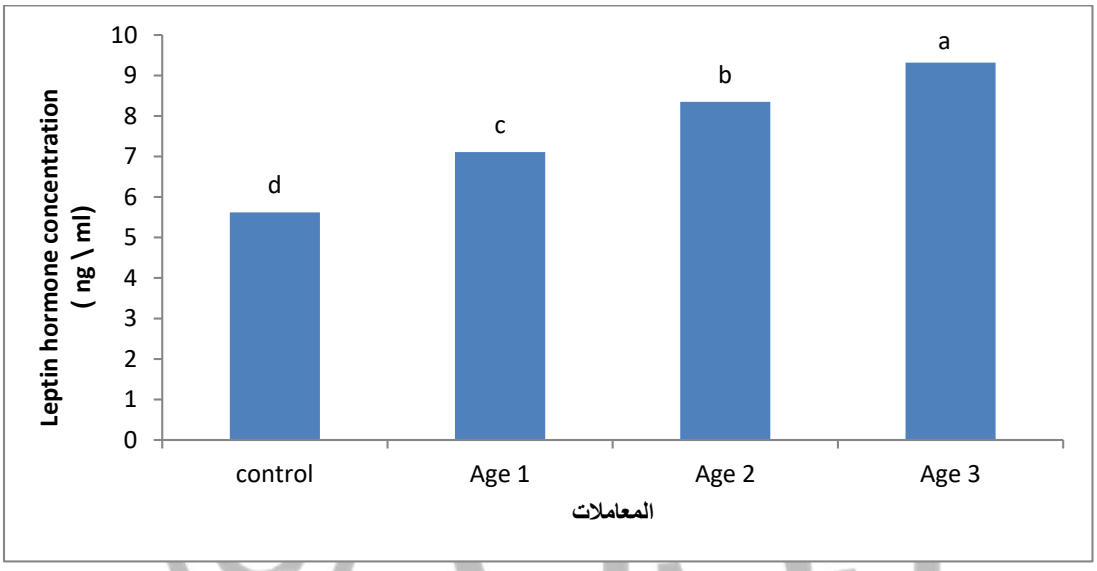
Upon analyzing the results and conducting statistical assessments, a pronounced and statistically significant decrease in Growth hormone concentration is evident with Age . This phenomenon underscores the adverse impact of getting older on Growth hormone concentration in infertile women when compared to the control group , Studies have shown that growth hormone is secreted from the pituitary gland after receiving stimulating signals, which is the hormone GnRH from the hypothalamus gland. The secretion of GnRH decreases with age, leading to a decrease in stimulating signals for the production of growth hormone and consequently a decrease in hormone levels as age advances (16). Due to the decrease in negative feedback regulation, the growth hormone stimulates the release of insulin-like growth factor 1 (IGF-1) from the liver and other tissues. As

age advances, tissue responsiveness to growth hormone and the regulatory negative feedback loop controlling growth hormone secretion becomes less effective, leading to a reduction in hormone secretion (17). Studies have confirmed that the decrease in growth hormone levels coincides with a decrease in the concentration of IGF-1, and both play a significant role in women's reproductive function, including the activation of primordial follicles, follicular development, Steroidogensis, ovulation, embryo implantation. Additionally, growth hormone enhances the response of granulosa cells to gonadotropic signals by regulating the expression of gonadotropic receptors, indicating an interaction between ovarian regulation and pituitary signals. Furthermore, growth hormone acts as an anti-aging agent at the cellular level, promoting cell repair, reducing oxidative stress, aiding in oocyte fertilization and embryo development, improving implantation and pregnancy rates, and enhancing immune response. This explains the decrease in fertility as women age. (18).

(19) confirmed in a study involving samples of infertile women with PCOS in China, that the concentration of growth hormone is inversely proportional to the decline in ovarian reserve in women as they age. Growth hormone also affects egg quality and the process of replacing eggs that are not suitable for ovulation. The results also agree with (18) and (20), who emphasized the role of growth hormone in directly controlling multiple reproductive functions, including the activation of primordial follicles, follicular development, ovarian Steroidogensis, oocyte maturation, and embryo implantation. Other studies have indicated that growth hormone enhances the response of granulosa cells to gonadotropic signals by regulating the expression of gonadotropic receptors (follicle-stimulating hormone receptor and luteinizing hormone receptor), suggesting an interaction between ovarian regulation and the endocrine signaling system (7)(21).

