Al-Qadisiyah Journal of Pure Science

ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514

DOI: 10.29350/jops

Al-Qadisiyah Journal of Pure Science

Effect of Frequent Use of Ovulation Stimulants on Total Protein, Total Cholesterol, LDL and VLDL

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Abstract

Background: Letrozole is an aromatase inhibitor that affects the ovaries, breasts, adipose tissue, and bone by preventing androgens from being converted to estrogens in the final step. The effects of estrogen are significantly lessened by this process. Estrogens have a significant impact on total protein, total cholesterol, LDL, and VLDL via regulating the metabolism of lipids and lipoproteins. FSH stimulation is used to induce ovulation in women who are not pregnant and can help individuals with pituitary dysfunction. In vitro fertilization produces more follicles when regulated stimulation of ovarian hyperstimulation is provided.

The aim: To assess the difference in the level (total protein, total cholesterol, LDL, and VLDL) of infertility treatment among the three study groups, the group that received HSF, the group that took LET, and the control group.

Material and Method: A research project was carried out from October 2022 to March 2023. Ninety women were engaged, split into three groups: those who received continuous injections of FSH for more than a month, those who took LET constantly for more than a month, and the control group, which did not use any medication to stimulate ovulation.

Results: Total Protein concentration showed no significant difference from the control group. However, there was a significant difference between the LET group and the control group, with the LET group's Additionally, there was a notable distinction between FSH and LET. Total cholesterol content measured during using of FSH and LET compared to the control group. FSH and LET did not differ significantly from one another. LDL cholesterol concentrations measured during the third month using FSH and LET compared to the control group. Additionally, there was a notable distinction between FSH and LET. VLDL cholesterol concentration measured for the third month using FSH and LET compared to the control group. FSH and LET did not differ significantly from one another

Keywords: *Ovulation; HSF; Letrzole; Total protein; Total cholesterol; LDL, VLDL*

1. Introduction

The long-term existence of mankind depends on its capacity for reproduction. But after engaging in unprotected sexual activity for a year or more, infertility can result from a variety of reproductive issues that impact both male and female partners [1]. Primary infertility refers to a couple who has never conceived, whereas secondary infertility is a pair that has conceived before but is having difficulty conceiving again [2].

Infertility in women is caused by a number of reasons, including sexually transmitted infections like syphilis and gonorrhea. The rising incidence of infertility has also been attributed to diseases including diabetes, hypertension, hypothyroidism, obesity, and addiction in young people [3]. It is significant to remember that women account for almost half of instances of infertility; ovulation problems, fallopian tube problems, endometriosis, and inexplicable reasons are among the contributing variables [4].

Women who are infertile have access to a wide range of treatment options, such as medication, surgery, assisted reproductive technology, and lifestyle changes. Superovulation and ovulation induction are the two main therapies for infertility. In cases of other forms of infertility, ovulation boost or controlled ovarian hyper stimulation is used to increase the number of follicles. However, the main objective of treatment for anovulatory women is to promote the creation of at least one follicle [5][6].

In order to pinpoint specific reasons and choose the best course of action, the first step in treating infertility is evaluation. Although a complete medical history and physical examination yield important information, ovulation triggers are frequently required. Among the medications that are most frequently used to induce ovulation are gonadotropins, Letrozole (LET), and clomiphene citrate [7].

For the treatment of women who are infertile owing to anovulation, clomiphene citrate has historically been the recommended medication [8]. However, because it stimulates the ovaries, using it might raise the likelihood of producing twins. It is important to exercise caution since excessive stimulation of the ovaries might result in medical intervention. To lower the chance of these adverse effects, Clomid should not be used again or the dosage raised without first talking to a doctor. LET has become a popular substitute for Clomid [8].

As an aromatase inhibitor, LET primarily affects the last stage of androgen-to-estrogen conversion. It reduces the availability of estrogen in a number of organs and tissues, such as the ovaries, breasts, adipose tissue, and bone, by inhibiting the aromatase enzyme by an average of 80 to 90%. LET dramatically lessens the effects of estrogens, making this mechanism especially critical when ovarian stimulation is involved. The regulation of fat and lipoprotein metabolism is largely dependent on estrogen. They have an impact on how these drugs are produced, used, and eliminated by the body. Thus, the markers of total protein, total cholesterol, low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) in the body may be impacted by the reduction in estrogen levels brought about by FSH, and LET treatment [9][10].

Lipid metabolism may alter as a result of LET as it decreases estrogen availability. Modulating the action of estrogen on fat and lipoprotein metabolism may lead to dysregulation of cholesterol markers [9]. To fully comprehend the impact of LET on lipid profiles and the consequences for metabolism in general, more study is required.

Follicle Stimulating Hormone (FSH) is also very beneficial for patients who do not have normal pituitary function and who require assistance in reaching ovulation. FSH has also been used to treat normally ovulating women who have not gotten pregnant with conventional techniques in addition to IUI. In this instance, FSH stimulation and intrauterine insemination are utilized to increase the amount of eggs that ovulate and, consequently, the likelihood of conception [11]. FSH is also used to achieve regulated ovarian hyper stimulation, which raises the quantity of follicles generated during in vitro fertilization. The fluid-filled sacs called folicles are where eggs develop [12].

Overall, LET's therapeutic success in a variety of diseases is attributed to its mode of action as an aromatase inhibitor and its capacity to decrease estrogen availability. It is important to take into account and vigilantly observe any possible impact on lipid markers when undergoing LET therapy.

2. Study Features

2.1 Total protein

Proteins are essential for the health, growth, and development of the body since they are the building blocks of all cells and organs. Two different kinds of proteins, albumin and globulin, have different functions in the circulation. By keeping blood vessels intact, albumin helps stop fluid from seeping out of blood arteries. Conversely, globulin proteins have a variety of roles in the immune system's operation [13].

Testing can determine the total protein level as well as the ratio of albumin to globulins. It is possible to utilize the A/G (albumin/globulin) ratio as a marker for possible underlying medical problems [14]. Protein-energy waste (PEW), which happens when the body's protein and energy stores decrease, frequently as a result of malnutrition, can be detected by measuring total serum proteins (TSP) [15].

TSP measurement is included in tests for kidney function and may also be used as a test for liver function, which helps diagnose liver diseases. Reduced TSP levels are commonly linked to disorders involving both renal and liver malfunction [16][17].

In the body, proteins are essential for tissue growth and repair. They serve as transporters for different chemicals entering the body and as enzymes, promoting chemical processes. Furthermore, proteins work as antibodies, supporting the immune system's proper operation and aiding in the body's fight against infections [18].

2.2 Total Cholesterol (TC)

After consumption, the liver and small intestine are principally responsible for the synthesis of total cholesterol, a form of lipid [17]. The body uses cholesterol as a precursor to produce hormones, vitamin D, bile acids, and a host of other chemicals. Despite moving throughout the bloodstream, cholesterol cannot move by itself. Rather, LDL and VLDL are the two primary lipoprotein subtypes that carry it through the blood [19].

A certain amount of cholesterol may build up and deposit itself in the artery walls when there is an overabundance of it in the blood. Plaque is formed over time by cholesterol deposits. Atherosclerosis, sometimes called hardening of the arteries or atherosclerosis, is a disorder where blood artery flexibility is decreased and restricted by plaque accumulation [20]. This raises the risk of cardiovascular illnesses by causing problems and disrupting normal blood flow. Maintaining cardiovascular health requires controlling cholesterol levels, especially the ratio of LDL to VLDL.

2.3 Low-Density Lipoproteins (LDL)

One of the four main types of lipoproteins is LDL. Its primary job is to move cholesterol from the liver to different parts of the body so that it may be absorbed and integrated into cell membranes. On the other hand, because LDL may build up in artery walls and induce atherosclerosis when present in excess, it is sometimes referred to as "bad" cholesterol. Since lower LDL cholesterol lowers the incidence of plaque development and associated cardiovascular problems, lower LDL cholesterol is generally thought to be good for vascular health [21] [22].

2.4 Very Low-Density Lipoproteins (VLDL)

Very low-density lipoprotein, or VLDL, is a specific kind of lipoprotein that the liver makes. It helps carry lipids and cholesterol through the bloodstream's aqueous environment. Triglycerides, cholesterol, and apolipoproteins combine in the liver to create VLDL. VLDL changes and becomes LDL when it travels through the circulation. Lipids are transported throughout the body via LDL, which makes it easier for lipids to reach different tissues and cells [21].

3. Methodology

Ninety women in all took part in this study between October 2022 and March 2023. During patient visits to the clinic or hospital, patient-brought paperwork were reviewed and oral interviews were conducted to get accurate information on the Women and Children Hospital in Al-Qadisiyah Governorate.

There were three groups of women, each with forty-five individuals. Women who took LET consistently for longer than a month made up the first group. Group 2 comprised ladies who received FSH injections on a regular basis for over a month. Thirty women made up the third group, often known as the control group, and they did not use any medication to promote ovulation. The age range of the ladies was 20 to 39.

Gel tubes were used to collect blood samples, which were then allowed to clot at room temperature for thirty to sixty minutes. After that, the tubes were kept in the main blood bank at -80 °C until they could be retrieved later, and they were centrifuged for 15 minutes at 3000 rpm.

3.1 Measurement of Lipid profile

A. Principle

The Sandwich-ELISA technique is employed by ELISA kits. An antibody that specifically targets TC has already been coated on the Microelisa strip plate that is included in the package. Standards or samples are combined with a particular antibody and applied to the proper wells on the Microelisa strip plate. Then, each well on the Microelisa strip plate receives an addition of a Horseradish Peroxidase (HRP)-conjugated antibody that is specific for TC, and the plate is incubated. After that, any loose parts are cleaned up.

Each well then receives an addition of TMB substrate solution. The only wells that will be blue are those that contain (TC) and HRP-conjugated TC antibody; these wells will become yellow when the stop solution is added. At 450 nm in wavelength, the optical density (OD) of the wells is determined with a spectrophotometer. The OD value and the TC concentration are exactly proportionate.

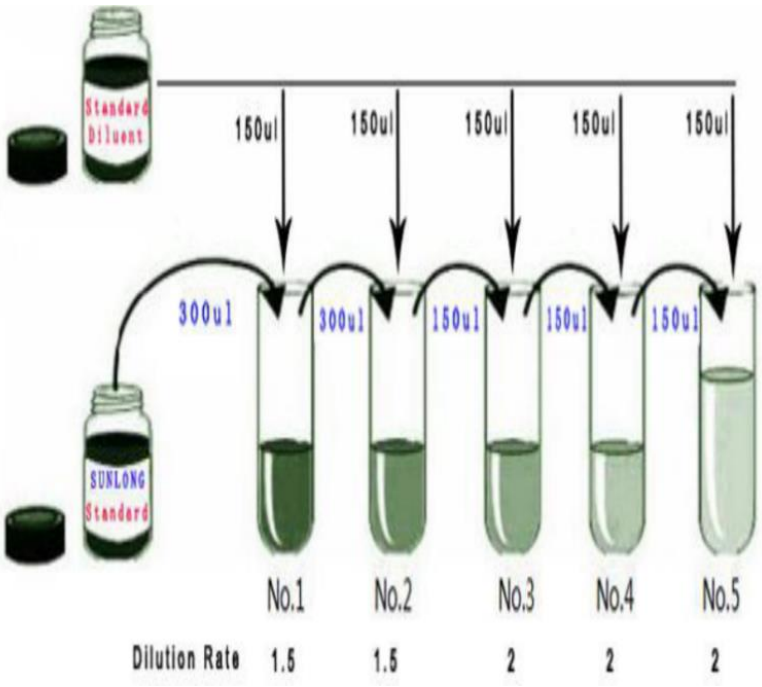
The OD of the samples is compared to the standard curve that comes with the kit in order to determine the concentration of TC in the samples. The concentration of TC in the samples may be determined by consulting the standard curve.

B. Procedure

Prior to pipetting the volume of 50ul from each tube into the microplate well—each tube utilizes two wells, for a total of ten wells—dilute the standard using tiny tubes.

Table 1. Dilution of Standards

1500µmol/L	**Standard No.1**	**300µl Original Standard + 150µl Standard diluents**
1000µmol/L	Standard No.2	300µl Standard No. 1 + 150µl Standard diluents
500µmol/L	Standard No.3	150µl Standard No. 2 + 150µl Standard diluents
250µmol/L	Standard No.4	150µl Standard No. 3 + 150µl Standard diluents
125µmol/L	Standard No.5	150µl Standard No. 4 + 150µl Standard diluents



TC: 2250µmol/L 1500µmol/L 1000µmol/L 500µmol/L 250µmol/L 125µmol/L

Figure 1. Dilution of Standards

One empty well labeled as the blank control should be included on the Microelisa strip plate. To create a dilution factor of 5, add 10 µl of the sample and 40 µl of dilution buffer to the sample wells. It's crucial to load the samples onto the well's bottom without coming into contact with the well wall. Shake gently to thoroughly combine, cover the plate with a closure plate membrane, and then incubate at 37°C for half an hour.