Effect of Age on Leptin hormone concentration in Infertile women

Figure 2 illustrates the effect of Age on Leptin hormone concentration The results show a significant increase in hormone concentration at the level of $P \leq 0.01$ in the Age 3 group (9.3 ± 0.7 ng/ml) compared to the Control group (5.6 ± 0.8 ng/ml). Additionally, a significant increase in hormone concentration was observed in the Age 2 group (8.3 ± 0.8 ng/ml) and the Age 1 group (7.1 ± 0.8 ng/ml) compared to the Control group.



The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of $p \leq 0.01$.

Figure 2 The effect of aging on Leptin hormone concentration (ng/ml) in the Control, Age 1, Age 2, and Age 3 groups.

Figures 2 show the relationship between age progression and its effect on Leptin hormone concentration in the blood serum, indicating a significant increase in hormone concentration in infertile women across different age groups compared to the control group. This increase in hormone concentration negatively affects a woman's fertility as she ages and is considered a vital indicator of a woman's fertility. The reason for the elevated Leptin hormone concentration may be attributed to the increase in fat levels, particularly in women approaching menopause, in addition to an increase in blood insulin and insulin resistance. This

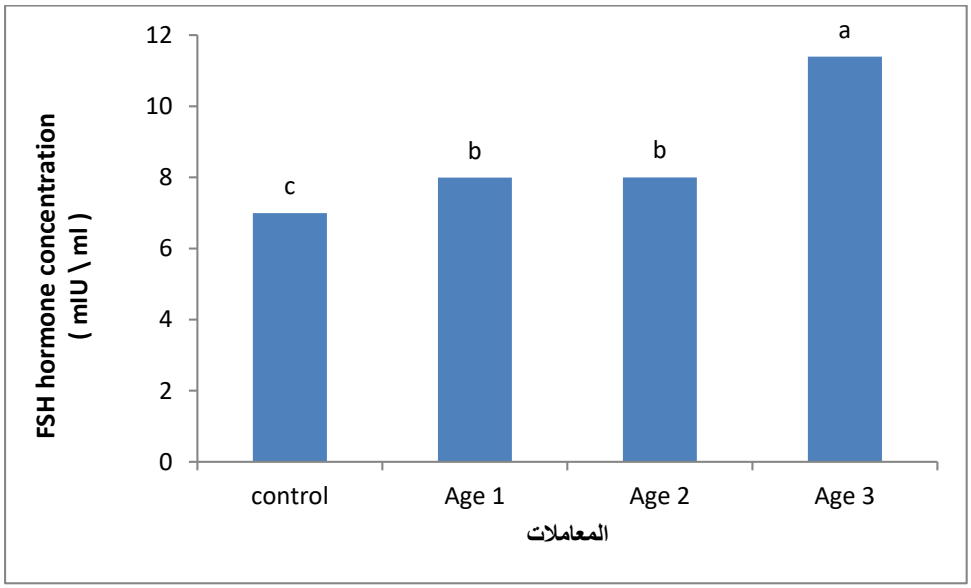
aligns with the findings of (22), as their results demonstrated a correlation between aging, increased fat mass, and central and peripheral Leptin resistance, as evident from their failure to reduce food intake, increase metabolism, and consequently stimulate weight loss. Leptin resistance is a common feature of aging and obesity. The results also align with a study by(23) and (24), which indicated an inverse relationship between the levels of Leptin hormone and anti-Mullerian hormone (AMH), a vital marker for the quality and quantity of ovarian follicles. A decrease in AMH is associated with aging and approaching menopause in women in general, and infertile women at younger ages in particular. Leptin inhibits the formation of ovarian steroids and the development of ovarian follicles.