For a 96-well plate, dilute the concentrated washing buffer 30 times; for a 48-well plate, dilute it 20 times. This will create the washing solution. Refill the wells with the diluted washing solution after carefully removing the Closure plate membrane and aspirating the wash solution. Once it has rested for thirty seconds, remove the wash solution and carry out this cleaning process five more times.

Pour 50 µl of HRP-Conjugate reagent into every well, except the one that is left blank. As instructed in Step 3, incubate. And follow Step 5's instructions for the washing process.

50 µl of Chromogen Solution A and 50 µl of Chromogen Solution B should be added to each well. Shake lightly to combine, then let it sit at 37°C for 15 minutes. Make sure that during the coloring process, the wells are shielded from light.

To end the reaction, add 50 µl of stop solution to each well. The wells' hue need ideally shift from blue to yellow. The absorbance (OD) at 450 nm may be measured using a Microtiter Plate Reader. It is recommended to set the blank control well's OD value to zero. The test must be completed within 15 minutes of applying the stop solution.

4. Results

4.1 Total Protein

The two groups' serum total protein concentration measurements in the first month following FSH administration showed a significant difference when compared to the control group: 5.854 ± 0.429 g/dL and 7.021 ± 0.591 g/dL, respectively. The LET group and control group showed no significant difference in their respective serum total protein concentration measurements, which were 7.053 ± 0.295 g/dL and 7.021 ± 0.591, as shown in figure (2). FSH and LET differed significantly from one another.

As seen in figure (3), the levels of serum total protein in the second month of FSH and LET use were significantly different from those in the control group. They were, respectively, 6.712 ± 0.091 g /dL, 6.52 ± 0.131 g /dL, and 7.021 ± 0.591 g /dL. FSH and LET did not differ significantly from one another.

The third month's FSH measurement of serum Total Protein concentration showed no significant difference from the control group; values were 6.987 ± 0.1308 g /dL and 7.021 ± 0.591 g /dL, respectively. However, there was a significant difference between the LET group and the control group, with the LET group's value being 5.694 ± 0.2547 g /Dl, as shown in figure (4). Additionally, there was a notable distinction between FSH and LET.

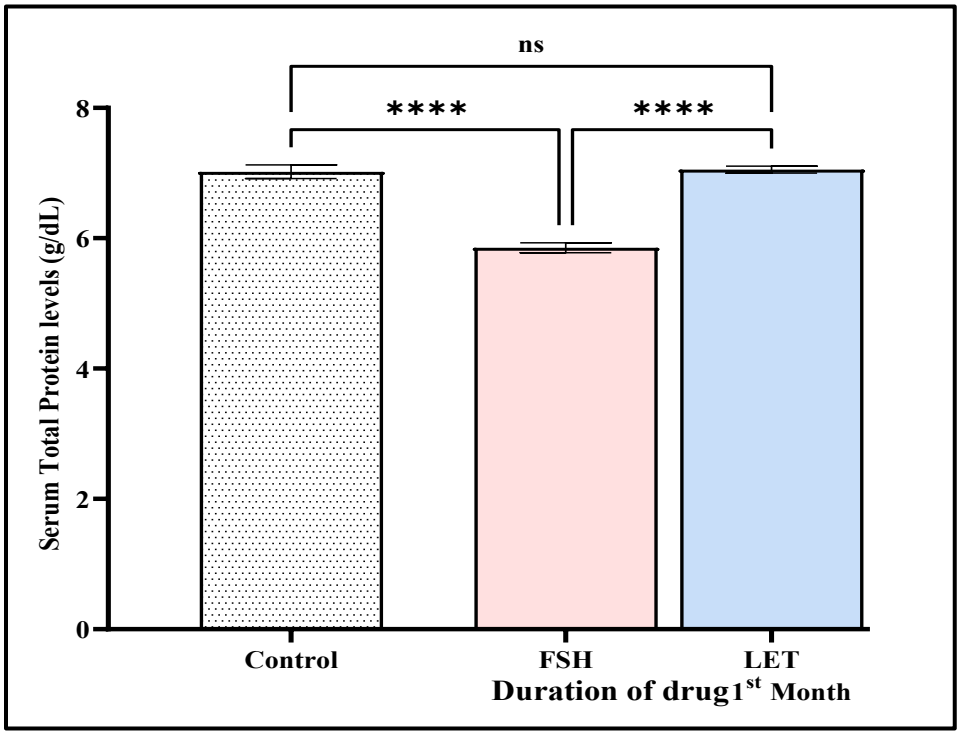


Figure 2. Estimation of serum Total Protein concentration in g/dL for the three groups in the first month.

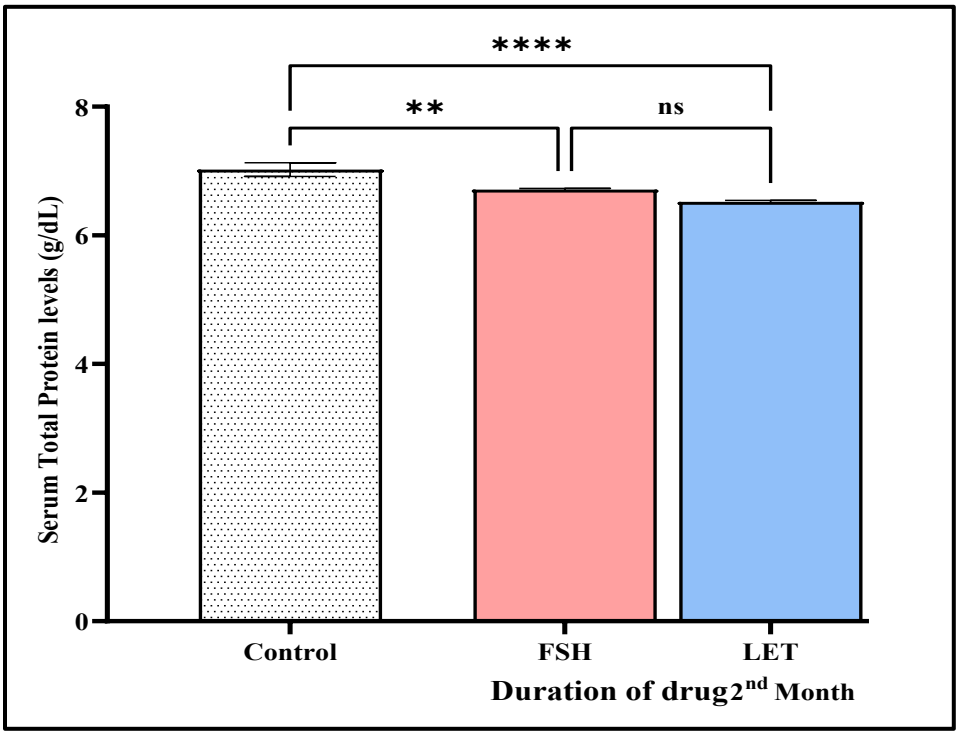


Figure 3. Estimation of serum Total Protein concentration in g/dL for the three groups in the second month.

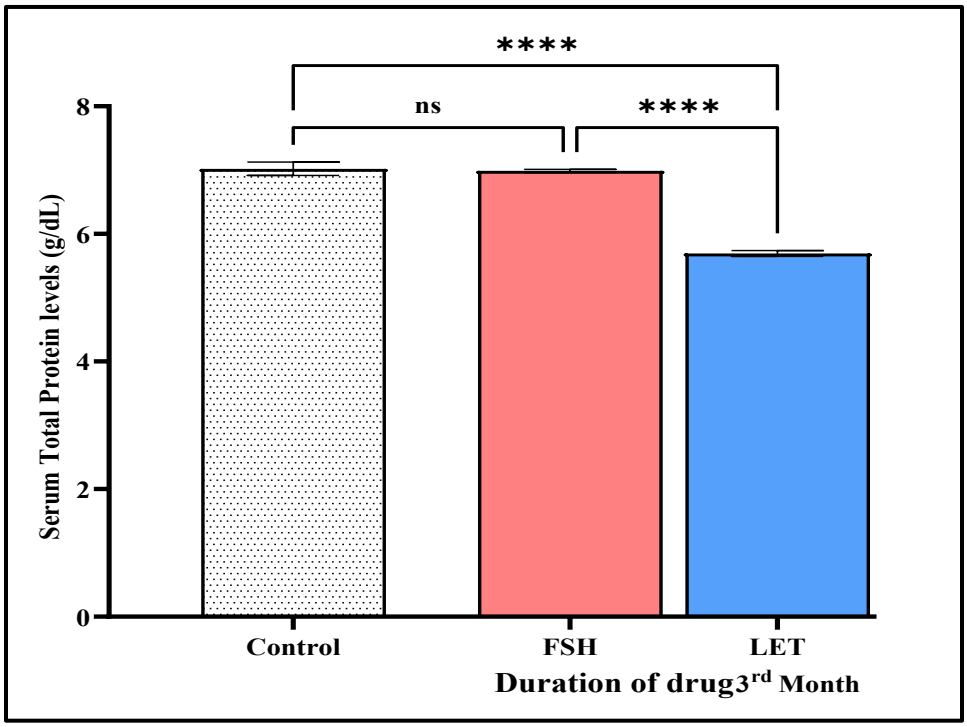


Figure 4. Estimation of serum Total Protein concentration in g/dL for the three groups in the third month.

4.2 Total Cholesterol levels (mmol/L)

As seen in figure (5), there was no discernible variation in the two groups' blood total cholesterol concentrations were measured one month following the administration of FSH and LET as compared to the control group. Additionally, there was no discernible difference between LET and FSH.

The levels of serum cholesterol in the second month of taking FSH and LET were significantly different from the control group; they were, respectively, 4.933 ± 0.347 mmol/L, 5.025 ± 0.443 mmol/L, and 4.66 ± 0.288 mmol/L, as shown in figure (6). Between FSH and LET, there was no discernible difference.

Figure (7) illustrates the considerable variation in the serum total cholesterol content measured during the third month using FSH and LET compared to the control group. The results were 5.26 ± 0.47 mmol/L, 5.29 ± 0.28 mmol/L, and 4.66 ± 0.288 mmol/L, respectively. FSH and LET did not differ significantly from one another.

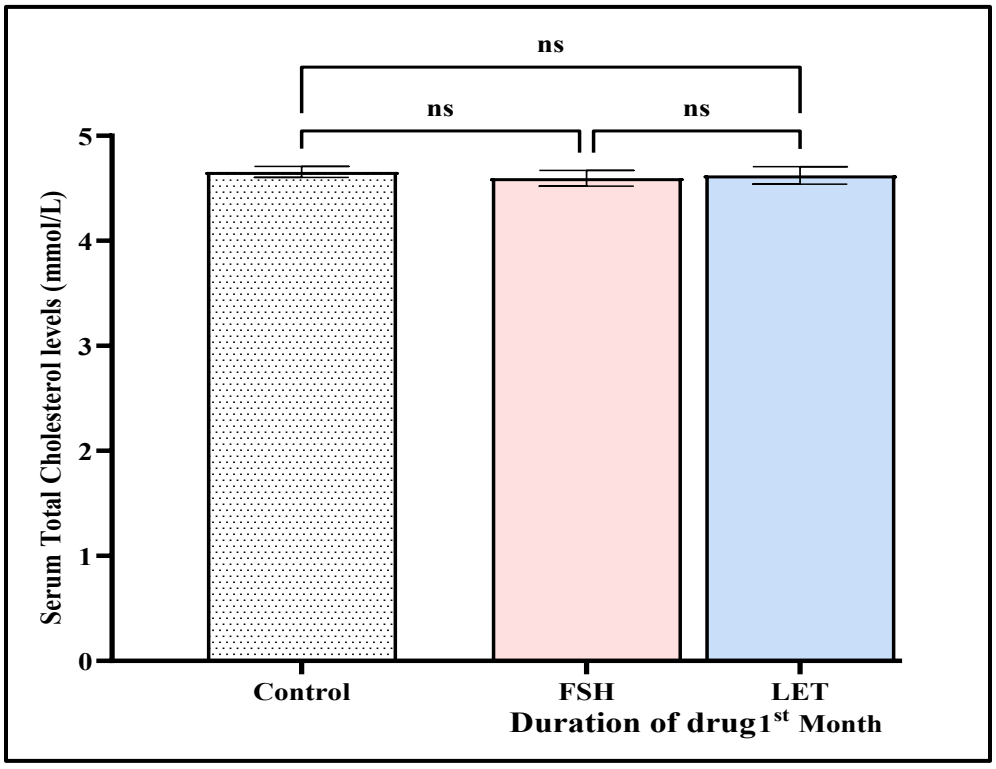


Figure 5. Estimation of serum total cholesterol concentration in mmol/L for the three groups in the first month.