Furthermore, a study conducted in the United States by (25) found an inverse relationship between leptin hormone and aging, with the hormone being found at higher levels during menopausal stages compared to the fertility stage. In a study conducted in China by (26), it was observed that as women aged and approached menopausal age, there was an increase in central fat distribution in the abdominal region, leading to an elevation in Leptin hormone levels secreted by fat cells, resulting in Leptin resistance (27). In another study, it was demonstrated that elevated levels of Leptin hormone have negative effects on a woman's fertility in all aspects. This increase was associated with higher concentrations of Prolactin hormone, Testosterone, FSH\LH ratio, and a decrease in estradiol and FSH hormone levels in the unexplained infertility (UI) group compared to healthy women (28). These findings are consistent with previous studies (29)(30) that showed a negative correlation between high Leptin hormone levels and decreased fertility in women as they age, due to increased fat deposition in the abdominal region resulting from the natural decline in estrogen hormone levels with age, and gradually approaching menopausal age, negatively impacting the balance of sex hormones.

Biochemical variables :

Effect of Age on FSH concentration in Infertile women

Figure 3 illustrates the effect of Age on FSH concentration , The significant increase in hormone concentration was evident in Age 3 group (11.39 ± 1.01 mIU/ml) with a probability level of $P \leq 0.01$ compared to the Control group (6.99 ± 0.83 mIU/ml). Similarly, the results showed a significant increase in FSH hormone concentration in Age 2 group (7.99 ± 0.91 mIU/ml) and Age 1 group (7.99 ± 0.85 mIU/ml) compared to the Control group.



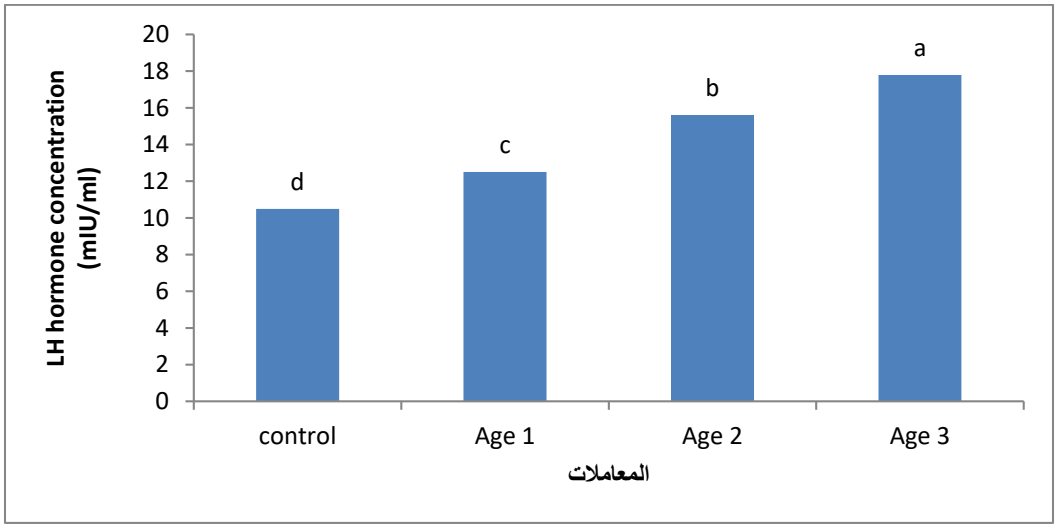
The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of $p \leq 0.01$.

Figure 3 The impact of age on the concentration of the FSH hormone (mIU/ml) in the Control, Age 1, Age 2, and Age 3 groups.

Effect of Age on LH concentration in Infertile women

Figure 4 illustrates the effect of Age on LH concentration ,The significant increase in hormone concentration was evident in the Age 3 group (17.78 ± 1.04 mIU/ml) with a probability level of $P \leq 0.01$ compared to the Control group (10.49 ± 0.71 mIU/ml) Similarly, a significant increase in hormone concentration was observed

in the Age 2 group (15.61 ± 1.41 mIU/ml) and the Age 1 group (12.50 ± 1.56 mIU/ml) compared to the Control group.



The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of $p \leq 0.01$.

Figure 4 The impact of age on the concentration of the LH hormone (mIU/ml) in the Control, Age 1, Age 2, and Age 3 groups.