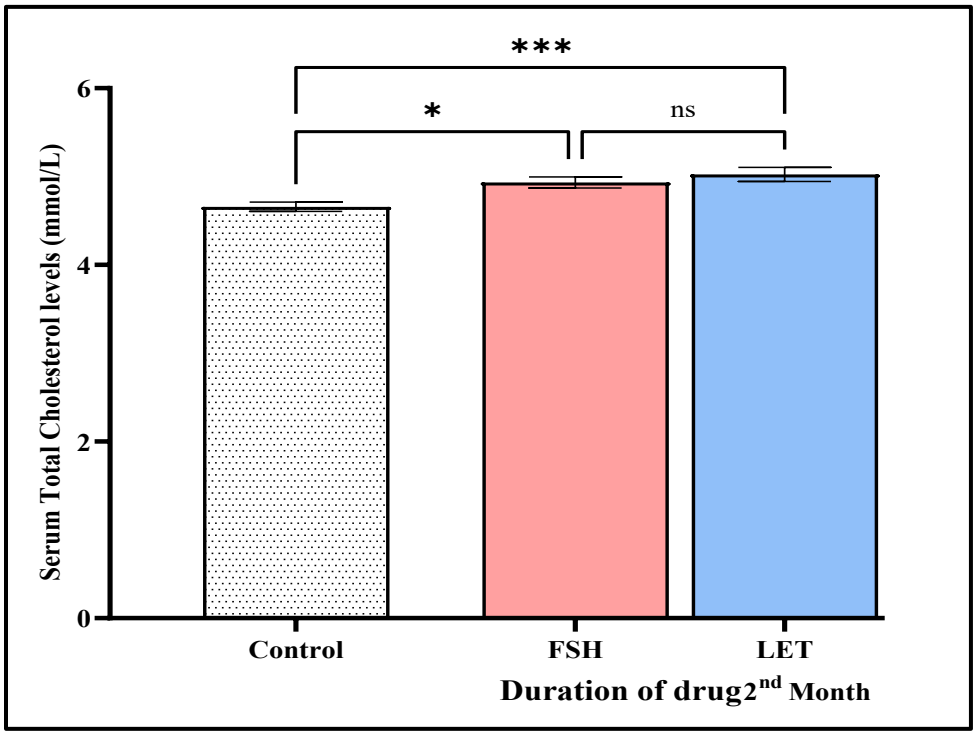


Figure 6. Estimation of serum total cholesterol concentration in mmol/L for the three groups in the second month.

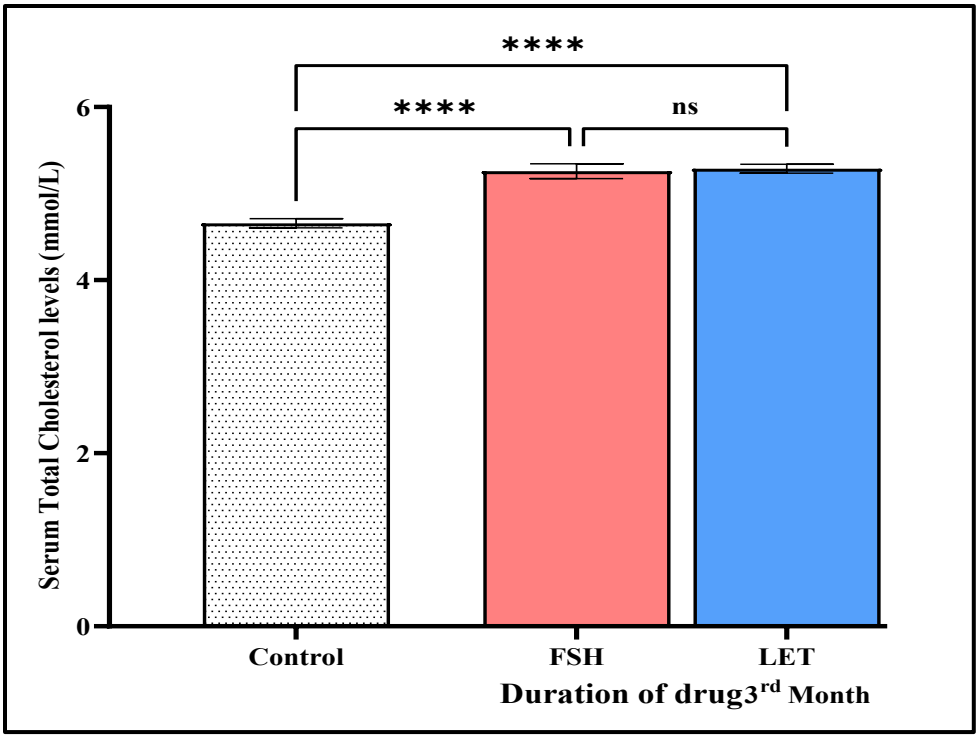


Figure 7. Estimation of serum total cholesterol concentration in mmol/L for the three groups in the third month.

4.3 LDL cholesterol

As seen in figure (8), there was no discernible variation in the two groups' blood LDL cholesterol concentrations were measured one month following the injection of FSH and LET as compared to the control group. Additionally, there was no discernible difference between LET and FSH.

During the second month of using FSH and LET, there was no significant difference between the LET and control group as shown in figure (9), but there was a significant difference between the FSH and control group's serum LDL cholesterol level, which was 3.211 ± 0.45 mmol/L, 2.89 ± 0.46 mmol/L, and 2.73 ± 0.37 mmol/L, respectively. Additionally, there was a notable distinction between FSH and LET.

Figure (10) illustrates the considerable variation in serum LDL cholesterol concentrations measured during the third month using FSH and LET compared to the control group. The results were 3.551 ± 0.5397 mmol/L, 3.288 ± 0.4787 mmol/L, and 2.73 ± 0.37 mmol/L, respectively. Additionally, there was a notable distinction between FSH and LET.