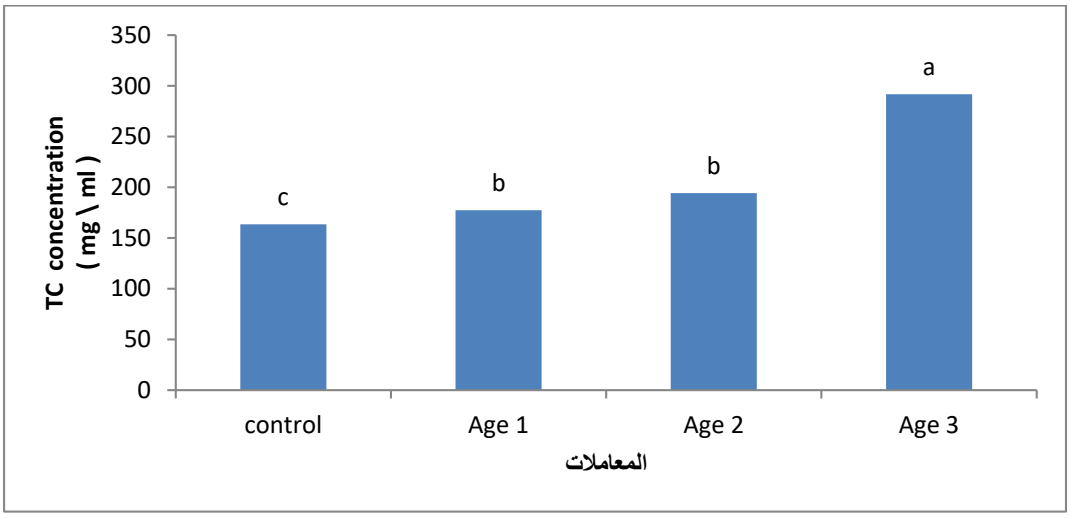
Figure 3 and 2 illustrates the impact of age on the concentration of FSH and LH hormones is clearly evident, showing a significant increase in hormone levels at a probability level of $P \leq 0.01$ as women affected by infertility age, compared to healthy women. These results are consistent with a study by (31) that included samples of infertile women aged between (17-54) years, where an increase in the levels of ovulation and follicle-stimulating hormones was observed as women advanced in age, reducing their likelihood of conception. Additionally, ovarian reserve (OR) depletion, as indicated by the Anti-Mullerian Hormone Test (AMH), was found to have an inverse relationship with decreased AMH levels and increased FSH and LH hormone levels, as well as the FSH/LH ratio, reflecting the age and reproductive function in females. Several studies have confirmed the natural increase in FSH and LH hormone concentrations in women with regular menstrual cycles as they age, with a significant rise observed after the age of forty,

indicating a clear decline in fertility, which may start early in some women (32). These findings are also in agreement with (33) a study confirmed a gradual increase in the concentration of FSH hormone and a decrease in AMH hormone with advancing age. Additionally, the study by (34) demonstrated an increase in FSH hormone concentration and its inverse relationship with decreased estrogen levels, which naturally decrease as women age, leading to menopause, increased adiposity, and bone fragility (35). A study in China showed an association between elevated FSH hormone levels in the pre-pregnancy stage, weakening ovarian functions and egg quality, and increasing the risk of miscarriage and premature births (36). Furthermore, (37) affirmed that a decrease in the sex hormone with age leads to decreased fertility. Although the mechanism linking sex hormones to fertility remains unclear, it is believed that nerve cells expressing GnRH may produce and respond to sex hormones, influencing the reproductive gland in the anterior part of the pituitary gland responsible for secreting FSH and LH hormones. These findings align with the study by (38), which included samples of infertile women from Najaf city in Iraq, demonstrating a positive correlation between elevated FSH hormone levels and age advancement in infertile women, leading to hormonal imbalance and hindering the ovulation process. The results also correspond with (39) through a study of women with primary infertility in Sulaymaniyah city in Iraq, showing a significant increase in FSH and LH hormone levels as infertile women age, particularly in the age range of (26-37) years, compared to healthy women, indicating a clear decline in fertility with age. The increase in LH levels is attributed to the decreased quality and quantity of eggs as women age, indicating ovarian dysfunction, while the increase in FSH levels points to poor follicle development and the occurrence of anovulatory cycles.

Effect of Age on Lipid profile concentrations in infertile women

Effect of Age on Total cholesterol concentration.

The figure 5 shows the effect of age on total cholesterol concentration, with a significant increase in TC concentration observed in Age 3 group (291.80 ± 1.1 mg/dl) at a probability level of P ≤ 0.01) compared to the Control group (163.50 ± 3.4 mg/dl) . The results also indicate a significant increase in TC concentration in Age 2 group (194.30 ± 2.4 mg/dl) and Age 1 group (177.40 ± 3.7 mg/dl), with no significant difference between Age 1 and Age 2 groups.

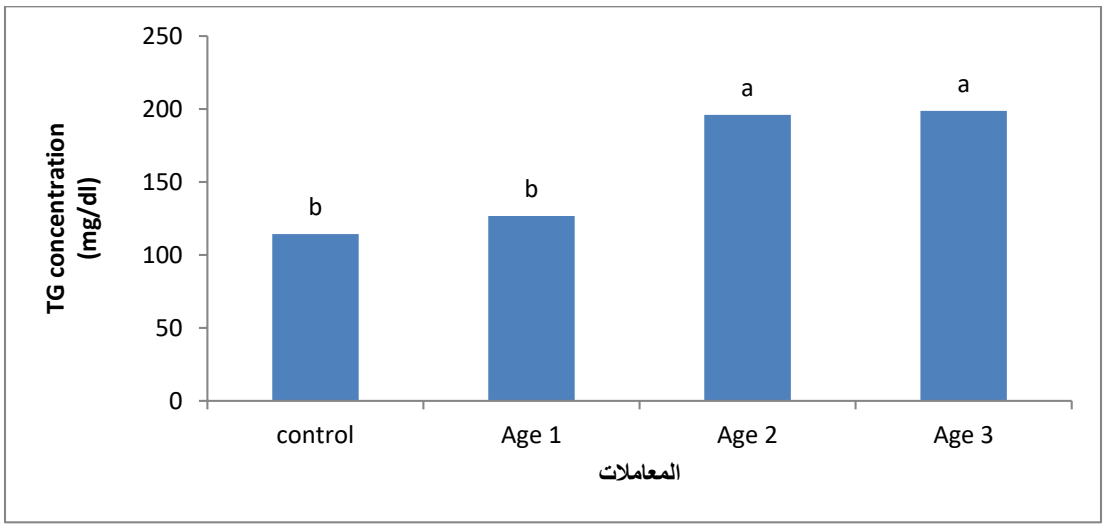


The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of p ≤ 0.01.

Figure (5)The effect of age on total cholesterol concentration (mg/dl) in the Control, Age 1, Age 2, and Age 3 groups.

Effect of Age on Triglycerides concentration.

Figure (6) Clearly shows the effect of age on triglycerides concentration, with a significant increase in TG concentration at a probability level of P ≤ 0.01 in the Age 3 group (198.70 ± 3.4 mg/dl) and the Age 2 group (196.00 ± 3.4 mg/dl) compared to the Control group (114.30 ± 1.3 mg/dl), and no significant difference between the Age 1 group (126.70 ± 8.1 mg/dl) and the Control group.