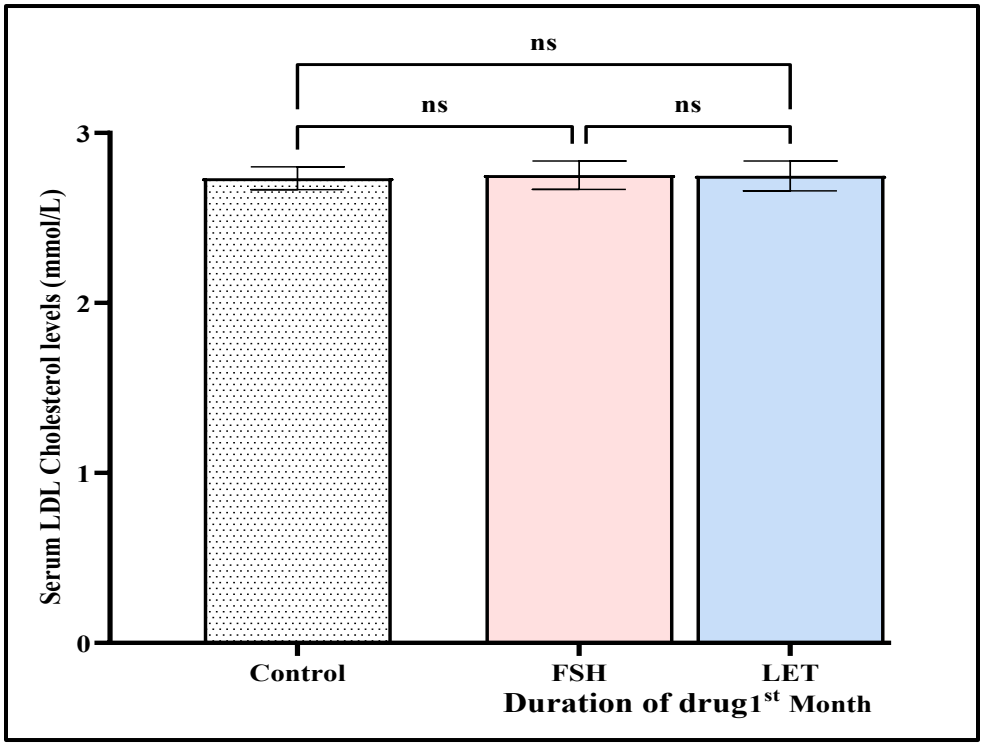


Figure 8. Estimation of serum LDL cholesterol concentration in mmol/L for the three groups in the first month.

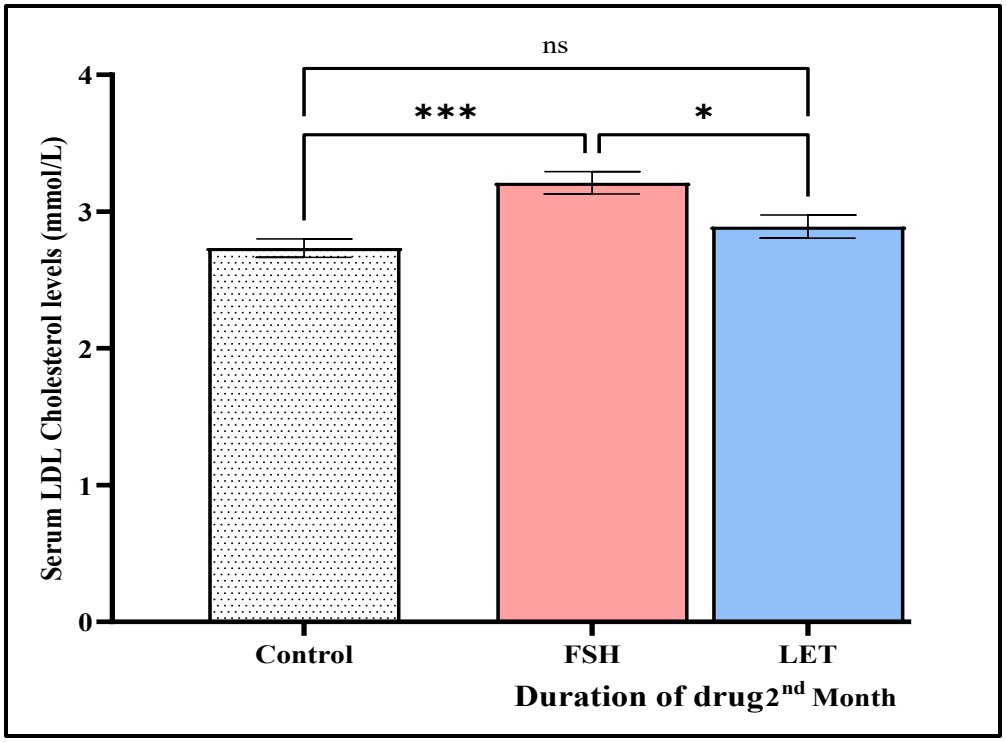


Figure 9. Estimation of serum LDL cholesterol concentration in mmol/L for the three groups in the second month.

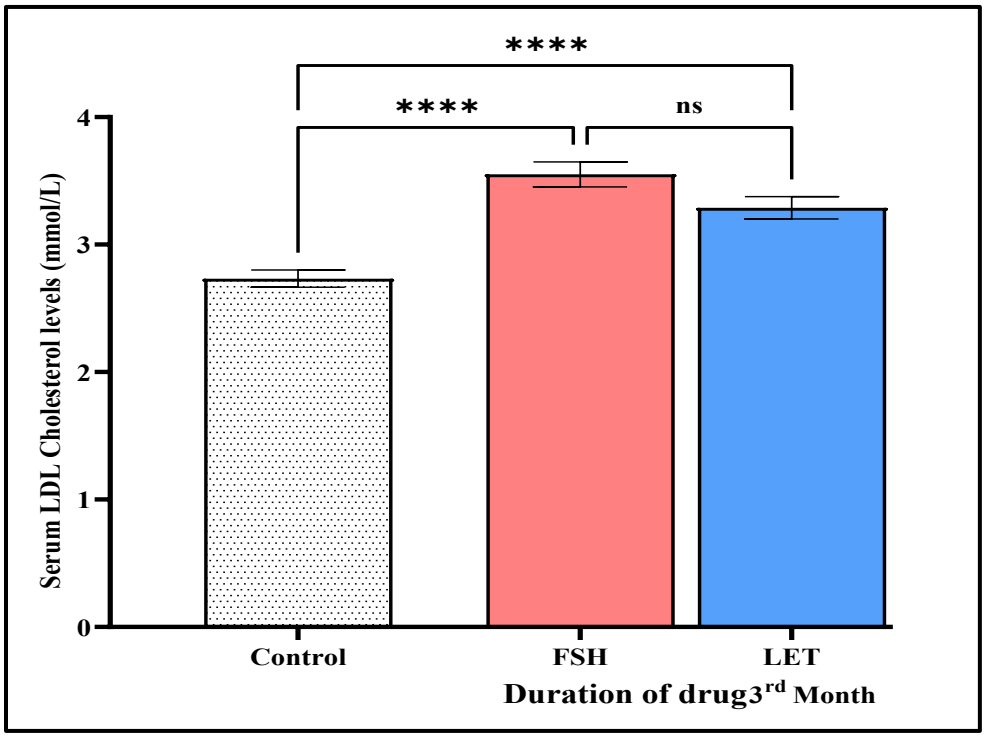


Figure 10. Estimation of serum LDL cholesterol concentration in mmol/L for the three groups in the third month.