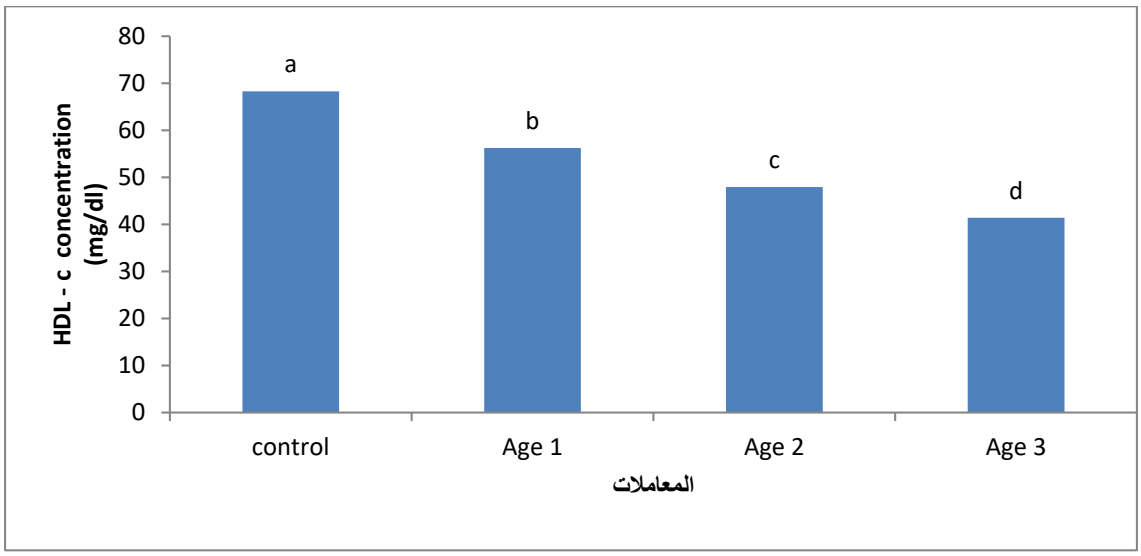


The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of $p \leq 0.01$.

Figure (6)The effect of age on Triglycerides concentration (mg/dl) in the Control, Age 1, Age 2, and Age 3 groups.

Effect of Age on (HDL _c) concentration

Figure (7) Shows the effect of age on high-density lipoprotein cholesterol (HDL-c) concentration, indicating a significant decrease in HDL-c concentration at a probability level of $P \leq 0.01$ in the Age 3 group (41.43 ± 2.79 mg/dl) compared to the Control group (68.30 ± 5.90 mg/dl), as well as a significant decrease in the Age 2 group (47.94 ± 3.89 mg/dl) and the Age 1 group (56.24 ± 4.25 mg/dl) compared to the Control group.

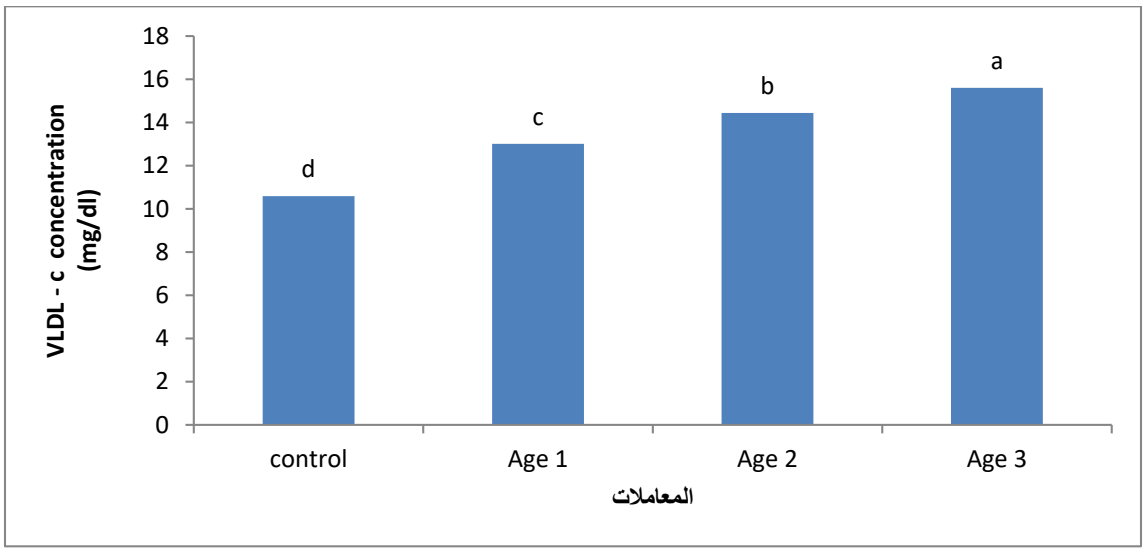


The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of $p \leq 0.01$.

Figure (7)The effect of age on HDL-c concentration (mg/dl) in the Control, Age 1, Age 2, and Age 3 groups.

Effect of Age on (VLDL _c) concentration

The figure (8) demonstrates the effect of age on very low-density lipoprotein cholesterol (VLDL-C) concentration, showing a significant increase in VLDL-C concentration at a probability level of $P \leq 0.01$ in the Age 3 group (15.60 ± 0.9 mg/dl) compared to the Control group (10.59 ± 0.7 mg/dl). Additionally, it also shows a significant increase in the Age 2 group (14.44 ± 1.0 mg/dl) and the Age 1 group (13.01 ± 0.20 mg/dl) compared to the Control group.