4.4 VLDL cholesterol

As seen in figure (11), there was no discernible variation in the two groups' blood VLDL cholesterol concentrations were measured one month following the administration of FSH and LET as compared to the control group. Additionally, there was no discernible difference between LET and FSH.

During the second month of using FSH and LET, the levels of serum VLDL cholesterol were 0.322 ± 0.037 mmol/L for HFS, 0.329 ± 0.0412 mmol/L for LET, and 0.304 ± 0.0261 mmol/L for the control group, as shown in figure (12). However, there was no significant difference between FSH and the control group. FSH and LET did not significantly differ from one another.

According to figure (13), there was a significant difference in the serum VLDL cholesterol concentration measured for the third month using FSH and LET compared to the control group. The values were, respectively, 0.3705 ± 0.04449 mmol/L, 0.3712 - 0.04014 mmol/L, and 0.304 ± 0.0261 mmol/L. FSH and LET did not differ significantly from one another.

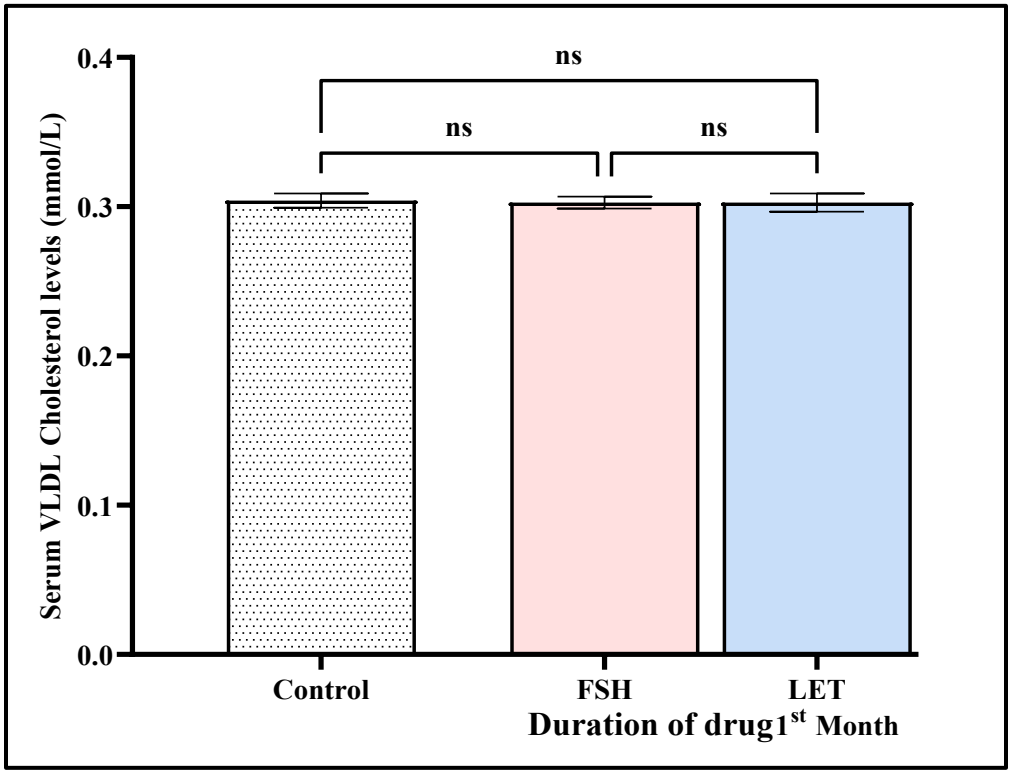


Figure 11. Estimation of serum VLDL cholesterol concentration in mmol/L for the three groups in the first month.

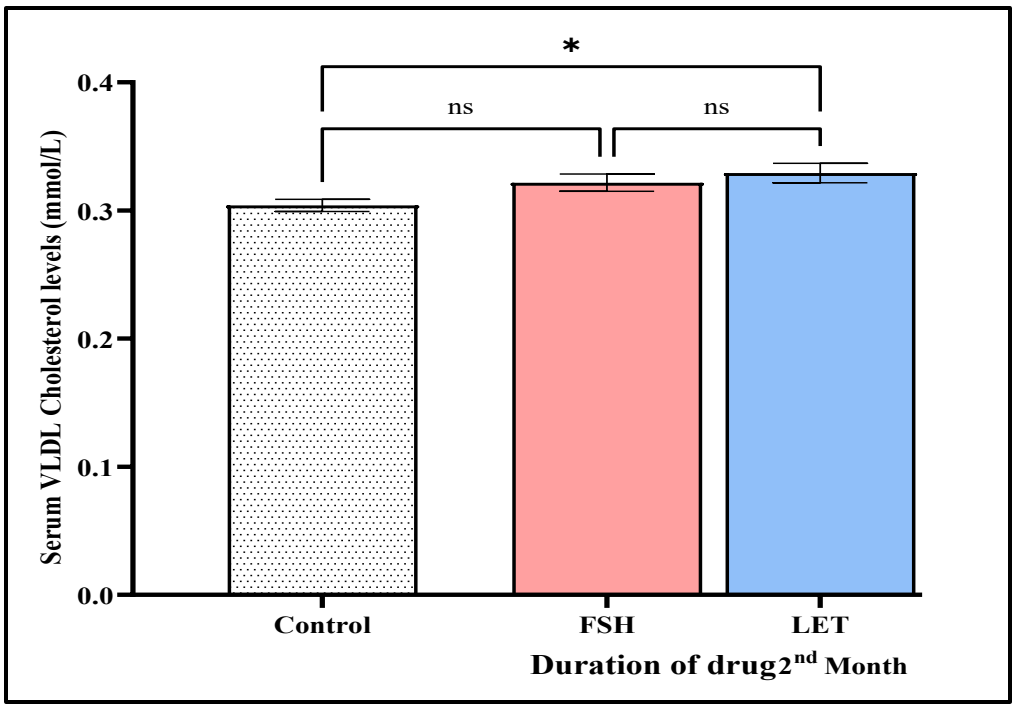


Figure 12. Estimation of serum VLDL cholesterol concentration in mmol/L for the three groups in the second month.