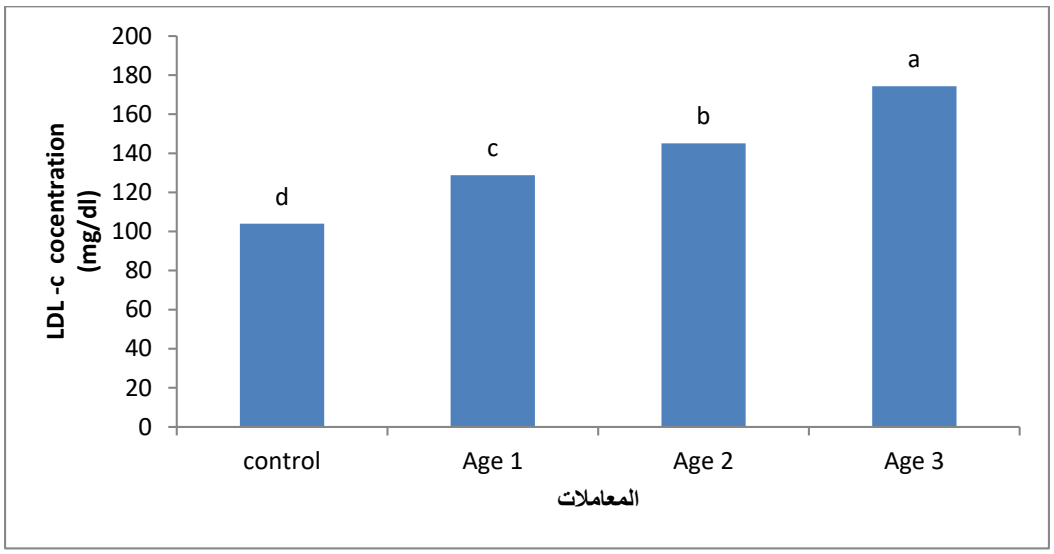


The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of $p \leq 0.01$.

Figure (8)The effect of age on (VLDL-c) concentration (mg/dl) in the Control, Age 1, Age 2, and Age 3 groups.

Effect of Age on (LDL _c) concentration

The figure (9) illustrates the effect of age on low-density lipoprotein cholesterol (LDL-c) concentration, indicating a significant increase in LDL-c concentration at a probability level of $P \leq 0.01$ in the Age 3 group (174.30 ± 1.1 mg/dl) compared to the Control group (104.00 ± 2.4 mg/dl). It also shows a significant increase in the Age 2 group (145.10 ± 1.0 mg/dl) and the Age 1 group (128.80 ± 2.8 mg/dl) compared to the Control group.



The values are expressed as the mean (±) standard deviation, and the sample size is 10. Different letters associated with figures indicate statistically significant differences at a significance level of $p \leq 0.01$.

Figure (9)The effect of age on (LDL-c) concentration (mg/dl) in the Control, Age 1, Age 2, and Age 3 groups.

The results shown in Figures (5), (6), (7), (8), and (9) showed the effect of age on the concentration of (TC) , (TG), (HDL-c), (VLDL-c), and (LDL-c), as the results show a significant increase in the levels of TC, TG, VLDL-c, and LDL-c in infertile women as they grow older, at a probability level $P \leq 0.01$ compared to the control group, and the decrease in HDL-c level appears as the woman gets older at a probability level of $P \leq 0.01$ compared to the control group. The results are consistent with (10) through the study conducted to compare the level of fats in women in the fertile stage and the premenopausal stage, it was observed that changes in hormone levels, especially estrogen, gradually affect the balance of fats and metabolism in the body. This is associated with a decrease in the concentration of c-HDL and an increase in TC and LDL-c. Additionally, a study by (34) showed that the increase in FSH hormone concentration is negatively correlated with the decrease in estrogen levels, which naturally decrease as women age and reach menopause, causing disturbances in fat metabolism. Treatment with periodic oral estrogen sulfate may help stimulate the secretion of HDL-C, causing

positive effects, as it plays an important role in Reverse Cholesterol Transport and cellular cholesterol homeostasis (40). Additionally, hepatic lipase activity decreases due to hormonal changes (41). The reason for the increase in triglycerides may be attributed to the decrease in Spexin hormone, which gradually decreases with age, as demonstrated in a study by (42), showing the inverse relationship of Spexin with age, body mass, fat metabolism, and triglyceride levels.

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