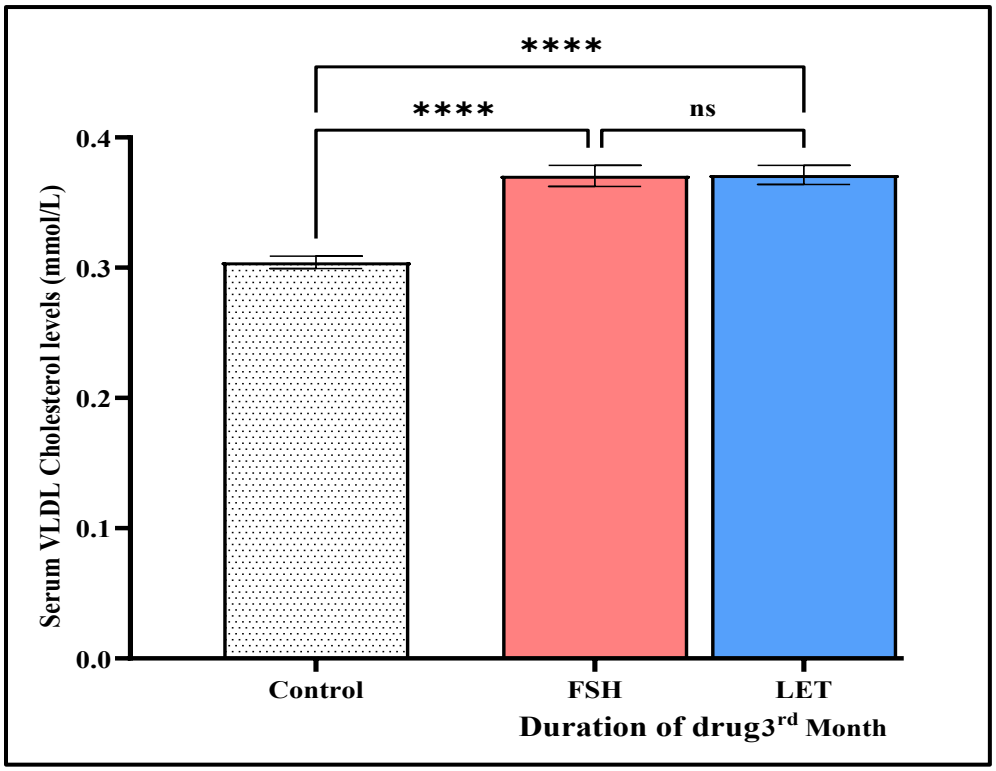


Figure 13. Estimation of serum VLDL cholesterol concentration in mmol/L for the three groups in the third month.

5. Discussion

The human body needs three basic components to ensure its functions; proteins, fats and antioxidants. For women with fertility issues, problems are often associated with these three components.

The effect of LET on protein, cholesterol, LDL, and VLDL in women may vary from person to person and depends on the individual response to the drug and individual health conditions.

In this study, it was found that cholesterol levels decrease as estrogen levels rise, especially after using clomiphene citrate for the third month of LET treatment.

In comparison to the control group, there is a substantial difference in the two groups' blood total protein concentrations measured one month after FSH and LET treatment. Additionally, there was a notable distinction between FSH and LET. The level of serum total protein differed significantly from the control group in the second month of taking FSH and LET. Additionally, there was a notable distinction between FSH and LET. When comparing the third month's serum Total Protein concentration to the control group, FSH and LET measurements show a significant change. Additionally, there was a notable distinction between FSH and LET.

Michael J. et al. found that while oral HRT regimens might increase albumin synthesis rates, there was no positive correlation between hormone replacement therapy (HRT) and total body protein turnover rates. Additionally, there was no negative correlation between ovarian hormone deficiency and the postmenopausal state [23].

In this investigation, there was no discernible difference between the two groups' blood total cholesterol concentrations measured one month after FSH and LET treatment and the control group. Additionally, there was no discernible difference between LET and FSH. The level of blood cholesterol differed significantly from the control group in the second month of taking FSH and LET. FSH and LET did not differ significantly from one another. When comparing the third month's blood total cholesterol concentration to the control group, FSH and LET measurements show a significant change. FSH and LET did not differ significantly from one another.

According to Volodymyr I. Lushchak's (2014) findings, which also indicated no correlation between high estrogen levels and hypocholesterolemia, cholesterol levels declined as estrogen levels increased, particularly during the third month of LEZ therapy when clomiphene citrate was used [24]. Inhibiting FSH signaling may be a novel strategy for treating hypercholesterolemia throughout the menopausal stage, particularly in premenopausal women who have elevated FSH injection levels alone [25]. This is according to Yangin et al. (2008).

There is no discernible difference between the two groups' blood LDL cholesterol concentrations measured one month after FSH and LET delivery and the control group. Additionally, there was no discernible difference between LET and FSH. The amount of blood LDL cholesterol differed significantly from the control group in the second month of taking FSH and LET. Additionally, there was a notable distinction between FSH and LET. When comparing the third month's blood LDL cholesterol concentration to the control group, FSH and LET measurements show a significant change. Additionally, there was a notable distinction between FSH and LET.

The present findings are consistent with the findings of Dmitry B. Zhurov et al. (2014) and Filippo Ciallo et al. (2017), who suggested that estrogen plays a crucial role in regulating cholesterol levels by quickening the degradation of low-density lipoprotein (LDL). In rat investigations, it has been discovered that pharmacological dosages of estrogen mediate LDL absorption and lower plasma LDL concentration [26][27]. According to Yang et al. (2021), FSH injection may interact with hepatocytes' LDLR receptors to

lower LDLR levels. This, in turn, may hinder LDL endocytosis, which would raise the amount of LDL in circulation [28].

There is no discernible difference between the two groups' blood VLDL cholesterol concentrations measured one month after FSH and LET delivery and the control group. Additionally, there was no discernible difference between LET and FSH. The level of blood VLDL cholesterol differed significantly from the control group in the second month of taking FSH and LET. Additionally, there was a notable distinction between FSH and LET. When comparing the third month's blood VLDL cholesterol concentration to the control group, FSH and LET measurements show a significant change. FSH and LET did not differ significantly from one another.

According to Cai et al., giving 2.5 mg/day LET for three months decreased cholesterol levels, but not VLDL-C or triglycerides [10].

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